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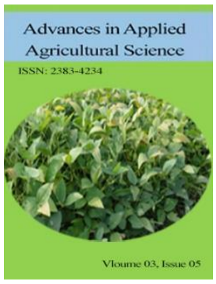

Research Article

Soybean frogeye leaf spot (*Cercospora sojina*): First economic damage threshold determination

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ABSTRACT

Frogeye leaf spot of soybean caused by *Cercospora sojina* is an economically important foliar disease in warm and humid regions. In Argentina, the severity and prevalence of FLS has increased during the last years. The main control strategies include the use of tolerant or resistant cultivars, seed and foliar fungicide treatment and crop rotation. Currently, the chemical control is the most frequent available strategy to avoid damages. In the present study, FLS epidemic progress was evaluated in a field trial with a natural intensity gradient created by combining dose of fungicide, number of applications and different application timings. The experimental design was Randomized Complete Block with four repetitions. Weekly assessments of disease severity was estimated as the average number of spots per central leaflet (NL) during eight evaluations. Area under the disease progress curves (AUDPC) and soybean grain yield were estimated. Damage functions were determined by linear regression analysis between grain yield as dependent variable (y) and FLS NL as independent variable (x) for growth stages R3, R4 and R5, for lower (L), upper (U) half of canopy and average between them (M). Equations were expressed on the basis of grain yield (Y) normalized to 1 ton/ha in the form Y = 1000 – a (NL). Where (a) is the damage coefficient (ton/lesion.ha) (Cd); Y is the grain yield normalized to 1 ton/ha, and NL is the FLS intensity (average number of lesions/central leaflet). For example, the equation obtained for R3 growth stage, Y = 1 -0,009 NL, represents a reduction of approximately 9 kg/ha per every average spot per central leaflet and for each ton of expected grain yield. The 9 equations obtained may be used to calculate the EDT for FLS in susceptible soybean cultivars. This is the first report of an EDT for FLS.

Frogeye leaf spot (FLS) of Soybean is a disease caused by *Cercospora sojina* Hara that has increased its importance in recent years, due to the expansion of soybean area, use of susceptible cultivars, conservation tillage, monoculture and favorable environmental conditions for its development (Carmona *et al*., 2009a). During the 2008/2009 season, it quickly spread throughout most of the Argentina's soy planting area, with intense attacks mainly in the provinces of Córdoba and Santa Fe. The disease was observed in almost all varieties of maturity groups III, IV and V. The incidence of affected plants in all areas of

Córdoba and Santa Fe provinces was 100%. In Buenos Aires province, incidence in the scouted fields was variable ranging from 0 (only one field) to 100%. The highest values of severity were found in fields of Cordoba with a severity equal to or greater than 30% (30-60%). In samples from Buenos Aires province, severity levels were ≤10% (Carmona *et al* 2009a). Estimated losses caused by FLS during the 2009/2010 soybean season in Argentina were about 2000 million dollars (Carmona, 2011).

Regarding FLS management, main control strategies include the use of tolerant or resistant cultivars, seed and foliar fungicide treatment and crop rotation. Use of soybean resistant varieties is the preferred measure for controlling this disease. However, it is supposed that the existence of races could change the genotype reaction. In Argentina, race 11 predominates in Buenos Aires, Córdoba and Santa Fe provinces (Scandiani *et al*., 2012). In Argentina, resistance genes were incorporated mainly in long-maturity groups because FLS initially developed in the northwest of the country (Carmona *et al*., 2010). Thus, varieties belonging to maturity groups VII and VIII are currently resistant. Approximately, 50% of the Pampas Region is planted with maturity groups III, IV and V, which are mostly susceptible (Carmona *et al*., 2010). Consequently, the only available strategy to avoid damages in these varieties is chemical control.

During the 2009/2010 season, it was observed that applications of fungicides early or late, with no epidemiological criteria, did not control FLS (Carmona *et al*., 2010). Therefore, to implement a FLS integrated management program based on scouting, resistant cultivars and epidemiological decisions for fungicide use, the economic damage threshold (EDT) must be developed. Thus, in practice, a simple model may be applied to estimate the damage caused to the host by a specific disease in function of the host's phenologic stage and disease intensity. The aim of the present study was to develop an EDT to guide the application of fungicides in soybean crops to manage FLS in Argentina.

