

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Disease warning systems for rational management of Asian soybean rust in
Brazil**

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Thesis presented to obtain the degree of Doctor
in Science. Area: Agricultural Systems
Engineering

**Piracicaba
2018**

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Disease warning systems for rational management of Asian soybean rust in Brazil

versão revisada de acordo com a resolução CoPGr 6018 de 2011

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To Ana Miskalo Beruski (in memoriam) and to Irene Castilho.

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RESUMO

Sistemas de alerta fitossanitário para o manejo racional da ferrugem asiática da soja no Brasil

A ferrugem asiática da soja (ASR), causada pelo fungo *Phakopsora pachyrhizi*, pode ocasionar elevados prejuízos às lavouras de soja. O controle da doença é realizado por meio de aplicações sequenciais de fungicidas em sistema calendarizado. Este, por sua vez, não considera a favorabilidade climática para recomendar pulverizações. A proposição de esquemas de pulverização mais eficientes pode ser obtida pelo uso de sistemas de alerta fitossanitário. Assim, objetivou-se avaliar o desempenho de diferentes sistemas de alerta fitossanitário, visando à determinação de esquemas de pulverização de defensivos químicos para o controle de ASR nos estados de São Paulo, Paraná e Mato Grosso, Brasil. O experimento foi conduzido em Piracicaba, SP, Ponta Grossa, PR, Campo Verde e Pedra Preta, MT, Brasil ao longo das safras de 2014/2015 e 2015/2016. Os tratamentos foram: Testemunha (sem aplicação); Aplicações calendarizadas a partir de R1, espaçadas em 14 dias (CALEND); Sistema de alerta baseado em dados de chuva limiar menos conservador (PREC_1 - 80% de severidade) e mais conservador (PREC_2 - 50% de severidade); Sistema de alerta baseado em dados de temperatura do ar e a duração do período de molhamento foliar com limiar menos conservador (TDPM_1 - 6 lesões cm²) e com limiar menos conservador (TDPM_2 - 9 lesões cm²). Os resultados obtidos confirmaram que as condições meteorológicas nas localidades estudadas foram favoráveis para o progresso da ASR. Verificou-se que a duração do período de molhamento foliar (DPM), temperatura do ar durante o molhamento e chuva acumulada influenciaram positivamente a ASR. Ao testar os sistemas de alerta no controle de ASR verificou-se que aqueles baseados em dados de chuva apresentaram os melhores desempenhos. O PREC_2 apresentou melhor desempenho em análise geral considerando todas as épocas de semeadura, ao passo que PREC_1 foi melhor quando em semeadura de outubro a novembro. Os sistemas TDPM, com ambos os limiares de ação, superestimaram os valores de ASR acusando um número maior de pulverizações comparada aos demais tratamentos. Modelos empíricos mostraram ser eficientes na estimação da DPM em Ponta Grossa, Campo Verde e Pedra Preta. Estimações pelo método de número de horas com umidade relativa acima de 90% (NHUR \geq 90%) apresentaram RMSE menor que 2,0 h viabilizando o uso da DPM estimada como variável de entrada de sistema de alerta. A rentabilidade do uso dos sistemas de alerta baseado em dados de chuva foi condicionada às variações no regime dessa variável nas localidades estudadas. PREC_1 e PREC_2 apresentaram maior ganho de produtividade em relação à CALEND durante o período com maior índice pluviométrico nas localidades de Piracicaba, Campo Verde e Pedra Preta. Em contrapartida os sistemas de alerta não foram efetivos no controle de ASR em Ponta Grossa.

Palavras-chave: *Phakopsora pachyrhizi*; *Glycine max*; Chuva; Manejo integrado de doenças

ABSTRACT

Disease warning systems for rational management of Asian soybean rust in Brazil

The Asian soybean rust (ASR), caused by the fungus *Phakopsora pachyrhizi*, may promote significant damages in soybean crop. The disease is mainly controlled by sequential applications of fungicides following a calendar-based system. However, this practice disregards the weather favorability to recommend spraying to ASR control. The proposition of fungicide schemes to make the ASR control more efficient can be reached by the use of disease-warning systems. Thus, the current study aimed to assess the performance of different disease-warning systems to determine better fungicide spraying schemes for the ASR control. The experiment was conducted in Piracicaba, SP, Ponta Grossa, PR, Campo Verde and Pedra Preta, MT, Brazil, over the 2014/2015 and 2015/2016 soybean growing seasons. The treatments were: Unsprayed check treatment; Calendar-based sprays in a 14-day interval from R1 stage (CALEND); Disease warning system based on rainfall data with less conservative threshold (PREC_1 - 80% severity cut-off); and more conservative threshold (PREC_2 - 50% severity cut-off); Disease warning system based on air temperature and leaf wetness duration with less conservative threshold (TLWD_1 - 6 lesions cm⁻²) and more conservative threshold (TLWD_2 - 9 lesions cm⁻²). The results confirmed that weather conditions in the field trials were favorable to ASR progress. Among the weather elements correlated to severity leaf wetness duration, cumulative rainfall and air temperature during leaf wetness duration influenced positively the ASR. By testing warning systems to control ASR it was evidenced that those based on rainfall data presented highest performances. PREC_2 showed a high performance considering all sowing dates; whereas, PREC_1 was better treatment during sowing dates between October and November. The TLWD disease-warning systems, with both thresholds, overestimated the ASR, recommending more sprays compared to other treatments. Empirical models were efficient for estimation of LWD in Ponta Grossa, Campo Verde and Pedra Preta. High performances in estimating LWD were identified by using number of hours with relative humidity above 90% (NHRH \geq 90%), being these able to be used as input in the disease-warning systems (RMSE less than 2.0 h). The profitability of use rainfall based warning systems was conditioned by variations in the rainfalls regimes at the studied sites. PREC_1 and PREC_2 presented the highest relative yield gains in relation to CALEND during the period with the highest rainfalls in Piracicaba, Campo Verde and Pedra Preta. However, in Ponta Grossa, the rainfall based warning systems were not effective to control ASR.

Keywords: *Phakopsora pachyrhizi*; *Glycine max*; Rainfall; Integrated disease management

1. INTRODUCTION

Brazil is an important agricultural country, being considered as a leader in the production of several commodities. The Brazilian economy is directly influenced by agriculture, mainly by soybean grain exports. The main Brazilian soybean importer is China that receives more than 50 % of the Brazilian production (USDA, 2017)

Soybean is grown all over the country, except in the Amazon Forest, Pantanal and semi-arid areas of the Northeast region. Over the last 10 years soybean area, production and yield have been increasing in Brazil with a tendency that will remain stable throughout the 2017/2018 growing season. According to the Brazilian Food Supply Agency (CONAB, 2017), for the 2017/2018 Brazilian soybean production, it is predicted increases in yield close to 8.6 %, resulting in a harvest of 114.1 millions of tons of grains. This outcome of yield is a consequence of the increased area, 2.7 % higher than the 2016/2017 growing season, along with yields of about 9.0 % of that obtained in the previous year (CONAB, 2017).

The states of Mato Grosso and Paraná have been contributing more expressively to soybean production in Brazil. In Mato Grosso, during 2016/2017 growing season, the soybean area reached 9.2 million ha, with a total production of 30.3 million tons and an average yield of about 3,277 kg ha⁻¹. In Paraná, the second largest producer, the soybean area in the same growing season was estimated at 5.2 million ha, along with a total production of 17.6 millions of tons, and an average yield of 3,360 kg ha⁻¹.

Soybean belongs to the family Fabaceae, genus *Glycine* with binomial name *Glycine max* (L.), Merrill (1917). Nowadays, soybean plants are erect, vigorous with an annual growth habit, which can be determined or undetermined. These morphological characteristics were obtained by means of advanced breeding techniques over the last four decades (GURIQBAL, 2010). In Brazil, soybean crop is sowed between October and December with harvests occurring between January and March, depending on the cultivar used and region of the country. Soybean crop cycle can range from 100 to 160 days, which varies according to the interaction between cultivar (maturity group) and climate conditions, mainly photoperiod duration and air temperature (CÂMARA et al., 1997).

Such as many other crops, soybean cultivation is closely related to geographic and climatic conditions. Based on that, weather variables, such as air temperature, rainfall, solar radiation, and photoperiod have major importance for this crop, directly affecting growth, development and yield (BATTISTI; SENTELHAS, 2015; CÂMARA et al., 1997;

HOLLINGER; CHANGNON, 1993), as well as its interactions with pests and diseases (DEL PONTE; ESKER, 2008; ROSENZWEIG et al., 2001).

Despite the direct effect of weather variables on soybean yield, local atmospheric conditions also bring about occurrences of pests, diseases, and weeds in production fields. As in Brazil soybean is grown in large areas all around the country, where weather conditions are normally favorable for diseases, the risk of epidemics is high but can vary according to climate variability.

Many diseases are able to affect and reduce soybean yield at a commercial scale; however since 2001 Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* Sydow & Sydow, has affected soybean crop at economical levels, with yield reductions of up to 70% (HARTMAN et al., 2016; FIALLOS; FORCELINI, 2013; CHRISTOVAM et al., 2010; PRADO et al., 2010; CUNHA; REIS; SANTOS, 2006). Such disease was firstly observed in Brazil fifteen years ago in the Paraná state and now is spread all over the country and also in South America (YORINORI et al., 2005).

ASR epidemics in soybean fields are frequently triggered by three main factors: local weather condition, which affects microclimate inside the canopy; inoculum concentration in the area; and crop management practices adopted by the growers in the region. Currently, in soybean fields, ASR epidemics are controlled by sequential fungicide sprays, especially fungicides composed by triazoles, strobilurins, their mixture, and carboxamides (TWIZEYIMANA; HARTMAN, 2016). Application of fungicides in soybean crop is based on a calendar schedule determined by a phenological stage (BIOFIX). In this particular case, sprays start on R1 stage (beginning of bloom) and continue throughout the entire reproductive stage of the crop (SCHERM et al., 2009).

According to Almeida et al. (2013), ASR control based on fungicide sprays is an effective method to assure disease management, reducing inoculum, suppressing sporulation and controlling spores spread in soybean fields. Although fungicide applications have been showing good results to mitigate the ASR damages, such a practice increases production costs and may impose impacts upon the environment and beneficial microorganisms. Moreover, in many cases, sprays have been done with no technical knowledge at all in order to rationalize the use of chemicals. As a result of that fungicides have been sprayed at inappropriate rates and timing, triggering thus significant reductions in soybean yield whenever disease control practices are to be necessary.

Another crucial triggering factor for ASR epidemics is the local weather conditions since they need to be favorable to allow for disease occurrence and development. Melching et

al. (1989) verified that leaf wetness duration between 6 and 12 h day⁻¹ and air temperature ranging from 15 to 28 °C are favorable to promote both infections and spread of ASR. However, more intense epidemic normally happens when leaf wetness duration is between 10 and 22 h day⁻¹ and air temperature between 20 and 25°C (ALVES et al., 2007; DEL PONTE et al., 2006a; MARCHETTI; MELCHING; BROMFIELD, 1976).

Leaf wetness duration and air temperature have a direct effect on ASR occurrence and dissemination, primarily by influencing the rate of infection (fungus germination and penetration) and sporulation processes (IGARASHI et al., 2014). Based on that, Igarashi et al. (2016) observed in a soybean crop that leaf wetness duration and air temperature were good predictors of ASR in southern Brazil.

Rainfall is another weather variable of high importance for ASR epidemics. Del Ponte et al. (2006a), by analyzing 21 experimental areas around soybean production fields in Brazil during two growing seasons, determined a high correlation between rainfall during the soybean reproductive phase and final ASR severity. A similar pattern was observed by Tschanz (1984) in Taiwan and by Stovold and Smith (1991) in Australia.

The effect of rainfall on ASR was also observed by Levy (2005) in southern Zimbabwe and by Yorinori et al. (2005) in Brazil, where disease values were lower under dry and hot weather. Strong correlations observed between rainfall and ASR severity are mainly caused by the influence of raindrops on urediniospores spread (ALMEIDA et al., 1997). Urediniospores tend to remain fixed inside the uredias; therefore it is difficult to be spread by wind. Whenever an episode of rainfall occurs, drops of water favor the release of urediniospores into the environment (ALMEIDA et al., 1997). Moreover, high rainfall amount promotes leaf wetness duration and reduces air temperature within the canopy, making the microclimate favorable for ASR progress (DEL PONTE et al., 2006a).

Considering the influence of weather conditions on ASR, mathematical models were developed to express this relationship. These models were used to evaluate disease severity and risk of occurrence, which served as the basis for developing or improving disease-warning systems (KELLY et al., 2015; DIAS; LI; YANG, 2014; DEL PONTE et al., 2006b).

According to Gleason et al. (2008), disease-warning systems are decision-making tools that help growers to manage plant disease, defining the better moment for spraying the crops. Disease-warning systems are initially developed based on relationships between weather variables and disease development under environmental controlled conditions (growth chambers) or for specific regions, which require test and validation of them under specific commercial fields (DUTTWEILER et al., 2008).

Weather variables used as input in the disease-warning systems are basically defined according to the disease characteristics; however, the most frequent ones are air temperature, rainfall, relative humidity, solar radiation and leaf wetness duration, which represent better the risk of outbreaks (ROWLANDSON et al., 2014; DUTTWEILER et al., 2008; GLEASON et al., 2008). For ASR, weather variables such as rainfall (DEL PONTE et al., 2006a; TAN; YU; YANG, 1996), sunlight (DIAS; LI; YANG, 2014; DIAS et al., 2010), air temperature and leaf wetness duration (REIS; SARTORI; CÂMARA, 2004; MELCHING et al., 1989; MARCHETTI; MELCHING; BROMFIELD, 1976) are the most common used in the disease-warning systems already available.

The first disease-warning systems for recommending sprays for ASR control were developed by Melching et al. (1989) and Marchetti, Melching and Bromfield (1976). These authors correlated daily ASR infection with weather variables measured along the soybean cycle. Recently, Del Ponte et al., (2006b) developed four linear and quadratic regression models with the objective of determining ASR occurrence at two distinct Brazilian sites. All of these models proposed by the aforementioned authors had as an input cumulative rainfall measured at the month shortly after the ASR identification in the field.

Based on the soybean yield reduction caused by ASR, scientific investigations are required to determine the favorable weather conditions that affect ASR occurrence and progress in different Brazilian regions, aiming to develop efficient decision support systems for its control. Therefore, the hypothesis of the present study is that the use of disease-warning systems based on rainfall or on the interaction between air temperature and leaf wetness duration, can make ASR control more efficient, rationalizing fungicides use and reducing production costs.

1.1. Objectives

1.1.1. General objectives

The general objective of this study was to test and to evaluate different Asian soybean rust warning systems, for a better recommendation of fungicide sprays schemes to control ASR for soybean in Brazil.

1.1.2. Specific objectives

Specific objectives of this study were:

- a) To identify and quantify the influence of weather variables on ASR disease progress at different Brazilian locations;
- b) To test and evaluate different disease-warning systems to recommend fungicide sprays for controlling ASR, with two different thresholds, and compared them with unsprayed and calendar based system;
- c) To assess the feasibility of leaf wetness duration estimated by different models as an input data for an ASR warning system;
- d) To evaluate the soybean yield and profitability in response to the different schemes for ASR control (calendar based x warning systems).

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2. ASIAN SOYBEAN RUST EPIDEMICS AS AFFECTED BY WEATHER CONDITIONS

ABSTRACT

The Asian soybean rust (ASR) (*Phakopsora pachyrhizi*) can reduce soybean production drastically. ASR epidemics are often triggered by weather conditions, which interfere actively on the disease progress. Therefore, weather variables can be used to estimate the risk of occurrence and severity of ASR outbreaks. This research aimed to determine the influence of different weather variables on ASR progress in different field trials in Brazil. Field experiments were conducted during 2014/2015 and 2015/2016 soybean growing seasons. For the first season, field trials were installed at Piracicaba, state of São Paulo, Ponta Grossa, state of Paraná, and Campo Verde, state of Mato Grosso. For the second season, besides the three field trials described above an additional experiment was also carried out in Pedra Preta, state of Mato Grosso. For all sites and seasons, soybean crop was drilled with 0.45 m row spacing and 12 plants per linear meter, totalizing 266,666 plants ha⁻¹. In order to create different environmental conditions, a sequential sowing date, nearly of 30-day intervals, was done. At each experimental site, an automatic weather station (AWS) was installed close to the crop (from 5 to 15 m lateral distance) in order to monitor weather conditions. In all areas a susceptible cultivar was sowed and no fungicide sprays were applied to ensure the natural disease occurrence. In Piracicaba and Ponta Grossa after disease identification, ASR severity assessments were done based on a diagrammatic scale proposed by Godoy; Koga; Canteri (2006). Furthermore, at Campo Verde and Pedra Preta, disease severity assessment was performed at the R5.5 soybean phenological stage, and at the end of the soybean growing season, a canopy defoliation caused by ASR was determined. Severity data were analyzed by disease progress curves, which were adjusted by the fitting of the experimental data to the following regression models: exponential, logistic, monomolecular and Gompertz. A simple linear regression analysis was performed between meteorological variables and ASR progress rate to identify those climatic variables of major influence on the disease. For Piracicaba and Ponta Grossa, the logistic model had the best fit to the ASR severity. In Piracicaba, Ponta Grossa, Campo Verde, and Pedra Preta the main weather variables influencing ASR were leaf wetness duration - LWD (R = 0.340), air temperature during LWD (R = 0.313), and cumulative rainfall (R = 0.304). The final severity was assessed only at Piracicaba and Ponta Grossa, and it was mainly influenced by LWD (R = 0.643).

Keywords: *Glycine max*; *Phakopsora pachyrhizi*; Epidemiology; Rainfall; Leaf wetness duration

2.1. Introduction

Many factors contribute to reduce soybean production and among them, diseases are one of most importance, which might be caused by fungus, bacteria, and virus (STRANGE; SCOTT, 2005). However, for the disease establishment, it is necessary the existence of a susceptible host and favorable environmental conditions, which characterizes the disease triangle. Environmental conditions should be seen as the main triggering factor to promote disease occurrence in conjunction with the pathogen and host populations (KRANZ; ROTEM, 2012; STRAND, 2000).

Air temperature, relative humidity, rainfall, leaf wetness duration, solar radiation, and wind are the main weather variables that affect the infection process and disease spread in soybean fields (CAO et al., 2014; JONES et al., 2010). Weather regimes characterize the local climate, which can originate favorable or unfavorable environmental conditions for disease occurrence. Furthermore, weather variables are straightly linked with fungicide spray effectiveness, affording a better or lower disease control (STEFANELLO et al., 2016).

A soybean disease called Asian soybean rust (ASR) (*Phakopsora pachyrhizi* Syd. & P. Syd) is impinged upon the weather variables and has been controlled by fungicide spray. The ASR is one of the most important soybean diseases. This pathogen is an obligate parasite fungus that infects all plant tissues, especially leaves, causing premature defoliation, early maturity, and yield reductions up to 90% under favorable conditions and with inefficient disease management (HARTMAN et al., 2016; YORINORI et al., 2005).

Air temperature can strike the whole ASR infection cycle by interfering and regulating the pathogen metabolic reactions (SENTELHAS; GILLESPIE, 2008). At an optimum temperature range, metabolic reactions can be accelerated; as a consequence a reduction in the infectious cycle occurs, increasing the favorability of disease occurrence (AGRIOS, 2005a).

Singh and Thapliyal (1977), by analyzing soybean breeding for ASR in India, determined that the optimum air temperature for the infection process was of 25°C. By scrutinizing ASR occurrence in China Tan; Yu and Yang (1996) observed that the ideal air temperature for infection ranged from 15 to 26°C. More recently, Bonde et al. (2007), using ASR isolates from Taiwan, Zimbabwe, Hawaii and Brazil, observed that air temperature between 17°C and 28°C were optimum for the urediniospore germination, germ tube growth, and ASR initial infection.

Under Brazilian weather conditions Vale; Chaves and Zambolim (1985) examined the effect of sowing dates on ASR incidence and observed that the highest number of lesions per cm² on the leaves were obtained under air temperature of 20°C. In the same way Alves et al. (2007) evidenced a more intense ASR infection under air temperature around 20°C, culminating in a lower ASR infection when air temperature remained below 15°C or above 28°C. More recently, Danelli and Reis (2016) determined that air temperature conditioned ASR growth and development, having the largest number of spores, lesions, uredia and uredias per lesions when temperatures were between 22°C and 25°C.

ASR occurrence in soybean crop is also influenced by local humidity conditions, whereas rainfall, irrigation, dew and leaf wetness are to be the main source of moisture in soybean production fields. Air humidity interferes with the initial development and establishment of ASR once it affects the pathogen germination and penetration processes (ROWLANDSON et al., 2014; SENTELHAS et al., 2008; ALVES et al., 2007). Moreover, high air humidity makes the host tissue thin, increasing the host susceptibility to pathogen infection (AGRIOS, 2005a). For fungal pathogens, i.e. *P. pachyrhizi*, the presence of free water on the host tissue is essential to spores longevity and viability, germination, infection and sporulation (DEL PONTE; ESKER, 2008).

Leaf wetness duration (LWD) associated with air temperature during LWD events have a direct effect on ASR occurrence and dissemination, primarily by influencing the rate of infection (fungus germination and penetration) and sporulation (IGARASHI et al., 2014). A positive interaction between LWD and ASR infection probability was found by Hartman et al. (2016) and Vale; Zambolim and Chaves (1990), with the highest disease occurring during days with 24 hours of LWD. While, Melching et al. (1989) and Bonde et al. (2007) observed that wet periods exceeding 10 hours for several consecutive days are optimal for ASR occurrence.

Besides air temperature and LWD, rainfall can also affect ASR occurrence. Rainfall regime is considered to be an epidemic triggering factor of ASR in soybean production fields, by creating a favorable environment for the pathogen (MEGETO et al., 2014; DEL PONTE et al., 2006a) and unfavorable for fungicide spray application in order to control the ASR (STEFANELLO et al., 2016; FIFE; NOKES, 2002).

Field experiments in soybean areas in Taiwan evidenced that rainfall regime is the main weather variable for ASR occurrence (TSCHANZ, 1984). Likewise, in Australian soybean areas, ASR epidemics only occurred when there were a high rainfall volume and frequency throughout the soybean growing season (STOVOLD; SMITH, 1991). Influence of

rainfall on ASR progress was also observed in Brazil, where areas with rainfall between 250 and 450 mm showed final severity upper to 70%; whereas areas with rainfall lower than 125 mm presented severity below 30% (DEL PONTE et al., 2006b). Megeto et al. (2014), using weather data from different field trials located all over Brazil, observed a high influence of rainfall on ASR occurrence. Similar results were obtained by Minchio et al. (2016).

According to Melching; Bromfield and Kingsolver (1979), the high correlation observed in several studies between rainfall and ASR occurrence is mainly related with urediniospores spread. Urediniospores tended to clump and stick together and rain splash could facilitate detachment of urediniospores from uredias (DEL PONTE et al., 2006a). Furthermore, rainfall has a remarkable influence on LWD patterns by promoting leaves wetness and increasing soil moisture, raising relative humidity and causing longer dew periods (DEL PONTE; ESKER, 2008).

Taking into account the interference of weather variables on ASR disease progress, the current study aimed to characterize the influence of weather variables (air temperature, relative humidity, rainfall and leaf wetness duration) on ASR disease progress at four different locations in Brazil: Piracicaba, state of São Paulo; Ponta Grossa, state of Paraná; Campo Verde and Pedra Preta, both in the state of Mato Grosso.

2.2. Material and Methods

Field experiments were conducted during 2014/2015 and 2015/2016 growing seasons. For the first season field trials were installed at Piracicaba, state of São Paulo (Lat. 22°42' S, Long. 47°37' W, Alt.: 567m, climate classification Cwa); Ponta Grossa, state of Paraná (Lat. 25°05' S, Long. 50°09' W, Alt.: 969 m, climate classification Cfb); and Campo Verde, state of Mato Grosso (Lat. 15°24' S, Long. 55°5' W, Alt.: 689m, climate classification Aw). For the second season, besides the three field trials described above an additional experiment was also carried out in Pedra Preta, state of Mato Grosso (Lat. 16°83' S, Long. 54°04' W, Alt.: 744m, climate classification Aw) - Brazil. Köppen's climate classification of each site was based on Alvares, et al. (2013).

