

Incidence of Charcoal Rot (*Macrophomina phaseolina*) on Soybean in Northwestern Argentina

Incidencia de la podredumbre carbonosa de la soja (*Macrophomina phaseolina*) en el Noroeste Argentino

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RESUMEN

El hongo polífago de suelo *Macrophomina phaseolina* (Tassi) Goid es el agente causal de la podredumbre carbonosa de la soja [*Glycine max* (L.) Merr.]. Esta es una enfermedad de importancia económica en todo el mundo, aunque solo en los últimos años se ha convertido en una gran preocupación para los productores de soja en el Noroeste Argentino. Este trabajo tuvo como objetivo evaluar la incidencia de la podredumbre carbonosa de la soja en esta región de Argentina y analizar su progreso en diferentes localidades. La incidencia de la enfermedad fue evaluada desde la campaña 2008/2009 hasta la 2012/2013 en 18 lotes de soja comerciales ubicados en 11 localidades geográficamente distintas en tres provincias del Noroeste de Argentina. Durante las tres primeras campañas, el valor máximo de incidencia de la podredumbre carbonosa fue inferior al 1%, pero aumentó al 30% en la campaña 2011/2012 y alcanzó el 90% en el ciclo 2012/2013, estas últimas dos campañas caracterizadas por altas temperaturas y sequía. Las curvas de progreso de la enfermedad variaron según la localidad, presentando pérdidas importantes de rendimiento cuando el gran incremento de incidencia de la enfermedad ocurrió durante las primeras etapas reproductivas. Los valores del área bajo la curva de progreso de la enfermedad (ABCPE) variaron según la localidad y los mayores valores, 725 y 650, se presentaron en Las Lajitas y Pichanal (Salta), respectivamente, en donde la podredumbre carbonosa de la soja se detectó en etapas reproductivas tempranas del cultivo. Estos resultados confirman la importancia creciente de la podredumbre carbonosa de la soja en el Noroeste Argentino y será de utilidad para comprender mejor la interacción soja-*Macrophomia phaseolina* y, por lo tanto, desarrollar mejores estrategias de manejo de la podredumbre carbonosa de la soja en esta región.

Palabras clave: *Macrophomina phaseolina*, *Glycine max*, progreso de la enfermedad, ABCPE.

ABSTRACT

The soilborne polyphagous fungus *Macrophomina phaseolina* (Tassi) Goid is the causal agent of charcoal rot of soybean [*Glycine max* (L.) Merr.]. This is an economically important disease worldwide, though only in recent years has become a major concern for soybean farmers in northwestern Argentina. The present work was aimed to evaluate the incidence of charcoal rot of soybean in this region of Argentina and to analyze its progress in different locations. Incidence of the disease was evaluated from the 2008/2009 to the 2012/2013 growing seasons in 18 commercial fields from 11 geographically distinct locations in three provinces in northwestern Argentina. During the first three seasons, highest charcoal rot incidence was less than 1%, but increased to 30% by 2011/2012 and reached 90% in 2012/2013, in two seasons characterized by high temperatures and drought. Disease progress curves varied per location, showing important yield losses when the steep increase in disease incidence occurred during early reproductive stages. The area under the disease progress curve (AUDPC) values varied by location and the greatest values, 725 and 650, were obtained in Las Lajitas and Pichanal (Salta), respectively, where charcoal rot increased in the early reproductive stages. These results confirmed the increasing importance of charcoal rot in northwestern Argentina and will help understand better the soybean-*Macrophomia phaseolina* interaction and thus help develop improved charcoal rot management practices in this region.

Key words: *Macrophomina phaseolina*, *Glycine max*, disease progress, AUDPC.

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INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the main crops in northwestern Argentina (NWA). This region includes the provinces of Tucumán, Salta, Jujuy, Santiago del Estero and Catamarca and is located between 22° and 29° south latitude and 63° and 68° west longitude. In NWA, 950,000 ha were planted in the 2016/2017 season (Bolsa de Cereales, 2017). In Tucumán province, 203,430 ha were planted (Fandos *et al.*, 2017) with a production of 540,000 t (Pérez *et al.*, 2017).

Diseases are one of the main factors that limit soybean production. In the past 20 years disease levels have gradually increased due to the use of susceptible cultivars, lack of crop rotation, and the widespread adoption of conservation tillage systems (Ploper, 2004). Since the occurrence of severe epidemics in the 1990s and 2000s, diseases are being considered as high risk factors for soybean production in NWA (Ploper, 2011).

