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Relationship between Late Soybean Diseases Complex and Rain in Determining Grain Yield Responses to Fungicide Applications

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Abstract

Wide distribution of soybean monoculture associated with no tillage has contributed to enhance damages caused by late diseases complex (LDC) in Argentina. LDC is a complex of diseases where *Septoria glycines* and *Cercospora kikuchii* are regarded as the major problem. Even though the use of foliar fungicides has increased, there is no rational and economic guide for their use. This is the main reason why the response to foliar fungicide applications is unpredictable. One of the main factors that contribute to the development of LDC is rainfall. The objective of this study was to evaluate the impact of rainfall during several growing seasons and different soybean growth stages on LDC severity and yield. We carried out 18 field experiments during three growing seasons (2004–2006) at several locations in the Argentine Pampas Region, to examine the relationship between rain and yield response to single fungicide applications (quinone outside inhibitors and demethylation inhibitors) at growing stages R3 and R5. The strongest associations ($R^2 = 0.81–0.84$; $P < 0.001$) were observed between accumulated rainfall from R3 to R5 and yield response to fungicides applied in R3 or R5. Our results suggest that a minimum of 65–90 mm rainfall during R3–R5 is required to justify fungicide application, with high probability that the use of fungicide will increase soybean yield as a consequence of disease control. These findings could lead to a simple model, useful as decision support system for use in planning and scheduling spray applications for LDC management in soybean crops.

Introduction

Soybean (*Glycine max* (L.) Merr.), the most important crop in Argentina, has a cultivated area of 18.13×10^6 ha, and during the 2009/2010 growing

season produced 52.68×10^6 tonnes, which represents the most significant source of income for the country (MINAGRI 2010). Argentina is the most important exporting country for soybean oil and meal providing 41 and 36.5% of the world trade in these commodities, respectively (Rossi 2004, 2009; Garff and Correnti 2008). The sown area and the total production have increased significantly during the last 10 years due to international demand and widespread use of no-tillage cultivation and glyphosate-resistant cultivars. The north of the Pampean Region (Buenos Aires, Santa Fe and Córdoba provinces) accounts for 82.7% of total Argentine production.

At present, the most important diseases of soybean in Argentina are the late diseases complex (LDC) (Díaz et al. 2005; Oerke 2006; Hartman and Hill 2010). Soybean monoculture using no-tillage technique has contributed to increase damages caused by these diseases (Carmona et al. 2004). The LDC affects yield generation and causes yield reduction. In addition, it may also affect the quality of the harvested seeds (e.g. seed decay caused by *Phomopsis sojae* Lehman and purple stain by *Cercospora kikuchii* T. Matsu & Tomoyasu, Gardner). This leads to a decrease in crop profitability and an increase of inoculum in the fields (Carmona et al. 2004; Gally et al. 2004; Molina et al. 2004). The most frequent LDC in the Pampean Region are brown spot (*Septoria glycines* Hemmi), *Cercospora* blight and purple stain (*C. kikuchii*), pod and stem blight (*P. sojae*) 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), causing yield damage up to 30% (Wrather et al. 2001; Carmona et al. 2004).

The critical period for determining soybean yield is between growth stages R1 (flowering) and R5–R6