At Piracicaba and Ponta Grossa throughout the 2014/2015 crop season, the field trial was conducted with Brasmax Potência RR® (indeterminate growth, and maturity group 6.7), during 2015/2016 soybean season the experiments were cultivated with Monsoy 6410 RR® IPRO® (indeterminate growth, and maturity group 6.4). At Campo Verde and Pedra Preta, a soybean cultivar TMG 132 RR® (determinate growth, and maturity group of 8.5) was sowed

for the two growing seasons. For all sites and seasons, soybean crop was drilled with 0.45 m row spacing and 12 plants per linear meter, totalizing 266,666 plants ha⁻¹. In order to create different environmental conditions sequential sowing dates, nearly of 30-day intervals, were done (Table 2.1).

Table 2. 1. Site, growing season and sowing dates for each experimental site.

Site	Growing season *	Sowing date	Growing season *	Sowing date
Piracicaba, SP	2014/2015	23/Oct/2014	2015/2016	22/Oct/2015
		18/Nov/2014		19/Nov/2015
		12/Dec/2014		18/Dec/2015
		20/Jan/2015		20/Jan/2016
		19/Feb/2015		19/Feb/2016
Ponta Grossa, PR	2014/2015	16/Oct/2014	2015/2016	08/Oct/2015
		11/Nov/2014		21/Nov/2015
		18/Dec/2014		11/Dec/2015
		15/Jan/2015		23/Jan/2016
Campo Verde, MT	2014/2015	21/Oct/2014	2015/2016	23/Oct/2015
		13/Nov/2014		07/Nov/2015
		12/Dec/2014		09/Dec/2015
Pedra Preta, MT	2014/2015	--	2015/2016	06/Nov/2015
		--		03/Dec/2015

* In Piracicaba and Ponta Grossa throughout the 2014/2015 the cultivar sowed was the Brasmax Potência RR® (indeterminate growth, and maturity group 6.7) while during 2015/2016 the Monsoy 6410 RR® IPRO® (indeterminate growth, and maturity group 6.4) was sowed. At Campo Verde and Pedra Preta, a soybean cultivar TMG 132 RR® (determinate growth, and maturity group of 8.5) was sowed for the two growing seasons.

In all field trials, the experimental design adopted was randomized blocks, with four replications and treatments arranged in a factorial scheme. Treatments were the combination of sowing dates with soybean growing seasons. For Piracicaba and Ponta Grossa, plots were comprised of five sowing rows with four meters length. At Campo Verde and Pedra Preta, plots presented five sowing rows with seven meters length.

Seed treatment was done with 200 ml of fungicide and pesticide (Pyraclostrobin, MetilTiofanato, and Fipronil) for each 100 kg of seeds. Soybean seeds were inoculated with *Bradyrhizobia* bacteria (*Bradyrhizobium japonicum*, strains SEMIA 5079 e 5080) with 5 x 10⁹ colony forming unit (CFU) ml⁻¹. Inoculation was performed by mixing seeds with liquid

inoculants and prior to the sowing of the seeds. Mineral fertilization was done based on soil analysis and considering the expected yield. In Piracicaba and Ponta Grossa field trails, soybean crop was fertilized with 100 kg of P_2O_5 ha⁻¹ and 100 kg of KCl ha⁻¹. In Campo Verde and Pedra Preta, the soybean fertilization was done with 152 kg ha⁻¹ of NPK (12-46-00), plus 7% of sulfate and 150 kg of KCl ha⁻¹.

At each experimental site, an automatic weather station (AWS) was installed close to the crop (from 5 to 15 m) in order to monitor meteorological variables. The AWS was equipped with calibrated sensors to measure: air temperature (AirT) and relative humidity (RH) (HMP35C probe, Vaisala); rainfall (TR-525M rain gauge, Texas Instruments) and leaf wetness duration (LWD) (Cylindrical Sensor, Weather Innovations). These sensors were connected to dataloggers (Models CR10X and CR1000, Campbell Scientific, Logan UT), which were programmed to perform readings at each minute and store averages (AirT and RH) or totals (rainfall and LWD) at every 15 minutes. All LWD sensors were previously tested and calibrated under laboratory and field conditions (SENTELHAS et al., 2004; SENTELHAS; MONTEIRO; GILLESPIE, 2004).

To ensure ASR occurrence, susceptible cultivars were sowed in all field trials. Furthermore, no fungicide sprays to control ASR were done. As the pathogen is spread all over the Brazilian agricultural lands and considering that weather conditions are constantly favorable to ASR occurrence, it was not necessary to perform inoculation so that the disease could naturally occur in the field.

Disease assessments were made in three central rows of each plot. At Piracicaba and Ponta Grossa shortly after ASR identification, assessments were made at every 10 days throughout the entire soybean growing season till the beginning of maturation. In order to determine ASR severity, soybean plants were separated in three parts based on plant height: a) lower third, a region close to the soil surface; b) middle third, a central region of the soybean plant; c) upper third, in the higher part of the canopy. For each part four leaflets were randomly selected and severity notes were ascribed based on a diagrammatic scale proposed by Godoy; Koga and Canteri (2006). For canopy with defoliation owing to ASR, the severity level was considered as 100%.

At Campo Verde and Pedra Preta, disease assessments were performed at the R5.5 soybean phenological stage, which turns out to be characterized by pods between 75% and 100% of grain granulation. Moreover, at the end of the soybean growing season, a canopy defoliation caused by ASR was determined. The defoliation assessments were realized when soybean plants reached the R7 phenological stage (beginning of the maturation); therefore,

visual evaluations were made following the defoliation severity scale proposed by Hirano et al. (2010).

Based on ASR severity data collected in Piracicaba and Ponta Grossa, the area under the disease progress curve (AUDPC) (SHANER; FINNEY, 1977) was calculated by the following expression:

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

where: n represents the number of observations; y_i is the disease severity measured at the “i” observation; t_i is the time in days of “i” observation; $(y_i + y_{i+1})$ corresponds to the mean height of the rectangle between points y_i and y_{i+1} ; $(t_{i+1} - t_i)$ is the difference of the rectangle base between points t_{i+1} and t_i , corresponding to the time interval in days between two consecutive evaluations.

Moreover, a temporal epidemiological analysis was made from the plot of cumulative severity data as a function of time. In order to determine the ASR apparent infection rate the disease progress curves were plotted and then fitted to the Exponential, Logistic, Monomolecular, and Gompertz models (Table 2.2) (MADDEN; HUGHES; BOSCH, 2007; CAMPBELL; MADDEN, 1990).

Table 2.2. Differential and integrated equations and their linearized forms to estimate the ASR growth at different field trials throughout 2014/2015 and 2015/2016 soybean growing seasons. Adapted from Xu (2006).

Model	dy/dt	y	Linear transformation
Exponential	$r_E y$	$B \exp(r_E t)$	$\ln(y)$
Logistic	$r_L y \left(\frac{K - y}{K} \right)$	$\frac{K}{1 + B \exp(-r_L t)}$	$\ln \left(\frac{y}{K - y} \right)$
Monomolecular	$r_M (K - y)$	$K [1 - B \exp(-r_M t)]$	$\ln \left(\frac{K}{K - y} \right)$
Gompertz	$r_G y [\ln(K) - \ln(y)]$	$K \exp[-B \exp(-r_G t)]$	$-\ln \left[-\ln \left(\frac{y}{K} \right) \right]$

K = maximum level of disease or asymptote of disease progress curve, y = disease at the time of observation, B = a parameter related to the level of initial disease or point of inflection, r = rate of disease increase and t = time. The subscripts E, L, M, and G depict, respectively, the Exponential, Logistic, Monomolecular and Gompertz models.

The determination of the best model was made based on the plot of the standard residue obtained by the difference between the observed ASR and the predicted ASR obtained

between each interval of disease assessment and also taking into consideration the coefficient of determination (R^2) obtained by means of a simple linear regression between observed and predicted disease values, and whether or not patterns exist in the residue chart versus predicted disease values (MADDEN; HUGHES; BOSCH, 2007; CAMPBELL; MADDEN, 1990). In addition, in order to compare observed and predicted disease data, the following statistical parameters were adopted: mean error (ME), mean absolute error (EAM) and root mean square error (RMSE).

To determine the weather influence on ASR epidemics a correlation analysis was performed to identify variables associated with final disease severity and AUDCP for Piracicaba and Ponta Grossa along with final defoliation for Campo Verde and Pedra Preta. Furthermore, the effects of weather conditions on ASR epidemics were performed confronting severity assessed in R5.5 stage and final severity with the mean air temperature during LWD (Air_LWD), LWD, cumulative Rainfall (C_Rainfall), mean air temperature (Air_T) and rainfall events (Rainfall_E). Correlation analyses were made using disease values with weather conditions measured 30 days before the severity assessment in soybean fields.

2.3. Results and Discussion

2.3.1. Weather characterization

The weather conditions during the soybean growing seasons in different sites are presented in Figures 2.1, 2.2, 2.3 and 2.4.

In Piracicaba, the mean air temperature for each sowing date remained between 22.9 and 24.7 °C, which are within the optimum range for ASR development, considered as the interval between 20 and 25 °C (DEL PONTE; ESKER, 2008; ALVES et al., 2007; MELCHING et al., 1989; MARCHETTI; MELCHING; BROMFIELD, 1976). However, air temperature above 25 °C during the two growing seasons was observed (Figure 2.1). Despite the permanence of air temperature above the upper favorable threshold for disease occurrence, such condition was not able to reduce ASR final severity, which reached 94.1 % in the first and 89.5 % in the second growing season (Table 2.3). For the fifth sowing date in 2015/2016, ASR was not observed, probably due to the occurrence of low temperatures combined with the absence of moisture, which was caused by the intense drought that occurred in this year.

Evidences that air temperature is not a limiting factor for ASR occurrence in soybean production fields of Brazil were also reported by Del Ponte et al. (2006b). In contrast, a

drawback in ASR epidemics due to high air temperature might be related in several studies. Alves et al. (2007) examined under tropical field conditions that urediniospores from different isolates do not germinate and appressoria does not grow in environments with air temperatures above 30 °C. Also in Brazil, Danelli and Reis (2016) verified no infection process whenever air temperature reached values higher than 30 °C.

The high-temperature effects on the ASR epidemics were also demonstrated in studies conducted in the Southeastern USA. Bonde; Nester and Berner (2012) certified that air temperatures of 29, 33 and 39 °C for one hour per day might reduce the urediniospore formation in 39, 19 and 0 %, respectively, compared to temperatures within an optimum range of ASR development. Another factor that might interfere with ASR development is the frequency of high temperatures above 33 °C. At different counties located in southern USA air temperatures above 33 °C decreased the ASR epidemics. Such effects were prominent whenever three consecutive days with air temperatures of 35 °C were observed, and in this case 50 % less urediniospore were produced (BONDE; NESTER; BERNER, 2013).

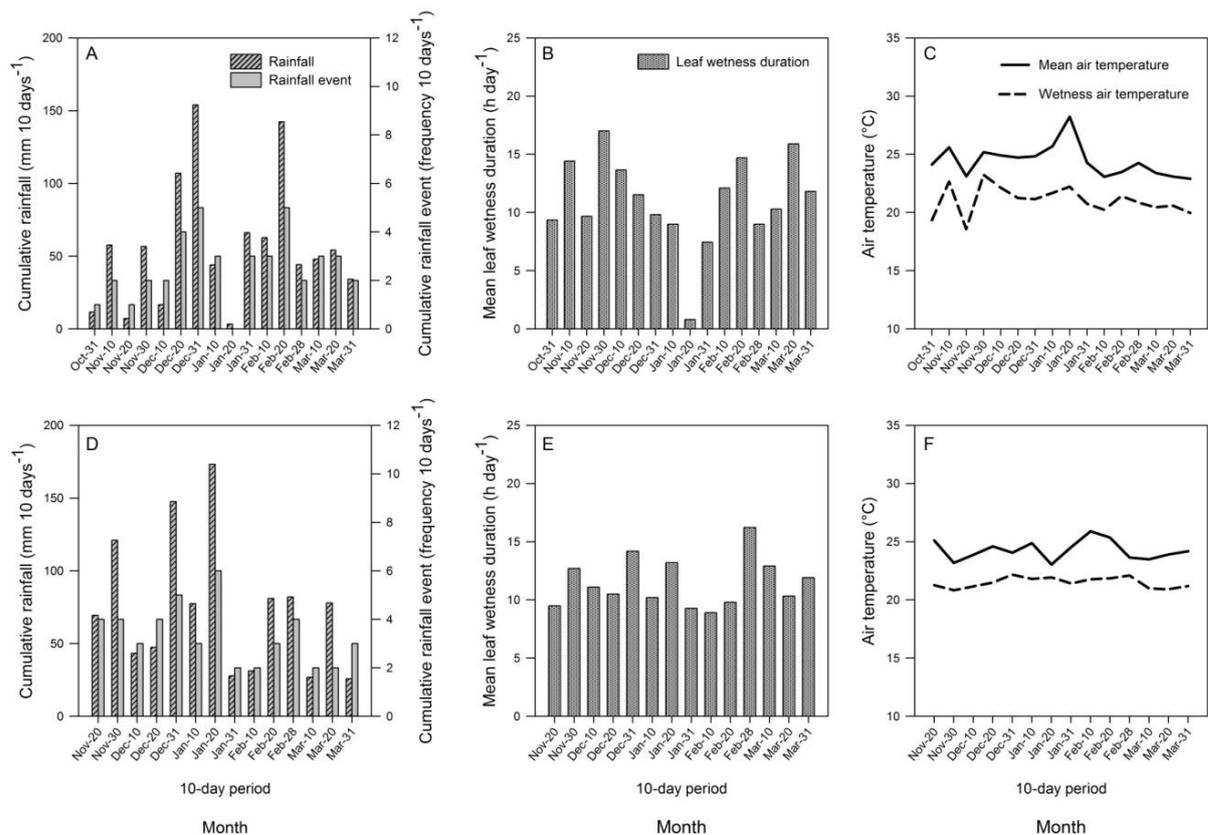


Figure 2. 1. Weather conditions in Piracicaba, SP, during 2014/2015 (A, B and C) and 2015/2016 (D, E and F) soybean crop growing seasons.

Under the thermal point of view, the favorability to ASR occurrence at Piracicaba can be corroborated by analyses of the air temperature during LWD (Figure 2.1). Considering all sowing dates for the 2014/2015 growing season, air temperature during LWD ranged from 18.2 °C to 23.2 °C. Throughout the 2015/2016 growing season, such variable varied from 14.4 °C to 22.2 °C, whereas air temperatures below 15 °C, a lower threshold of optimum range of temperature to ASR development, were found with the highest frequency throughout the fifth sowing date.

Table 2. 3. Final severity and area under the disease curve progress (AUDCP) caused by Asian soybean rust in Piracicaba and Ponta Grossa throughout the 2014/2015 and 2015/2016 soybean growing seasons at different sowing dates.

Piracicaba – 2014/2015			Ponta Grossa – 2014/2015		
Sowing date	Final Severity (%)	AUDCP	Sowing date	Final Severity (%)	AUDCP
23/Oct/2014	92.2 a	2287.5 a	16/Oct/2014	66.8 a	1676.8 a
18/Nov/2014	100.0 a	1926.9 ab	11/Nov/2014	18.6 b	555.5 c
12/Dec/2014	100.0 a	1655.7 b	18/Dec/2014	71.3 a	1073.7 b
20/Jan/2015	84.4 a	837.1 c	15/Jan/2015	81.0 a	1946.8 a
19/Feb/2015	94.1 a	1726.2 b	--	--	--
CV	8.1%	11.4%	CV	14.5%	14.2%
Piracicaba – 2015/2016			Ponta Grossa – 2015/2016		
Sowing date	Final Severity (%)	AUDCP	Sowing date	Final Severity (%)	AUDCP
22/Oct/2015	65.9 b	1969.3 a	08/Oct/2015	73.7 a	577.2 a
19/Nov/2015	97.2 a	1827.2 a	21/Nov/2015	16.4 b	243.9 bc
18/Dec/2015	95.1 a	1144.9 b	11/Dec/2015	12.1 b	101.2 c
20/Jan/2016	100.0 a	780.1 b	23/Jan/2016	11.5 b	277.3 b
19/Feb/2016	1.9 c	26.1 c	--	--	--
CV	9.8%	24.2%	CV	8.1%	22.7%

The final severity and AUDCP data followed by different letters are statistically different by the Tukey's test ($\alpha = 0.05$)

At Ponta Grossa the air temperature during LWD most of the time remained under an optimum range of ASR development (Figure 2.2). Throughout the 2014/2015 soybean crop growing season air temperature ranged from 14.9 °C to 21.6 °C, whilst throughout the 2015/2016 growing season air temperature during LDW stayed between 17.3 °C and 20.7 °C

(Figure 2.2). In spite of air temperatures remained lower than an optimum range, studies demonstrated that the infectious process may occur under temperatures varying from 15 to 28°C (ALVES et al., 2007; DEL PONTE et al., 2006a; MELCHING et al., 1989).

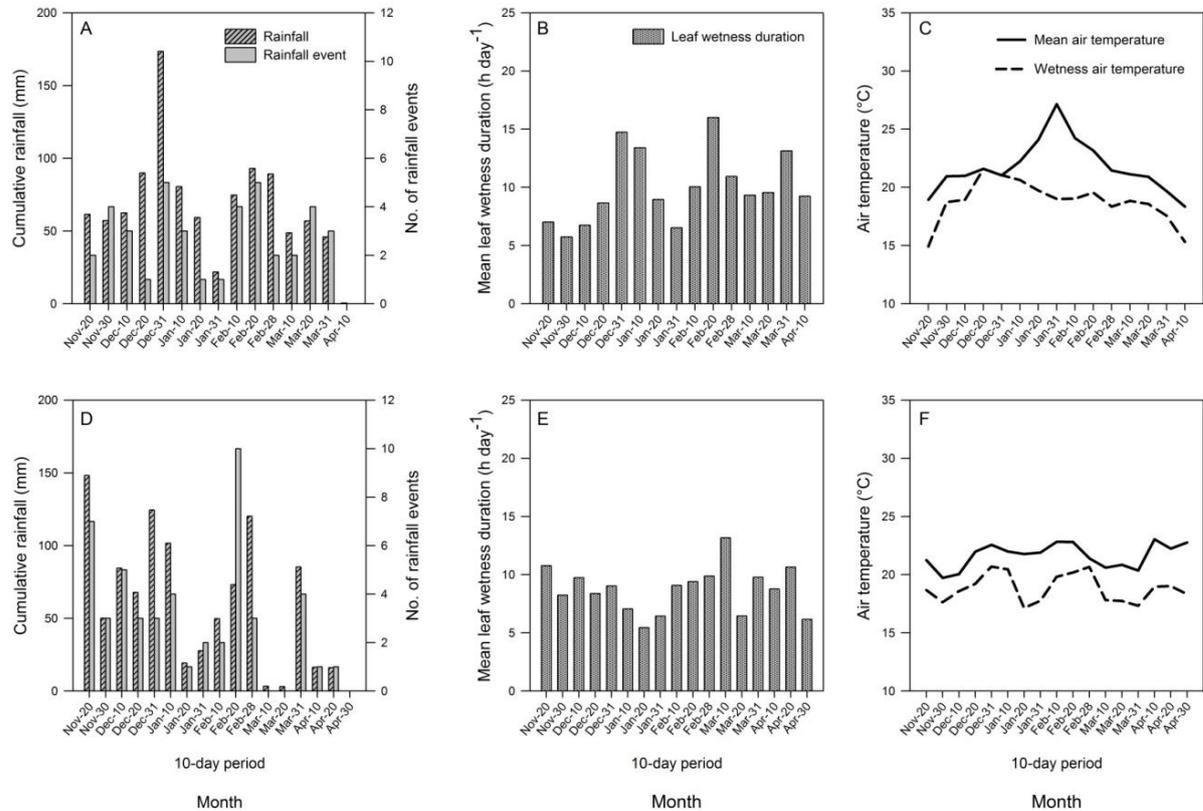


Figure 2. 2. Weather variables in Ponta Grossa, PR, throughout the 2014/2015 (A, B and C) and 2015/2016 (D, E and F) soybean growing seasons.

Although air temperature has remained within a favorable thermal range for the ASR development it was characterized that lower air temperatures were measured at Ponta Grossa, a fact that might explain low values of final disease severity and AUDCP on some sowing dates (Table 2.3). Despite air temperature has been considered a no-limiting factor for ASR epidemics in Brazil (DEL PONTE et al., 2006b), the occurrence and establishment of pathogens, e.g. *P. pachyrhizi*, can be significantly conditioned by the thermal regime (DANELLI; REIS, 2016; CAO et al., 2014; ALVES et al., 2007; BONDE et al., 2007; MELCHING; BROMFIELD; KINGSOLVER, 1979). Such effects were identified by Danelli and Reis (2016), who found shorter latent period of *P. pachyrhizi* under a temperature of 25 °C compared to 15 °C.

In Campo Verde, it is possible to see that air temperatures during LWD were favorable to ASR development at this site (Figure 2.3), despite of the fact that for the majority of the periods in analysis the mean air temperature remained down below 25 °C. Throughout

the first growing season, it was not observed air temperatures out of the ASR favorable range, being the mean air temperature throughout the three crop growing seasons corresponding to 23.2 °C, whilst air temperature during LWD remained at 20.8 °C (Figure 2.3). During the second experimental year, air temperature during LWD varied from 21.1 °C to 23.1 °C.

Although air temperature has been above 25 °C in some 10-day periods, most of the time this variable remained within the favorable range for the ASR occurrence. The favorable environmental condition for ASR occurrence in Campo Verde resulted in high intensity of defoliation, of almost 100 % in most of the assessments (Table 2.4).

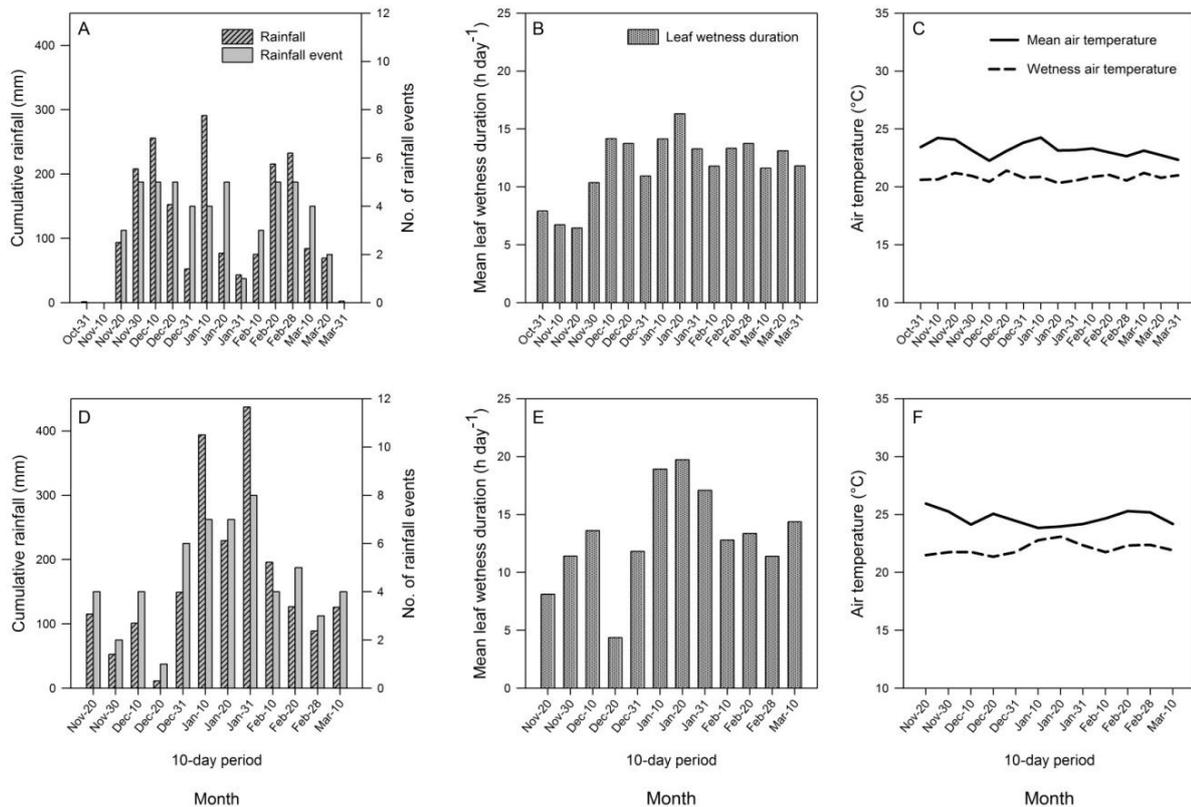


Figure 2.3. Weather conditions in Campo Verde, MT, during 2014/2015 (A, B and C) and 2015/2016 (D, E and F) soybean growing seasons.