Charcoal rot of soybean is caused by the polyphagous fungus *Macrophomina phaseolina* (Tassi) Goid., that infects nearly 500 species in more than 100 plant families around the world (Mihail & Taylor, 1995). *M. phaseolina* has wide morphological, physiological, genetic, and pathogenic variability that allowed its adaptation to different environmental conditions and hence wide geographic distribution (Su *et al.*, 2001).

The fungus survives as hard, black microsclerotia in the soil; these fungal structures germinate between 20° and 40°C, and infect root tissue (Crous *et al.*, 2006). Soybean seedlings can be infected at emergence through the early vegetative stages, but symptoms are not typically observed until the R5 to R7 growth stages (Fehr *et al.*, 1971; Meyer *et al.*, 1974; Mengistu *et al.*, 2015). The symptoms in soybean seedlings affected by *M. phaseolina* are reddish brown lesions in the hypocotyl region that later become ash-gray and change to black discoloration (Wyllie, 1989). In mature plants the disease produces chlorotic lesions in leaves, followed by death of leaves that remain attached to the stem, and premature plant death (Ploper & Scandiani, 2009). The best diagnostic symptom is found when the epidermis is peeled away from the stem exposing numerous small, black bodies of microsclerotia that are frequently produced in the xylem and pith of the stem and may block water flow (Wyllie, 1989; Mengistu *et al.*, 2015). After soybean plants are harvested, microsclerotia in the plant residue return to the soil and survive for almost 2 year on residue at or below the soil surface. Reis *et al.* (2014) indicated that microsclerotia could survive for almost 35 months on infected crop residue in Brazil, but their pathogenicity decreased after 6 months.

Soybean plants with charcoal rot symptoms are more frequently observed in stressed areas of the field such as compacted soils, field edges and hillsides. Drought and high temperatures favor disease development (Meyer *et al.*, 1974; Mihail, 1989; Singleton *et al.*, 1992).

Charcoal rot of soybean is an economically important disease in different countries of the world and can reduce yield and seed quality (Smith & Carvil, 1997; Wyllie, 1989). Yield losses by *M. phaseolina* worldwide were estimated at 4.18% in 2006 (Wrather *et al.*, 2010). The exact measurement of yield loss conducted under field experimental plots was estimated to be 30% (Mengistu *et*

al., 2011). In Argentina, hot and dry weather conditions prevailed in the 2000/2001 growing season, which favored charcoal rot development on soybean. The provinces of Catamarca, Chaco, Córdoba, Entre Ríos, Santa Fe, Salta, Santiago del Estero and Tucumán were affected with varying levels of yield losses, including total losses in some fields (Ploper *et al.*, 2001).

The objective of this research was to analyze the incidence of charcoal rot of soybean in northwestern Argentina during five growing seasons and to analyze its progress in selected locations.

MATERIAL AND METHODS

Field evaluation of charcoal rot.

Incidence of charcoal rot on soybean was evaluated from the 2008/2009 to the 2012/2013 growing seasons in 18 commercial fields from 11 geographically distinct locations in three provinces in NWA. In Tucumán province, three fields were evaluated in La Cocha, one in Puesto del Medio, one in San Agustín, two in La Cruz and one in La Virginia. In Salta province, five in General Mosconi, one in Pichanal, one in Las Lajitas, and one in Metán, and finally in Santiago del Estero province, one in Arenales, and one in Rapelli (Figure 1). All the cultivars planted in the fields included in this study, as well as the great majority that are currently planted in the region, are susceptible to charcoal rot.

Disease incidence was evaluated every fifteen days beginning at VE stage through the R7 stage (Fehr *et al.*, 1971). A modified 'W' sampling design was used to select ten points arbitrarily in each field (Delp *et al.*, 1986). At each sampling point, 3.12 m² (two 3 m-rows spaced 0.52 m apart) were evaluated to determine the incidence of the disease (percentage of plants with charcoal rot symptoms). To confirm the diagnosis, the epidermis was peeled away from the stem to expose the small, black bodies of microsclerotia (Mengistu *et al.*, 2015).

To establish the relationship between environmental conditions and charcoal rot incidence, air temperature and precipitation data were obtained during the five growing seasons from weather stations located in three sites which are representative of the soybean area in the province of Tucumán (La Cruz located in the North of the province, San Agustín in the Center and La Cocha in the South), data provided by Sección Agrometeorología EEAOC.

