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Development and validation of a fungicide scoring system for management of late season soybean diseases in Argentina

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ABSTRACT

The use of foliar fungicides is a common disease control practice among soybean producers around the world, yet there is still no clear understanding about the timing and opportunity of fungicide applications to manage late season diseases (LSD) in soybean crops. The unnecessary use of fungicides in extended areas increases production costs, risk of resistance and risk of negative environmental impact. The objective of this study was to develop and validate a scoring system to guide the application of fungicides in soybean crops to manage LSDs in Argentina, with special reference to Septoria brown spot and Cercospora leaf blight. To develop the scoring system, a risk matrix with weighted epidemiological risk factors was developed based on previous research data. The scoring system recommends application of foliar fungicides based on the total points accumulated from the risk factors. Scoring greater than 33 indicates a higher probability of obtaining a positive yield response, whereas scoring below 23 indicates no expected response and thus no need for fungicide applications. Intermediate values indicate that the application of fungicides would produce uncertain results thus detailed analysis of risk factors would be required. To validate the scoring system, 19 field trials were carried out over five growing seasons in three Argentinian provinces. The fungicide used in all trials was a mixture of a quinone outside inhibitor and a demethylation inhibitor fungicide. In most cases, the scoring system resulted in appropriate decisions to apply the fungicide within the so-called "window of opportunity", which lies between the R3 and R5 soybean developmental stages. The greatest yield responses were achieved when the scoring system recommended the fungicide application at R3 or R4 or R5, depending on the obtained sum of points. In all cases, except when the scoring system recommended no application of the fungicide, disease severity values were significantly greater in untreated than in treated plants. Regarding net income, phenology-based applications showed negative margins in cases where the scoring system recommended no applications, demonstrating that in such situations the use of fungicide caused losses rather than yield advantages. In contrast, when the scoring system recommended the application of fungicide regardless of timing (R3, R4 or R5), net margins were always positive and generally provided a higher income. The scoring system presented in this study can be a valuable tool to reduce the number of fungicide applications in soybean crops, especially in seasons when conditions for LSD development are not favorable.

1. Introduction

Late season soybean diseases (LSDs), a combination of various diseases that affect soybean leaves, stems, pods and seeds, cause premature senescence and reduce grain yield and seed quality (Hartman et al., 1999; Wrather et al., 2004; Sweets et al., 2008). In the Pampean Region of Argentina, the main LSDs are Septoria brown spot (*Septoria glycines* Hemmi), Cercospora leaf blight and purple stain (*Cercospora kikuchii* T. Matsumoto & Tomoyasu), pod and stem blight (*Phomopsis sojae* (Lehman) Wehm) and anthracnose [*Glomerella glycines* (Hori) Lehman & Wolf; *Colletotrichum truncatum* (Schw.) Andrews & WD Moore], with *S. glycines* and *C. kikuchii* being the prevalent pathogens (Carmona et al., 2010).

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Severity of LSDs has increased in recent years in Argentina, mainly due to soybean monocropping and conservation tillage (Wrather et al., 2010). This has led to an increased fungicide use to reduce LSD damage (Carmona, 2011; Carmona et al., 2011b). Although the use of fungicides is a common disease control practice in soybean worldwide, there is still no clear understanding about the right application timing to manage LSDs (Martins, 2003; Ortiz-Ribbing et al., 2008; Bestor, 2011). Currently, not only in Argentina but also in many other soybean producing countries, fungicide applications for control of LSDs are based on varying criteria, the most common being the application according to a fixed crop growth stage, typically between R3 and R5 (Fehr and Caviness, 1977; Díaz et al., 2005; Schneider et al., 2008; Swoboda and Pedersen, 2009; Bestor et al., 2010; Cruz et al., 2010; Soto-Arias and Munkvold, 2011; Allen, 2012; Chanda et al., 2014). This phenology-based criterion, has widely and presumably been adopted due to the lack of technical information on fungicide application timing (Ward et al., 2013) and the ease of implementation, as it requires no disease scouting or diagnosis (Reis, 2013). Clear understanding of host phenology and the critical period when grain yield is defined is very important (Board et al., 2011; Owen et al., 2013), but should not be the only information to decide on fungicide applications. The main effect of a fungicide occurs when it interacts with the target fungus. Therefore, the criterion guided by crop phenology is inconsistent when considered alone. Environmental variables, especially the occurrence and amount of rainfall from R3 to R5, are crucial to define yield response when fungicides are used to control LSDs (Fitt et al., 1989; Carmona et al., 2010, 2011a).

One of the most important aspects to consider when developing a scoring system for LSD management is the length of the incubation and latent phases of the disease (Kulik, 1984; Sinclair, 1991; Larran et al., 2002; Klingelfuss and Yorinori, 2001). LSD symptoms become evident at the end of the soybean growing season (mainly at R6-R7), when fungicide applications rendered late (Klingelfuss and Yorinori, 2001; Chanda et al., 2014); yield components are normally defined between R3 and R5 (Board et al., 2011; Egli, 1997; Jiang and Egli, 1993, 1995; Owen et al., 2013). Unlike what happens with diseases that have short incubation and latent periods, such as Asian soybean rust (*Phakopsora pachyrhizi*) and soybean frogeye leaf spot (*Cercospora sojina*), with LSDs there is low correlation between symptoms and severity at growth stages where fungicides should be applied.