(beginning of grain filling–maximum seed size) (Fehr et al. 1971), with the interval between R3 and R5.5 being particularly sensitive (Kantolic et al. 2003; Kantolic and Slafer 2007). The increase and maintenance of healthy leaf area index and the duration of healthy leaf area determine the quantity of photosynthetic active radiation intercepted and absorbed during this critical period (Díaz et al. 2005). On the other hand, LDC pathogens have long incubation and latency periods; thus generally, when symptoms become visible, mainly towards the end of the growing season at stage R6 or R7, it is already too late for chemical control (Klingelfuss and Yorinori 2001). This is the reason for the weak correlation found between severity of the LDC during earlier stages (R3, R4 and R5) where fungicides should have been applied and yield response (Cagnin Martins 2003; da Costa 2005). Nevertheless, final severity observed in stages R6 and R7 is a good indicator to detect differences and analyse epidemics. Thus, severity estimation at those stages (and not in previous ones) is normally recommended. Therefore, decision making cannot be based on disease severity because diagnosis is usually complex and unclear and can only be performed accurately at the end of the growing season when damage by LDC has already happened. There is little information to guide decisions for foliar fungicide application aimed at controlling this complex of diseases in Argentina. Current practice in fungicide application is not well based on scientific criteria. Farmers have adopted the application of foliar fungicides during the critical soybean period (calendar-based fungicide sprays programmes) as a means to manage LDC. However, the efficiency and profitability of this practice seems to be directly related to weather conditions (Backman et al. 1984; Ploper et al. 2001; Carmona et al. 2003, 2004, 2005, 2006). Fungicides applied during drought do not increase yield as in those conditions that disease and damage levels are low (Backman et al. 1979). One of the main variables that determine the spread of epiphytias is the amount and distribution of rainfall; therefore, it may be used to decide on chemical treatments (Carmona et al. 2010) together with information about further environmental conditions and assessment of disease levels (Swoboda and Pedersen 2009).

The objective of this study was to explore the association between rainfall, severity of LDC and soybean yield response to fungicide application in different soybean growth stages.

Materials and Methods

Six trials per season (a total of 18) were conducted at several locations (Armstrong, Marcos Juárez, Cruz Alta, Arteaga, Godeken, Monje and Montes de Oca) in southern Santa Fe and south-eastern Córdoba provinces, in the core of the Argentine Pampas Region, during the 2003/2004, 2004/2005 and 2005/2006 growing seasons. Experiments were carried out in commercial soybean fields that were previously sown with soybean using a no-tillage system (direct

drilling). Soils were typical argiudols with 3.5% organic matter. Cultivars were glyphosate tolerant and susceptible to LDC. Soybean was sown at 0.52 m between rows with a seeding rate aimed to achieve 42 plants/m². Fertilization and crop protection, except for disease control, were performed according to the usual practices carried out in the Pampean Region. Reproductive growth stages were deemed to have been achieved when 50% of the plants in the plot had reached that stage.

Experiments were designed as randomized complete blocks with four replicates. Plots were 20 m² (10 × 2 m). Treatments consisted of single fungicide applications at two growth stages R3 (beginning of pod growth) and R5 (beginning of seed growth). They included quinone outside inhibitor (QoI) fungicides (strobilurins) mixed with demethylation inhibitor (DMI) fungicides (triazoles), DMI fungicide mixtures and methyl benzimidazole carbamates fungicides alone or mixed. Fungicides were used as tools to evaluate a system of fungicide application timing in relation to the effect of LDC and rainfall and not to determine the specific efficacy of their active ingredients. Different groups of active ingredients were considered: type A (QoI + DMI) and type B (DMI mixtures) (Table S1). Fungicides were applied using a CO₂ backpack sprayer equipped with three full cone nozzles (Lurmark 30 HCX4, Hypro Lurmark Nozzles: New Brighton, MN, USA) on a handheld boom, at a pressure of 3.16 kg/cm² and 150 l/ha.

Severity of the LDC (percentage of diseased leaf area) was visually estimated at stage R7 (da Costa 2005; Díaz et al. 2005; Niero et al. 2007). To this end, twenty randomly selected plants per plot were chosen and dissected into the top, middle and bottom sections. The disease severity was differentially scored for each pathogen by visual estimation of the percentage of chlorotic and necrotic leaf area, and severity values for each plant part were averaged to obtain the disease severity for each plot.

To identify and confirm the causal agents of plant disease in the experiments, an additional analysis of symptomatic leaf, stem and pod tissue was performed. Five samples per plot were taken at stage R7, incubated in a humid chamber for 7 days at 23–26°C under alternating cycles of 12-h darkness and 12-h near ultraviolet light and then examined under stereomicroscope and microscope for identification.

After maturity, grain yield expressed in kg/ha was determined in each experimental plot and adjusted to 130 g/kg moisture content. Rain was collected daily with a simple rain gauge (pluviometer) in each field during the entire growing season.