In addition, disease progress was assessed at 18 locations from Salta, Santiago del Estero and Tucumán provinces during the 2012/2013 cropping season. The Area Under the Disease Progress Curve (AUDPC) was calculated using the formula of Madden *et al.* (2007):

$$AUDPC = \sum_{i=1}^n \left(\frac{x_{i+1} + x_i}{2} \right) (t_{i+1} - t_i)$$

where x_i = plant affected (disease incidence) at the i th observation, t = time (days), and n = total number of observations. Σ is the sum of all of the individual trapezoids or areas from i to $n - 1$. $i + 1$ represent observations from 1 to n .

Statistical analysis

The statistical analysis of AUDPC data was performed with the InfoStat software (Di Rienzo *et al.*, 2017), using ANOVA and LSD test at $\alpha = 0.05$ for mean comparison. Data on AUDPC were square root transformed before the statistical analysis.

The relationship between precipitation (mm) and values of charcoal rot incidence (%) was determined using a linear regression analysis ($\alpha = 0.05$) with the InfoStat software (Di Rienzo *et al.*, 2017).

RESULTS

Field evaluation of charcoal rot.

Disease incidence from 18 fields at 11 locations in NWA over a period of five growing seasons (2008/2009, 2009/2010, 2010/2011, 2011/2012 and 2012/2013) are summarized in Table 1. During the first three seasons, charcoal rot incidence remained low and did not exceed 1%. However, significant increases ($P=0.001$) in charcoal rot incidence were observed between the first three seasons (2008/2009, 2009/2010, 2010/2011) and the following two seasons, with values up to 30% in 2011/2012 and up to 90% in 2012/2013. The greatest incidence (90%) was recorded in the 2012/2013 at General Mosconi, Salta (Table 1).

Mean monthly air temperature deviations and total monthly precipitation deviations from the 30-year means at La Cocha, La Cruz and San Agustín (Tucumán) from 2008/2009 through the 2012/2013 growing seasons are presented in Table 2. Seasons with the lowest precipitation exhibited the greatest charcoal rot incidence at R7, as observed for La

Cocha (258.5 mm) and San Agustín (314.3 mm) during the 2012/2013 season, with incidences of 25% and 30%, respectively. A linear regression analysis between precipitation (mm) and values of charcoal rot incidence (%)