To date, no practical approach has yet been developed anywhere to apply fungicides based on severity of LSDs. The prevailing approach is based on crop phenology (Hoffmann et al., 2004; Reis, 2013). In the U.S.A., protocols to determine the timing to control *C. kikuchii* are still being developed (Ward et al., 2013).

The aforementioned difficulties determine the need for an efficient scoring system to predict LSD development in the field without relying solely on visual symptoms. In consequence, fungicides are used only during the critical period when the crop must be protected against LSD epidemics. The key in the development of a reliable scoring system is to determine environmental and agronomic conditions that are conducive to severe LSD epidemics, thus to avoid unnecessary fungicide applications (Stuckey et al., 1984; TeKrony et al., 1985).

Last, one of the important benefits of using a rational scoring system for management of LSDs would be to avoid or delay the development of pathogen resistance to fungicides (Mueller and Bradley, 2008; Cruz et al., 2010). Fungicide resistance is one of the most important issues in modern agriculture (Deising et al., 2008) and has been increasingly reported in recent years in soybean crops (Zhang et al., 2012; Xavier et al., 2013; Zeng et al., 2014).

The objective of this study was to develop and validate a scoring system to guide the application of fungicides in soybean crops to manage LSDs in Argentina. Special reference is made to Septoria brown spot and Cercospora leaf blight.

2. Materials and methods

The scoring system was developed from preliminary work reported by Carmona et al. (2011a,b), Carmona and Reis (2012) and Carmona (2013), and was based on the impact of weather, disease pressure and other factors useful to estimate the probability of expected next return to fungicide treatment. In order to generate the scoring, the relative contribution of each factor was weighted according to their epidemiological impact on LSDs. A score or risk value between 0 and 10 was then assigned. The process was based mainly on our previous research and field experience in fungicide trials (Carmona and Reis, 2009, 2012; Carmona et al., 2010, 2011a,b; Reis, 2013; Smirnoff et al., 2013, 2014), as well as on literature on scoring systems for soybean (Backman et al., 1984; Stuckey et al., 1984; TeKrony et al., 1985; Malvick, 1998; Hoffmann, 2002; Martins, 2007) and on LSDs (Backman et al., 1979; TeKrony et al., 1983; Kulik, 1984; Franco Neto and West, 1989; Sinclair, 1991; Schuh, 1993; Wrather et al., 1993, 2004, 2010; Guerzoni, 2001; Klingelfuss and Yorinori, 2001; Larran et al., 2002; Martins, 2003; Hoffmann et al., 2004; Díaz et al., 2005; Cruz, 2008; Swoboda and Pedersen, 2009; Cruz et al., 2010; Bestor, 2011; Grichar, 2013; Kyveryga et al., 2013). Based on the compiled and examined data, it was found that the most important risk factors for LSD development for the Argentina soybean production areas are rainfall (amount and type) between R3 and R5 growth stages; inoculum source (particularly, infested residue from soybean monoculture) and detection of LSDs (symptoms) during early crop stages. As discussed throughout the manuscript, decisions regarding LSD management should be based on risk analysis and thus the scoring for each epidemiological factor was fixed according to the impact of weather, disease pressure and other factors, in order to estimate the probability of expected net return to fungicides treatment.

From all the agronomic and weather variables identified, ten were selected for the Argentina soybean growing conditions. Those factors determine greater risk related to the epidemiology of LSDs:

A) Rainfall (both occurred and predicted) between R3 and R5: This is one of the most important factors because it is related to the frequency and duration of leaf wetness, as well as to fungal spore release, dispersal, germination, and penetration (Fitt et al., 1989). To determine this factor, the accumulated rainfall from R3 to R5 is calculated. Then the risk rating for rainfall was assigned considering the mm of rain that are associated to the response to fungicide application at R3 or R5, as previously described in detail in work by Carmona et al. (2011a).
B) Intensity of rainfall between R3 and R5: Rain level above a 7-mm threshold (excluding drizzle and light rain) could contribute to producing horizontal and vertical conidial dispersion from pycnidia of *S. glycines* and to ensuring the wetness needed for infection by *Cercospora* conidia (Schuh, 1993; Carmona et al., 2010). The risk of this factor was assigned based on the > 7-mm rain threshold that is associated with the LSD severity estimated at R7, according to the results of previous research (Carmona et al., 2010).
C) Crop rotation: Fields rotated with LSD non-host crops have lower inoculum density than fields in monoculture (Hoffmann et al., 2004). Risk rating for crop rotation was assigned from data by Smirnoff et al., 2013, 2014, which indicates damage and premature senescence due to LSDs in monoculture.

D) Tillage system (conventional tillage or no-tillage –direct seeding): LSDs are caused mostly by necrotrophic pathogens that survive in crop residue. The more crop residue accumulated on the soil surface under conservation tillage the higher the risk for LSD epidemics (Wrather et al., 1993; Hartman et al., 1999).