Throughout most of the 2015/2016 growing season, the air temperature at Pedra Preta remained above 25 °C, an upper optimum air temperature threshold for ASR occurrence. However, air mean temperature during the leaf wetness duration (LWD) was always lower than 25 °C and under the presence of a free water film sitting on the plant surface reached a better range for the ASR to occur in the field (Figure 2.4). According to Del Ponte and Esker (2008), the urediniospores germination with subsequent success on infection process is possible under free water conditions on the plant surface. Therefore, considering the presence of such a free water film on the plant surface during the nocturnal period, air

mean temperature during LWD was favorable to both infection and establishment of ASR in soybean plants, a fact that promoted high defoliation at the end of the soybean growing season in Pedra Preta (Table 2.4).

Table 2. 4.Defoliation caused by Asian Soybean Rust in Campo Verde and Pedra Preta, MT, throughout the 2014/2015 and 2015/2016 growing seasons and at different sowing dates.

Campo Verde				Pedra Preta	
2014/2015		2015/2016		2015/2016	
Sowing date	Defoliation (%)	Sowing date	Defoliation (%)	Sowing date	Defoliation (%)
21/Oct/2014	97.8 b	23/Oct/2015	97.8 b	06/Nov/2015	99.8 a
13/Nov/2014	100.0 a	07/Nov/2015	100.0 a	03/Dec/2015	99.5 a
12/Dec/2014	100.0 a	09/Dec/2015	100.0 a	--	--
CV	0.3%		0.3%		0.4%

The defoliation data followed by different letters are statistically different by the Tukey's test ($\alpha = 0.05$)

The influence of night air temperatures on the ASR occurrence was demonstrated in Taiwan through the 1980's. Tschanz; Wang and Tsai (1983) determined that ASR epidemics were correlated with the mean night air temperatures, evidencing that when such temperature was around 14 °C no rust lesions occurred at all and that ASR epidemics were retarded. In contrast, whenever the high mean night temperature was recorded (≈ 25 °C), high rust lesions were observed in all field trials.

Air humidity is also an important triggering factor to disease occurrence. Water vapor in the environment is directly related to rainfall, irrigation and dew, which can promote effects on leaf wetness duration (LWD) in order to galvanize a disease initial development, particularly concerning both germination and penetration processes (ROWLANDSON et al., 2014; DEL PONTE; ESKER, 2008; GLEASON et al., 2008; SENTELHAS et al., 2008). According to Melching et al. (1989) the optimum range of LWD to assure ASR infection and spread is between 6 and 12 hours per day. For all stages of the infectious process is necessary a LWD ranging from 10 to 22 hours per day (ALVES et al., 2007; DEL PONTE et al., 2006a; MARCHETTI; MELCHING; BROMFIELD, 1976).

By analyzing the LWD regime in Piracicaba it was observed that this weather variable remained within an optimum range for ASR occurrence throughout the two soybean growing seasons, except for the fifth sowing date during the 2015/2016 season (Figure 2.1). On the fifth sowing date a drought occurred in Piracicaba reducing the LWD values in at least

three hours per day. As a consequence, in average, LWD during the fifth sowing date was 7.0 h per day as opposed to LWD ranging from 10.1 to 12.1 h per day on the other sowing dates (Figure 2.1).

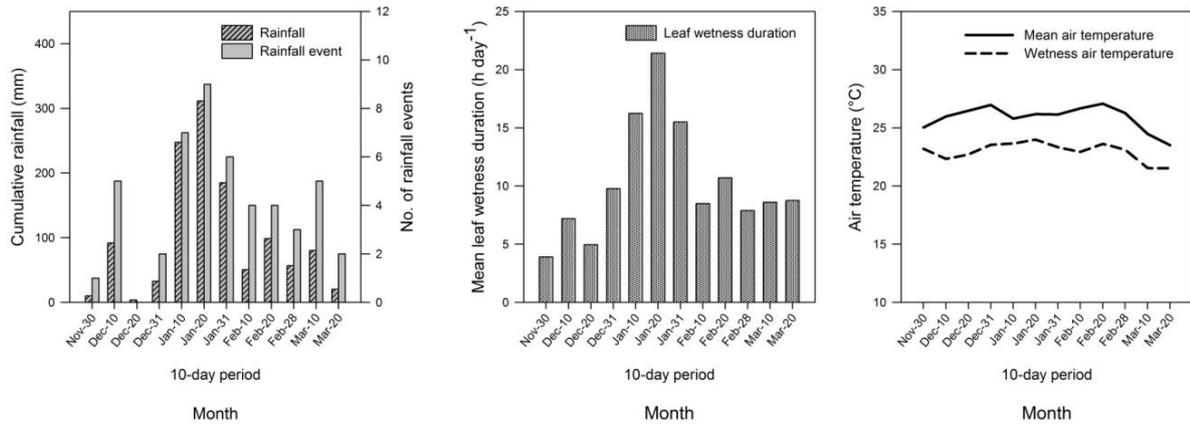


Figure 2.4. Weather conditions at Pedra Preta, MT, throughout the 2015/2016 soybean growing season.

Alves et al. (2007), examining ASR intensity in different soybean cultivars under specific air temperature and LWD regimes, determined a high ASR severity whenever LWD was above a threshold of 12 h day⁻¹. On the other hand, when LWD was of 6 h day⁻¹ ASR severity was lower. A similar condition was observed herein during the last sowing date for the 2015/2016 growing season under a mean measured LWD of 7.0 h day⁻¹. As a consequence of a drop in LWD there were reductions in the ASR final severity (1.9 %) and AUDCP (26.1) (Table 2.3).

The effects of drought on ASR occurrence were also reported by Yorinori et al. (2005) in Brazil and Paraguay throughout the 2001/2002 soybean growing season. In this particular case, low epidemics intensity in soybean fields in Paraguay was found compared to disease intensity in Brazilian soybean fields due to drought. Differently from the lack of an epidemic ASR in Paraguay throughout the same crop growing season in Brazil, significant yield losses as a function of ASR occurrence exceeded 60 %, reaching values of 75 % in soybean fields located at the central-east region of Brazil.

For the remaining sowing date in Piracicaba, the ASR favorability might be evidenced and confirmed by the final severity data along with the AUDCP, which presented higher values compared to the drought period. Throughout the 2014/2015 crop growing season, final severity remained between 84.3 and 100% and AUDCP ranged from 837.1 to 2287.5. For the 2015/2016 growing season, final severity varied from 65.9 to 100% and AUDCP shifted from 780.1 to 1969.3 (Table 2.3).

Increases in final severity values and AUDCP as a function of LWD may be realized because high air humidity regimes make the host tissue more susceptible to ASR infection, and as a result disease values can rise (AGRIOS, 2005b). Moreover, for plant diseases caused by a fungus, such as ASR pathogen, the presence of free water on the plant surface is strongly related to the spore formation, longevity, germination, and sporulation (DEL PONTE; ESKER, 2008).

In Ponta Grossa, opposite patterns of LWD comparing the two soybean growing seasons were noticed herein. Throughout the 2014/2015 soybean growing season LWD remained within a favorability range for ASR occurrence, whilst during 2015/2016 for all sowing dates the LWD remained below the lowest optimum threshold for ASR occurrence (Figure 2.2).

Except for the second soybean growing season of 2014/2015 in Ponta Grossa, the LWD ranged from 9.8 to 11 h per day and environmental favorability interfered positively in the values of final severity of the disease, which varied from 66.8 % to 81.0 %, whereas AUDCP oscillated between 1073.7 and 1946.8 (Table 2.3). The opposite outcome was observed on the second sowing date with an ASR final severity equals to 18.6 % along with an AUDCP corresponding to 555.5. Reductions in the disease severity in soybean fields might be related to depletion on the availability of free water for the pathogen infection process. It was evidenced a significant reduction in LWD as soybean plants entered into the reproductive phenological stage, considered to be as the most susceptible phase for disease occurrence (XAVIER et al., 2017; YOUNG et al., 2011). During this phenological stage mean LWD reduced from 13.4 to 9.0 h, reaching an average of 6.5 h on the third 10-day period of January (Figure 2.2), making the environment rather unfavorable to ASR occurrence.

Similar environmental conditions described previously occurred throughout the 2015/2016 season in Ponta Grossa. In general a mean LWD below 9.2 h per day was observed in this trail (Figure 2.2). Thus, the lowest values of final severity and AUDCP were obtained on the second, third and fourth sowing dates, and this might be associated with the LWD regime (Table 2.3). Only on the first sowing date of the current crop growing season high values of final severity (73.6 %) and AUDCP (577.2) were observed (Table 2.3).

Since LWD takes into consideration the presence of free water over the plant tissues under field conditions, changes in LWD might be brought about mainly by the rainfall regime (SENTELHAS et al., 2008). Thus, in the current study, it was identified that rainfall frequency and volume were very similar for the periods with low or high LWD in all field trials

Nevertheless, in Ponta Grossa the 2014/2015 soybean growing season regular rainfall regimes during most of a 10-day period were observed, providing in turn a favorable weather condition to ASR occurrence. At this crop growing season only on the second sowing date the lowest final severity and AUDCP were obtained (Table 2.3). Such a reduction in ASR occurrence might be ascribed to irregular rainfall distribution during the second and third 10-day period in January, totaling 20 days and comprising two rainfall events recorded at Ponta Grossa (Figure 2.2). Effects of rainy days on ASR severity were reported by Del Ponte et al. (2006a).

Several studies reported that rainfall itself is considered to be an epidemic triggering factor for ASR occurrence (MEGETO et al., 2014; DEL PONTE et al., 2006a; STOVOLD; SMITH, 1991; TSCHANZ, 1984) since it enhances time periods with free water over the plant surfaces. Once rainfall is strongly related to within-canopy spore dispersal, such a variable has a remarkable influence on the performance of fungicide sprays applied to ASR control (STEFANELLO et al., 2016; FIFE; NOKES, 2002).

The influence of rainfall on diseases indices scrutinized in the current research might be corroborated by using field data collected from 2015/2016 in Ponta Grossa. For such a period the highest disease values were recorded throughout the first soybean crop growing season, coinciding with a period of a heavy and regular frequency of rainfall (Figure 2.2). From the second sowing date lower disease values were observed, getting more than a twofold decrease compared to the first sowing date (Table 2.3). Reductions in disease values may be attributed to dry periods during January, February and March in Ponta Grossa region (Figure 2.2).

In Campo Verde regular rainfalls occurred throughout the two soybean growing seasons, making the environment more favorable to ASR occurrence. At this site for both crop growing seasons, high defoliation values were assessed, having been probably the target of a great influence of a high number of rainfall episodes. Such data shown by season and frequencies above 30 rainfall events per sowing date can be seen in Figure 2.3.

The impact of rainfall on ASR progress was also evaluated in Australia, where Stovold and Smith (1991) verified that severe ASR epidemics happen only in soybean fields under a high rainfall amount. Levy (2005) draws the conclusion that at soybean production areas located in the Southern Zimbabwe ASR infection is less severe compared to other Zimbabwe's agricultural fields, due to hot and dry weather in this region.

Effects of uneven rainfall episode and drought periods can be observed on the fifth sowing date throughout the 2015/2016 soybean growing season at Piracicaba. In this

particular season two rainfall events were measured above 1 mm, totalizing 8.6 mm throughout the growing season. The consequence of a dry weather pattern might be built in the disease index measured in the field, whose final severity was of 1.9 % and AUDCP was of 26.1 (Table 2.3).

Drought periods also had a considerable influence on LWD at Pedra Preta during the first sowing date of the 2015/2016 growing season. In such a period it was possible to identify a low LWD corresponding to 3.9 h per day. LWD reached values close to an optimum range for the ASR occurrence only at the end of the fourth 10-day period of the growing season, within which a mean value of 9.8 h per day was observed.

At Pedra Preta low values of LWD at beginning of the growing season were not to be sufficient to affect the ASR in soybean production fields (Table 2.3). During the period with a low LWD, soybean plants were in the vegetative phase, and at this time soybean plants had a low susceptibility to ASR (BONDE; NESTER; BERNER, 2013). Thus, throughout the crop growing season in Pedra Preta neither the weather conditions nor the host characteristics did favor the ASR occurrence in soybean production fields. However, by the time wet environmental conditions prevailed over again and remained in compliance with a favorability range for ASR occurrence, soybean plants were at the beginning of the bloom, coinciding therefore with a period of a high susceptibility to the ASR infection, and as a result disease progress turned out to be favored in the field (Table 2.4) (XAVIER et al., 2017; YOUNG et al., 2011).

Throughout the second soybean growing season at Pedra Preta and for all field trials located in Campo Verde, LWD conditions were favorable to ASR occurrence, especially during the 2015/2016 growing season in Campo Verde, within which the mean LWD varied from 13.1 to 15.4 h per day (Figure 2.3). The environment favorability at these sites caused the crop to garner high defoliation values, always above 97 %.

2.3.2. Epidemiological analyses

The epidemiological analyses were performed for Piracicaba and Ponta Grossa because in these particular field trials disease assessment was made at different moments throughout the soybean crop growing seasons. For the two field trials in Mato Grosso, there was no possibility of performing epidemiological analyses since severity was assessed only in the R5.5 phenological stage.

For Piracicaba and Ponta Grossa the logistic model was the one with the best fit to describe the ASR progress curve (Table 2.5) when compared to the exponential, monomolecular and Gompertz models (Annex A, B and C, respectively).

Studies using mathematical models to predict plant disease epidemics were conducted by Vanderplank (1963) and since then the logistic model is recommended as the most appropriate to predict disease severity, including ASR epidemics. According to Kim; Wang and Yang (2005), the logistic model can be used to predict few parameters of ASR epidemic; however it requires precise and quantitative assessments of disease development in order to obtain good accuracy and precision of the model. Because of such requirement the aforementioned authors developed a fuzzy logic-based model making use of Taiwan data to estimate ASR apparent infection rate.

Despite modifications applied to mathematical models proposed to estimate plant disease epidemics, an excellent performance of the logistic model has been observed to predict ASR epidemics, especially in field experiments conducted in Brazil. By studying ASR epidemics at two distinct sites located in the state of Paraná, Brazil, studies determined a good performance of the logistic model to predict ASR occurrence with a mean $R^2 = 0.96$ for both experiments (MESQUINI et al., 2011; TSUKAHARA; HIKISHIMA; CANTERI, 2008).

A good performance of the logistic model for describing the temporal progress of ASR was also found by Moreira et al. (2014) in field trials conducted in Mato Grosso, Brazil, from 2009 to 2011. In this research, R^2 values ranged from 0.97 to 0.99. The use of the logistic model to calculate the infection rate was applied by Xavier et al. (2017) in order to determine the relationship between soybean leaf/plant age and susceptibility to infection caused by *P. pachyrhizi*.

By comparing measured with estimated severity during 2014/2015 soybean growing season in Piracicaba, the logistic model resulted in a R^2 higher than 0.93 for the five sowing dates. Considering all sowing dates the mean calculated R^2 was of 0.97 ($P = 0.009$). Besides presenting better fitting, the logistic model also led to lower errors (ME = 0.020, MAE = 0.057 and RMSE = 0.092) compared to other models (Table 2.5).

In the second experimental year at Piracicaba, 2015/2016, a strong correlation between measured and estimated ASR severity by means of the logistic model for the first three sowing dates was obtained, with R^2 equal to 0.99, 0.99 and 0.90 for the first, second and third sowing dates, respectively. For the sowing dates aforementioned the mean p-value was of 0.017 and ME = 0.020, MAE = 0.038 and RMSE = 0.058. A reduction in accuracy and precision was observed with the use of logistic model to predict ASR progress on the fourth

and fifth sowing dates, taking into account lower values of R^2 and higher values of ME, MAE and RSME (Table 2.5).

Table 2. 5. Epidemiological analysis and statistical coefficients for the logistic model to describe the Asian soybean rust severity progress in Piracicaba and Ponta Grossa, Brazil.

Site	Growing Season	Sowing Date	ASR infection rate	Initial inoculum	R^2	p-value	ME	MAE	RMSE
Piracicaba	2014/2015	1st	0.124	0.018	0.97	0.002	-0.019	0.035	0.068
		2nd	0.170	0.002	0.93	0.008	0.053	0.059	0.138
		3rd	0.171	0.001	0.97	0.016	0.052	0.063	0.094
		4th	0.148	0.004	0.98	0.008	-0.015	0.075	0.091
		5th	0.110	0.005	0.97	0.015	0.029	0.055	0.072
	2015/2016	1st	0.229	0.051	0.99	0.002	-0.003	0.018	0.018
		2nd	0.154	0.001	0.99	0.000	0.013	0.018	0.038
		3rd	0.172	0.001	0.90	0.051	0.051	0.079	0.118
		4th	0.304	0.011	0.82	0.096	0.090	0.109	0.191
		5th	0.057	0.008	0.66	0.393	0.000	0.003	0.003
Ponta Grossa	2014/2015	1st	0.093	0.017	0.93	0.008	-0.014	0.052	0.077
		2nd	0.087	0.002	0.86	0.247	0.003	0.030	0.036
		3rd	0.103	0.005	0.93	0.008	0.004	0.055	0.113
		4th	0.136	0.006	0.98	0.011	-0.020	0.054	0.061
	2015/2016	1st	0.123	0.060	0.84	0.027	0.003	0.083	0.098
		2nd	0.072	0.030	0.95	0.005	-0.0004	0.011	0.012
		3rd	0.050	0.032	0.77	0.050	-0.003	0.016	0.21
		4th	0.007	0.130	0.52	0.278	-0.0001	0.005	0.005

At Ponta Grossa, except for the second sowing date of the 2014/2015, R^2 was higher than 0.93, representing a high accuracy and precision of the logistic model to estimate ASR severity. For the first, third and fourth sowing dates, the following errors were obtained: ME = -0.010, MAE = 0.054 and RMSE = 0.083. On the second sowing date the calculated statistical parameters were the following: $R^2 = 0.86$ ($P = 0.247$), ME = 0.003, MAE = 0.030 and RMSE = 0.036 (Table 2.5).

Reduction in statistical indices was also observed for the 2015/2016 growing season at Ponta Grossa. Only for the second sowing date a high R^2 (0.95) was obtained, even with low calculated errors (ME = -0.0004, MAE = 0.011 and RMSE = 0.012). However, for the other sowing dates, R^2 values were lower than 0.84. Even faced with a low precision of the

logistic model to estimate ASR severity in Ponta Grossa, there was no significant increase in ME, MAE and RMSE, which presented the following respective values: -0.0001; 0.029; and 0.0413.

By making use of the epidemiological analysis, values of apparent infection rate and initial inoculum of ASR were also determined for both sites (Table 2.5). In general, it was observed that the highest disease rates were obtained for experiments set up at Piracicaba. In a comparative analysis of growing seasons in Piracicaba, it was determined a highest apparent infection rate in 2015/2016, with a mean value of 0.183 day^{-1} , being 20% higher than the average of infection rate obtained for the field experiment carried out in 2014/2015. At Ponta Grossa, there were lower mean apparent infection rates, with values of 0.105 day^{-1} and 0.063 day^{-1} for 2014/2015 and 2015/2016, respectively.

Oliveira et al. (2015), determining the efficiency of potassium phosphite and potassium silicate to control ASR, obtained apparent infection rates by using the logistic model of 0.144 day^{-1} . Similar results were found by Moreira et al. (2014) with logistic infection rates ranging from 0.05 to 0.14 day^{-1} .

By examining the initial inoculum values obtained by the logistic model throughout the 2014/2015 growing season in Piracicaba and Ponta Grossa, similar values of initial inoculum were found, corresponding, in average, to 0.006 and 0.007, respectively. For the 2015/2016 growing season there was an increase in the initial inoculum at both experimental sites, however this increase was higher in Ponta Grossa, with a value of 0.063. On the other hand in Piracicaba the increase was lower, with an initial inoculum value equals to 0.014 (Table 2.5).

2.3.3. Relationship between weather variables and Asian soybean rust severity

The severities obtained at R5.5 stage for each plot in all field trials were correlated with weather variables (Table 2.6). For Piracicaba, Ponta Grossa, Campo Verde, and Pedra Preta it was possible to observe significant R values ($P < 0.05$) for the correlations with LWD ($R = 0.34$), Air_LWD ($R = 0.31$), and C_Rainfall ($R = 0.30$) (Table 2.6). When the relationships were performed with final severity, higher R values were obtained for LWD ($R = 0.64$). For the other weather variables, similar patterns in relation to severity at R5.5 were obtained, mainly for Air_T and Rainfall_E (Table 2.6).

The effects of moisture in the soybean canopy on ASR occurrence were also determined by several researchers (MINCHIO et al., 2016; IGARASHI et al., 2014; MEGETO et al., 2014; NARVÁEZ et al., 2010; ALVES et al., 2007; DEL PONTE et al.,

2006a; MELCHING et al., 1989). Moisture in the environment is mainly affected by rainfall, which also affects air temperature and leaf wetness duration. All these weather variables affect most fungal diseases, including ASR (ROWLANDSON et al., 2014).

Table 2. 6. Pearson's correlation coefficient for the relationship between weather variables and Asian soybean rust severity assessed at R5.5 phenological stage and at the end of the soybean crop cycle.

Severity	n	Pearson correlation values				
		Air_T	Air_LWD	LWD	C_Rainfall	Rainfall_E
R5.5	96	-0.05 ^{ns}	0.31 ^{**}	0.34 ^{**}	0.30 ^{**}	0.09 ^{ns}
Final	60	0.26 [*]	0.39 ^{**}	0.64 ^{**}	0.30 [*]	0.40 ^{**}

R5.5 soybean phenological stage, which turns out to be characterized by pods between 75% and 100% of grain granulation; n = number of observations; Air_T = mean air temperature (°C); Air_LWD = mean air temperature measured during leaf wetness (°C); LWD = mean leaf wetness duration (hours day⁻¹); C_Rainfall = cumulative rainfall (mm in 30 days); Rainfall_E = rainfall events (days with rain in 30 days). ^{ns} represents that Pearson coefficient is not significant; ^{*} represents that Pearson coefficient is significant at $P \leq 0.05$ and ^{**} represents that Pearson coefficient is significant at $P \leq 0.01$.

In this study, assessed ASR severities were more influenced by rainfall and leaf wetness duration, similar to what was obtained by Del Ponte et al. (2006a) when analyzing 34 ASR epidemics all over Brazil. These authors observed that final ASR severity was mainly correlated to total rainfall ($R = 0.95$) and number of days with rain >1 mm ($R = 0.93$). On the other hand, air temperature was less correlated to the ASR epidemics ($R = 0.47$). Rainfall increases leaf wetness duration, which favors infection and sporulation processes (DEL PONTE et al., 2006a; FITT; MCCARTNEY; WALKLATE, 1989), also promoting production of spores and their survival (NARVÁEZ et al., 2010; DEL PONTE; ESKER, 2008).

Using ASR data collected from different Brazilian regions, Megeto et al. (2014) also identified a positive correlation between ASR and variables derived from rainfall. More recently, Minchio et al. (2016) obtained a Pearson coefficient of 0.87 by correlating ASR epidemics in Southern Brazil with accumulated rainfall throughout the soybean cycle. Also, a positive impact of average weekly rainfall on the maximum ASR incidence ($R = 0.39$) and severity ($R = 0.38$) was observed by Young et al. (2011) when analyzing ASR epidemics in sentinel plots in Florida, USA.

As mentioned previously, rainfall affects LWD, thus this variable is an important triggering factor to ASR development, as observed in Table 2.6. The effects of the LWD on ASR were initially mentioned by Melching et al. (1989). These authors observed that soybean

plants when inoculated with *P. pachyrhizi* urediniospores presented high ASR intensity under 12 h per day of wetness for three days ($R^2 = 0.97$).

The influence of the LWD on ASR development in soybean plants was also observed by Narváez et al. (2010). In this study, the authors generated different LWD periods by using an irrigation system and observed that 18 h of LWD increased disease severity and the rate of disease spread in the upper canopy.

Therefore, disease factors related to environmental moisture, such as rainfall and LWD, are potential variables to be used as inputs of the disease-warning system to recommend ASR epidemics control in soybean fields in Brazil. Another weather variable that showed positive influence on ASR severity was the air temperature during the leaf wetness duration (Air_LWD), which is also a promising variable to be incorporated into a disease-warning system. Mean air temperature (Air_T) showed low correlation with ASR severity, only presenting significant correlation for final severity (Table 2.6). Similar results were observed by Del Ponte et al. (2006a), which, according to such authors are associated to the non-limiting mean temperatures for ASR occurrence during the soybean growing season in the Brazilian producing regions.

2.4. Conclusions

According to the analyses performed with data from different Brazilian regions, it was possible to conclude that under favorable weather conditions and without any disease control, Asian soybean rust presented a high destructive potential reflecting in defoliation and final severity close do 100 %.