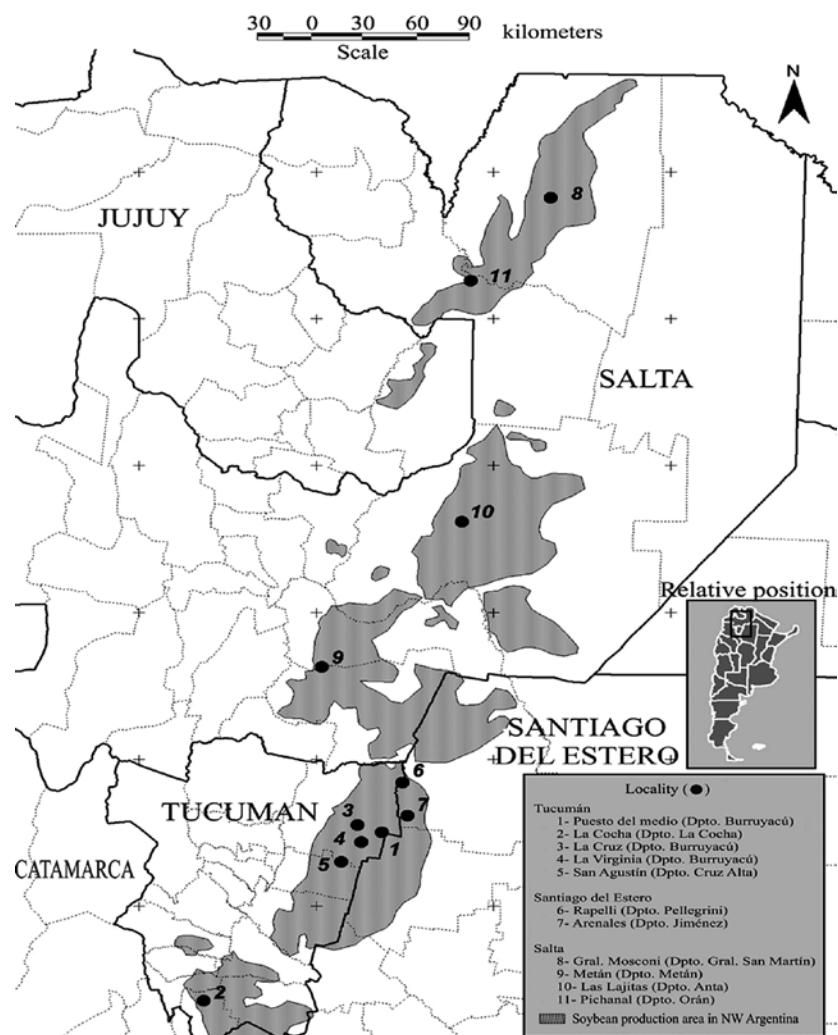


Figure 1. Location of field trials monitored for charcoal rot (*Macrophomina phaseolina*) in soybean during the 2008/2009, 2009/2010, 2010/2011, 2011/2012 and 2012/2013 seasons in northwestern Argentina.

Table 1. Charcoal root rot (*Macrophomina phaseolina*) maximum incidence values at the R7 growth stage from 18 commercial soybean fields at 11 locations in northwest Argentina in five growing seasons.

Province	Location, Department (# of fields)	Charcoal rot incidence (%) at the R7 growth stage				
		2008/09	2009/10	2010/11	2011/12	2012/13
Tucumán	Puesto del Medio, Burruyacú (1)	<1	<1	<1	5	25
Tucumán	La Cocha, La Cocha (3)	<1	<1	<1	10	25
Tucumán	La Cruz, Burruyacú (2)	<1	<1	<1	5	2
Tucumán	La Virginia, Burruyacú (1)	<1	<1	<1	5	15
Tucumán	San Agustín, Cruz Alta (1)	<1	<1	<1	5	30
Salta	General Mosconi, San Martín (5)	<1	<1	<1	30	90
Salta	Metan, Metán (1)	<1	<1	<1	5	5
Salta	Las Lajitas, Anta (1)	<1	<1	<1	10	45
Salta	Pichanal, Orán (1)	<1	<1	<1	10	40
Santiago del Estero	Arenales, Bobadal (1)	<1	<1	<1	5	20
Santiago del Estero	Rapelli, Pellegrini (1)	<1	<1	<1	5	25

Table 2. Mean monthly air temperature and total monthly precipitation at three locations in Tucuman province during five growing seasons (2008/2009, 2009/2010, 2010/2011, 2011/2012 and 2012/2013). Values of *Macrophomina phaseolina* (Mp) incidence at beginning maturity (R7) stage are also presented for each season and location.

La Cocha							La Cruz (Burruyacú)							San Agustín (Cruz Alta)						
2008/ 2009	2009/ 2010	2010/ 2011	2011/ 2012	2012/ 2013	30-year average		2008/ 2009	2009/ 2010	2010/ 2011	2011/ 2012	2012/ 2013	30-year average		2008/ 2009	2009/ 2010	2010/ 2011	2011/ 2012	2012/ 2013	30-year average	
Air temp (°C)																				
Dec	25.3	24.1	25.1	24.7	26.3	25.0	Dec	25.5	24.4	25.0	26.1	26.2	24.9	Dec	25.7	24.4	25.0	25.4	26.5	25.5
Jan	24.3	25.1	21.5	26.6	26.6	25.5	Jan	24.1	25.4	24.5	26.5	26.0	25.1	Jan	24.4	25.4	24.9	24.7	26.5	25.9
Feb	23.8	24.5	23.1	25.8	25.2	24.4	Feb	23.7	24.3	22.5	26.3	24.5	23.7	Feb	23.8	24.7	23.0	27.3	25.2	24.5
Mar	23.1	23.6	21.3	22.7	21.3	22.2	Mar	23.0	23.2	22.7	24.7	20.8	22.4	Mar	23.0	23.4	21.5	23.8	21.5	22.9
Apr	21.2	19.0	19.1	18.9	19.9	18.6	Apr	21.4	18.1	19.6	18.9	20.0	19.4	Apr	21.3	18.0	19.5	19.2	20.8	19.7
Precipitation (mm)																				
Dec	169.0	138.0	77.5	110.0	23.1	107.5	Dec	225.0	222.0	207.0	114.0	58.9	169.7	Dec	116.0	258.0	159.0	84.1	25.1	154.4
Jan	53.3	190.0	148.0	84.8	83.8	165.8	Jan	120.0	205.0	447.0	72.9	127.0	232.6	Jan	84.3	178.0	209.0	84.6	103.0	187.1
Feb	65.0	128.0	219.0	198.0	81.0	144.6	Feb	121.0	276.0	216.0	34.5	136.0	164.4	Feb	94.0	179.0	162.0	81.3	127.0	136.4
Mar	134.0	31.0	71.6	41.1	54.6	168.2	Mar	175.0	210.0	114.0	74.4	75.4	173.1	Mar	174.0	69.3	109.0	71.4	40.9	138.6
Apr	56.9	35.3	63.5	125.0	16.0	79.9	Apr	30.2	119.0	41.1	115.0	16.3	74.8	Apr	36.6	43.6	46.0	93.0	18.3	62.2
Total	478.2	522.3	579.6	558.9	258.5	666.0		671.2	1032.0	1025.1	410.8	413.6	814.6		504.9	727.9	685.0	414.4	314.3	678.7
Mp Incidence (%)																				
	<1	<1	<1	5	25		<1	<1	<1	5	2		<1	<1	<1	5	30			