E) LSD presence in soybean crop residue from previous season: This is an indirect way to estimate the source of inoculum for the previous cropping season.

F) Sanitary quality of the seed: Pathogens that are associated to seed and that can be transmitted to seedlings are considered (Wilcox, 1973; Walters, 1980; McGee, 1983; Franca Neto and West, 1989; Hartman et al., 1999; Dalbir Singh and Mathur, 2004). Health testing or seed treatment can reduce the probability of introducing or increasing pathogens that cause LSDs when seed is infected with causal pathogens of LSD.

G) Length of growing season: In the studied region, most planted soybean crops are genotypes with different maturity groups and varying crop cycle length. The longer the crop cycle the greater the probabilities of a crop to undergo LSD epidemics. For this factor, the assigned score increased according to the length of the growing season (longer seasons = greater score). This is a low-weight item in the system in comparison with rainfall or monoculture.

H) Grain destination: If the harvested seed is intended for sowing then disease control should be prioritized as to improve seed health quality (Soto-Arias and Munkvold, 2011).

I) Field potential productivity: The average yield of each field in seasons without adverse weather conditions or other abnormal factors should be considered. Yield potential measurement refers to the ability of the field to allow economic return after a fungicide application.

J) Presence of LSD symptoms in the field: some of the LSDs such as *S. glycines* and *C. kikuchii* may develop symptoms earlier in the growing season, even at vegetative stages (Sinclair, 1991). These symptoms pre-announce the epidemiological triggering of LSDs. The visual presence or absence of LSDs is valuated as a risk factor, which is an indicator that the inoculum source is present in the field. LSD symptoms must be observed during scouting.

2.1. Validation of the scoring system

To validate the generated scoring system, 19 field experimental trials were carried out during the 2007/2008 (5 trials), 2008/2009 (4 trials), 2009/2010 (4 trials), 2010/2011 (4 trials) and 2011/2012 (2 trials) seasons in the Argentina provinces of Buenos Aires, Entre Ríos and Tucumán.

Trials were conducted in commercial fields under soybean monoculture from direct planting of a wide range of susceptible varieties to LSDs. Treatments consisted of a mixed commercial fungicide (trifloxystrobin + cyproconazole) applied at R3, R5, and at the growing stage indicated by the scoring system when not in agreement with the R3 and R5 stages. An untreated control was also included. For the particular environmental and production conditions in each trial, the scoring system indicated the action to be performed: sometimes the recommendation was not to apply the fungicide whereas other times the recommendation was to apply the fungicide at R3, R5, or at R4.

The experimental design implemented at each location was a randomized complete block design with four replications, with the experimental unit being a 10 × 2-m plot with soybean rows spaced at 52 cm. Fungicide was applied with a carbon dioxide backpack sprayer equipped with four 50-cm apart, full-cone spray nozzles (Lurmark HCX4 30) and providing an overall pattern width of 2 m. The sprayer was operated at a pressure of 3.16 kg/cm^2 and at a water volume rate of 150 l/ha. Daily rainfall was measured *in situ* and all risk factors suggested by the scoring system were recorded for each trial. Grain yield (kg/ha) was measured at physiological maturity in each plot and adjusted to 130 g/kg moisture content.

Field experiments were located at: 36°30′30.3″S, 64°04′29″W (Daireaux 2008); 35°37′36.36″S; 61°19′45.90″W (Carlos Casares 2008); 34°5′36.54″S, 60°25′19.29″O (Pergamino 2008); 33°55′56.69″S, 60°27′59.49″O (Pergamino SM 2009); 33°55′55.40″S, 60°28′6.61″O (Pergamino SM 2010); 33°47′23.80″S, 60°51′19.32″O (Pergamino SF 2010); 33°17′44.87″S, 60°40′38.83″O (Pergamino SN 2011); 34°54′17.76″S, 61°53′49.98″W (Lincoln 2008); 35°18′38.90″S, 61°30′0.27″W (Lincoln 2009); 34°49′44.40″S, 62°51′39.42″W (Villegas 2008); 32°56′47.94″S, 58°32′26.03″W (Gualeguaychú 2009, 2010, 2011); 35°32′38.30″S, 63°06′53.33″W (América LD 2009); 35°32′38.30″S, 63°06′53.33″W (América 2010); 35°32′54.77″S, 63°05′53.91″W (América LG 2011); 35°35′38.9″S, 62°59′3.7″W (América ME 2011); 31°51′07.4″S, 60°32′18.6″W (Paraná 2012); 26°49′13.19″S, 64°51′12.60″W (Tucumán 2012).

Severity of *S. glycines* and *C. kikuchii*, measured as the percentage of diseased leaf area, was visually estimated at R6-R7 on all leaves of 20 plants chosen arbitrarily in each plot (Díaz et al., 2005; Da Costa, 2005). The estimated severity of all leaves was averaged to obtain a single value per plot.