Materials and Methods

A field trial was conducted during the 2009/2010 season at El Trébol in Santa Fe province. A FLS-susceptible soybean cultivar (NA4990RG) was planted with an inter-row spacing of 42 cm. A natural gradient of FLS intensity was created by combining dose of fungicide and number of applications at different times (Casa *et al*., 2009; Carmona *et al*., 2009b, 2012). Applications of trifloxystrobin + cyproconazole, including mineral oil at doses of 300 cm3/ha, started at first FLS symptoms (one lesion per leaflet). The ten treatments resulted from the combination of: a) fungicide dose (cm3/ha): 75, 150, 300; b) number of applications: 0, 1, 2 and 3 and c) application timing: R2 (05/02/2010), R4.5 (25/02/2010), R5.5 (17/03/2010), as follows: T1 = untreated control; T2 = first application when first symptoms were observed at R2, second application 20 days after first application, at R4.5 and third application after 20 days second application (R5.5), using half the recommended dose (75 cm3/ha); T3 = one application at R4.5 with half recommended dose (75 cm3/ha); T4 = two applications at R4.5 and R5.5 with half the recommended dose (75 cm3/ha); T5 =three applications at R2, R4.5 and R5.5 with recommended dose (150 cm3/ha); T6 =one application at R4.5with recommended dose (150 cm3/ha); T7 = two applications at R4.5 and R5.5with recommended dose (150 cm3/ha); T8 = three applications at R2, R4.5 and R5.5 double recommended dose (300 cm3/ha); T9 = one application at R4.5 double recommended dose (300 cm3/ha); T10 = two applications at R4.5 and R5.5 double recommended dose (300 cm3/ha). The experimental design was Randomized Complete Block with four repetitions. Experimental unit consisted of one plot (10 m x 2,5m). In order to confirm the causal agent of the lesions observed in the field, conidia from sporulating lesions obtained from 25 leaflets randomly collected, were cultured on autoclaved potato dextrose agar (PDA) medium. Inoculated PDA medium was incubated at 25 °C with a 12h photoperiod under NUV (320–420 nm, 36•5 μmol m−2 s−1) supplied by two 40 W black light fluorescent tubes positioned 50 cm. Isolates obtained were seeded into the centers of petri dishes (9 cm in diameter) containing PDA medium and incubated in a growth chamber at 25°Cunder permanent NUV light for 20 days. The cultures were observed microscopically identifying isolates based on their micromorphological characters. Lesion size was quantified by measuring with precision rule the diameter of the lesions from 5 to 6 central leaflets of the top and bottom half of the canopy separately. The diameter of 50 randomly selected spots were averaged. Disease severity was estimated as the average number of lesions per central leaflet (NL), differentiating between upper (U) and lower (L) half of the canopy. Weekly assessments of disease severity were performed on central leaflets of all leaves from the main stem excluding branches of 10 fixed plants (identified and marked with seals), in the three central lines of each experimental unit. Eight evaluations were performed in R3 (12/02/10), R4 (19/02/10), R5 (01/03/10), R5.5 (08/03/10), R6 (16/03/10), R6.5 (22/03/10), R7 (29/03/10) and R8 (09/04/10). Area under the disease progress curves (AUDPC) was calculated (Vanderplank, 1963). Soybean grain yield was estimated by harvesting the four central rows of each plot by eight meters long using an experimental combine. Statistical analyses were carried out using INFOSTAT software (InfoStat/Professional version 1.1, Universidad Nacional de Cordoba, Cordoba, Argentina). Data were analyzed using ANOVA and differences between means were tested using a post-hoc Tukey test. Damage functions were estimated by linear regression analysis between grain yield as dependent variable (y) and FLS severity (average number of spots per central leaflet) as independent variable(x) for growth stages R3, R4 and R5. Equations were expressed on the basis of grain yield (Y) normalized to 1 ton/ha in the form Y = 1000 – a (NL). Where "a" is the damage coefficient (ton/lesion.ha) (Cd); Y is the grain yield normalized to 1 ton/ha, and NL is the FLS intensity (average number of lesions/leaflet). The economic damage threshold (EDT) was calculated using the Mumford & Norton (1984) formula modified for diseases (Casa *et al*., 2009; Carmona *et al*., 2012): EDT = [Cc/(Pp*Cd)]*Ec; where EDT = disease intensity (average number of lesions per central leaflet); Cc = cost of control (USD/ha); Pp = soybean price (USD/ton); Cd = damage coefficient (calculated based on the potential yield); Ec = control efficiency of fungicide used estimated based on fungicide fields trials (Dashiell & Akem, 1991; Mwase & Kapooria, 2000), equal to 70%. To determine the action threshold (AT), a decrease of 20% of EDT obtained was proposed (Carmona *et al*., 2012).