A factorial analysis of covariance (ANCOVA) was applied to the observed yield values to detect the main effects and interactions using the yield of non-treated plots as covariate. Three main factors were considered, season (2004, 2005 and 2006); timing of application (R3 and R5 growth stages); and type of fungicide (type A = QoI + DMI = azoxystrobin + cyproconazole and type B = DMI mixtures = cyproconazole + dife-

noconazole in 2006 and 2005 and difenoconazole + propiconazole in 2004). When the ANCOVA analysis indicated significant treatment effects, differences between means were subjected to the Tukey test.

Linear regression analysis was performed to assess the relationships between yield response (yield of treated plots minus yield of controls) and accumulated rain between selected growth stages (R3 to R5, R4 to R6, R4 to R6.5, R3 to R6.5, R1 to R3, VE to R5 and VE to R6.5).

Results

Pathogens identified in R7 in all plots in all the three seasons were *S. glycines* Hemmi, *C. kikuchii* T. Matsumoto & Tomoyasu, Gardner, *C. truncatum* (Schwein) Andrews & W.D. Moore, *G. glycines* (Hori) Lehman & Wolf, *P. sojae* Lehman, *Diaporthe phaseolorum* var. *sojae* (Lehman) Wehm, *Pseudomonas savastanoi* pv. *glycinea* (Janse 1982) Gardan et al. 1992 and *Xanthomonas axonopodis* pv. *glycines* (Nakano 1919) Vauterin et al. 1995. Disease intensity and severity of LDC was different in different years and localities, depending on weather conditions (Table S1).

Yield responses to fungicide applications differed significantly among seasons. All tested fungicides (both DMI fungicides and their mixtures with QoI fungicides) showed efficient control of LDC. Total rainfall in the R1–R5 interval was similar in all seasons (Table 1). In contrast, rainfall accumulated during R3–R5 interval exhibited a gradient across years (Table 1). This interval was wettest in 2005 and driest in 2004. The greatest yield responses were achieved in 2005, when precipitation accumulated between stages R3 and R5 was the highest (Table 1).

In all cases, treated plots had significant lower severity levels than controls (Table S1), which indicates that yield benefits were the result of control of LDC by fungicides. Although in 2004 there was less rain during the R3–R5 interval, the yield of both treated and non-treated plots was higher than in 2005 and 2006. This shows that less rain during the R3–R5 interval reduced LDC development but did not affect grain determination as good soil moisture conditions existed and soybean crops did not suffer drought stress.

Linear correlation between yield of fungicide-treated and untreated plots throughout the three seasons was

high ($R^2 = 0.88$, Fig. 1) and unaffected by environmental conditions. This indicates that differences in yield responses (yield of treated plots minus yield of controls) were due to the fungicide activity in reducing LDC. Main effects of both season ($P < 0.001$) and application timing ($P = 0.004$) were significant but application timing did not interact with season or type of fungicide. The only significant interaction was season with type of fungicide ($P < 0.05$). Type A fungicide (QoI + DMI) was more effective for the control of LDC in seasons with high precipitation during the R3–R5 interval (Fig. 2a). Although no relationship was found between the time of application and other factors, there was a tendency to obtain higher yield responses with one fungicide application at stage R3. This tendency was more marked with higher levels of accumulated rain between R3 and R5 (Fig. 2b).

Linear relationships between yield responses and rainfall in the R3–R5 interval were significant for fungicide application at both stages (Fig. 3). Coefficients of determination for this relationship for intervals R1–R3, VE–R5 and VE–R6.5 were non-significant (Table 2) and the ones between R4–R6, R4–R6.5 and R3–R6.5, although closer to the values for R3–R5, were generally lower (Table 2). Overall, these data suggest that the relationships for the R3–R5 interval is the most appropriate, with more than 80% of variation in yield response explained by the amount of rainfall accumulated between R3 and R5 ($P < 0.001$, Fig. 3).