Grain yield response to fungicide application and net economic return were calculated and expressed as kg/ha. Net economic return from fungicide application was calculated as grain yield response − fungicide application cost (expressed as kg/ha); grain yield response = yield from treated plots − yield from untreated plots; and fungicide application cost = fungicide average cost plus application average cost (equal to 25 USD/ha)/soybean average price (equal to 270 USD/Tn) = equal to 92.6 kg/ha. Fungicide and application costs, and Argentina's soybean ton price were obtained from the average values of all seasons included in this validation study.

To evaluate the performance of the scoring system, trials were grouped according to the application decision suggested by the scoring system, namely: i) application at R3, ii) application at R5, iii) application at R4, or iv) no application. Each set of trials was analyzed for yield and disease severity separately. The on-farm trials were conducted as randomized complete block designs (Schabenberger and Pierce, 2002) with four blocks on each trial (or farm by year combination). The experiment in each trial was analyzed as a factorial mixed model with trial, application time and trial × application time interaction as fixed factors and with block nested within trial as a random effect. In all cases in which the treatment interaction was not significant, main effects were significant and the test for the treatment means were compared using Fisher's LSD test. The Gaussian and homoscedasticity assumptions were verified with graphical analysis of the model residuals and Levene and Kolmogorov–Smirnov tests (Type I error rate = 0.05). The assumptions held true in all cases.

3. Results

3.1. Development of the scoring system for chemical control of LSD

The scoring system interface is shown as a table with different factors, where users can quickly answer questions or select available options to obtain a guiding recommendation on whether or not to apply a fungicide within the R3-R5 growth stages as well as on whether or not to identify value in inducing a yield response

(Table 1). With this tool, the user will have a grid with several items to answer and according to the responses, the user will get an individual score for each item. The sum of all items will result in a final score that will guide the application decision. Depending on the obtained sum of points, each field is classified into three possible categories: i) high probability of yield response after fungicide application, ii) need to re-evaluate and discuss the technical and economic convenience of the fungicide application decision, including rainfall forecast consideration, and iii) low probability of yield response, thus no recommendation for fungicide application. Producers and advisors can operate the system as needed or when any changes that affect crop health or production occur.

The classification of the final decision based on the sum of all items is as follows: if the result is ≥ 33 points, it is highly probable to obtain yield response to fungicide application; if the result is < 23 points, it is unlikely to obtain yield response to fungicide application; between 23 and 32 points, discussion with a technical advisor about the economic and technical advantages of the use of fungicide will be needed (the closer to 33, the greater the probability to obtain yield response). In these cases, the rainfall forecasts should be also considered. For example, if the obtained score is between 23 and 32, but rainfall forecast suggests periods of rain, provided the crop is in good growing conditions, fungicide application is recommended. Conversely, if the forecast predicts light rain or no rain, or the crop is in significant thermal or water stress, application of fungicides should be postponed or even avoided. The decision to apply fungicides may be at R3, R4 or even at R5, when the scoring is greater than 33, which indicates a higher probability of obtaining a positive yield response. When producers have different fields that reach the same total score, fungicide application should be prioritized in those where LSD symptoms have been observed during scouting.

Table 1
Scoring system proposed to assist the decision-making process to apply a foliar fungicide for management of LSDs.

Risk factor for LSD epidemics	Factor levels	Scoring
A. Rainfall occurred between R3 and R5	80 mm or more	10
	65 to 80 mm	6
	50 to 65 mm	2
	50 mm or less	0
B. Intensity of rainfall that occurs between R3 and R5, rain events of 7 mm or greater	75% or more	5
	<75%	0
C. Crop rotation	Two or more seasons of previous soybean crops	5
	One year of soybean crop	3
	No soybean crop in the previous season	2
	No soybean crop in the last two growing seasons	0
D. Tillage system	Direct drilling or minimum tillage	4
	With stubble removal or burial	0
E. LSD presence in soybean crop from last season (LSD presence in crop residue)	YES	6
	NO	0
F. Sanitary quality of the seed	Untreated seeds	3
	Seeds treated with fungicide	0
G. Length of growing season (days to R8)	Full season soybean varieties (>145d)	4
	Mid-season soybean varieties (134–145d)	3
	Early season soybean varieties (<134)	2
H. Production destination as seed	YES	5
	NO	0
I. Field potential productivity	More than 3000 kg/ha	4
	from 2500 to 2999 kg/ha	3
	from 2000 to 2499 kg/ha	1
J. Presence of LSD symptoms in the field	YES	6
	NO	0

3.2. System validation

In the 19 validation trials, Septoria brown spot (*S. glycines*) and Cercospora leaf blight (*C. kikuchii*) were the predominant diseases (Table 2) thus confirming they are the most frequent foliar diseases found in Argentina (Díaz et al., 2005). The trials were grouped according to the application decision suggested by the scoring system: a) no application (three trials), b) application at R3 (six trials), c) application at R4 (six trials) and d) application at R5 (four trials) (Table 2). Treatments guided by the scoring system resulted in the highest economic returns regardless of the location and year in 90% of the cases (17 of the 19 trials). Yield responses and net economic margins followed similar trends (Table 2). The highest yield response was 1047 kg/ha in the América location, 2009/2010 season.