Considering data from Piracicaba and Ponta Grossa, temporal ASR progress was better described by the Logistic model. This model enables to predict future ASR epidemics based on the disease apparent infection rate.

Epidemics of ASR till R5.5 phenological stage were mainly influenced by leaf wetness duration, cumulative rainfall and air temperature during the leaf wetness duration. When ASR severity at the end of the soybean cycle was considered, it was conditioned mainly by leaf wetness duration. Therefore, any disease warning system for ASR should include the variables above mentioned, together or individually, as inputs.

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3. ASIAN SOYBEAN RUST WARNING SYSTEMS – IMPACTS ON NUMBER OF SPRAYS AND DISEASE INTENSITY

ABSTRACT

Asian soybean rust (ASR) is controlled by sequential applications of fungicides following a calendar-based system. This approach takes into account only crop aspects, disregarding the influence of local weather conditions on disease progress. This study evaluated the performance of disease-warning systems to control ASR in different Brazilian regions. The experiments were conducted in the municipality of Piracicaba, São Paulo State, (Lat. 22°42' S, Long. 47°30' W, Alt.: 546m), in Ponta Grossa, Paraná State (Lat. 25°05' S, Long. 50°09' W, Alt.: 969 m), in Campo Verde, Mato Grosso State (Lat. 15°24' S, Long. 55°5' W, Alt.: 689m.) and in Pedra Preta, Mato Grosso State (Lat. 15°24' S, Long. 55°5' W, Alt.: 689m.), Brazil, throughout 2014/2015 and 2015/2016 soybean growing seasons. In each field trial, at least two sowing dates were adopted to impose different weather conditions for the disease. Soybean seeds were drilled with row spacing of 0.45 m and plant population of 266,666 plants per ha. The experiments were conducted under a randomized block design allotted in split plots with four replications. The treatments were: UNS - unsprayed check treatment; CALEND - calendar-based sprays in a 14-day interval from R1 stage; PREC_1 - system based on rainfall with threshold 1 (80% severity as cut-off); PREC_2 - system based on rainfall with threshold 2 (50% severity as cut-off); TLWD_1 - system based on Air_T and LWD with threshold 1 (9 infection probability as cut-off); TLWD_2 - system based on Air_T and LWD with threshold 2 (6 infection probability as cut-off). The fungicide used in all treatments was a commercial mixture of QoI and SDHI (Azoxystrobin + Benzovindiflupyr) at a dosage of 150 grams per hectare. Weather data were measured with automatic weather stations in the experimental sites. For all locations, the UNS exhibited higher disease levels. Considering all sowing dates and locations, the system PREC_2 presented better performance when compared with the other treatments. However, at sowing dates in October and November, being classified as the optimum for soybean cultivation in Brazil, the treatment PREC_1 showed a satisfactory ASR control with a low number of sprays compared with CALEND. On the other hand, in December, PREC_2 showed a good performance to control ASR. The disease-warning systems TLWD determined a high number of sprays to control ASR, possibly due to threshold values used to recommend sprays adopted herein. The results showed that disease-warning systems based on rainfall data were effective to control ASR, promoting lower disease levels with a reduction in the number of sprays. Thus, PREC_1 and PREC_2 can be effectively used to manage ASR in Brazilian soybean areas.

Keywords: *Phakopsora pachyrhizi*; *Glycine max*; Rainfall; Leaf wetness duration; Integrate disease management

3.1. Introduction

Soybean production in Brazil became highly dependent on fungicide application after the identification of the fungus *Phakopsora pachyrhizi* Sydow & Sydow (YORINORI et al., 2005). According to Food and Agriculture Organization (FAO), between 2001 and 2002, when the pathogen was identified in Brazil, fungicide consumption in soybean producing areas in Brazil was 17.2 tons of active principle. The disease progress and establishment in all Brazilian agricultural areas three-folded the uptake of such active ingredients throughout the 2010/2011 crop season, totaling 55.5 tons (FAO, 2014).

P. pachyrhizi is the pathogen that causes Asian soybean rust (ASR), a disease that has caused damages and considerable losses in soybean crops worldwide. ASR occurrences under high severity conditions may drastically decrease soybean yield up to 70 %; however, yield losses can reach 90 % under favorable environments for the fungus, with inefficient disease control (HARTMAN et al., 2016; DALLA LANA et al., 2014; YORINORI et al., 2005). Due to the lack of ASR-resistant soybean cultivars, the disease control is performed by fungicide sprays. The sprays follow a calendar-based approach starting at the beginning of bloom (phenological stage R1 according to Fehr et al. (1971) with fungicide sprays based on disease episodes in the field (GODOY et al., 2016; YORINORI et al., 2005).

After ASR identification in Brazil, fungicides belonging to triazole (DMI), strobilurins (QoI), and their mixtures showed efficiency for ASR control, as demonstrated by Scherm et al. (2009) when fungicides reduced disease severity by up to 58 % and increased yield by 44 %. The efficiency of azoxystrobin use associated to cyproconazole also was identified in Londrina, Paraná State, throughout the 2005/2006 crop growing season, with ASR control efficiency of 89 % compared to the treatment with no fungicide sprays (GODOY et al., 2009).

However, due to a wide sowing window for soybean cultivation in Brazil, a large number of fungicide sprays is needed to control ASR. According to Godoy et al. (2016) and Fiallos and Forcelini (2013), the number of sprays may range from two to six within the crop cycle. Under frequent exposures of the pathogen to DMI fungicides since the 2007/2008 crop growing season, a significant loss of efficiency was observed. Furthermore, six years after, fungicides belonging to QoI group also demonstrated to be less effective to control ASR in Brazil (SCHMITZ et al., 2014). Recent fungicides of the carboxamide (SDHI) group were expected to be more efficient in ASR management. However, after three years of a massive usage of SDHI fungicides in the field, in the 2016/2017 crop season growers also reported

efficiency reduction of this group of fungicides (FRAC, 2017; GODOY et al., 2017; SIMÕES et al., 2017).

An issue in the use of fungicides to control ASR is that sprayings are performed in a curative manner. Under several situations, eradicating doses or underdoses of products are used; besides, application technologies are inadequate, and most importantly, the correct application time based on local meteorological conditions are not taken into account by soybean growers (MEGETO et al., 2014). The consequence is a reduction of sensitivity of the pathogen to chemical products, making its control inefficient, increasing production costs and disease pressure (ALVIM et al., 2009a, 2009b).

A rational recommendation for fungicide sprayings to control ASR is based on the adoption of disease-warning systems (DWS). DWS are decision-making tools to help growers to manage crop diseases in production fields (GLEASON et al., 2008). Frequently, DWS start with the selection of meteorological variables that positively affect the occurrence and progress of diseases. Subsequently, the relationship between local meteorological variables and disease progression is represented by mathematical equations in order to determine better spray times (KELLY et al., 2015; ROWLANDSON et al., 2014; GLEASON et al., 2008; DEL PONTE et al., 2006a).

Scientific researchers have already been conducted to investigate the influence of local meteorological variables on ASR progress. Preliminary studies determined that the ideal temperature for the ASR infectious process ranges from 20 to 25 °C (ALVES et al., 2007; BONDE et al., 2007; MARCHETTI; MELCHING; BROMFIELD, 1976). On the other hand, such process is negatively influenced by air temperatures below 15 °C (DEL PONTE et al., 2006a; MELCHING et al., 1989) and above 30 °C (DANELLI; REIS, 2016; BONDE; NESTER; BERNER, 2013; ALVES et al., 2007). ASR occurrence and development was also correlated with variables related to water regime conditions. In these studies, environments with leaf wetness duration over 10 hours per day (HARTMAN et al., 2016; VALE; ZAMBOLIM; CHAVES, 1990; MELCHING et al., 1989), along with a high and well distributed rainfall regime, is conducive to ASR occurrences (MEGETO et al., 2014; DEL PONTE et al., 2006a, 2006b).

Identification of local meteorological variables that affect occurrence and progress of ASR allows to propose and incorporate prediction equations of ASR into DWS. Statistical analyses conducted by Reis; Sartori; Câmara (2004), based on experimental data published by Melching et al. (1989), revealed that the interaction between leaf wetness duration and air temperature during leaf wetness period allows to estimate the number of rust lesions per cm²

of the leaf. Under climatic Brazilian conditions, Del Ponte et al. (2006a) identified a high correlation between ASR final severity and frequency and volume of rainfall. More recently, using data of ASR epidemics in Brazilian agricultural areas, Dias; Li; Yang (2014) evidenced a positive dependence relationship between ASR epidemics and cloudiness under specific site conditions, allowing to define a linear model to predict the final severity of the disease.

Although disease prediction models have been largely reported in the literature, research on testing and validation of such models at field scales are scarce, mainly concerning alert systems proposed for the soybean crop. The lack of such a practice creates a considerable gap between DWS proposition and its implementation in soybean production fields. Kelly et al. (2015) described an example that represents the validation of prediction equations of ASR under field conditions. The authors assessed two distinct DWS in northern Florida and confronted them to the calendar-based approach. The authors reported that for trials carried out throughout the 2011 growing season, DWS based on precipitation data showed to be more efficient to control ASR, reducing the number of sprays compared to the calendar-based approach.

The frequent losses of fungicide efficiency for ASR chemical control associated with a strong dependence of soybean producers on the use of chemicals have led to the need of protocols to rationalize the use of chemicals for ASR control in soybean fields. Therefore, the hypothesis of this study is that ASR chemical control can be improved by using locally adjusted and validated DWS. Based on that, the aim of this study was to evaluate the performance of two ASR warning systems, based on different decision thresholds and weather variables, and compared them to the calendar-based spray system, often used by the growers, in order to define the best spray time for controlling ASR in three main Brazilian producing regions.

3.2. Material and Methods

3.2.1. Methods for recommending Asian soybean rust control

For ASR control at the different sites, three methods were used to determine the time to perform fungicide sprays. The first was a calendar-based system and was the control treatment, as it is the procedure adopted by soybean growers. The second and third ones were classified as alternative, based on disease-warning systems, which consider the regime of

different weather variables for determining spray recommendations to control ASR. The description of each method is presented below.

Calendar-based System – This system is adopted by growers to control ASR and considers that fungicide sprays should start when soybean plants reach R1 phenological stage, which is characterized by the beginning of blossom, i.e., when the plants need to have at least one flower open at any node of the branches (FEHR et al., 1971). From the first fungicide spray, the remaining sprays were performed at intervals of 14 days (GODOY et al., 2009) until when soybean plants reached R7 phenological stage, i.e., the beginning of crop maturation. Therefore, the critical period for ASR control adopted in this study ranges from R1 and R7 (GODOY et al., 2009; LEVY, 2005).

Rainfall Warning System (PREC) – This disease-warning system is based on the model proposed by Del Ponte et al. (2006a) to estimate the final ASR severity as a function of rainfall and number of rainy days throughout 30 days before crop maturity. Such a model was applied in two different ways (Equations 3.1 and 3.2), accounting for the weight of the previous time on ASR severity:

$$SEV_1 = -2.1433 + 0.1811 * (PREC_{1-15}) + 1.2865 * (DP_{1-15}) \quad (3.1)$$

$$SEV_2 = -2.1433 + 0.1811 * (PREC_{16-30}) + 1.2865 * (DP_{16-30}) \quad (3.2)$$

where: SEV_1 shows severity accumulated within 15 days before the calculation day (1-15); SEV_2 represents severity estimated between 16 and 30 days prior to the calculation day (16-30); $PREC_{1-15}$ and DP_{1-15} refer to accumulated rainfall throughout the 15-day period prior to the calculation day and days with rainfall ($> 0.5\text{mm}$) for the same period, respectively; and $PREC_{16-30}$ and DP_{16-30} represent accumulated rainfall and days with precipitation throughout the period between 16 and 30 days prior to the final severity calculation.

The equation initially proposed by Del Ponte et al. (2006a) was used to calculate final severity as a function of a 30-day series; therefore, SEV_1 and SEV_2 values were multiplied by two, since such values considered only 15 days as input variables. To estimate the final severity of ASR, the aforementioned equation was modified to estimate daily disease severity values in % (DSV). Moreover, to use the model that shows the need for spray, different weights were ascribed to two 15-day periods immediately before the calculation day of DSV (equation 3.3):

$$DSV = (SEV_1 * 0.7) + (SEV_2 * 0.3) \quad (3.3)$$

Different weights for SEV_1 and SEV_2 values were determined based on weather conditions for 15 days nearer the estimation date of disease severity as it showed a greater effect on the infectious process and progress of ASR epidemics as opposed to the period between days 16 and 30.

The need for fungicide spray to control ASR was determined based on critical DSV, according to the average of three days of DSV established as a threshold for decision making concerning fungicide sprays in soybean crops to control ASR. The trials allowed to adopt two action thresholds for the spray recommendation, one less conservative and another one more conservative (Table 3.1).

After indicating a need for fungicide sprays, the monitoring of weather continued; however, the next sprays were recommended only after the active period of the fungicide in the field, with a time interval of no less than 14 days.

Table 3. 1. Daily disease severity values (DSV) of Asian soybean rust obtained from the average of three days based on rainfall volume and frequency necessary for recommendations of sprays in compliance with action thresholds calculated by the disease-warning system proposed by Del Ponte et al. (2006a).

Criterion	Sprays			
	1 st	2 nd	3 rd	4 th or over
	Mean DSV for three days (%)			
Threshold 1 – Less conservative	80	60	55	50
Threshold 2 – More conservative	50	45	35	30

Temperature and Leaf Wetness Duration Warning System (TLWD) – The second disease-warning system was proposed by Reis; Sartori; Câmara (2004), using experimental data published by Melching et al. (1989). The disease-warning system is based on an equation that expresses the interaction between air temperatures during the period when the leaf wetness occurs (Air_LWD, in °C) and leaf wetness duration (LWD, in h) in order to calculate the number of lesions per cm² of plant tissue:

$$NL = 12.6118 * \exp \left(-5 * \left(\left(\frac{LWD - 21.409}{4.2205} \right)^2 \right) + \left(\left(\frac{Air_LWD - 14.1368}{2.427} \right) \right)^2 \right) \quad (3.4)$$

where: NL is the number of lesions per cm^2 of plant tissue. Equation 3.4 was used to determine daily values of infection probability (DVIP), with 0 indicating no infection; 1 intensity from 0.1 to 3.0 lesions per cm^2 ; 2 intensity from 3.1 to 6.0 lesions per cm^2 ; 3 intensity from 6.1 to 9.0 lesions per cm^2 (Table 3.2).

Table 3. 2. Daily values of infection probability (DVIP) for Asian soybean rust based on mean air temperature during leaf wetness duration (Air_LWD) along with leaf wetness duration (LWD). Source: Reis et al. (2004).

Air_LWD (°C)	DVIP			
	0	1	2	3
	LWD (h day ⁻¹)			
<14.9	< 11.0	11.1 – 14.0	> 14.1	--
15 – 19.9	< 7.0	7.1 – 13.0	13.1 – 17	> 17.1
20 – 24.9	< 7.0	7.1 – 10.0	10.1 – 17	> 17.1
>24.9	< 7.0	7.1 – 11.0	11.1 – 17	> 17.1

The recommendation for fungicide sprays was based on the sum of a critical DVIP (Σ DVIP critical), given by the sum of seven days. The 7-day period computing weather data was determined based on the mean latent period for the pathogen to cause ASR epidemics in soybean (DANELLI; REIS, 2016; GODOY et al., 2016; MARCHETTI; MELCHING; BROMFIELD, 1976). Therefore, the recommendation for the first spray to control ASR was kept whenever the sum of seven days of DVIP reached a value equal to six (more conservative threshold) or nine (less conservative threshold). As considered for the disease-warning system proposed by Del Ponte et al. (2006a), after the first spray recommendation, the remaining sprays were only indicated after the period of fungicide action in the field (14 days).

3.2.2. Field trial description

Field experiments were carried out at the following Brazilian sites: Piracicaba, SP, (Lat. 22°42' S, Long. 47°37' W, Alt. 567m); Ponta Grossa, PR, (Lat. 25°05' S, Long. 50°09' W, Alt. 969 m); Pedra Preta, MT (Lat. 16°51' S, Long. 54°01' W, Alt. 745 m); and Campo Verde, MT (Lat. 15°23' S, Long. 55°03' W, Alt. 667 m). The climates of these locations are respectively classified as Cwa (humid subtropical, with dry winter and hot summer), Cfb

(humid subtropical, without dry season and temperate summer) and Aw (tropical with dry winter), according to Alvares et al. (2013).

For the sites of Piracicaba, Ponta Grossa and Campo Verde, field trials were conducted throughout the 2014/2015 and 2015/2016 crop seasons, while in Pedra Preta, field trials were only conducted throughout the 2015/2016 crop season. In Piracicaba and Ponta Grossa, Brasmax® Potência RR cultivar (6.7 maturation group) and MonSoy-6410® Ipro cultivar (6.4 maturation group) were sowed during the 2014/2015 and 2015/2016, respectively. For both locations in the Mato Grosso State, the cultivar used was TMG® 132 RR (8.5 maturation group). For all sites, the final plant population was 266,666 plants per hectare. For Piracicaba and Ponta Grossa, the experimental units comprised five rows spaced at 0.45 m, with a 4-m length. In Campo Verde and Pedra Preta, the plots had five rows spaced at 0.45 m and with a length of 7 m.

In all sites, field trials were sowed at different dates to induce weather variability, generating distinct environmental conditions for ASR occurrence and development in the field. The number of sowing dates were: Piracicaba – five; Ponta Grossa – four; Campo Verde – three; and Pedra Preta - two. Details of the sowing dates are presented in Table 2.1.

For each site, field trials were conducted under a randomized block design allotted in split plots with four replications. At the different experimental sites, the main factor comprised the different sowing dates and the secondary factor referred to methods used for ASR control.

Prior to sowing, in the different experimental areas, weeds were dissected with specific herbicides according to infesting species and infestation degree. Soil fertilization (P and K) for Piracicaba and Ponta Grossa was based on soil chemical analysis and expected yield of soybean crop (EMBRAPA, 2013). In Campo Verde and Pedra Preta, soil fertilization was performed following the recommendations of Fundação MT for achieving high yields. In order to meet N requirements of the soybean crop in all field experiments, the seeds were inoculated with N-fixing bacteria. For inoculation, each kilogram of seed was inoculated with 2 ml of *Bradyrhizobium japonicum* inoculums (lineages SEMIA 5079 and 5080) at a concentration of 5×10^9 units forming colonies (UFC ml⁻¹).

Soybean seeds were treated with a mixture of fungicide and insecticide of protecting action (Pyraclostrobin), systemic (Thiophanate-methyl), contact and ingestion (Fipronil) at a dosage of 200 mL for each 100 kg of seeds. After treatment, the seeds were sown mechanically in the experimental sites, using a tractor coupled to a seeder disc.

The treatments adopted in all experimental units were:

- 1- Unsprayed check treatment (UNS) – with no fungicide sprays to control ASR;
- 2- Calendar-based system (CALEND) – with the market standard methodology considered. In this method, fungicide spray starts when soybean plants reach R1 phenological stage (FEHR et al., 1971). After the first spray, the new sprays will be applied every 14 days until the plants reach R7 phenological stage (FEHR et al., 1971);
- 3- Disease-warning system based on rainfall data (PREC_1) – with sprays recommended in accordance with the prediction equations of final severity proposed by Del Ponte et al. (2006a), and daily values of severity assessment starting when soybean plants reach V3 phenological stage (FEHR et al., 1971). This particular treatment takes into account threshold 1 (less conservative) as described in Table 3.1.
- 4- Disease-warning system based on rainfall data (PREC_2) – follows the same procedure presented for PREC_1 reported previously; however, taking into account the threshold 2 (more conservative) as described in Table 3.1;
- 5- Disease-warning system based on temperature and leaf wetness duration data (TLWD_1) – an alert system based on the equation proposed by Reis; Sartori; Câmara (2004), with DVIP determination starting when soybean plants reach V3 stage. Spray recommendations are required whenever the sum of DVIP reaches 9 (less conservative);
- 6- Disease-warning system based on temperature and leaf wetness duration data (TLWD_2) – follows the same procedure presented for TLWD_1, but with spray recommendations done when the sum of DVIP reaches 6 (more conservative).

Except for unsprayed check treatment (no fungicide), the control of ASR was performed with the fungicide composed by a mixture of QoI and SDHI (Azoxystrobin + Benzovindiflupyr, respectively) at a dosage of 150 g ha^{-1} . After each fungicide application in soybean plots a 14-day period with no fungicide application was taken into consideration, under the assumption that fungicide residues on leaves would continue to suppress ASR infection effectively during this period.

In the four different sites, meteorological data were collected from automatic weather stations (AWS) located approximately 10 m away from the experiments. The AWS had electronic sensors to measure global solar radiation (Pyranometer CM3, Kipp & Zonen trademark), air temperature and relative humidity (HMP35C, Vaisala trademark), wind speed and direction (03001, Young trademark), precipitation (TR-565M model), and leaf wetness

duration (cylinder sensors Weather Innovation trademark). All sensors were coupled to Campbell Scientific Inc data acquisition systems (CR-10x and CR-1000 models), which were programmed to record variables at each minute and store averages at 15-min intervals. Meteorological data were collected daily always in the morning to allow the use of prediction algorithms for ASR at the experimental areas, and consequently, to allow sprays in the same or in the next day.

When needed, sprays to control ASR in the field was conducted by a pressurized costal sprayer of carbon dioxide (CO₂), from Herbicat® trademark, with an application bar containing four sprayers (TEEJET XR/11002, VP) spaced at 0.45 m. Sprayers were regulated at a pressure from 2 to 2.5 bar, with applications performed at a speed of 4.5 km h⁻¹ for distributing approximately 200 L of fungicide per hectare.

In Piracicaba and Ponta Grossa, disease evaluations were made along the three central rows at each experimental unit. After identification in the experimental site, ASR severity assessments were made every 10 days, collecting 12 leaflets of soybean plants. For a good representation of disease in soybean plants, the canopy was stratified into three parts (lower, medium, upper thirds), with four leaflets collected from each third to obtain a mean severity value. At each ASR severity evaluation, scores were attributed according to the diagrammatic scale proposed by Godoy; Koga; Canteri (2006), with values ranging from 0.6 to 78.5%. In case of defoliation caused by ASR, a value of 100% severity was considered.

In Campo Verde and Pedra Preta, assessments of disease severity were made only when soybean plants were at R5.5 phenological stage (FEHR et al., 1971), for that the protocol to evaluate the ASR severity was the same adopted in Piracicaba and Ponta Grossa. Furthermore, in both sites aforementioned were also determined the percentage of defoliation caused by ASR at the end of soybean crop cycle (HIRANO et al., 2010).

In all field trails soybean yield was obtained from the weight of all soybean grains harvested from each useful plot, which were the three central rows. After harvest the soybean grain data was corrected by a water content of 130 g kg⁻¹ (13%).

With soybean yield and ASR data obtained in all field trails, the area under the disease progress curve (AUDPC) and final severity were determined for Piracicaba and Ponta Grossa. For the two sites in Mato Grosso, defoliation percentage in soybean plants was also investigated. These variables were therefore compared with the number of sprays recommended for each ASR control method. Variables obtained throughout the field trials in each site for different sowing dates were subjected to analysis of variance with the application of the F test. The means were compared at the level of 5% of significance by the Tukey's test.

3.3. Results and Discussion

In the analysis considering all locations, soybean growing seasons, and sowing dates, the number of sprays recommended to control ASR was distinct for each method of control recommendation (Table 3.3). The disease-warning system (DWS) based on rainfall data with a less conservative threshold (PREC_1) recommended 1.8 sprays on average, at least two sprays less than other treatments. Despite fewer sprays, soybean plants managed following PREC_1 showed higher final severity values (43.4 %) and AUDCP (635.2) in Piracicaba and Ponta Grossa than the other methods, but the same level of defoliation measured in Campo Verde and Pedra Preta. As a consequence, the average soybean yield following the PREC_1 recommendations was 2439.6 kg ha⁻¹, lower than other treatments with fungicide sprays recommendation (Table 3.3).

Regarding DWS based on rainfall data with a more conservative threshold (PREC_2), the number sprays was 3.5 on average. Comparing with the PREC_1, the number of sprays increased due to the threshold considered for spray recommendation. The effect of increasing the number of sprays reflected in a high disease control and soybean yield. Comparing the treatments PREC_2 and PREC_1, the final severity was reduced by 18.9 %, while the AUDCP was reduced by 246.4 units and the yield was increased by 444.4 kg ha⁻¹ (Table 3.3).