Discussion

Our results show that the potential benefits of foliar fungicide action on soybean yield are dependent on the rainfall registered in the R3–R5 interval. Even though the rainfall between these growth stages happened after the R3 fungicide applications and before the R5 applications, the effect on yield increase due to fungicide activity was the same for both timing of application treatments (Fig. 3). This suggests that rainfall had an important effect on the rate of development of the LDC epidemic during the most critical period of grain yield determination. This response could be associated with the increase in dissemination and infection of the main LDC pathogens observed in this study (*S. glycines*, *C. kikuchii*, *C. truncatum* and

Table 1

Yield responses of soybeans to fungicide applications (yield of treated plots minus yield of controls, in kg/ha) at growth stage R3 or R5 and mean accumulated rainfall for periods R1–R3, R3–R5 and R1–R5 in three growing seasons. Means are derived from data from all localities within a year and treatment

Seasons	Growth stage of application	Mean yield of treated plots (kg/ha) ^a	Mean yield of control plots (kg/ha) ^a	Yield response (kg/ha)	Mean rain R1–R3 (mm)	Mean rain R3–R5 (mm)	Mean rain R1–R5 (mm)
2004	R3	4236 ± 197	4137 ± 182	99	161	64	225
	R5	4235 ± 199		98			
2005	R3	4039 ± 98	3508 ± 114	531	92	139	231
	R5	3936 ± 105		428			
2006	R3	3968 ± 134	3639 ± 146	329	129	116	245
	R5	3889 ± 139		250			

^aStandard errors of mean yields are shown.

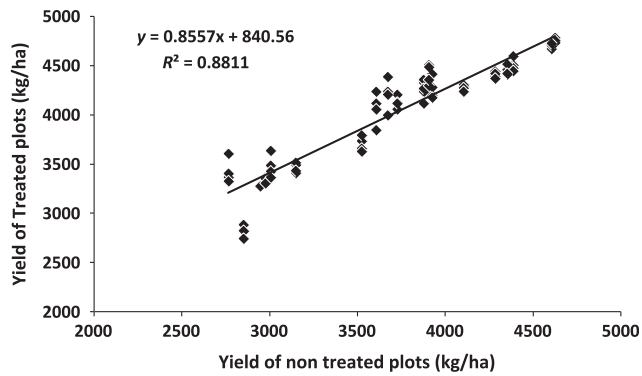


Fig. 1 Relationship of soybean yields in fungicide-treated and untreated plots. Means are derived from data from all localities, all seasons, all timings of application and all fungicides

P. sojae). Most causal agents of soybean LDC have hydrophilic fructifications, and rainfall has an important role in removal, dispersal and inoculation of pathogen inocula (*Septoria*, *Colletotrichum* and *Phomopsis*) (Fitt et al. 1989; Maude 1996; Carmona et al. 2009). This explains the effect of rain on the development of these diseases, as it helps their spread in two ways: (i) rain-splash physically moves spores throughout the crop canopy by carrying them from diseased plant tissues or infected crop residues to healthy plants (Fitt et al. 1989); and (ii) rain provides the moisture and leaf wetness duration required for spore germination and penetration of the fungus into plants (Tekrony et al. 1985). These epidemiological aspects of the LDC allow a better understanding of the association between amount of rainfall and the soybean yield response to fungicide application.

Although relative humidity and duration of leaf surface wetness were not considered for this analysis, it is assumed that higher levels of rain provide higher levels of both these variables. The various localities where trials were carried out belong to the same agro-ecological zone (Hall et al. 1992), where climate conditions are homogeneous. Also due to the short distance between trials (23 km), temperatures did not vary too much and so were not a factor causing differences on LDC development between trials.

In spite of physiological effects of QoI fungicides having been well documented, especially in cereals (Grossmann et al. 1999; Bayles and Hilton 2000), it is assumed (as demonstrated by Swoboda and Pedersen 2009) that QoI fungicides do not produce non-fungicidal physiological effects or associated yield improvement in soybean and that fungicides should only be used for disease management.