3.3. Recommendation to apply the fungicide at R3

There was no significant treatment × location interaction in any of the trials for yield, but there was for severity. In six trials where the scoring system suggested applications to be made at R3, yields and economic margins obtained in the treatments were significantly higher ($P < 0.05$) than those in the control and in applications made at R5 (Fig. 1A; Table 2). Average leaf severity also showed significant differences between treatments with lower values corresponding to the R3 application recommended by the scoring system (Fig. 1B; Table 2). Statistical analysis showed significant yield differences among the trial locations ($P < 0.05$; Table 2), with the highest yield achieved in Lincoln and the lowest in Gualeguaychú. Nevertheless, there were no significant differences in the economic return among locations (Table 2). The analysis for severity also detected differences among locations, with the lowest values found in Gualeguaychú, América and Pergamino and the highest in Carlos Casares and América (Table 2).

3.4. Recommendation to apply the fungicide at R4

In six trials where the scoring system recommended fungicide application at R4, yields were the highest and differed significantly from the other treatments (Fig. 2A). The applications at R3 and R5 showed yields that differed from those obtained at R4 or from the control (Fig. 2A). There was no significant treatment × location interaction for yield. For severity, there was a significant treatment × location interaction and statistically significant differences among R4 applications, which had the lowest values, and the non-treated, which had the highest values (Fig. 2B; Table 2). The analysis showed significant differences among locations (Table 2). Gualeguaychú and Villegas showed the highest yields ($P < 0.05$) and Lincoln the lowest ($P < 0.05$) (Table 2). However, when analyzing the severity by location, Paraná and Lincoln had the lowest values and Villegas the highest (Table 2).

3.5. Recommendation to apply the fungicide at R5

In four trials where the scoring system suggested fungicide application at R5, yields were the highest and the only ones that differed significantly from the control (Fig. 3A). The application at

Table 2
Season, location, variety, planting date, treatment (application timing), yield (kg/ha), yield response (kg/ha), scoring system outcome and net economic margin for 19 validation trials conducted in Argentina. (No Application not to proceed with fungicide application).

Season	Location	Variety	Planting date	Application timing	Yield (kg/ha)	Yield response (kg/ha)	Scoring system outcome and recommendation	Net economic return (kg/ha)	Severity (%) *Septoria + Cercospora*
2012	Tucumán	A8000RG	14/12/2011	Non-treated	2,077	0	–	0	34
				R3	2,065	−13	22=>No Application	−105	30.5
				R5	2,160	83	22=>No Application	−9	34.5
2009	Pergamino SM	DM4800	30/12/2008	Non-treated	1,634	0	–	0	14.25
				R3	1,567	−67	22=>No Application	−159	11.25
				R5	1588	−46	22=>No Application	−136	9.75
2009	América LD	DM4670	16/10/2008	Non-treated	4,044	0	–	0	14.25
				R3	4,086	42	28=>No Application	−51	14
				R5	4,036	−7	28=>No Application	−100	13.75
2008	Carlos Casares	DM3700	05/11/2007	Non-treated	4,000	0	–	0	51.7
				R3	4,111	111	35=>R3	19	31.7
				R5	4,222	222		130	53.3
2008	Lincoln	DM4870	01/11/2007	Non-treated	4,917	0	–	0	50.0
				R3	5,217	300	31=>R3	207	25.7
				R5	5,133	217		7	36.7
2009	Gualeguaychú	N6411	10/11/2008	Non-treated	2,325	0	–	0	70.9
				R3	3,058	733	32=>R3	640	9.3
				R5	2,677	352		259	14.6
2010	América	DM4670	18/10/2009	Non-treated	3,836	0	–	0	33.8
				R3	4,882	1,047	44=>R3	954	18.3
				R5	4,239	403		311	23.5
2010	Pergamino SM	DM4970	29/10/2009	Non-treated	3,781	0	–	0	34.3
				R3	4,299	518	35=>R3	426	15.5
				R5	3,838	57		−35	23
2011	América LG	DM4670	13/10/2010	Non-treated	4,011	0	–	0	46.9
				R3	4,366	355	36; R3	263	24.3
				R5	4,098	87		−6	51.3
2008	Villegas	DM4870	04/11/2007	Non-treated	3,787	0	–	0	55.0
				R3	3,773	−14		−107	51.7
				R4	4,233	446	35; R4	354	20.0
				R5	4,076	289		197	30.0
2009	Lincoln	DM4870	21/10/2008	Non-treated	1,657	0	–	0	26.0
				R3	1,731	74		−19	20.7
				R4	1,991	333	34, R4	241	18.0
				R5	1,917	259		167	17.3
2010	Gualeguaychú	N6126	28/10/2009	Non-treated	3665	0	–	0	56.1
				R3	4,203	538		445	18.5
				R4	4,215	550	30; R4	457	19.4
				R5	3,903	238		145	29.4
2011	América ME	DM3700	25/10/2010	Non-treated	2,420	0	–	0	35.5
				R3	2,328	−92		−185	36.8
				R4	2,546	126	42, R4	34	21.3
				R5	2,202	−218		−310	29.3
2011	Gualeguaychú	RA625	15/11/2010	Non-treated	4,051	0	–	0	60.7
				R3	4,520	469		376	15.5
				R4	4,350	299	44, R4	206	20.7
				R5	4,252	201		109	28.6
2012	Paraná	NA5909RG	29/12/2011	Non-treated	3,014	0	–	0	11.8
				R3	3,214	200		108	10.3
				R4	3,345	331	35; R4	238	12.5
				R5	3,309	295		203	9.7
2008	Pergamino	DM3700	30/10/2007	Non-treated	3,632	0	–	0	27.5
				R3	3,731	98		6	21.0
				R5	3,846	214	30; R5	121	20.0
2008	Daireaux	DM4870	02/11/2007	Non-treated	4,127	0	–	0	41.7
				R3	4,280	154		61	31.7
				R5	4,478	351	35; R5	258	22.7
2010	Pergamino SF	4613	09/11/2009	Non-treated	3,013	0	–	0	30.3
				R3	3,251	237		145	24.5
				R5	3,328	314	41, R5	222	19.5
2011	Pergamino SN	A4613	26/12/2010	Non-treated	2,191	0	–	0	26.3
				R3	2,317	126		34	24.0
				R5	2,503	313	32, R5	220	25.8