Comparing PREC_2 with the calendar based approach (CALEND) and disease DWS based on air temperature and leaf wetness duration with a less conservative threshold (TLWD_1) a smaller number of sprays were identified in PREC_2. On average, for CALEND and TLWD_1 four fungicide sprays were recommended to control ASR (Table 3.3). Reductions of at least 0.5 sprays, on average, were obtained by CALEND and TLWD_1 systems which resulted in similar values of final severity, AUDCP and soybean yield. It shows that the use of PREC_2 is effective for ASR control, promoting reduction in the number of sprays recommended for different locations.

Currently, management of plant disease, including ASR, is carried out mainly by fungicide sprays (SCHERM et al., 2009), which are applied following a calendar-based approach having plant phenology as the only aspect considered for guiding the sprays. Therefore, this methodology can be considered very conservative, as it does not take into account environmental conditions, consequently, sprays can be indicated at an inappropriate

time to control ASR (ABULEY; NIELSEN, 2017; KELLY et al., 2015; MEGETO et al., 2014; DEL PONTE et al., 2006a).

Table 3. 3. Average number of sprays, final severity, area under the disease curve progress (AUDCP), percentage of defoliation and yield obtained by different Asia soybean rust control methods in field trials conducted in Piracicaba, SP, Ponta Grossa, PR, Campo Verde and Pedra Preta, MT, Brazil, during the 2014/2015 and 2015/2016 growing seasons.

Treatments	Spray number	Final severity ^{1*}	AUDCP ^{2*}	Defoliation ^{3**}	Yield
		%		%	Kg ha ⁻¹
UNS	--	65.7 a	1146.3 a	99.4 a	1904.6 d
CALEND	4.3 a	20.7 cd	332.3 c	91.9 b	3048.7 ab
PREC_1	1.8 c	43.4 b	635.3 b	90.8 bc	2439.6 c
PREC_2	3.5 b	24.5 c	388.8 c	83.4 d	2884.0 b
TLWD_1	4.0 ab	19.7 cd	285.8 cd	87.0 cd	3048.7 ab
TLWD_2	4.5 a	16.8 d	213.6 d	85.5 d	3229.2 a
CV	26.0 %	28.9 %	30.8 %	10.2 %	20.8%

¹Transformed data using $(x+k)^{1/2}$ with $k = 1$; ² Transformed data using $(x+k)^{1/2}$ with $k = 10$; ³ Transformed data using $\sin^{-1}((x/100)^{1/2})$; *Variables calculated in Piracicaba and Ponta Grossa field trials; **Variables obtained in Campo Verde and Pedra Preta. Spray number, final severity AUDCP and defoliation data followed by different letters are statistically different by the Tukey's test ($\alpha = 0.05$)

Treatments PREC_1 and PREC_2 considered the same input variables to recommend sprays for ASR control. The difference between both was the threshold considered to recommend sprays. The difference in performance observed for PREC_1 and PREC_2 to control ASR confirms the necessity of parameterization, adjustments, and validation in the models to predict disease control accurately under field conditions. According to Magarey and Isard (2017), the first challenge for using a DWS is to define the criteria to recommend sprays in the fields. Fundamentally, the criteria to determine the thresholds to recommend sprays should change from region to region, mainly because of variations of weather conditions which change disease patterns of occurrence, and then incidence and severity. Such environmental variations will result in different moments for the first disease observation (RACCA et al., 2010).

The prediction equation used in PREC_1 and PREC_2 refers to an empirical model to predict ASR final severity (DEL PONTE et al., 2006a). Empirical models have high dependence with the local conditions since its fundamental principle is a correlation between weather conditions and plant disease variables. Thus, prior to the DWS implementation,

empirical relations need to be validated, passing through possible parameterizations to avoid errors under operational scale (ROWLANDSON et al., 2014; GLEASON et al., 2008).

The need for adjustments in empirical DWS due to different regions can be observed in the management of the disease sooty blotch and flyspeck (SBFS) in apple trees. This was first proposed for Kentucky, USA where fungicide sprays were recommended when the threshold of 182 hours with LWD was accumulated after the first fungicide sprays (HARTMAN, 1993). In order to ensure the DWS performance for SBFS control in North Carolina, USA the threshold had to be increased to 273 hours (BROWN; SUTTON, 1995). Batzer et al. (2008) and Duttweiler et al. (2008) proposed a modification in this DWS, which was tested by Rosli et al. (2017) in Iowa, USA, who obtained a good performance to control SBFS with fewer sprays and high disease control compared with the calendar-based approach.

Improvements in DWS performance was also observed under management practices of *Alternaria solani* for different crops. Initially, such a DWS was developed to control the pathogen on tomato crop being called FAST (Forecaster of *Alternaria solani* on Tomato). In the test of the FAST, in Pennsylvania, US, an efficient fungicide schedule was obtained with at least three sprays less than calendar-based approach, which in turn recommended 10 sprays (MADDEN; PENNYPACKER; MACNAB, 1978). After seven years of field trials modifications were proposed in the FAST system making possible the prediction of more anthracnose and Septoria leaf spot on tomato. Faced with such modifications the DWS was renamed as TOMCAST (GLEASON et al., 1995). In order to be used with accuracy on potato crop in Denmark, Abuley and Nielsen (2017) incorporated different maturation groups of potato into TOMCAST and verified a reduction of 50 % in fungicide sprays required to control early blight compared with the conventional method used by growers in that region.

As mentioned above, PREC_1 did not show a good performance considering all locations, soybean growing seasons, and sowing dates (Table 3.3). However, for sowing dates in October and November, the best sowing period for soybean in Brazil, PREC_1 controlled ASR effectively during 2014/2015 and 2015/2016 soybean growing seasons in Piracicaba, Ponta Grossa and Campo Verde, reducing fungicide sprays compared to CALEND (Tables 3.4, 3.5 and 3.6).

In Piracicaba, during the 2014/2015 growing season, the soybean sowed on October 23 required two sprays by PREC_1, while CALEND prescribed six sprays (Table 3.4). Treatment PREC_1 presented final severity of 34.1 % and AUDPC of 695.4 with four sprays less than CALEND (Figure 3.1). Although the final severity and AUDPC obtained with CALEND system were lower than that with PREC_1, such response variables values were

statistically similar ($P \leq 0.05$) between both treatments (Table 3.4). In Piracicaba, for 2015/2016 season, the soybean crop sowed on October 22 and November 19 required at least two sprays less in the treatment PREC_1 as opposed to CALEND. In both sowing dates, final severity and AUDCP assessed for PREC_1 and CALEND were statistically similar ($P \leq 0.05$) (Table 3.4 and Figure 3.1 and Figure 3.2).

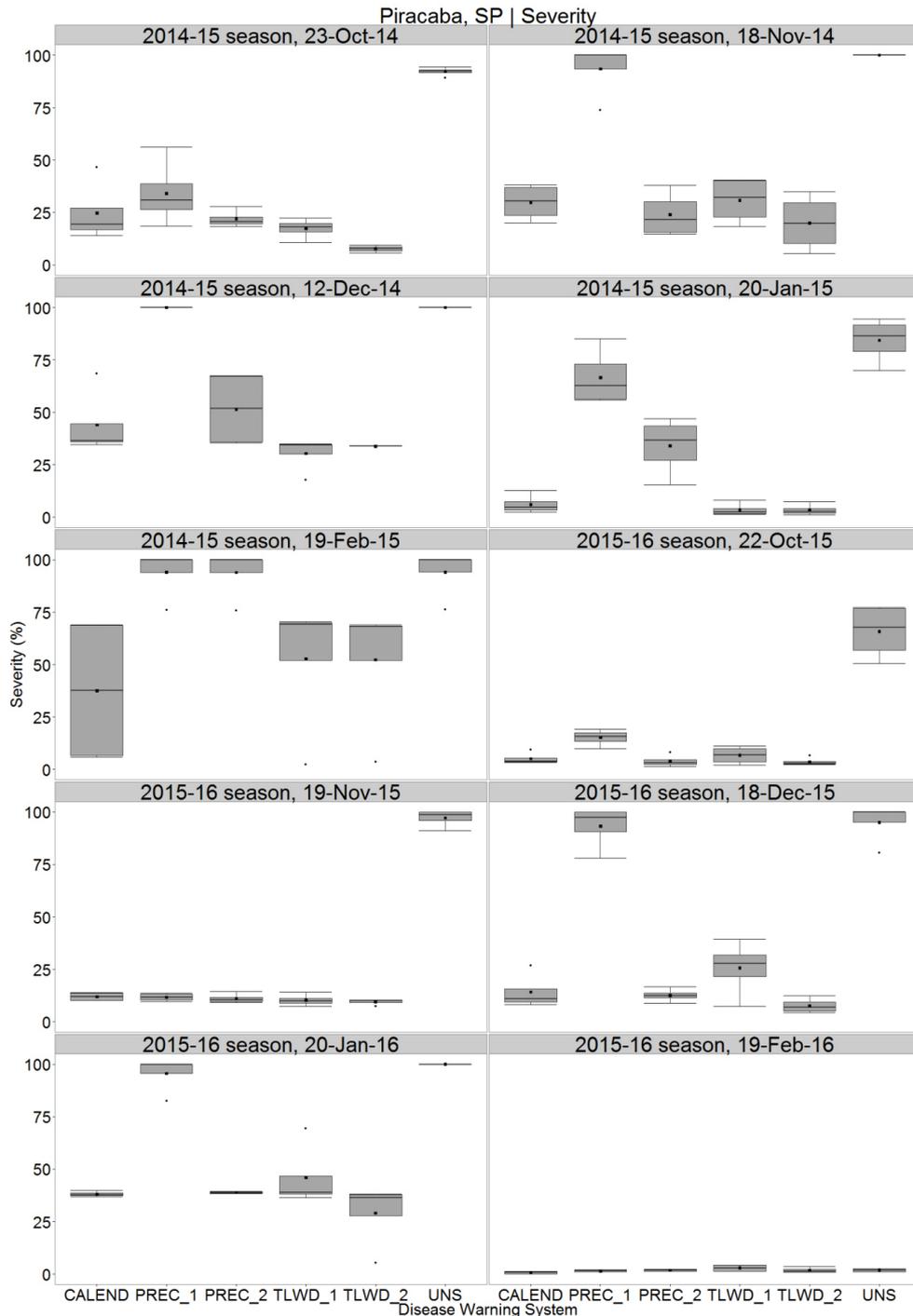


Figure 3.1. Final Asian soybean rust severity considering different sowing dates and the adoption of different control systems in Piracicaba, SP, Brazil, during 2014/2015 and 2015/2016 growing seasons.

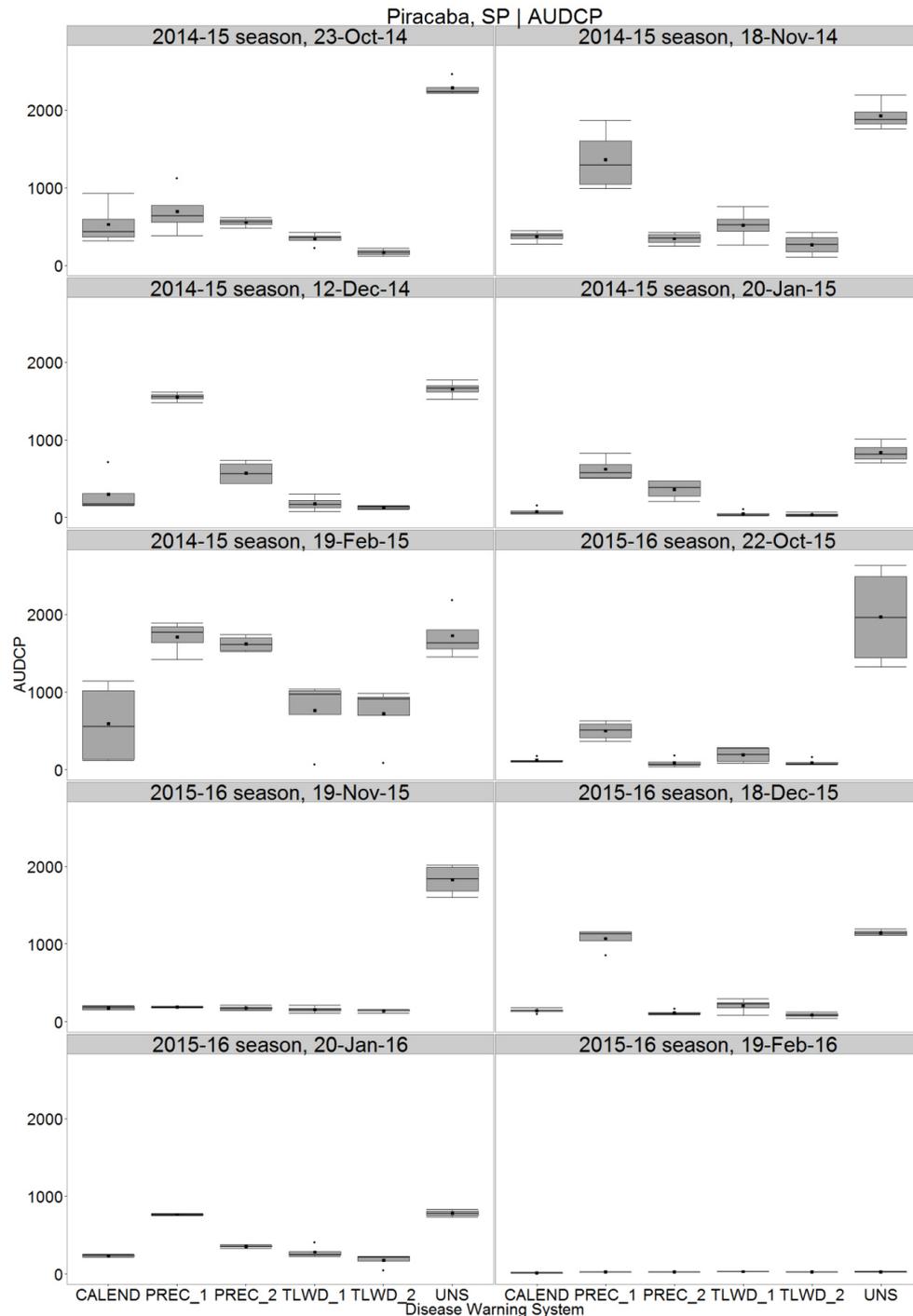


Figure 3.2. Area under disease curve progress (AUDCP) for Asian soybean rust considering different sowing dates and the adoption of different control systems in Piracicaba, SP, Brazil, during 2014/2015 and 2015/2016 soybean growing seasons.

Data from soybean sowed on October 16 of 2014 in Ponta Grossa showed that PREC_1 recommended three sprays, while the other treatments recommended four (TLWD_1), five (CALEND and PREC_2) and six sprays (TLWD_2). For all treatments with fungicide spray application, ASR final severity ranged from 28 % to 41 % (Figure 3.3), and

the values were similar ($P \leq 0.05$) (Table 3.5). The same results were observed for AUDCP, corroborating that ASR control following sprays recommendations under PREC_1 was statistically similar.

Table 3. 4. Number of sprays, final severity and area under disease curve progress (AUDCP) considering different sowing dates and the adoption of different disease-warning systems to control Asian soybean rust in Piracicaba, SP, Brazil, during 2014/2015 and 2015/2016 growing seasons.

Treatment	2014/2015					2015/2016						
	Sowing date	Spray	Final Sev. %	AUDCP		Sowing date	Spray	Final Sev. %	AUDCP			
UNS		0	92.1	a	2287.5	a		0	65.8	a	1969.2	a
CALEND		6	24.7	bc	529.1	bc		5	5.1	b	119.9	b
PREC_1	23-Oct	2	34.1	b	695.4	b	22-Oct	2	15.2	b	499.2	b
PREC_2		5	21.9	bc	556.2	bc		5	3.9	b	87.4	b
TLWD_1		5	17.3	bc	340.6	cd		5	6.7	b	188.0	b
TLWD_2		6	7.74	c	167.8	d		5	3.6	b	90.4	b
UNS			0	100.0	a	1926.9		a		0	97.1	a
CALEND		5	29.8	b	373.2	c		5	11.9	b	177.3	b
PREC_1	18-Nov	0	93.4	a	1361.3	b	19-Nov	3	11.7	b	189.1	b
PREC_2		5	24.0	b	345.2	c		4	11.0	b	173.9	b
TLWD_1		5	30.8	b	517.0	c		5	10.2	b	156.8	b
TLWD_2		5	19.9	b	267.4	c		6	9.4	b	142.2	b
UNS			0	100.0	a	1655.6		a		0	95.1	a
CALEND		5	44.0	b	301.9	bc		5	14.1	b	139.9	b
PREC_1	12-Dec	0	100.0	a	1554.2	a	18-Dec	0	93.2	a	1070.5	a
PREC_2		3	51.5	b	573.8	b		5	12.5	b	114.3	b
TLWD_1		5	30.4	b	179.2	c		5	25.6	b	207.1	b
TLWD_2		6	33.8	b	129.9	c		5	7.6	b	84.9	b
UNS			0	84.3	a	837.0		a		0	100.0	a
CALEND		4	6.1	c	76.9	c		4	38.1	b	235.0	bc
PREC_1	20-Jan	0	66.6	a	623.4	a	20-Jan	0	95.6	a	764.4	a
PREC_2		1	33.9	b	360.7	b		1	38.9	b	354.0	b
TLWD_1		4	3.5	c	48.0	c		3	45.9	b	280.3	bc
TLWD_2		4	3.4	c	37.3	c		3	29.0	b	174.1	c
UNS		19-Feb	0	94.0	a	1726.1		a	19-Feb	0	1.8	ab
CALEND		4	37.5	b	592.3	c		2	0.7	b	11.9	b
PREC_1		0	94.0	a	1709.5	a		0	1.4	b	22.7	a
PREC_2		0	93.9	a	1622.2	ab		0	1.8	ab	23.0	a
TLWD_1		3	52.8	ab	759.8	bc		0	2.8	a	28.2	a
TLWD_2		4	52.2	ab	721.6	bc		1	1.8	ab	23.1	a

Final severity (Final sev.) and AUDCP data followed by different letters are statistically different by the Tukey's test ($\alpha = 0.05$)

At Ponta Grossa similar performance of PREC_1 was observed for sowing on October 08 2015; however this treatment recommended only one spray, three less than CALEND. Even with fewer sprays, ASR final severity with PREC_1 was 6.4 %, 0.2 % lower compared with CALEND (Figure 3.3). In contrast, AUDCP obtained in PREC_1 was higher compared with CALEND (Figure 3.4), but they were not different ($P \leq 0.05$) (Table 3.5).

Number of sprays and percentage of defoliation in Campo Verde, MT (Table 3.6), showed that PREC_1 recommended fewer fungicide sprays for the first sowing dates of 2014/2015 and 2015/2016, compared to the other treatments. For the first and second sowing dates of 2014/2015 and 2015/2016 growing seasons, PREC_1 recommended four and three sprays, respectively, one less than CALEND. Even with fewer fungicide sprays required to control ASR, PREC_1 presented similar values of defoliation in relation to CALEND, as well as to other DWSs. A higher number of spray reductions by PREC_1 was observed for the first sowing date of 2015/2016, when PREC_1 recommended only two sprays, two less than any other methods (Table 3.6).

Table 3. 5. Number of sprays, final severity and area under disease curve progress (AUDCP) considering different sowing dates and the adoption of different disease-warning systems to control Asian soybean rust at Ponta Grossa, PR, Brazil, during 2014/2015 and 2015/2016 growing seasons

Treatment	2014/2015					2015/2016						
	Sowing date	Spray	Final Sev. %	AUDCP		Sowing date	Spray	Final Sev. %	AUDCP			
UNS		0	66.8	a	1676.7	a		0	73.5	a	577.2	a
CALEND		5	32.8	b	691.7	b		4	6.6	b	89.7	b
PREC_1	16-Oct	3	28.0	b	617.0	b	8-Oct	1	6.4	b	155.6	b
PREC_2		5	32.8	b	650.1	b		4	7.3	b	115.1	b
TLWD_1		4	37.7	b	589.6	b		3	7.8	b	125.6	b
TLWD_2		6	41.0	b	545.0	b		3	7.3	b	94.5	b
UNS			0	18.5	a	555.4		a		0	16.4	a
CALEND		4	9.5	ab	476.7	ab		5	3.0	d	92.3	d
PREC_1	11-Nov	4	7.3	b	206.8	bc	21-Nov	2	11.6	b	176.2	b
PREC_2		5	11.7	ab	326.4	abc		5	5.8	c	120.4	cd
TLWD_1		6	8.0	b	407.0	abc		4	7.0	c	142.6	bc
TLWD_2		6	7.2	b	147.5	c		5	5.9	c	129.4	c
UNS			0	71.2	a	1073.6		a		0	12.0	a
CALEND		4	48.3	b	672.3	b		4	3.7	d	73.5	ab
PREC_1	18-Dec	0	48.6	b	672.9	b	11-Dec	1	10.3	ab	92.1	ab
PREC_2		3	15.8	d	422.1	c		3	6.4	c	83.3	ab
TLWD_1		3	16.8	d	351.6	c		4	8.5	bc	86.9	ab
TLWD_2		4	31.8	c	435.5	c		4	3.3	d	67.5	b
UNS			0	81.0	a	1946.7		a		0	11.5	ab
CALEND		3	53.1	bc	1152.6	b		4	3.1	b	173.6	b
PREC_1	15-Jan	0	46.4	bcd	768.3	c	23-Jan	0	17.2	a	255.4	a
PREC_2		1	63.4	ab	866.2	c		0	4.1	b	203.4	b
TLWD_1		2	36.6	cd	550.9	d		2	6.2	b	183.6	b
TLWD_2		2	29.8	d	402.4	d		3	6.1	b	184.2	b

Final severity (Final Sev.) and AUDCP data followed by different letters are statistically different by the Tukey's test ($\alpha = 0.05$)

In different Brazilian regions where soybean is cultivated, the growing season starts in September, frequently after a free-host period (GODOY et al., 2016). However, sowing dates with lower climatic risks range from October to December (BATTISTI; SENTELHAS, 2014). According to the planting and harvesting schedule of soybean crops in Brazil

(CONAB, 2017), throughout the 2014/2015 and 2015/2016 season, about 80% of soybean areas were sown between October and November. This period matches with planting dates where PREC_1 showed good performance to control ASR in Piracicaba, Ponta Grossa, and Campo Verde (Tables 3.4, 3.5 and 3.6).

To recommend sprays, PREC_1 have to accumulate the threshold of 80 % of DSV. This threshold is reached only with the occurrence of high volume and frequency of rainfall. Based on rainfall data observed in Piracicaba during the first sowing date of 2014/2015, the threshold was reached after 280 mm of rainfall recorded within the interval of 30 days prior to the date of DSV calculation. Thus, in Piracicaba, Ponta Grossa and Campo Verde the good performance of PREC_1 coincided with periods with higher rainfall volumes, measured throughout November and December, when soybean plants sown in October and November were under monitoring.