Identifying the critical period for disease control is one of the most important tools for integrated disease management (Kantolic et al. 2003). The number of soybean pods and seeds is determined during a period that begins around flowering (R2–R3) and extends through to seed filling (R5–R6) (Egli and Zhen-wen 1991; Egli 1993; Jiang and Egli 1993). According to Kantolic et al. (2003) and Kantolic and Slafer (2007),

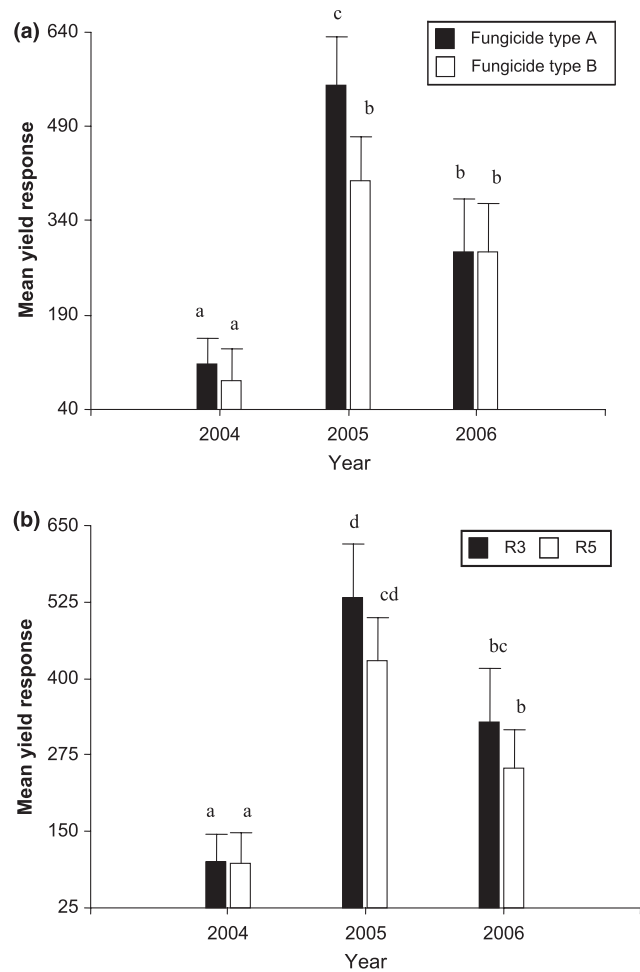


Fig. 2 Mean yield responses of soybeans to fungicide application (yield difference between fungicide-treated and untreated plots in kg/ha): (a) for three seasons and two fungicide types: type A = azoxystrobin + cyproconazole; type B = cyproconazole + difenoconazole in 2006 and 2005, difenoconazole + propiconazole in 2004; (b) applied at each of two growth stages (R3 and R5) for the three seasons. Different letters at the top of each bar correspond to significant differences at the $P = 0.05$ confidence level

soybean green leaf area index (GLAI) and its duration during this period are important for grain yield determination. Thus, it is not surprising that, if conditions for the establishment of an LDC epidemic coincide with this developmental interval, yield responses to fungicide application should be found. This is why the application of fungicides in field trials is based only on the growth stage of the soybean crop (Backman et al. 1979, 1984; Pataky and Lim 1981; Phillips 1984). Although many are in agreement that fungicides should be applied during the critical period for yield determination (Pataky and Lim 1981; Phillips 1984; Akem 1995; Cagnin Martins 2003; Wrather et al. 2004), we were not able to find information about proper timing for fungicide application within those application windows. This lack of information could be explained by the unpredictable yield response to fungicide applications obtained in several experiments carried out to explore the performance of foliar