indicated a significant linear correlation of $Y = -0.06x + 36.71$ ($P = 0.0061$) and $R^2 = 0.61$.

The AUDPC values from the 2012/2013 season for 18 commercial fields in 11 locations in Salta, Tucumán and Santiago del Estero provinces are presented in Table 3. The greatest AUDPC values of 725 and 650 were obtained in Las Lajitas and Pichanal (Salta), respectively, followed by General Mosconi-Plot 4 (Salta) and Puesto del Medio (Tucumán) with 470 and 255, respectively. These AUDPC values differed significantly ($P \leq 0.05$) within and between locations. The lowest AUDPC value (25) was observed in La Cruz-Plot 2 (Tucumán), significantly lower ($P \leq 0.05$) than all other sites (Table 3).

Disease progress curves and the growth stage at which disease incidence increased varied per location, but the curve shapes could be grouped in four patterns. Only one example of each of these patterns (Puesto del Medio, General Mosconi, Pichanal and Las Lajitas) are presented in Figure 2.

The disease progress data from Puesto del Medio and General Mosconi, followed an exponential model with adjusted R^2 : 0.9998 and 0.9968, and with AUDPC values of 255 and 470, respectively. At both locations, disease incidence rapidly increased after the R5 growth stage, reaching 25% and 90% at growth stage R7 for Puesto del Medio and General Mosconi, respectively. These were the maximum incidence values recorded in these locations (Figure 2). However, since the disease increased after R5, the plants were still able to produce seeds, though the seeds were smaller and lighter in weight than those from healthy plants.

Disease progress data from Pichanal and Las Lajitas followed a sigmoidal-logistic model with adjusted R^2 : 0.9929 and 1.0000, and with AUDPC values of 650 and 725, respectively. However, the steep increase of disease incidence occurred in early reproductive growth stages, R2 (full bloom) and R4 (full pod) for Pichanal and Las Lajitas, respectively. Although in both of these cases

incidence values remained below 50% at growth stage R7 (beginning maturity), symptomatic plants died prematurely during pod filling stage and did not produce grain. These results indicated that AUDPC, and also yield loss (data not shown), are more related to the growth stage in which charcoal rot increases its incidence than to the highest values of incidence reached at the R7 growth stage.

Table 3. Area under the disease progress curve (AUDPC) values for charcoal rot (*Macrophomina phaseolina*) of soybean during the 2012/2013 season from 18 soybean commercial fields at 11 locations in northwestern Argentina.

Province	Location*	Soybean genotype ^v	AUDPC ^z
Tucumán	Puesto del Medio	NA8000RG	255 d
Tucumán	La Cocha	NA7636	80 f
Tucumán	La Cocha	DM7.8	175 e
Tucumán	La Cocha	NA5909	65 fgh
Tucumán	La Cruz	NA8000RG	50 hi
Tucumán	La Cruz	NA8000RG	25 j
Tucumán	La Virginia	n.d.	80 f
Tucumán	San Agustín	NA8000RG	160 e
Salta	General Mosconi	NA8000RG	48 hi
Salta	General Mosconi	NA8000RG	70 fg
Salta	General Mosconi	NA8000RG	55 ghi
Salta	General Mosconi	NA8000RG	470 c
Salta	General Mosconi	NA8000RG	45 i
Salta	Metán	n.d.	45 i
Salta	Las Lajitas	n.d.	725 a
Salta	Pichanal	n.d.	650 b
Santiago del Estero	Arenales	n.d.	70 fg
Santiago del Estero	Rapelli	NA8000RG	160 e
p-value			<0.0001

^vnd=not determined; ^zMeans in each column followed by the same letter are not significantly different (LSD, $\alpha = 0.05$). *The listed names under location refer to the fields where disease incidence data were collected.