Table 3. 6. Number of fungicide sprays and percentage of defoliation considering different sowing dates and the adoption of different disease-warning systems to control Asian soybean rust in Campo Verde, MT, Brazil, during 2014/2015 and 2015/2016 growing seasons

Treatment	2014/2015			2015/2016				
	Sowing date	Spray	Defoliation %	Sowing date	Spray	Defoliation %		
UNS		0	97.7	a		0	100.0	a
CALEND		5	79.5	b		5	93.0	b
PREC_1	21-Oct	4	80.5	b	23-Oct	2	96.8	ab
PREC_2		5	80.0	b		4	92.5	b
TLWD_1		5	82.5	b		4	96.8	ab
TLWD_2		5	83.2	b		4	93.5	ab
UNS		0	100.0	a		0	91.3	a
CALEND		5	92.0	b		4	67.5	b
PREC_1	13-Nov	5	93.7	b	7-Nov	3	71.3	b
PREC_2		5	95.5	b		4	67.5	b
TLWD_1		6	94.5	b		5	68.8	b
TLWD_2		6	92.7	b		5	68.8	b
UNS	12-Dec	0	100.0	a	9-Dec	0	100.0	a
CALEND		3	100.0	b		3	91.3	b
PREC_1		3	100.0	b		4	91.3	b
PREC_2		5	68.7	c		4	94.3	b
TLWD_1		5	76.2	b		4	92.5	b
TLWD_2		5	77.5	b		4	90.8	b

Defoliation data followed by different letters are statistically different by the Tukey's test ($\alpha = 0.05$)

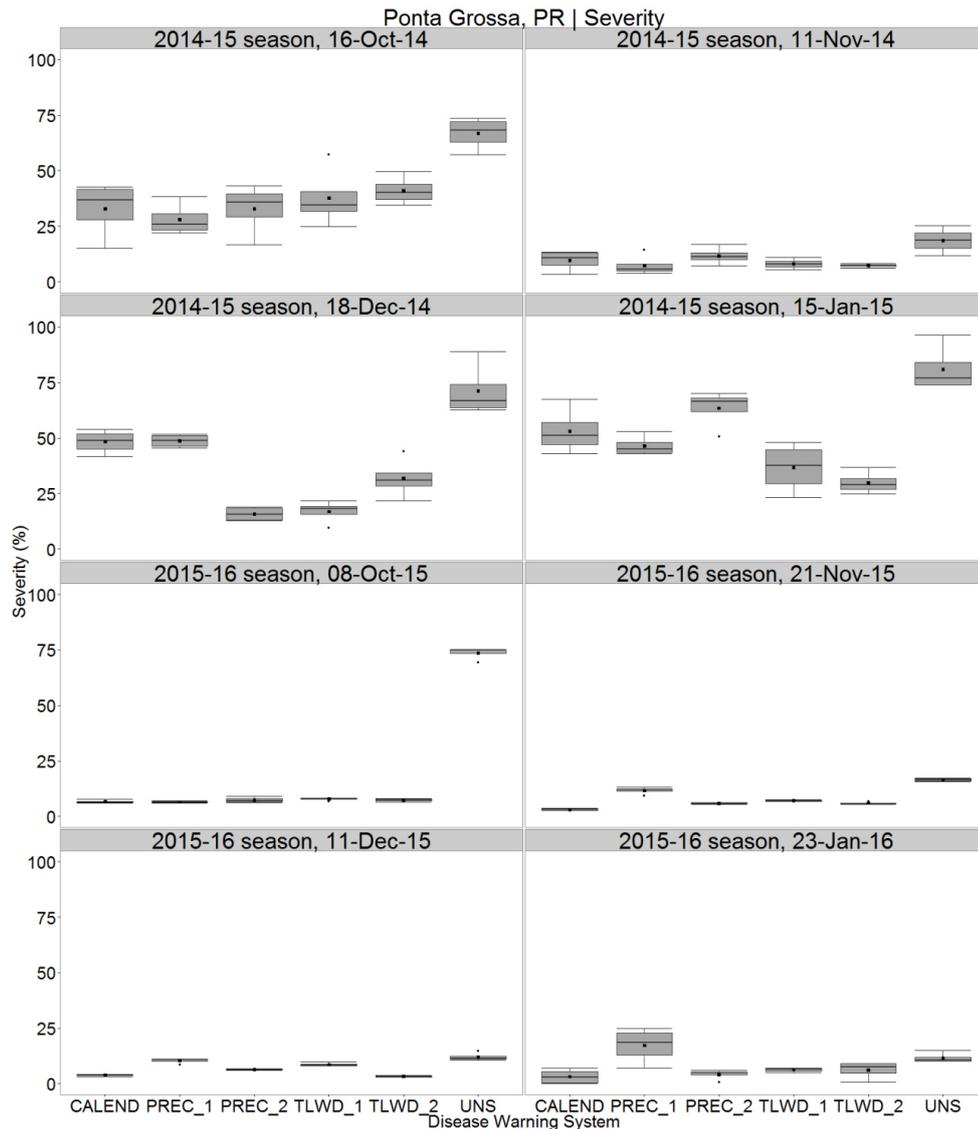


Figure 3.3. Final Asian soybean rust severity considering different sowing dates and the adoption of different control systems in Ponta Grossa, PR, Brazil, during 2014/2015 and 2015/2016 growing seasons.

In the empirical model obtained by Del Ponte et al. (2006a) the disease progress was influenced by weather conditions that determine LWD, especially rainfall events. The authors reported that the strong correlation between ASR severity and rainfall was observed for sowing dates ranging from October to early January of the following year, when high rainfall volumes with good distribution occurred. This strengthens the viability of PREC_1 to manage ASR to the sowings in October and November in Piracicaba, Ponta Grossa and Campo Verde, because the action threshold was reached due to high rainfall indices, with spraying indications for ASR control when environment conditions are favorable to the disease.

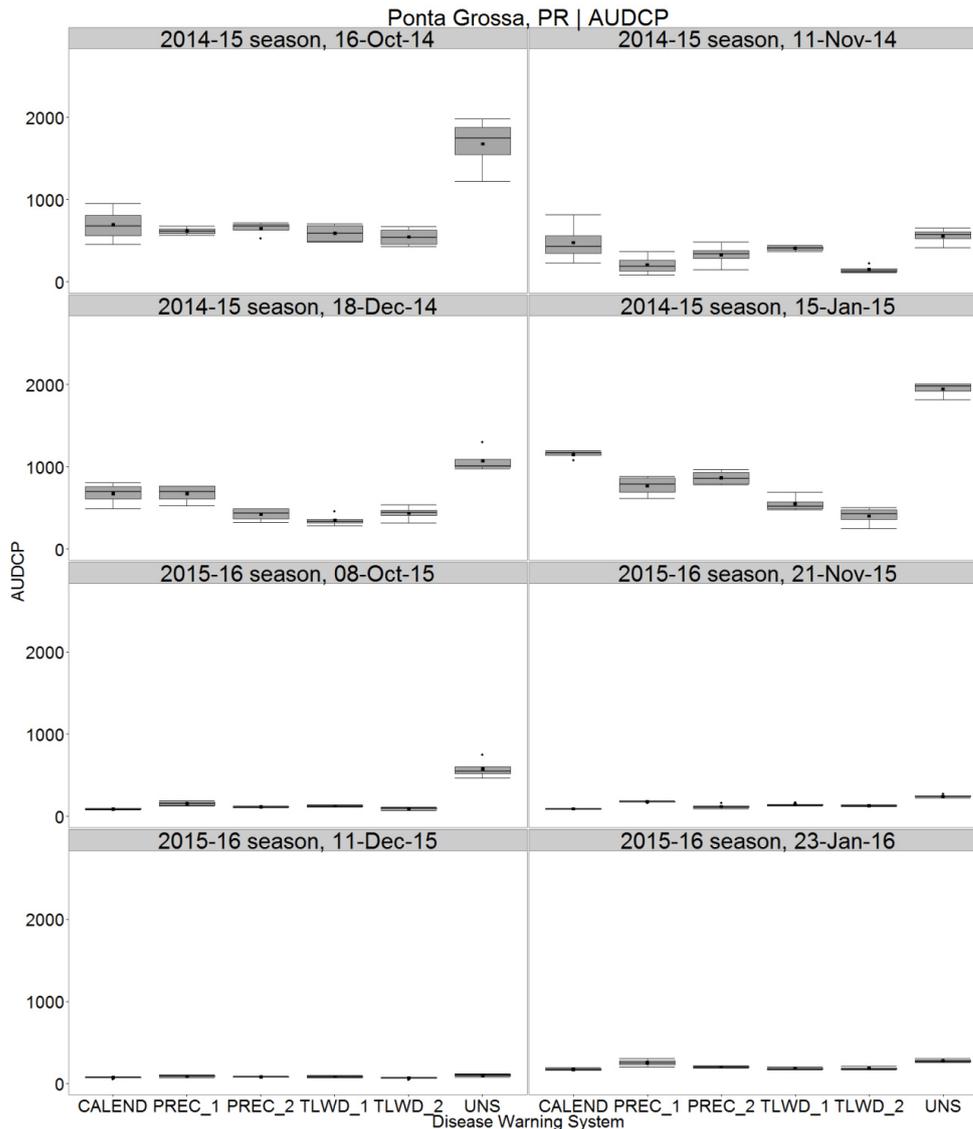


Figure 3.4. Area under disease curve progress (AUDCP) for Asian soybean rust considering different sowing dates and the adoption of different control systems in Ponta Grossa, PR, Brazil, during 2014/2015 and 2015/2016 soybean growing seasons.

Kelly et al. (2015) conducted a study based on empirical relations between ASR severity and rainfall events used to define parameters for fungicide sprays to control ASR in soybean and kudzu plants in USA. Between the sowing dates of 2010 and 2011, the DWS based on rainfall data reduced at least one spray, compared with the calendar-based approach for which two sprays were always applied. Although the DWS was tested under low disease pressure, the authors mentioned that the DWS is a promising tool for ASR control, allowing to ensure a better time to make sprays, resulting in fewer sprays in soybean fields compared with the conventional method adopted by the growers.

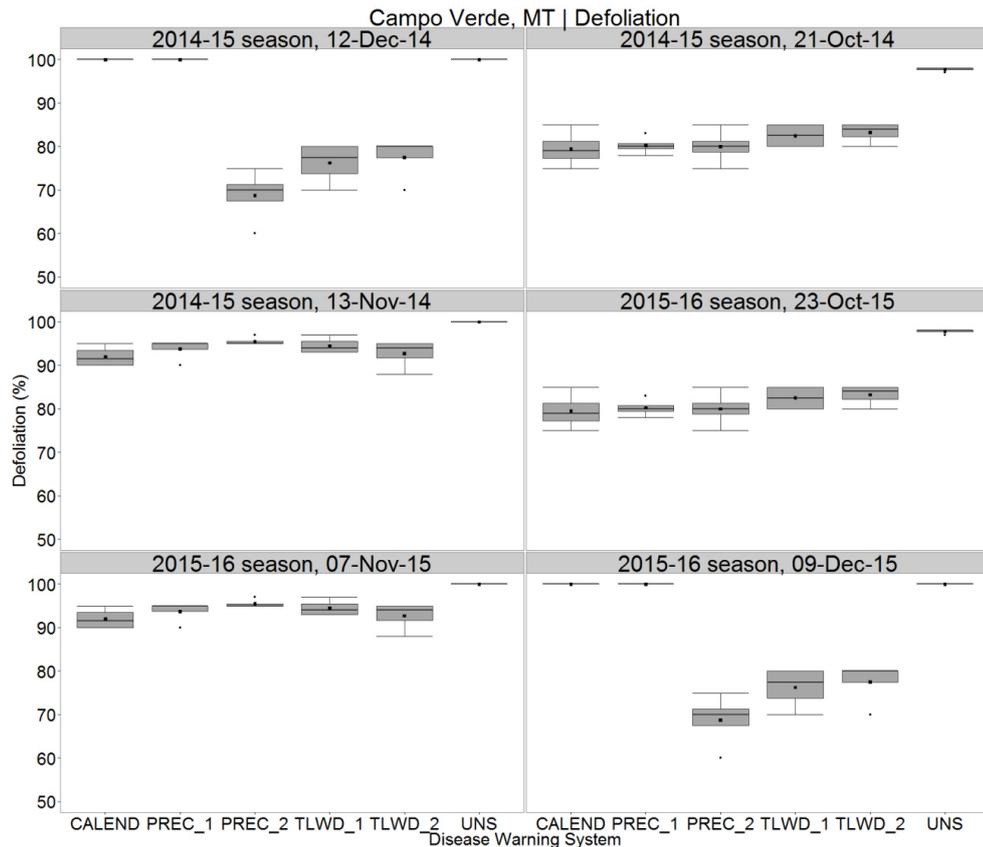


Figure 3.5. Final defoliation for Asian soybean rust considering different sowing dates and the adoption of different control systems in Campo Verde, MT, Brazil, during 2014/2015 and 2015/2016 soybean growing seasons.

As mentioned previously, soybean cultivation in Brazil extends until December, classified as a late sowing period. For such a period, treatment PREC_2 presented a better performance compared with other treatments, since it reduced fungicide sprays associated with a good disease control. In Ponta Grossa, throughout both soybean growing seasons, PREC_2 saved one spray compared with CALEND. Moreover, in Piracicaba, PREC_2 saved two and three sprays in comparison to CALEND for December 12, 2014 and January 20, 2016 sowing dates, respectively.

The better performance of PREC_2 on sowing dates described above is linked to adjustment in DWS threshold to recommend sprays. In contrast with PREC_1, for PREC_2 the action threshold to determine the first spray was 50 %, against 80 % adopted in PREC_1. In Piracicaba and Ponta Grossa during 2014/2015 season, 100 mm less of rainfall was observed than in December and January, confirming the need for adjustment in the threshold to recommend sprays by DWS. In both sites, during the period, a heat and dry period occurred

causing a reduction in frequency and volume of rainfall, therefore DWS based on rainfall with the less conservative threshold may not recommend sprays when it is necessary to be applied.

The effects of rainfall reductions become more prominent when January and February sowing dates are analyzed, especially for Piracicaba and Ponta Grossa, where the DWS based on rainfall did not recommend fungicide sprays (Tables 3.4 and 3.5). Influence of changes in the rainfall regime on the performance of DWS proposed by Del Ponte et al. (2006a) was also verified by Sentelhas et al. (2012) in field trials conducted at different Brazilian regions. The authors verified the strong correlation between DSV values and rainfall pattern in Rondonópolis, MT, Londrina, PR and Passo Fundo, RS. Throughout the three years of field experiments, the DSV values were above 50 % in Rondonópolis. The opposite was observed in Passo Fundo where each DSV was under 50 %, for both sites the DSV varied according to variability of rainfall levels. In Passo Fundo, a drought occurred and interfered on DSV values. In contrast, during 2010/2011 in Rondonópolis and Londrina, a high occurrence of rainfall kept the DSV above 70 % (SENTELHAS et al., 2012).

During sowing dates with low rainfall, only treatments based on air temperature and leaf wetness duration (LWD) recommended sprays (Tables 3.4 and 3.5). Under Brazilian climate conditions, LWD may be caused by two sources, rainfall or dew (SENTELHAS et al., 2008). Thus, in periods without rainfall, LWD was determined by dew deposition mainly during the night, which resulted in spray recommendation by DWS proposed by Reis; Sartori; Câmara (2004).

The analysis of all field trials showed that the TLWD system presented good ASR control, which can be observed in the values of final severity, AUDCP, and defoliation, similar to or lower than CALEND recommendations. The effective ASR control by TLWD is related to the direct influence of air temperature and LWD on the ASR infection process. Basically, air temperature affects the whole infection process and is responsible for regulating metabolic reactions of the pathogen (GILLESPIE; SENTELHAS, 2008). Similarly, LWD affect the initial establishments of the pathogen, because it influences the germination and penetration processes (ROWLANDSON et al., 2014; GILLESPIE; SENTELHAS, 2008; SENTELHAS et al., 2008).

The equation used in TLWD was applied to predict the first ASR symptoms in Londrina, Paraná State, during two soybean seasons and showed a good performance (IGARASHI et al., 2016). In the current study, TLWD with both thresholds were also able to predict ASR epidemics in different sites; however, an overestimation was verified in the prediction, as TLWD required higher number of sprays compared with CALEND, PREC_1

and PREC_2, except for the sowing dates of January in Ponta Grossa (Table 3.5) and February in Piracicaba (Table 3.4), and in both sowing dates in Pedra Preta (Table 3.7).

Similar results were reported by Kelly et al. (2015) by using the same DWS under analysis for ASR management in Florida, USA. In 2009, the DWS recommended six sprays to control ASR in kudzu plants, while the DWS based on rainfall data and the calendar-based approach determined applications of four and two sprays, respectively. A similar situation was observed in 2010 and 2011 whenever DWS based on LWD data recommended at least two sprays for ASR management in kudzu and soybean plants.

In a comparative analysis between TLWD_1 and CALEND in Pedra Preta for sowing date in November 6, 2015, the DWS saved one spray comparing with CALEND and promoted reductions in the defoliation index. A similar situation was observed for the second sowing date in the same field trial, where TLWD_1 recommended three sprays, while CALEND prescribed four (Table 3.7).

Table 3. 7. Number of sprays and percentage of defoliation considering different sowing dates and adoption of different disease-warning systems to control Asian soybean rust at Pedra Preta, MT, Brazil, during 2015/2016 growing season.

Treatment	Sowing date	Spray number	Defoliation	
			%	
UNS	6-Nov	0	100	a
CALEND		4	92.8	ab
PREC_1		4	89.0	b
PREC_2		4	92.5	ab
TLWD_1		3	91.5	ab
TLWD_2		5	90.3	ab
UNS	3-Dec	0	100.0	a
CALEND		4	100.0	a
PREC_1		4	89.5	b
PREC_2		5	86.0	b
TLWD_1		3	98.0	a
TLWD_2		5	87.0	b

Defoliation data followed by different letters are statistically different by the Tukey's test ($\alpha = 0.05$)

For the fourth sowing date at Ponta Grossa, 2014/2015, the TLWD with both thresholds determined two sprays, one less than CALEND. In this period, the good performances of DWSs were confirmed by the values of final severity and AUDCP, which was 36.6 % and 550.9 for the less conservative threshold, as well as 29.8 % and 402.4 for the more conservative one, being such values lower than those calculated for CALEND (Table 3.5). For the fifth growing season in Ponta Grossa, values of final severity and AUDCP were similar among TLWD_1, TLWD_2 and CALEND; however, the DWSs saved one (TLWD_2) and two (TLWD_1) sprays in relation to CALEND (Table 3.5).

For Piracicaba, during 2014/2015, TLWD_1 showed a good performance in plots sown on February 19, where DWS recommended three sprays, saving one in relation to CALEND; however with similar final severity and AUDCP. Also for Piracicaba, but in 2015/2016 season, for sowing date of January 20, an efficient disease control was observed using TLWD_1 and TLWD_2, which recommended three fungicide sprays, one less than that recommended by CALEND (Table 3.4).

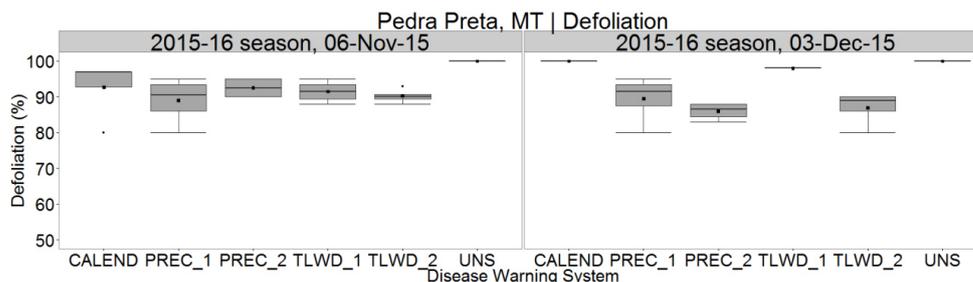


Figure 3.6. Final defoliation for Asian soybean rust considering different sowing dates and the adoption of different control systems in Pedra Preta, MT, Brazil, during 2015/2016 soybean growing seasons.

Although the TLWD treatment failed to save sprays on specific sowing dates for the locations assessed, studies using DWS based on air temperature and LWD were conducted and showed a high performance to control diseases and to reduce sprays. For example, Zhang (2015) validated a DWS to control *Colletotrichum acutatum* and *Botrytis cinerea* in strawberry in Iowa, USA, and observed that the assessed system reduced two sprays in relation to the conventional method adopted by growers. The same DWS was tested and validated in Florida State, USA, and it saved half of the fungicide sprays when compared to the weekly spray schedule (PAVAN; FRAISSE; PERES, 2011).

In this study, a high number of sprays required by TLWD treatments is probably related to the action threshold, which was 6 and 9 DVIP, respectively, for the most and less conservative levels. Juliatti et al. (2006), using the equation proposed by Reis; Sartori; Câmara (2004), verified that 15 DVIP was the value that better indicated the appearance of ASR lesions. On the other hand, Igarashi et al. (2016) observed the first ASR symptoms in soybean plants when the sum of DVIP was corresponding to 9. Thus, there is still a need for adjustments on the TLWD action thresholds for a better spray recommendation for ASR control.

3.4. Conclusion

The results of the current study evidenced that:

- PREC_2 presented a better performance for ASR control considering all locations, soybean growing seasons and sowing dates. In this general analysis, PREC_2 provides an effective ASR control with fewer fungicide sprays;
- PREC_1 worked well on the sowing dates of October and November, except for sowing date of November, 2014 in Piracicaba, enabling its use in periods with higher rainfall volumes. Thus, PREC_1 may be a good alternative to control ASR in soybean crops when sowed early at the season in the main Brazilian soybean regions;
- TLWD proved to be efficient to control ASR epidemics; however, on most occasions, such a DWS indicated a greater number of sprays as opposed to CALEND, PREC_1 and PREC_2, which reveals that further adjustments are required.

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4. EVALUATING THE PERFORMANCE OF EMPIRICAL LEAF WETNESS DURATION ESTIMATION METHODS ON ASIAN SOYBEAN RUST SPRAY DECISION

ABSTRACT

Asian soybean rust (ASR) is controlled by sequential applications of fungicides following a calendar-based system. This approach considers only aspects of the crop, disregarding the influence of local weather conditions on disease progress. The relationship between local weather and disease progress can be represented by mathematical methods, which have been the basis of warning systems for indicating precise spray timing for disease control. A limitation for widespread implementation of ASR warning systems is the scarcity of leaf wetness duration (LWD) data. An alternative way to make LWD data available is to estimate it by physical or empirical methods. The objective of this study is to assess the feasibility of using LWD estimated by different empirical methods (Relative Humidity Threshold Method, Depression Point Method and CART Method) as input data for an ASR warning system. The study was carried out with data obtained from field experiments conducted in Ponta Grossa, PR, Campo Verde, MT and Pedra Preta, MT, throughout the 2014/2015 and 2015/2016 growing seasons. The estimated LWD values were used as input in an ASR warning system, proposed by Reis et al. (2004). More reliable estimations of LWD using the NHRH were obtained with 90 % and 89 % as thresholds. By the adoption of the $\text{NHRH} \geq 90\%$ high estimation errors were observed in Campo Verde (RMSE = 1.04 h) compared with Pedra Preta (RMSE = 0.89 h) and Ponta Grossa, PR (RMSE = 1.00 h). Even considering the estimate errors of the $\text{NHRH} \geq 90\%$ method, it was the most suitable as input for running ASR warning system (RMSE less than 2.0 h). Using estimated LWD in Campo Verde resulted in EDSV higher than MDSV in the beginning of the period; however, after the 56th day, EDSV was lower than MDSV. In Ponta Grossa, EDSV was higher than MDSV during all growing season, and in Pedra Preta, EDSV was lower than MDSV for all assessed period. For the more conservative threshold, five sprays per season were recommended for all experimental sites and the fractions of correct estimates (θ_1) in Ponta Grossa ($\theta_1 = 0.884$ and $\theta_1 = 0.869$ for 2014/2015 and 2015/2016, respectively) were higher than those obtained in Pedra Preta and Campo Verde. On the other hand, for the less conservative spray threshold, a reduction of one spray was observed in Ponta Grossa, compared to Pedra Preta and Campo Verde, where five sprays were still recommended. In this case values of θ_1 for Campo Verde was 0.783 and 0.750, respectively for 2014/2015 and 2015/2016, for Pedra Preta was 0.826, and in Ponta Grossa, θ_1 was 0.878 for 2014/2015 and 0.946 for 2015/2016. According to these results, empirical $\text{NHRH} \geq 90\%$ method has enough accuracy and precision for being used as input in the ASR warning system in the most traditional soybean regions of Brazil.

Keywords: *Phakopsora pachyrhizi*; *Glycine max*; Leaf wetness; Disease warning system; Relative humidity

4.1. Introduction

Asian soybean rust (ASR) (*Phakopsora pachyrhizi* Sydow & Sydow) was first observed in Brazilian soybean fields during the 2001/2002 growing season, and since then it has been considered a major problem for the crop in the country (YORINORI et al., 2005). The damage caused by this disease varies with prevailing weather conditions and management actions during the growing season. Under favorable environmental conditions and in the absence of control, the disease can reduce yield by up to 70 % (HARTMAN et al., 2016; DALLA LANA et al., 2014; BROMFIELD, 1984).

In countries where ASR can cause severe epidemics, disease control is based on a calendar schedule determined by a crop phenological stage (SCHERM et al., 2009). In this case, fungicide sprays start at the R1 stage, which is the beginning of bloom (FEHR et al., 1971). The number of sprays per season depends on disease severity in soybean fields. In many Brazilian soybean fields more than three fungicides sprays per season is common (GODOY et al., 2016; SIQUERI, 2005; YORINORI et al., 2005).

Although the calendar-based system is convenient for soybean growers because sprays can be pre-scheduled, such a method disregards the influence of local weather conditions on disease risk. Thus, both the number of sprays and their timing could be inappropriate, reducing control efficiency, increasing crop management cost, and thereby suppressing income for growers (MEGETO et al., 2014).