Season: crop season; Location: trial location; Variety: soybean variety; Treatment: consisted in applying a mixture of strobilurin and triazole (trifloxystrobin + cyproconazole) at three different timings: a) at the fixed growing stage R3; b) R5 and c) at the growing stage indicated by the scoring system (in the cases it did not coincide with R3 or R5). A control plot without application was also included. Scoring system outcome: In this column the total sum points is presented followed by the growth stage when the scoring system indicated to proceed with fungicide application. Net economic margin: return from fungicide application was calculated as: grain yield response − fungicide application cost (expressed as kg/ha); being grain yield response = yield from treated plots − yield from untreated plots; and fungicide application cost = fungicide average cost plus application average cost (equal to 25 USD/ha)/soybean average price (equal to 270 USD/Tn) = equal to 92.6 kg/ha; Septoria + Cercospora Severity (%): Severity of *S. glycines* and *C. kikuchii* was visually estimated at R6-R7 on all leafs of 20 plants per plot randomly chosen.

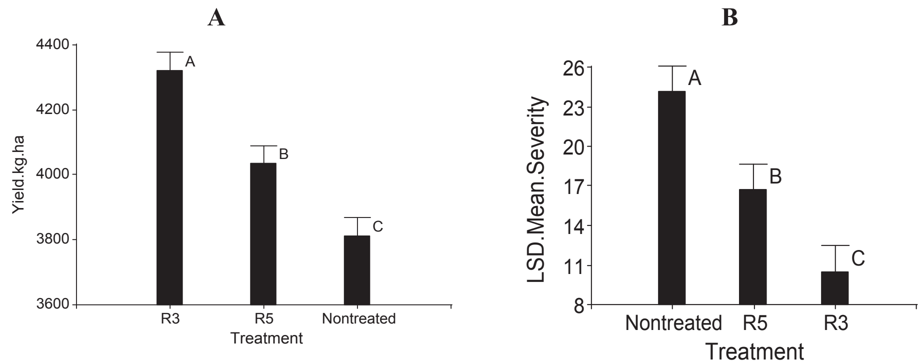

Fig. 1. A. Yield and B. Severity in untreated plots and fungicide-treated plots at R3 and R5 in six trials in which the scoring system recommended fungicide application at R3.

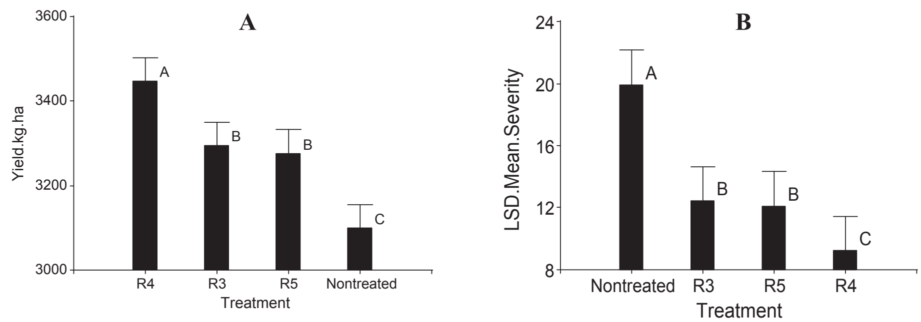

Fig. 2. A. Yield and B. Severity in untreated plots and fungicide-treated plots at R3 and R5 in six trials in which the scoring system recommended fungicide application at R4.

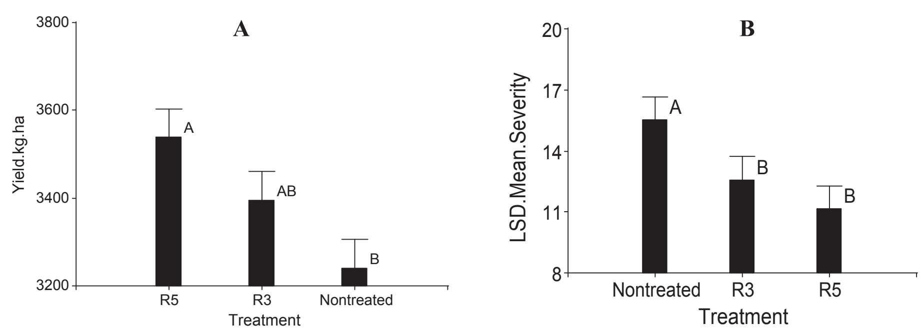

Fig. 3. A. Yield and B. Severity in untreated plots and fungicide-treated plots at R3 and R5 in six trials in which the scoring system recommended fungicide application at R5.