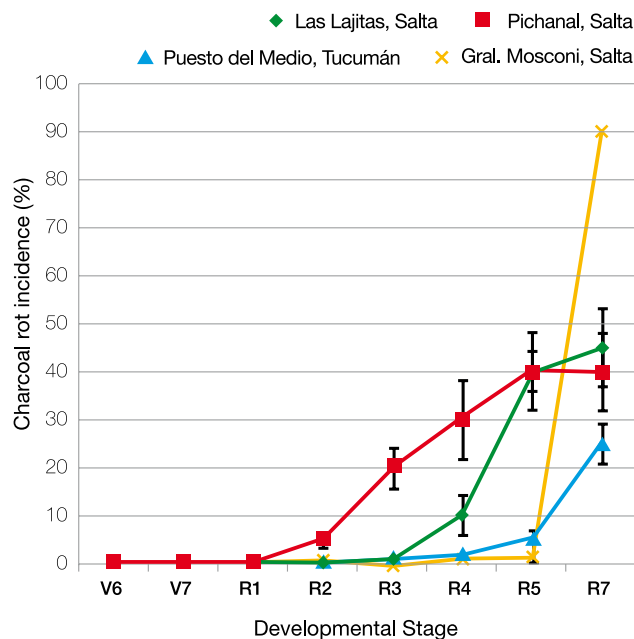


Figure 2. Charcoal rot (*Macrophomina phaseolina*) progress curves according to growth stage in soybean during the 2012-2013 season in four locations of northwest Argentina.

DISCUSSION

During the five growing seasons, there were variations in environmental parameters (temperature, rainfall, etc.) and a wide range in disease incidence. Our data indicated that disease incidence of charcoal rot in northwest Argentina was low (< 1%) during the first three seasons, but increased reaching a maximum of 30% in the 2011/2012 season, and 90% in the 2012/2013 season. There was an average of 300 mm of water deficit and an increase in temperature of 2.8°C above the 30-year average that favored disease development during the last two seasons analyzed. This indicates that charcoal rot can be an important disease in northwestern Argentina during growing seasons characterized by high temperature and drought. These results are in accordance with previous reports (Cardona *et al.*, 1998; Ploper *et al.*, 2001; Mengistu *et al.*, 2011; Mengistu *et al.*, 2013) that showed great incidence of the disease and high values of colony forming units (CFU) when high air and soil temperatures were accompanied by low water potential in soil. In Paraguay, charcoal rot has caused severe problems with a prevalence of 100% in 48 localities evaluated from April to August 2008 (Orrego Fuente and Grabowski, 2009).

Our results showed that fields at Pichanal and Las Lajitas in the province of Salta exhibited the greatest AUDPC values (650 and 725) during early reproductive growth stages (R2 and R4, respectively), and the field located in General Mosconi, Salta, showed the highest disease incidence of 90% at R7 indicating that the level of disease incidence varied between locations.

Charcoal rot of soybean was very important when the steep increase in disease incidence occurred during early soybean reproductive grown stage (R1 to R4) because symptomatic plants died prematurely during pod filling stage and did not produce grain. Our results are in accordance with Ploper *et al.* (2001) that reported total

yield losses in some fields during the 2000/2001 growing season in northwestern Argentina; season characterized by hot and dry weather conditions. When the significant increase in disease incidence occurred at or after R5, AUDPC values were lower and plants continued to produce seeds, though smaller, due to the reduced duration of the filling stage.

The charcoal rot progress data from Puesto del Medio and General Mosconi, followed an exponential model with low AUDPC values (255 and 470, respectively) than Pichanal and Las Lajitas (650 and 725) that follow a sigmoidal-logistic model. Almeida *et al.* (1994) quantified the temporal progress and spatial pattern of soybean bud blight in Brazil during two seasons using AUDPC values. On the other hand, Yang *et al.* (2015) used AUDPC values to find soybean genotypes resistances to *Colletotrichum truncatum*, the resistance genotypes have low AUDPC values than the susceptible genotypes.

This is the first study to establish the significance of charcoal rot in northwestern Argentina using epidemiological parameters. These results should be useful to understand better the soybean-*Macrophomina phaseolina* interaction and thus help develop improved charcoal rot management practices in northwestern Argentina.

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