In contrast with the calendar-based approach, epidemiological research has linked environmental variables to the risk of occurrence and severity of plant disease outbreaks under field conditions. These relationships, represented by mathematical methods, have been incorporated into disease-warning systems in order to indicate more efficient and effective spray timing for Asian soybean rust control (KELLY et al., 2015; DIAS; LI; YANG, 2014; DEL PONTE et al., 2006a).

According to Gleason et al. (2008), disease-warning systems are decision tools that help growers to manage plant diseases. Disease-warning systems are often developed initially based on relationships between weather variables and disease development under controlled conditions (growth chambers) or for specific climate condition. As a result, they need to be tested and validated in field trials, especially in climates that differ from those in which the systems were initially developed (DUTTWEILER et al., 2008).

Weather variables used as inputs in disease-warning systems are defined based on disease characteristics. Frequently, air temperature, rainfall, relative humidity, solar radiation

and leaf wetness duration have been used to assess risk of outbreaks (ROWLANDSON et al., 2014; GLEASON et al., 2008). For ASR, weather variables such as rainfall (DEL PONTE et al., 2006b; TAN; YU; YANG, 1996), sunlight (DIAS; LI; YANG, 2014; DIAS et al., 2010), air temperature, and leaf wetness duration (REIS; SARTORI; CÂMARA, 2004; MELCHING et al., 1989; MARCHETTI; MELCHING; BROMFIELD, 1976) are the most used in the disease-warning systems.

Leaf wetness duration (LWD) and air temperature during the night or during LWD events have a direct effect on ASR occurrence and dissemination, primarily by influencing the rate of infection (fungus germination and penetration) and sporulation (IGARASHI et al., 2014). According to Bonde et al. (2007) and Melching et al. (1989), air temperatures between 18 and 26 °C during wet periods exceeding 10 h for several consecutive days are optimal for ASR occurrence.

LWD is defined as the presence of free water on aerial plant surfaces. Under field conditions, LWD may be caused by rain, fog, irrigation, dewfall from the atmosphere, or distillation from the soil (SENTELHAS et al., 2008; MONTEITH; UNSWORTH, 1990). In some climates, dew is a primary contributor to LWD. Dew results from condensation of atmospheric moisture on plant surfaces that cool from radiative heat loss to a clear sky during calm nights (SENTELHAS et al., 2008; LUO; GOUDRIAAN, 2000).

Considering the importance of LWD as a major driving force for crop disease epidemics, several warning systems use it as an input variable (HUBER; GILLESPIE, 1992). Despite its importance, LWD is difficult to measure in weather stations (ALVARES et al., 2015). According to Madeira et al. (2002), LWD is not measured at most conventional or automatic weather stations worldwide; therefore measurements of LWD are often unavailable to act as inputs to disease-warning systems.

Another problem associated with LWD measurements is the lack of a standard for its measurement (DALLA MARTA; MAGAREY; ORLANDINI, 2005; SENTELHAS et al., 2004). LWD is a difficult variable to measure because it has a high level of spatial heterogeneity depending on canopy structure and leaf position and arrangement (BATZER et al., 2008; GLEASON et al., 2008; SENTELHAS et al., 2005).

An alternative way to make LWD data available, and therefore allow for its use as an input to disease-warning systems, is by means of methods which estimate LWD as a function of weather variables that are frequently available at weather stations (ROWLANDSON et al., 2014). LWD methods are classified into different categories, including physical, empirical or mixed approach (ALVARES et al., 2015; KIM et al., 2010). Physical methods are based on

energy balance principles, are highly accurate, and can be used in a broad range of geographical and climatic data. A potential limitation of physical methods is that they tend to be complex and require inputs of weather variables that are not monitored by many weather stations, such as net radiation or cloud cover (SENTELHAS; GILLESPIE, 2008; SENTELHAS et al., 2006).

Empirical methods, in contrast, simulate LWD by using statistical methods to relate environmental variables to LWD. These methods are based on decision rules that are optimized by statistical best fit procedures (GLEASON, 1994). A simplest empirical method considers the presence of wetness whenever relative humidity (RH) exceeds a particular threshold. This method inputs the number of hours per day during which RH is equal or above a specific threshold, such as 80, 85, 90 or 95 % (SENTELHAS et al., 2008).

Hybrid methods or mixed approach incorporate into the same equation physical principles of the environment with parameters determined empirically. According to Kim et al. (2010), this approach has been developed to overcome limitations of empirical and physical methods. Mixed approach due to incorporate physical principles might be portable from region to region; moreover as this calculation uses empirical parameters it is necessary less weather variables compared with physical methods to determine LWD (KIM et al., 2010; KIM; TAYLOR; GLEASON, 2004; GLEASON, 1994).

Despite the simplicity of empirical relative humidity-based methods for LWD estimation, it is necessary to calibrate the RH threshold from region to region, by adjusting it to the local climatic conditions in order to optimize such a method performance. Sentelhas et al. (2008) observed that the optimum threshold for the RH method differed among regions. For Ames, Iowa, USA, the calibrated RH threshold was 83%, similar to 82 % reported by Kim et al. (2010) for the same site. In both cases the thresholds differed considerably from the original one, considered as 90 % (SENTELHAS et al., 2008).

Even taking into account the different approaches to estimate LWD, currently there is no parameterization for estimating this variable. According to Rowlandson et al. (2014), even the World Meteorological Organization (WMO) lacked a standard method to estimate LWD, a fact that triggers great difficulties for defining the best way to determine LWD with accuracy. The absence of a standard method results in imprecise and inaccurate estimates of this variable, reflecting a risk to those who make use of estimated values, when measurements are missing.

Errors related to the lack of parameterization can increase the risks for growers who incorporate warning systems using these input data into their disease management practices

(ROWLANDSON et al., 2014). Such errors can lead to excessive or insufficient sprays whenever LWD is wrongly estimated. According to Mueller et al. (2009), the major factor that defines the success or failure in ASR control is optimization of the timing of fungicide sprays. As a result, precise determination of LWD is of high importance for assuring sustainability of soybean crop when using an ASR warning system.

Considering the scarce availability of weather stations that measure LWD, and that even where LWD sensors are available, these have no parameterization for standard measurements, therefore the hypothesis of the present study is that the use of LWD estimated by different empirical methods can be used as input to run ASR warning systems, with enough accuracy and precision. Based on that, the aim of this study was to assess the feasibility of LWD estimated by different methods, having temperature, relative humidity and wind speed as variables, and evaluate the impact of such input data on the number of sprays recommended by an Asian soybean rust warning system in Brazil.

4.2. Material and Methods

The experiments were carried out in Ponta Grossa, Paraná State, Campo Verde and Pedra Preta, both in Mato Grosso State, Brazil. At Ponta Grossa and Campo Verde the weather data were collected during two soybean growing seasons (2014/2015 and 2015/2016), whilst at Pedra Preta they refer only to 2015/2016 growing season (Figure 4.1). According to the Köppen climate classification, Ponta Grossa belongs to the Cfb type, characterized as a mesothermal, subtropical climate, and lacking a dry season, with a mean temperature for the hottest month below 22°C (ALVARES et al., 2013). In contrast, for both locations in Mato Grosso State the climate formula is Aw, megathermal, known as a climate of savannahs, with a dry winter and rainy summer (ALVARES et al., 2013). The climate conditions at each site during the monitoring periods are shown in Table 4.1.

At each experimental site, an automatic weather station (AWS) was installed close to the soybean fields (from 5 to 15 meters lateral distance) in order to monitor weather conditions. The AWS was equipped with calibrated sensors to measure: air temperature (AirT) and relative humidity (RH) (HMP35C probe, Vaisala); rainfall (TR-525M rain gauge, Texas Instruments); wind speed (Model 03001, Campbell Scientific, Logan, UT) and leaf wetness duration (LWD) (Cylindrical Sensor, Weather Innovations). Such electronic sensors were connected to Dataloggers (Models CR10X and CR1000, Campbell Scientific, Logan,

UT), which were programmed to perform readings at each minute and store averages at every 15 minutes.

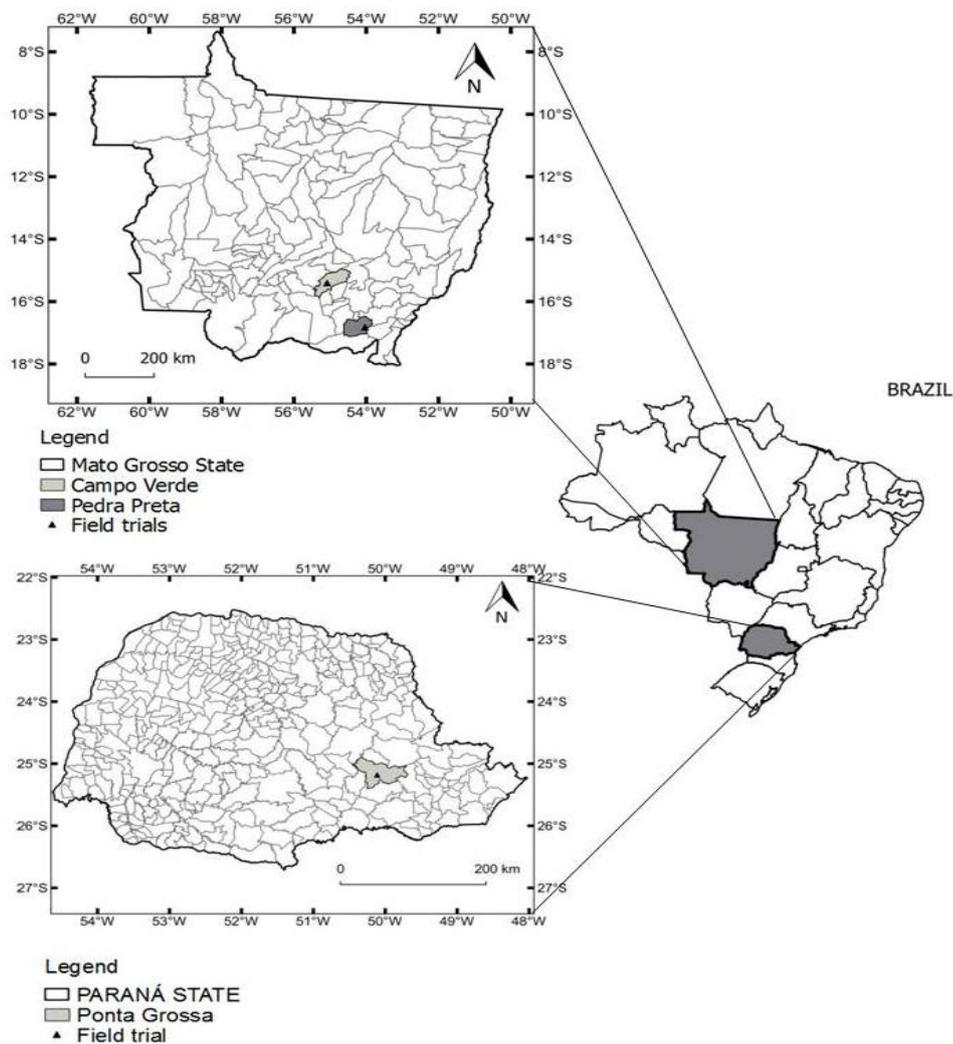


Figure 4.1. Soybean field trial locations where Asian soybean rust was assessed along with automatic weather stations to collect meteorological data for LWD estimation (Figure elaborated by Neves, 2017).

Table 4.1. Mean atmospheric conditions at different sites throughout the crop growing season in Brazil.

Sites	Air T (°C)		RH (%)		Rainfall (mm)		Wind speed (m s ⁻¹)	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
Campo Verde	23.3	24.5	85.1	84.9	1708.9	1843.5	2.2	2.0
Pedra Preta	--	25.3	--	84.9	--	1171.6	--	1.5
Ponta Grossa	21.3	21.1	76.9	80.4	873.7	1075.6	2.3	2.1

All LWD sensors were previously tested and calibrated under laboratory and field conditions. Following recommendations of Sentelhas et al. (2004) and Sentelhas; Monteiro; Gillespie (2004) the sensors were painted with an off-white latex paint to increase their ability to detect small amounts of wetness and mimic the thermal conditions of the leaves. For each location, two LWD sensors were mounted over mowed turfgrass, or over the soybean crop at 30-cm height above the canopy, with an inclination of 30° and facing south (SENTELHAS et al., 2008).

Daily air T and RH data were obtained by averaging. Daily rainfall totals were obtained by summing up rainfall events for a 24-h period. To obtain daily LWD data, the sum was made considering 24 hours, starting at 12h15 of day “n” and finishing at 12h00 of day “n + 1”. For each site the weather data were considered according to the soybean season. The data series started on November 1st of year “n” and finished on March 1st of year “n + 1”.

Three empirical methods to estimate LWD were evaluated: (I) number of hours with relative humidity equals or greater than a given threshold ($NHRH \geq RH \%$) (SENTELHAS et al., 2008); (II) dew point depression (DPD) (GILLESPIE; SRIVASTAVA; PITBLADO, 1993); and classification and regression tree (CART) (GLEASON, 1994).

The $NHRH \geq RH \%$ method uses only relative humidity values to estimate LWD. This method takes into account that wetness itself is occurring whenever RH is above or equal to a given threshold. In this study, RH thresholds considered for LWD estimation ranged from 80 to 95 %, with a 1 % interval.

The DPD method is based on AirT and RH. Basically, LWD is calculated by the difference between air temperature and dew point temperature, and considers that wetness is occurring whenever the difference between such temperatures is less than an established threshold. The thresholds tested were 2.0 °C for dew beginning and 3.8 °C for dew finishing.

The CART method is a nonparametric method that combines classification and a regression tree analysis with stepwise linear discriminate analyses. This procedure takes RH, DPD, and wind speed as inputs.

LWD estimates were obtained for each site and year, and analyzed separately for rainy days (days with overall rainfall $> 1 \text{ mm day}^{-1}$) and for dry days. Such a procedure was adopted to differentiate dew from rain as sources of wetness and to isolate the impact of each source on method's performance (Figure 4.2).

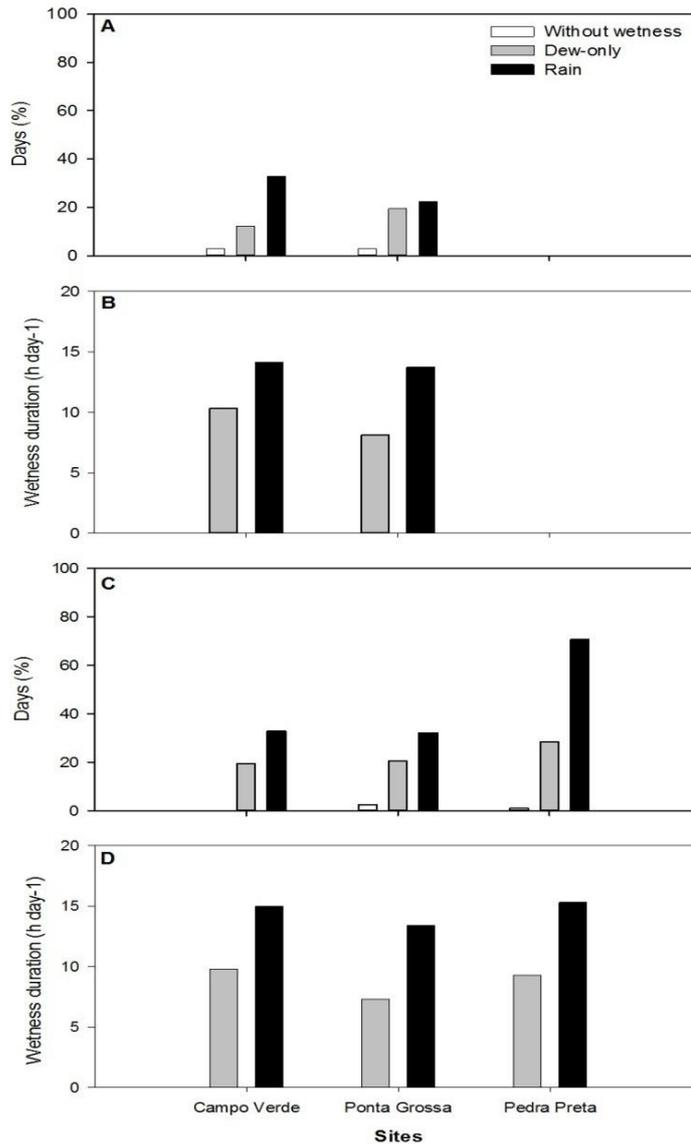


Figure 4.2. Percentage of days classified as rainy, dew-only and without wetness (A and C), and wetness duration per day (B and D) throughout both 2014/2015 (A and B) and 2015/2016 (C and D) soybean growing seasons at Ponta Grossa, PR, Campo Verde and Pedra Preta, MT, Brazil.

Method-derived estimates of LWD were compared to measurements from cylindrical sensors by means of regression analyses. The following statistical parameters were used to evaluate the performance of the methods: coefficient of determination (R^2), as a measure of precision; and Willmott agreement index (d), as a measure of exactness (WILLMOTT et al., 1985). The overall performance of the estimates was assessed by the c index (CAMARGO; SENTELHAS, 1997), which is obtained by multiplying d and the correlation coefficient (r). Values of c range from 0 for low confidence to 1 for high confidence (Table 4.2). To quantify

estimates errors, mean error (ME), mean absolute error (MAE) and root mean square error (RMSE) were calculated.

Table 4.2. Confidence index (c) proposed by Camargo and Sentelhas (1997) used to evaluate the overall performance of empirical LWD methods.

c index values	Performance
> 0.85	Excellent
0.76 – 0.85	Very good
0.66 – 0.75	Good
0.61 – 0.65	Median
0.51 – 0.60	Tolerable
0.41 – 0.50	Bad
≤ 0.40	Terrible

After the determination of the most accurate and precise method to estimate the LWD for each site and weather condition, measured and estimated LWD were used in the ASR warning system

The ASR warning system used herein was proposed by Reis; Sartori; Câmara (2004), using data published by Melching et al. (1989). The ASR warning system is based on the interaction between mean air temperature (AirT) during the LWD occurrence and LWD to determine disease severity values (DSV) (Table 4.3). When observed LWD was considered, it was determined the measured DSV (MDSV) and when estimated LWD was considered, it was determined the estimated DSV (EDSV).

Table 4.3. Asian soybean rust severity values obtained by the combination between the mean air temperature during the LWD occurrence and measured LWD. Adapted from Reis; Sartori; Câmara (2004).

Mean air temperature during LWD (°C)	DSV			
	0	1	2	3
	LWD (h)			
< 14.9	< 11.0	11.1 – 14.0	> 14.1	--
15 – 19.9	< 7.0	7.1 – 13.0	13.1 – 17	> 17.1
20 – 24.9	< 7.0	7.1 – 10.0	10.1 – 17	> 17.1
> 24.9	< 7.0	7.1 – 11.0	11.1 – 17	> 17.1

Daily DSV represents the probability of ASR infection on soybean, as follows: 0 = no infection probability; 1 = low infection probability; 2 = medium infection probability; and 3 = high infection probability.

Determination of MDSV and EDSV were made based on Brazilian soybean growing seasons. For each site simulations comprised 90 days during the susceptible period for ASR infection. For each site five sowing dates were adopted to start the simulations at 10-day intervals from November 10 through December 20, of 2014 to 2016.

Spray recommendations were determined by summing up MDSV and EDSV for seven preceding days (\sum MDSV and \sum EDSV, respectively). According to Reis; Sartori; Câmara (2004), the first fungicide application is recommended whenever the sum of daily severity values reaches 6 (more conservative threshold) or 9 (less conservative threshold). After sprays, a spray-free period of 14 days was adopted, under the assumption that fungicide residues on leaves would continue to suppress ASR infection effectively during this period. Just after the spray-free period, the calculation of MDSV and EDSV was restarted.

Differences between daily probability of ASR infection on soybean calculated using MDSV and EDSV, with LWD estimated by the most accurate method, for each experimental site, were compared using the following statistical indices and errors: R²; d; c; ME; MAE; and RMSE.

The performance of both DSV and number of sprays determined by the warning system with estimated LWD was also evaluated based on a four-cell contingency table (Table 4.4) and having MDSV as the truth.

Table 4.4. Four-cell contingency table used to classify the performance of the Reis; Sartori; Câmara (2004) Asian soybean rust warning system using estimated and measured LWD as input variables for recommending sprays.

Input data source	Spray (EDSV)	No Spray (EDSV)
Spray (MDSV)	Hits (H)	Misses (M)
No Spray (MDSV)	False alarms (F)	Correct negatives (N)

H and N denote hits by the method. H = sprays were recommended by the estimated inputs when necessary according to the measured inputs (true positive outcome); N = no spray was recommended using the estimated weather inputs, and the MDSV threshold using measured weather inputs did not recommend a spray (true negative). M and F represent errors by the method. M means sprays that were recommended by estimated LWD inputs when they were not required by using measured LWD inputs (false positive); F signifies when using estimated-LWD inputs did not result in a spray recommendation, whereas using the measured-LWD inputs recommended spraying (false negative).

Based on that, the performance of the estimates was determined by the fraction of correct estimates (θ_1) (KIM et al., 2010; KIM; TAYLOR; GLEASON, 2004). This index is

the ratio between true positives (H) and correct negative (N) events, which were considered to be the sprays recommended in the same day, as follows below:

$$\theta_1 = \frac{H + N}{H + M + F + N} \quad (4.1)$$

The LWD data was recorded under different weather conditions in Brazil, which make possible LWD to occur in both dry and wet conditions, making that one condition to occur more times than other. As a consequence, it is possible for an LWD method to obtain high accuracy just by predicting the preponderance of hours in which wetness was absent. To correct the situation described above, the k agreement index was calculated complying with a method proposed by Dietterich (2000), as follows:

$$k = \frac{\theta_1 - \theta_2}{1 - \theta_2} \quad (4.2)$$

where θ_2 is an estimate of the probability of the ASR control recommendation based on measured and estimated LWD agree by chance, given the observed counts in the contingency table, which is calculated as follows:

$$\theta_2 = \frac{(H + M) \cdot (H + F)}{(H + M + F + N)^2} + \frac{(F + N) \cdot (M + N)}{(H + M + F + N)^2} \quad (4.3)$$

4.3. Results

4.3.1. LWD methods performance

The comparison of the different methods to estimate LWD allowed to identify that NHRH was the most accurate method for all sites and growing seasons. More reliable estimations of LWD were obtained using 90% and 89% as thresholds, resulting in MAE of 1.61 and 1.54 h, respectively. Also, good performances of LWD estimation using NHRH were obtained adopting 86 %; 88 %; and 92 % as thresholds.

These results emphasize the importance of testing and validating empirical LWD empirical methods before their use as inputs in warning systems or any other agricultural management tool. According to Gleason et al. (2008), one reason for the lack of

implementation of disease-warning systems is the inaccuracy of LWD estimation. Therefore, the determination of a correct threshold for the NHRH method is important to reduce LWD errors and, consequently, errors in DSV and number of sprays.

Table 4.5. Statistical indices and errors for the comparison between measured and estimated LWD using three empirical methods throughout 2014/2015 and 2015/2016 soybean growing seasons in Campo Verde, MT, Brazil.

Sites	Methods	Threshold	R ²	d	c	EM	MAE	RMSE
						(h)		
Campo Verde	NHRH	80 %	0.684	0.777	0.643	4.036	4.070	1.445
		81 %	0.693	0.797	0.664	3.728	3.788	1.412
		82 %	0.706	0.819	0.688	3.376	3.463	1.374
		83 %	0.729	0.843	0.720	3.014	3.140	1.335
		84 %	0.728	0.858	0.731	2.666	2.889	1.302
		85 %	0.731	0.875	0.748	2.244	2.614	1.260
		86 %	0.737	0.892	0.766	1.852	2.391	1.221
		87 %	0.739	0.906	0.779	1.413	2.200	1.175
		88 %	0.735	0.912	0.782	1.088	2.095	1.143
		89 %	0.730	0.919	0.785	0.662	2.015	1.099
		90 %	0.712	0.918	0.774	0.072	2.010	1.037
		91 %	0.712	0.915	0.772	-0.490	2.061	0.983
		92 %	0.699	0.901	0.753	-1.145	2.237	0.917
		93 %	0.635	0.878	0.700	-1.043	2.777	0.912
		94 %	0.589	0.840	0.645	-1.988	3.289	0.826
	95 %	0.568	0.796	0.600	-3.082	3.899	0.727	
	DPD	--	0.724	0.914	0.778	0.852	2.075	1.120
	CART	--	0.400	0.768	0.486	-2.216	3.475	0.864

Campo Verde was the site with the highest LWD errors. The NHRH ≥ 90 % method was the most accurate for estimating LWD in Campo Verde, MT (EM = 0.07 h; MAE = 2.01 h; RMSE = 1.04 h), with good precision (R² = 0.712) and high exactness (d = 0.918), and method performance classified as good (c = 0.774) (Table 4.5). In Ponta Grossa, PR, LWD estimation errors were lower than in Campo Verde; the most accurate estimates were obtained with NHRH having 89 % and 90 % as thresholds (Table 4.6).