R3 did not differ from that at R5 or from the control. Nevertheless, there were no significant differences in economic returns (Table 2). There was no significant treatment × location interaction for yield and severity. For severity, both chemical treatments had significantly lower values than the untreated control, but there were no differences between them (Fig. 3B; Table 2). Statistical analysis showed differences between the locations where the trials were conducted. Daireaux location had the highest yield and Pergamino SN the lowest ($P < 0.05$; Table 2). However, Daireaux presented the highest values of severity, differing significantly from the other locations.

3.6. Recommendation not to apply the fungicide

In three trials where the scoring system recommended not to apply the fungicide, yields did not differ significantly among treated plots (R3 and R5) and from the untreated control (Table 2). In addition, average foliar severity had no statistical differences between treatments and the untreated control (Table 2). There were significant differences ($P < 0.05$) among the locations for yield and disease severity. Thus, América LD, Pergamino SM and Tucumán differed significantly from each other regarding yield (Table 2). The highest yield average was obtained in América, whereas the highest severity was obtained in Tucumán, differing significantly from the other two locations. There was no significant treatment × location interaction and yield or between treatment × location interaction and severity. The net margins obtained from the fungicide application were all negative but not statistically significant (Table 2).

4. Discussion

The scoring system developed in the present study was useful to guide the timing for fungicide application to control LSDs according to the probability of yield response, thereby avoiding unnecessary applications and ensuring a significant crop yield response.

Profitable net margins depend on soybean and fungicide prices at the time of application, but in the present study positive net margins followed positive yield responses in applications guided by the scoring system. The results of this study are consistent with those of Backman et al. (1984), Shurtleff et al. (1980), TeKrony et al. (1985) and Malvick (1998). The systems proposed by those authors have in common the inclusion of rain as a factor. In our studies, rainfall was the most helpful factor to define crop yield response to fungicide application, which makes logical sense, as in general, fungicides applied during drought periods, when LDSs do not reach high epidemic levels, do not significantly increase yields (Backman et al., 1979; Hoffmann, 2002; Grichar, 2013; Kyveryga et al., 2013; Mueller et al., 2014). From a study on 282 on-farm evaluation trials conducted from 2005 to 2009 across Iowa, Kyveryga et al. (2013) concluded that site-specific observations of spring rainfall could be used to identify soybean fields that are either likely or unlikely to produce above break-even yield responses, and therefore, to help farmers avoid unnecessary foliar fungicide applications on soybean.

The scoring system developed in our study considers pluvial precipitations occurred between R3 and R5 as the best related to the use of fungicides thereby to yield response. In previous research (Carmona et al., 2010, 2011a), we found that a significant soybean yield increase in response to fungicide applications for control of LSDs was strongly associated with the amount of rainfall occurred between R3 and R5. Forecast weather data can provide farmers with advanced warning of LSD infection periods and allow them to anticipate risk (Magarey et al., 2001). Thus, and in accordance with Mueller and Bradley (2008) and Swoboda and Pedersen (2009), the decision to apply foliar fungicides to a specific field should be made only after considering current and future weather conditions, disease development, crop yield potential, and the economic return due to management with fungicides.

The scoring system herein proposed can be used by producers or crop advisors as many times as needed or when changes in some of the factors that integrate the system (e.g. rainfall, yield potential, onset of symptoms, etc.) are made. The scoring system will indicate whether or not to apply the fungicide regardless of the growth stage of the soybean crop because the system must function from R3 early on, but the decision to apply fungicides may be at R3, R4 or even at R5. For cases in which the system indicates uncertainty, i.e. a point sum between 23 and 32, producers and farm advisors may wait the occurrence of rainstorm to define the fungicide application or anticipate the application relying on forecasts, especially where more risks can be afforded or when difficulties arising from infrastructure and logistics are present, provided that the point sum is closest to 33, the forecast is accurate and the crop is growing under non-water or thermal stress conditions (Pfender et al., 2011).

The factors that integrate the scoring system and the relative weighting of each factor are not rigid and can suffer from subsequent modifications of new research and technology changes in the agricultural production system, changes in pathogen populations involved, etc. (Gent et al., 2011). Likewise, in the future, any of the factors that justifiably improve the performance of the scoring system may be added or removed.

Validation of a system is essential and mandatory to assess its usefulness and benefits and to detect its weaknesses. In addition, validation allows achieving continuous improvement and the possibility that the degree of adoption among farmers and advisors is maximized.