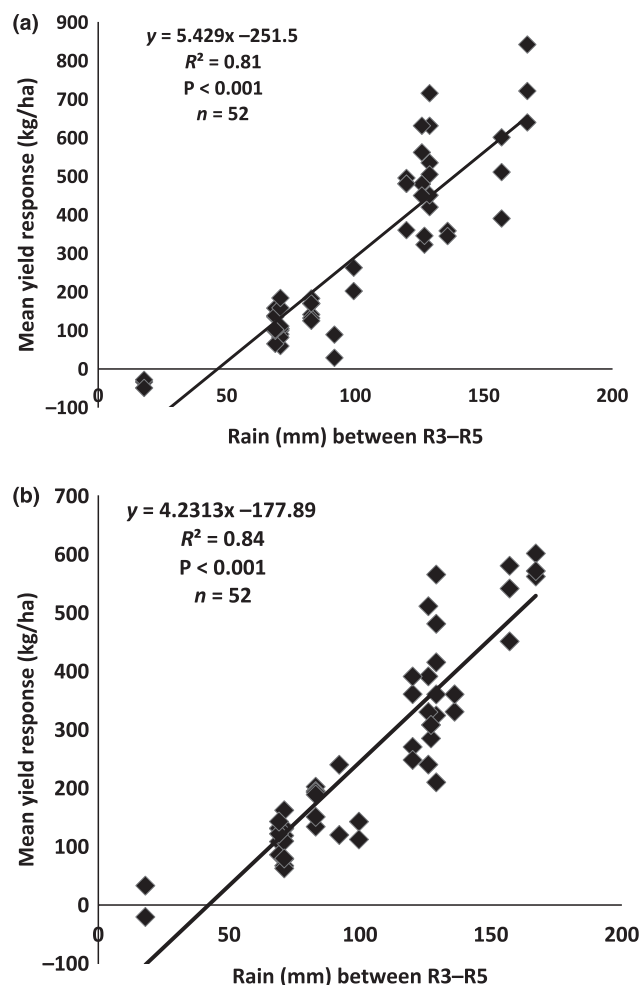


Fig. 3 Relationship between yield responses of soybeans to fungicide application (yield difference between fungicide-treated and untreated plots in kg/ha) and rainfall accumulated during growing stages R3–R5, for applications at (a) R3; (b) R5. All types of fungicides were included

Table 2

Coefficients of determination (R^2) for the linear regression analysis of yield responses of soybeans to fungicide applications (yield of treated plots minus yield of controls, in kg/ha) and cumulated precipitation (mm) between different soybean growth stages (R3 to R5, R4 to R6, R4 to R6.5, R3 to R6.5, R1 to R3, VE to R5 and VE to R6.5), for different fungicide mixtures applied at each of two different growth stages (R3 or R5). VE = emergence (Fehr and Caviness 1977)

Rain (mm)	Yield response (kg/ha)					
	QoI + DMI		DMI mixture		All fungicides	
	R3	R5	R3	R5	R3	R5
R3–R5	0.79	0.77	0.79	0.91	0.81	0.84
R4–R6	0.69	0.54	0.60	0.64	0.68	0.61
R4–R6.5	0.80	0.76	0.67	0.66	0.76	0.75
R3–R6.5	0.77	0.77	0.73	0.83	0.79	0.86
R1–R3	0.18	0.14	0.23	0	0.20	0.12
VE–R5	0.00	0.00	0.00	0.00	0.00	0.00
VE–R6.5	0.41	0.43	0.31	0.52	0.44	0.48

DMI, demethylation inhibitors; QoI, quinone outside inhibitors.

fungicides to control LDC in soybean. Phillips (1984) suggests that it would be helpful for growers to identify situations where the use of fungicides is most likely to be profitable.

Our experimental data showed that yield increments due to fungicide activity were clearly perceived in a certain and defined growth stage period (from R3 to R5) and were strongly associated with rainfall occurring from R3 onwards. Our results show that yield response to fungicide application does not depend only on the phenological growth stage. Plant disease epidemic development depends on a disease triangle (Agrios 2005), i.e. epidemics develop as a result of the timely combination of the same elements that result in plant disease: susceptible host plants, a virulent pathogen and a favourable environment. Thus, the recommendation for fungicide application should not be based on plant phenology alone. Environmental conditions and assessment of disease levels should be used as a guide for foliar fungicide application on soybean (Swoboda and Pedersen 2009). However, LDC, in particular, has long incubation and latency periods, and when symptoms generally become visible, mainly towards the end of the growing season at stage R6 or R7, it is already too late for chemical control (Klingelfuss and Yorinori 2001).