Results

All isolates were identified as C. sojina. FLS was expressed at levels that were considered epidemic (up to 99 spots per leaflet in average, in the upper half of the canopy). Both the seeded soybean cultivar and the environmental conditions contributed to the occurrence of disease. Meteorological variables recorded in the field trial showed a close link with those described as key to the requirements of infection and survival of *C. sojina* (Phillips, 1999). Soybean genotype NA4990RG proved susceptible allowing to detect differences in the number of lesions and grain yield between treatments. The average lesion size in the untreated control and in most of the treatments reached values 2-3 mm (in the lower half of the canopy the control came to 3.3 mm in the evaluation at R5). It was noted that at the end of the epidemic the FLS intensity (AUDPC) was always higher in the control plots in relation to any chemical treatments performed ($p < 0.05$). The mixture of trifloxystrobin + cyproconazole in various doses and times resulted in progressively reduced final disease severities when compared with untreated control plots. The lowest AUDPC value for the average canopy halves represented only between 12.5 to 14% compared to the control (AUDPC values of 209 and 240 in treatments 5 and 8, vs. 1672 in the control). Damage caused by the FLS were estimated by the decrease in yield by comparing the difference between the average of the treatments applied with fungicide and the untreated control being between 176-626 kg/ha, representing a loss of between 56 to 200 USD/ha (reference value 320 USD soybean Tn).

Damage functions, EDT and AT (R3, R4, R5) are shown in Table 1. The thresholds are always comprised within the critical period for grain yield determination (in soybean between R3-R5), allowing integration of the fungicide, the life of the host and epidemiological control criteria. According to the damage function Y [Tn/ha] = - 0.0452 x + 4.97, obtained in this work for the average leaf strata at the R3 growth stage (Table 1, column 3), per every average spot per central leaflet in the main stem a decreased of 45.2 kg/ha is produced for a potential grain yield of 4970 kg/ha. When this equation is adjusted to 1 Tn is expressed: Y = 1 - 0,009 NL (Table 1). This represents a reduction of approximately 9 kg/ha per every average spot per central leaflet and for each ton of expected grain yield. For example, a field with a potential grain yield of 4 tn/ha, cost of fungicide application equal to 25 USD/ha and a value of a ton of soybean equal to 320 USD/ton, fungicide should be applied on susceptible cultivars when field scouting observations for FLS detects an average value of 1.20 spots per central leaflet throughout the foliar stratum of the crop. It is noteworthy that the obtained EDTs in this study are just an example, calculated with the values previously indicated. EDT value will change depending on the potential grain yield, cost of fungicide application and soybean price.

Discussion

The coefficients of determination ranged 0.45-0.71 ($p < 0.05$), indicating a linear relationship between grain yield and disease severity. The EDT varied according to growth stage and half of the canopy considered (Table 1). Generally, the upper half of the canopy showed a lower EDT when compared to the lower, probably because the young leaves that generally are in the top have an increased susceptibility to FLS and the fungicide should be applied a little earlier. Nevertheless, the differences between EDTs and between canopy layers are not significant. Whereas the efficiency of chemical control should not allow the disease exceeds the UDE, the application must be made in advance, because both control implementation and fungicidal action, demands time (Zadoks & Schein, 1979). It is therefore proposed an AT 20% lower than EDT, based on previous experiences in other pathosystems (Carmona *et al*., 2009b, 2012).