The number of field trials and the experimental design used in the present study was considered adequate to fulfill the validation objective. The results coincide with those of TeKrony et al. (1985), who validated a scoring system using a methodological approach similar to that used in the present study. In our study, the validation process encompassed five different cropping seasons and it was strengthened and enriched with data from different regions. The design and number of trials were fit enough to generate a data matrix and allow detecting statistical differences between the tested treatments.

Across the analysis for yield response in the different groups, the treatment × trial interaction was not significant. This would imply that the scoring system is choosing scenarios that have homogeneous response to the treatment: within the groups generated by the system, the effects of the treatments on yield are similar among trials within each group. In most cases, the scoring system allowed obtaining higher yield responses and net margins to fungicide applications. An important aspect that emerges from this validation study is that the fungicide application was not always economically justified. Thus, when the scoring system recommended not to apply, soybean grain yields obtained at R3 and R5 did not differ significantly ($P < 0.05$) from the control, indicating that the scoring system led to the right decision (Table 2). For example, during the 2012 season, in Tucumán and Pergamino (SM) the total sum of the points was 22 and the decision was not to apply the fungicide, but in América (LD) the sum was 28, which corresponded to the interval of uncertainty (range 23–32). In this last situation, the decision was also not to apply because no rainfall forecasts announced upcoming rains that may change the LSD epidemiological scenario. In trials where the score was between 30 and 33, when assessing the existence of rainfall forecasts in conjunction with the crop state, the decision was to apply.

In general, the scoring system allowed to correctly recommend fungicide applications within the so-called “window of opportunity”. The highest yields were obtained as a function of the indication of the scoring system, recommending the application of fungicides at R3, R4 or R5 according to the score obtained (Table 2; Figs. 1A, 2A and 3A). While there were differences between locations, the scoring system allowed guiding chemical applications efficiently according to the particular assessment of each field where a trial was located. Even for the same locality as Pergamino, and the same cropping season (2009/2010), in one trial the scoring system recommended applying at R3 (Pergamino SM) and in another applying at R5 (Pergamino SF), displaying a sensitivity to detect differences in scoring factors (Table 2). The same happened in América, where during the 2010/2011 season, in one trial (América MM) the scoring system suggested the application of fungicides at R4, but suggested applying elsewhere in the same locality (América LG) at R3 (Table 2). Despite the geographic proximity, probably convective rains were concentrated in some fields and not in others. This factor can be quantified *in situ* with a rain gauge, and it can explain the differences in decisions within the same locality.

An important aspect to consider is the severity analysis of both *S. glycines* + *C. kikuchii*. In all cases, except where the scoring system recommended not to apply the fungicides, the severity values obtained in the untreated control were significantly greater than in the other treatments, indicating that applications of trifloxystrobin + cyproconazole significantly reduced LSD severity. However, there was no correlation between yield and severity (data not shown). While the disease severity was lower in plants treated with chemicals than in the non-treated plots in all trials (except for trials where it was recommended not to apply), no robust relationship between severity estimated at R6 -R7 and grain yield was apparent. Even when considering different locations, there was no relationship between higher yields and lower severity recorded in most of the examined cases.

Severity estimated from R6 to R7 cannot be used as a good estimator to predict yield damage, though it could be used to detect differences between/among treatments and untreated controls or to analyze the end of an epidemic. This claim is supported by several research (Guerzoni, 2001; Martins, 2003; Da Costa, 2005). Probably, sequential estimations of severity and assessment of other indicators, such as remaining green leaf area, healthy leaf area duration, defoliation, genotype × fungicide, reflectance and radiation should also be analyzed to better study this association.

Regarding net margin gain (Table 2), its variations followed the same trend as with yield responses. When the scoring system advised not to apply, phenology-based applications showed negative margins, demonstrating that in these cases the use of fungicide would have been detrimental rather than beneficial to the crop. In contrast, when the scoring system recommended to apply regardless of the growth stage, net margins were always positive and generally provided the greatest possible income.

In this study, we found that foliar fungicide application was not economically justifiable in every year at either studied location. Therefore, fungicide use should be reassessed each year given that profitability is year-specific, that is, it depends on yield potential, prices, and weather conditions that favor foliar disease occurrence. Research on the relationship between weather conditions and disease severity will be instrumental to improve the understanding of such relationship and to develop economic thresholds for fungicide treatment decision aids.

We consider that the scoring system presented in this study is simple and dynamic thus it can be easily adopted by farmers and crop consultants. The scoring system can be operated as a software and accessed from the internet, enabling soybean growers to easily access to the information necessary to make decisions on fungicide applications. This is a very important issue, as the research community often perceives adoption and use of predictive systems to be low, due to the so-called problem of implementation (Gent et al., 2011).

5. Conclusions

The foliar fungicide scoring system for LSD management presented in this paper can help soybean growers reduce the number of fungicide sprays and costs of production, especially in seasons when conditions for disease occurrence are not favorable. The benefits of such a tool is that growers can decide on fungicide applications only when conditions are favorable for LSD development, thus reducing the number of sprays and production costs without compromising disease control. Future validation field trials conducted in different agro-ecological zones and soybean production systems will help improve the efficiency and accuracy of the scoring system proposed and likely, will make it adaptable to other regions.

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