Accordingly, a risk model based on precipitation may be a useful tool when deciding fungicide application to manage LDC. It can help to predict likely responses to the use of fungicides as a function of the amount of rainfall collected from R3 onwards (Holden et al. 2007). In this respect, to apply a decision support system for fungicide application in most practical farm situations, we propose two options: (i) to measure rainfall amount from R3 onwards and when cumulated rainfall exceeds approximately 65–90 mm, apply the fungicide. Depending on weather conditions, fungicide application could be carried out at R3, R4 or R5; and (ii) to proceed based on an accurate rain forecast (time and volume). In this case, when the forecast predicts the occurrence of more than 65–90 mm rain between R3 and R5, apply the fungicide before the onset of rain. This way the tissues would be protected from infection. Thus, soybean growers, consultants and advisers should measure rainfall from R3 onwards and, with the help of rain forecasts and mathematical yield equations (as a function of rainfall and soybean prices), determine how many millimetres of rain would economically justify the use of fungicides in their fields, always assuming the presence of inocula in infected soybean residues under monoculture. For example, a yield increase of 400 kg/ha could be expected with 120 mm rain between R3 and R5 when the fungicide is applied at R3. With only a few millimetres less (100 mm), the predicted response would be 300 kg/ha (Fig. 3). The historical rainfall average (1994–2006) between growth stages R3 and R5 in Marcos Juarez, located in the core of the area where field trials were carried out, is around 80–90 mm. Considering this, the use of fungicides in a single

application represents an efficient strategy to manage soybean LDC in the cropping area of the Argentine pampas.

In the rainiest R3–R5 interval (2005), better responses were obtained with early applications (R3) and type A fungicides (QoI and DMI mixtures) (Fig. 2). Delaying application to R5 in wet seasons results in poorer control and slightly lower yields compared with treatments at R3 (Table 1 and Fig. 2). This indicates the need for further research to improve the definition of the window of time for a risk model based on precipitation that can be a useful tool to recommend fungicide applications. To control LDC, it is critical to take proper action at the right time and farmers ought to use a risk model in deciding time of application.

That simple model could be improved or combined with more specific incidences or thresholds of precipitation. Our findings are in accordance with Carmona et al. (2010), who found that rainfall during the R3–R5 growing stages correlated best with the severity of the LDC. They found that severity of LDC showed the highest correlation with the number of days with precipitations >7 mm, the total accumulation of daily precipitations >7 mm and the interaction between them. They concluded that by quantifying these rainfall variables in growth stages R3–R5, it was possible to predict the intensity of *S. glycines* and *C. kikuchii* in R7.

Our results showed that the mixture of azoxystrobin + triazole produced a better yield response in seasons with high precipitation during the R3–R5 interval. Probably, the combination of fungicides with different modes and mechanisms of action and the activity of QoI improve the control of LDC, particularly in rainy years when these pathogens are more active. Nevertheless, the different types of fungicide (QoI + DMI mixtures and DMI mixtures) had the same tendency in controlling LDC and reacted in the same way to the rainfall occurring during growing stages R3–R5.

Foliar fungicides are an important tool to help reduce the increase of disease inocula and to limit the spread of the disease. This is the first field study to assess the relationship between LDC, rainfall and yield increase by fungicide applications in different soybean growth stages in Argentina. The results obtained indicate that significant yield increases in response to fungicide applications could occur under wet periods with high rainfall during growth stages R3 to R5. The findings presented in this study can be useful as part of a decision support system for planning spray activities (Hansen et al. 1994; Holden et al. 2007; Burke and Dunne 2008; Kromann et al. 2009). Further studies considering agronomic aspects, genotypes and other climatic variables are necessary to develop a field tested point system with several parameters for scheduling the application of foliar fungicides, to prevent unnecessary applications and to increase grower's profits.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Growing season, locality, soybean cultivar, product name, active ingredients, and fungicide application rate used in the field trials, and severity of LDC at R7 for plots treated at R3 and plots treated at R5.

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