Table 1. *Damage function and Economic Damage Threshold for FLS during 2009/2010 soybean season in Santa Fe, Argentina.*

	R3			R4			R5		
	L	U	M	L	U	M	L	U	M
Damage function	y = -0,0348x + 5,0259	y = -0,058x + 4,867	y = -0,0452x + 4,9791	y = -0,0271x + 4,923	y = -0,0492x + 4.8344	y = -0,0372x + 4,908	y = -0,0306x + 5,0013	y = -0.0317x + 4.8042	y = -0.0319x + 4.9114
R^2	0,4584	0,5714	0,5201	0,5788	0,7117	0,6658	0,6285	0.6855	0.6735
p	0,0377	0,0244	0,0339	0,0167	0,0032	0,0088	0,0123	0.0056	0.0065
Damage Function adjusted to 1 Tn	Y = 1 – 0,0069 NL	Y = 1 – 0,0011 NL	Y = 1 -0,009 NL	Y = 1 -0,0055 NL	Y = 1 –0,01 NL	Y = 1 -0,0075 NL	Y = 1 -0,0061 NL	Y = 1 -0,0065 NL	Y = 1 -0,0064 NL
EDT	1,97	1,15	1,51	2,48	1,34	1,80	2,23	2,07	2,10
AT	1,58	0,92	1,20	1,99	1,07	1,44	1,79	1,66	1,68

Abbreviations: determination coefficient (R2), level of significance (p), economic damage threshold (EDT) and action threshold (AT) for a soybean crop with a 4 ton/ha potential grain yield, soybean price of 320 USD/ton, control cost of 25 USD/ha, efficiency of control equal to 70%, for lower (L), upper (U) and average between L and U (M) half of canopy. Calculations were performed for the reproductive phenological stages R3, R4 and R5 because are related with the critical period for grain yield determination in soybean.

The maximum EDT achieved was 2.48 average spots per central leaflet with an AT of 1.99 spots. The lowest value was 1.15 and 0.92 for the EDT and AT respectively (Table 1). In practice, the values of AT are the maximum economically tolerable disease prior to the application of fungicides. These values appear to be low to proceed with the fungicide application, but given the FLS epidemiological characteristics, damage and loss caused, they are probably adequate to manage this disease. The EDT should never be exceeded throughout the crop season. In this case, the application of fungicides is justified, because its benefits far outweigh the costs of its use.

Optimal fungicide application timings for FLS control were studied in other countries but based only on the phenological stage of soybeans. In these cases fungicides are applied depending on the phenological growth stages, without considering the level of severity of this disease. For example, Akem (1995) determined that fungicide applications from R1 to R3 reduced FLS severity and increased grain yield significantly. This is the reason for which this author recommends to begin with applications on susceptible cultivars from R1. This phenology-based criterion has widely been presumably adopted due to the lack of technical information on fungicide application timing based in EDT, and the ease of implementation, as it requires no disease scouting or diagnosis. In these cases it could happen that applications are unnecessary if the disease does not occur, generating not only environmental impact but also a decline in economic return and an increased selection pressure towards resistant strains (Zhang *et al.*, 2012; Standish *et al.*, 2014). It could also happen that the time of application based solely on the phenology not be correct because the levels of disease already present are high, and therefore the application is late and not achieve the expected results. Thus, clear understanding of host phenology and the critical period when grain yield is defined is very important (Owen *et al.*, 2013), but should not be the only information to decide on fungicide applications (Carmona *et al.*, 2015). Consequently, the principle of EDT as an indicator of the beginning of the sprays may constitute the cornerstone of integrated disease management because it involves both economic and epidemiological aspects.

Therefore, fungicide applications should be conducted in a sustainable and cost effective manner. This implies that EDTs and ATs are not fixed or rigid but variables depending on changes in the original factors that are used to calculate them (potential grain yield, soybean prices, application cost and efficiency of control). To the best of the authors' knowledge, this is the first report of an EDT for FLS.

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