Table 4.6. Statistical indices and errors for the comparison between measured and estimated LWD using three empirical methods throughout 2014/2015 and 2015/2016 soybean growing seasons in Ponta Grossa, PR, Brazil.

Sites	Methods	Threshold	R ²	d	c	EM	MAE	RMSE
						(h)		
Ponta Grossa	NHRH	80 %	0.927	0.978	0.942	0.605	1.094	1.110
		81 %	0.929	0.980	0.944	0.487	1.063	1.086
		82 %	0.930	0.980	0.946	0.407	1.047	1.074
		83 %	0.931	0.981	0.947	0.338	1.032	1.063
		84 %	0.931	0.982	0.947	0.272	1.009	1.052
		85 %	0.934	0.982	0.949	0.208	0.991	1.044
		86 %	0.933	0.982	0.949	0.158	0.989	1.038
		87 %	0.934	0.983	0.950	0.097	0.981	1.030
		88 %	0.935	0.983	0.951	0.044	0.966	1.022
		89 %	0.936	0.983	0.951	-0.018	0.952	1.012
		90 %	0.936	0.983	0.951	-0.085	0.955	1.002
		91 %	0.936	0.983	0.951	-0.153	0.956	0.995
		92 %	0.935	0.983	0.951	-0.208	0.966	0.989
		93 %	0.934	0.982	0.949	-0.284	0.983	0.978
		94 %	0.876	0.952	0.891	-1.329	1.612	0.845
95 %	0.876	0.952	0.891	-1.329	1.612	0.845		
	DPD	--	0.935	0.983	0.951	0.040	0.964	1.022
	CART	--	0.864	0.960	0.893	-0.669	1.431	0.946

In Pedra Preta, MT, DPD and NHRH, using 88% as a threshold, were the most accurate methods for estimating LWD. Such methods showed high precision ($R^2 = 0.927$) and exactness ($d = 0.981$) and c index was classified as excellent ($c = 0.944$) (Table 4.7).

The methods that evidenced a better performance for estimating LWD were those with $MAE = 1.335$ h and $RMSE = 1.015$ h. Based on Gleason (1994) and Sentelhas et al. (2008), all best methods revealed errors within an acceptable maximum MAE of 2 hours for operational use in warning systems.

By analyzing only the rainy days, in general, the NHRH methods were those with the highest accuracy for LWD estimation for all sites and growing seasons (Table 4.8 and 4.9).

Table 4.7. Statistical indices and errors for the comparison between measured and estimated LWD using three empirical methods throughout 2015/2016 soybean growing season in Pedra Preta, MT, Brazil.

Site	Methods	Threshold	R ²	d	c	EM	MAE	RMSE
						(h)		
Pedra Preta	NHRH	80 %	0.877	0.943	0.883	2.621	2.655	1.542
		81 %	0.891	0.950	0.897	2.388	2.422	1.472
		82 %	0.905	0.958	0.911	2.155	2.207	1.414
		83 %	0.915	0.964	0.923	1.871	1.974	1.342
		84 %	0.923	0.970	0.932	1.621	1.793	1.267
		85 %	0.921	0.972	0.932	1.379	1.707	1.213
		86 %	0.927	0.976	0.940	1.043	1.560	1.158
		87 %	0.926	0.979	0.942	0.716	1.457	1.091
		88 %	0.927	0.980	0.944	0.362	1.379	1.027
		89 %	0.926	0.981	0.944	-0.095	1.371	0.962
		90 %	0.913	0.976	0.932	-0.629	1.560	0.892
		91 %	0.887	0.965	0.908	-1.198	1.853	0.827
		92 %	0.855	0.947	0.876	-1.948	2.345	0.738
		93 %	0.835	0.931	0.851	-2.534	2.741	0.667
		94 %	0.797	0.903	0.806	-3.319	3.422	0.580
	95 %	0.750	0.861	0.746	-4.164	4.198	0.504	
		DPD	--	0.927	0.981	0.944	0.121	1.379
	CART	--	0.875	0.966	0.903	0.267	1.767	1.144

For Campo Verde, MT, estimated LWD was more accurate when $\text{NHRH} \geq 88\%$ was used ($R^2 = 0.650$), with the lowest errors and best performance indices ($d = 0.891$ and $c = 0.718$) (Table 4.8). Similar results for rainy days were obtained for Ponta Grossa, PR, where NHRH method with RH thresholds of 90 % and 91 % were those with the best accuracy ($R^2 = 0.915$) (Table 4.9).

In Pedra Preta, MT, the lowest LWD errors during the rainy days were obtained with the $\text{NHRH} \geq 88\%$ ($\text{EM} = 0.06$ h; $\text{MAE} = 1.72$ h; $\text{RMSE} = 1.03$ h). Also, such method presented good precision ($R^2 = 0.824$), high accuracy ($d = 0.952$) and, therefore, high confidence ($c = 0.864$) (Table 4.10). Despite the high errors comparing rainy days with errors calculated using rainy and dry days together (Table 4.7), they are within the limit considered

as acceptable for operational purpose in warning systems (SENTELHAS et al., 2008; GLEASON, 1994).

Table 4.8. Statistical indices and errors for the comparison between measured and estimated LWD using three empirical methods considering only rainy days throughout 2014/2015 and 2015/2016 soybean growing seasons in Campo Verde, MT, Brazil.

Sites	Methods	Threshold	R ²	d	c	EM	MAE	RMSE
						(h)		
Campo Verde	NHRH	80 %	0.573	0.724	0.548	3.424	3.477	1.316
		81 %	0.588	0.747	0.573	3.134	3.228	1.292
		82 %	0.610	0.776	0.606	2.804	2.939	1.263
		83 %	0.648	0.809	0.651	2.452	2.637	1.231
		84 %	0.637	0.826	0.660	2.080	2.406	1.202
		85 %	0.641	0.850	0.681	1.641	2.174	1.168
		86 %	0.652	0.872	0.704	1.231	2.015	1.131
		87 %	0.661	0.888	0.722	0.789	1.913	1.095
		88 %	0.650	0.891	0.718	0.478	1.901	1.070
		89 %	0.640	0.891	0.713	0.061	1.923	1.035
		90 %	0.613	0.878	0.688	-0.541	2.069	0.982
		91 %	0.619	0.869	0.684	-1.081	2.189	0.937
		92 %	0.599	0.842	0.652	-1.695	2.419	0.888
		93 %	0.516	0.803	0.577	-1.749	3.058	0.875
		94 %	0.469	0.749	0.513	-2.677	3.688	0.807
95 %	0.465	0.700	0.477	-3.757	4.423	0.723		
	DPD	--	0.637	0.888	0.709	0.279	1.920	1.053
	CART	--	0.281	0.657	0.348	-3.177	4.072	0.800

Results for dry days (Tables 4.11, 4.12 and 4.13) showed that NHRH method had less errors and better performance for estimating LWD than during rainy days (Tables 4.8, 4.9 and 4.10). Also, for dry days, it was possible to identify that more than one RH threshold resulted in a high accuracy to estimate LWD for each given experimental site and growing seasons. LWD estimations were closer to measurements whenever 89 %, 90 %, and 91 % thresholds were used, respectively, at Campo Verde, Ponta Grossa and Pedra Preta (Tables 4.11, 4.12 and 4.13). Obtaining more than one RH threshold reinforces the necessity of recalibration to reduce uncertainties in LWD estimation (ROWLANDSON et al., 2014).

Table 4.9. Statistical indices and errors for the comparison between measured and estimated LWD using three empirical methods considering only rainy days throughout 2014/2015 and 2015/2016 soybean growing seasons in Ponta Grossa, PR, Brazil.

Sites	Methods	Threshold	R ²	d	c	EM	MAE	RMSE
						(h)		
Ponta Grossa	NHRH	80 %	0.875	0.960	0.897	0.788	1.178	1.094
		81 %	0.884	0.964	0.907	0.666	1.129	1.081
		82 %	0.893	0.968	0.915	0.578	1.098	1.072
		83 %	0.898	0.970	0.920	0.493	1.073	1.063
		84 %	0.902	0.972	0.923	0.426	1.044	1.051
		85 %	0.906	0.974	0.927	0.355	1.020	1.044
		86 %	0.906	0.974	0.928	0.294	1.016	1.039
		87 %	0.910	0.976	0.931	0.224	0.994	1.030
		88 %	0.912	0.977	0.933	0.165	0.980	1.022
		89 %	0.913	0.977	0.934	0.098	0.968	1.015
		90 %	0.915	0.978	0.935	0.026	0.961	1.007
		91 %	0.915	0.978	0.935	-0.051	0.959	1.000
		92 %	0.914	0.978	0.935	-0.104	0.961	0.996
		93 %	0.912	0.977	0.932	-0.183	0.980	0.987
		94 %	0.816	0.933	0.843	-1.235	1.603	0.896
	95 %	0.817	0.933	0.844	-1.238	1.600	0.896	
		DPD	--	0.913	0.977	0.933	0.154	0.976
	CART	--	0.800	0.940	0.840	-0.684	1.496	0.952

Table 4.10. Statistical indices and errors for the comparison between measured and estimated LWD using three empirical methods considering only rainy days throughout 2015/2016 soybean growing season in Pedra Preta, MT, Brazil.

Sites	Methods	Threshold	R ²	d	c	EM	MAE	RMSE
						(h)		
Pedra Preta	NHRH	80 %	0.720	0.850	0.722	2.939	2.988	1.321
		81 %	0.741	0.867	0.746	2.732	2.805	1.297
		82 %	0.765	0.886	0.774	2.451	2.573	1.265
		83 %	0.787	0.906	0.804	2.085	2.329	1.226
		84 %	0.814	0.923	0.832	1.793	2.110	1.194
		85 %	0.812	0.929	0.838	1.537	2.024	1.172
		86 %	0.826	0.941	0.855	1.183	1.793	1.139
		87 %	0.821	0.948	0.858	0.646	1.768	1.085
		88 %	0.824	0.952	0.864	0.061	1.720	1.030
		89 %	0.823	0.948	0.860	-0.659	1.854	0.966
		90 %	0.811	0.933	0.840	-1.427	2.207	0.899
		91 %	0.792	0.912	0.812	-2.085	2.598	0.845
		92 %	0.772	0.880	0.773	-2.963	3.305	0.775
		93 %	0.774	0.857	0.754	-3.683	3.878	0.710
		94 %	0.750	0.816	0.707	-4.622	4.720	0.641
	95 %	0.717	0.762	0.645	-5.780	5.780	0.570	
	DPD	--	0.832	0.954	0.870	-0.305	1.744	0.999
	CART	--	0.735	0.913	0.783	-0.415	2.317	1.040

Table 4.11. Statistical indices and errors for the comparison between measured and estimated LWD using three empirical methods for dry days throughout 2014/2015 and 2015/2016 soybean growing seasons in Campo Verde, MT, Brazil.

Sites	Methods	Threshold	R ²	d	c	EM	MAE	RMSE
						(h)		
Campo Verde	NHRH	80 %	0.583	0.696	0.531	4.176	4.523	1.494
		81 %	0.606	0.727	0.566	3.844	4.185	1.448
		82 %	0.614	0.753	0.590	3.463	3.810	1.403
		83 %	0.643	0.784	0.629	3.094	3.443	1.353
		84 %	0.661	0.809	0.657	2.787	3.156	1.315
		85 %	0.667	0.831	0.679	2.396	2.844	1.269
		86 %	0.674	0.852	0.700	2.037	2.581	1.231
		87 %	0.677	0.871	0.717	1.604	2.375	1.175
		88 %	0.669	0.880	0.720	1.252	2.229	1.134
		89 %	0.678	0.894	0.737	0.812	2.068	1.081
		90 %	0.686	0.904	0.749	0.243	1.964	1.014
		91 %	0.672	0.898	0.736	-0.359	2.029	0.951
		92 %	0.665	0.882	0.719	-1.087	2.268	0.866
		93 %	0.605	0.840	0.653	-1.686	2.836	0.796
		94 %	0.568	0.787	0.593	-2.592	3.440	0.700
95 %	0.525	0.719	0.522	-3.631	4.258	0.596		
	DPD	--	0.665	0.885	0.722	1.030	2.198	1.107
	CART	--	0.572	0.840	0.635	-1.433	2.299	0.859

Table 4.12. Statistical indices and errors for the comparison between measured and estimated LWD using three empirical methods for dry days throughout 2014/2015 and 2015/2016 soybean growing seasons in Ponta Grossa, PR, Brazil.

Sites	Methods	Threshold	R ²	d	c	EM	MAE	RMSE
						(h)		
Ponta Grossa	NHRH	80 %	0.796	0.936	0.835	0.690	1.259	1.198
		81 %	0.802	0.941	0.842	0.561	1.204	1.156
		82 %	0.821	0.948	0.859	0.435	1.146	1.126
		83 %	0.843	0.956	0.878	0.307	1.068	1.098
		84 %	0.851	0.960	0.885	0.199	1.021	1.077
		85 %	0.860	0.963	0.892	0.109	0.984	1.060
		86 %	0.858	0.962	0.891	0.052	0.972	1.050
		87 %	0.856	0.962	0.890	-0.013	0.993	1.038
		88 %	0.861	0.963	0.894	-0.073	0.966	1.029
		89 %	0.863	0.963	0.895	-0.131	0.952	1.014
		90 %	0.861	0.962	0.893	-0.188	0.964	1.005
		91 %	0.861	0.962	0.893	-0.246	0.963	0.997
		92 %	0.858	0.960	0.890	-0.307	0.979	0.988
		93 %	0.855	0.959	0.887	-0.378	0.994	0.975
		94 %	0.756	0.903	0.785	-1.336	1.625	0.802
	95 %	0.755	0.903	0.784	-1.344	1.629	0.800	
	DPD	--	0.859	0.962	0.892	-0.071	0.973	1.029
	CART	--	0.756	0.925	0.805	-0.643	1.378	0.945

Table 4.13. Statistical indices and errors for the comparison between measured and estimated LWD using three empirical methods considering dry days throughout 2015/2016 soybean growing season in Pedra Preta, MT, Brazil.

Sites	Methods	Threshold	R ²	d	c	EM	MAE	RMSE
						(h)		
Pedra Preta	NHRH	80 %	0.847	0.875	0.805	3.765	3.765	2.174
		81 %	0.863	0.891	0.828	3.353	3.353	1.984
		82 %	0.880	0.904	0.848	3.059	3.059	1.853
		83 %	0.883	0.915	0.859	2.706	2.765	1.689
		84 %	0.899	0.926	0.878	2.382	2.500	1.502
		85 %	0.895	0.931	0.881	2.059	2.294	1.366
		86 %	0.892	0.943	0.891	1.529	2.176	1.242
		87 %	0.890	0.948	0.895	1.235	2.000	1.128
		88 %	0.881	0.953	0.895	0.765	1.882	0.987
		89 %	0.873	0.957	0.894	0.324	1.735	0.887
		90 %	0.845	0.952	0.875	-0.147	1.794	0.806
		91 %	0.777	0.932	0.822	-0.853	2.029	0.712
		92 %	0.740	0.913	0.785	-1.588	2.235	0.599
		93 %	0.701	0.882	0.739	-2.294	2.588	0.503
		94 %	0.612	0.821	0.642	-3.147	3.324	0.377
		95 %	0.484	0.742	0.516	-3.912	4.029	0.291
	DPD	--	0.880	0.956	0.897	0.559	1.794	0.949
	CART	--	0.815	0.922	0.833	1.735	2.324	1.462

Although several RH thresholds were classified as of high accuracy, precision and exactness for NHRH method, the majority converged to 90 %. Based on that, NHRH \geq 90 % method was admitted as the most suitable threshold to estimate LWD. Comparison between measured and estimated LWD using the NHRH \geq 90 % method in Campo Verde revealed periods of both overestimation and underestimation (Figure 4.3), resulting in a high dispersion of data around the 1:1 line, with R² = 0.712 (Figure 4.3). The NHRH \geq 90 % method was the most accurate for Ponta Grossa (Figure 4.4). The method slightly underestimated LWD during both 2014/2015 and 2015/2016 soybean growing seasons (slope of 0.971, R² = 0.936) (Figure 4.4). During 2015/2016 soybean growing season in Pedra Preta, the NHRH \geq 90 % method underestimated LWD but with a relatively high R² (0.912) (Figure 4.5).

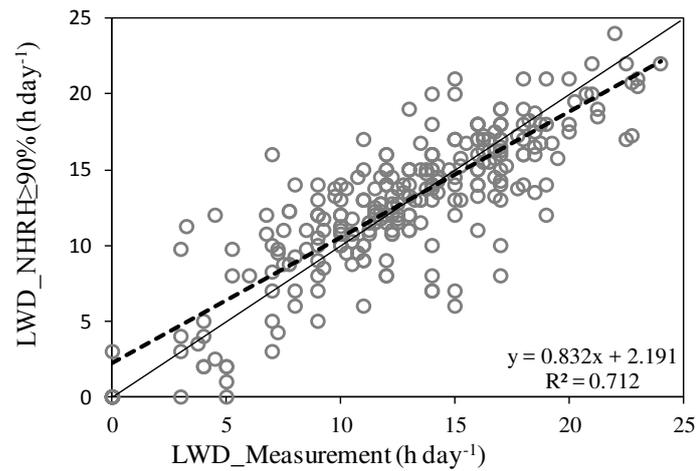


Figure 4.3. Relationship between measured and estimated LWD (NHRH \geq 90 %) for two soybean growing seasons in Campo Verde, Mato Grosso State, Brazil.

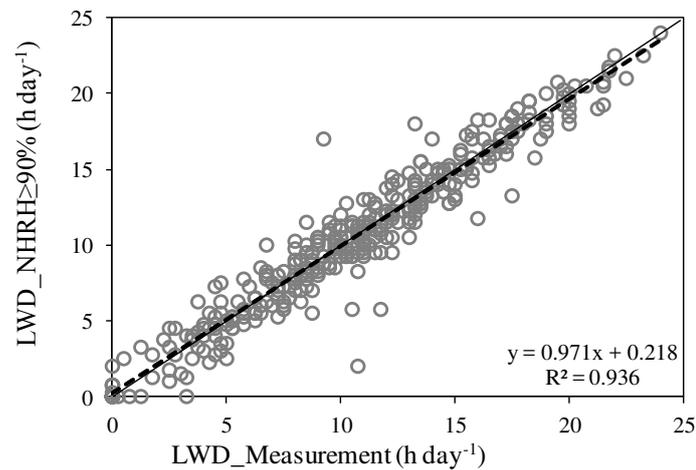


Figure 4.4. Relationship between measured and estimated LWD (NHRH \geq 90 %) for two soybean growing seasons in Ponta Grossa, Parana State, Brazil.

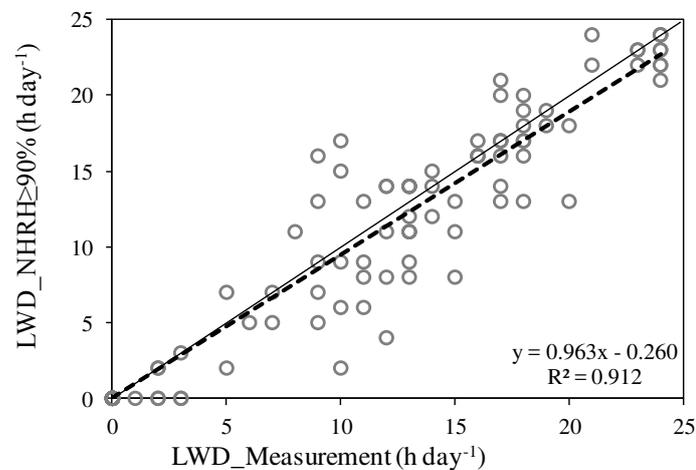


Figure 4.5. Relationship between measured and estimated LWD (NHRH \geq 90 %) for one soybean growing season in Pedra Preta, Mato Grosso State, Brazil.

Even considering the errors of the $\text{NHRH} \geq 90\%$ method, it was the most suitable for being used as input for running ASR warning system, to predict disease severity values and spray timing.

4.3.2. Influence of estimated LWD on ASR warning system

MDSVs were calculated for all sites, using two soybean growing seasons and based on five sowing dates. Considering all sowing dates, higher values of MDSV were obtained for both sites located at the State of Mato Grosso. In average, by analyzing different growing seasons and sowing dates in Campo Verde and Pedra Preta, MDSV was equal to 167 and 182, respectively. Ponta Grossa, however, showed a lower MDSV compared to both sites in Mato Grosso, reaching an average value of 116 for all growing seasons and sowing dates (Figure 4.6).

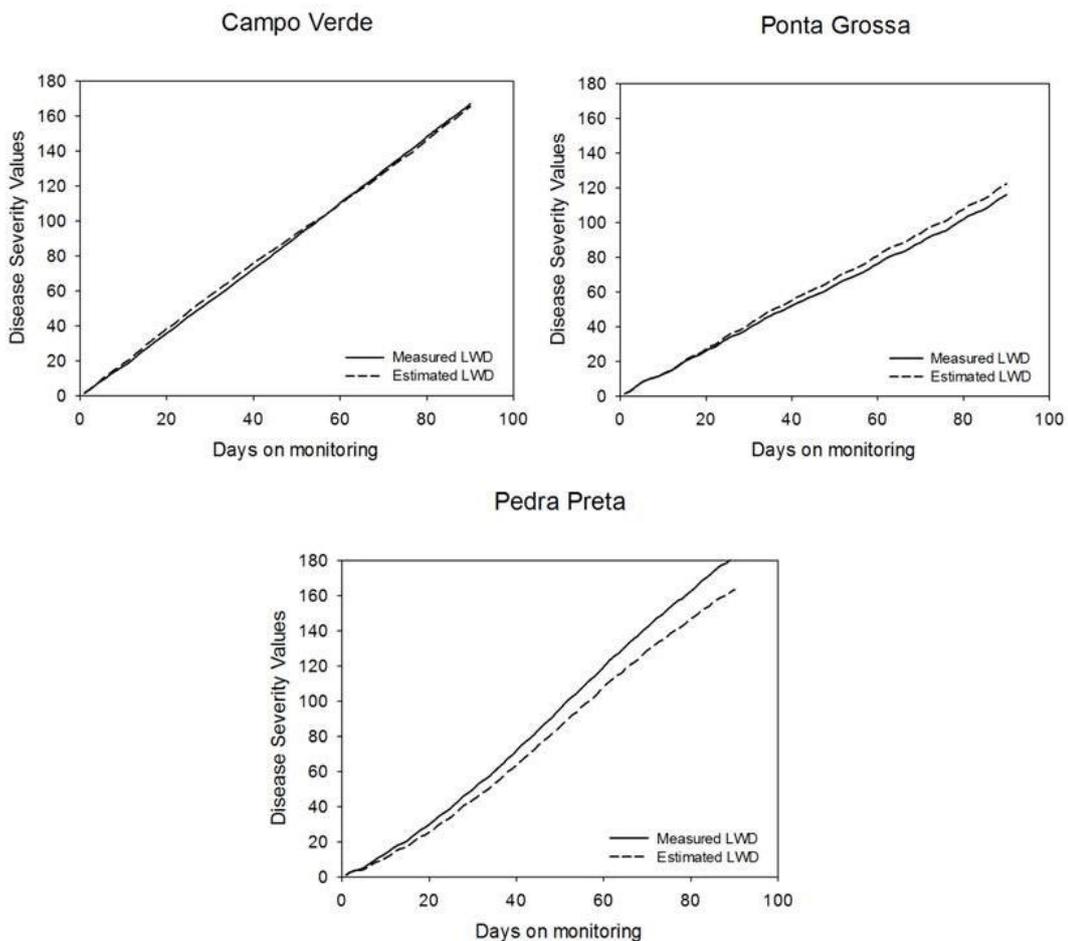


Figure 4.6. Average cumulative disease severity values from five sowing dates obtained as a function of LWD measured and estimated by $\text{NHRH} \geq 90\%$. Disease severity values were calculated for Campo Verde, MT, Ponta Grossa, PR, and Pedra Preta, MT, Brazil.

Based on the disease severity values obtained by means of LWD measured and estimated for all sowing dates, it was possible to identify for the period before the 55th day of monitoring in Campo Verde that EDSV was higher than MDSV. After the 56th day, however, EDSV was lower than MDSV, and this pattern remained until the last day of monitoring. In Ponta Grossa, EDSV exceeded MDSV during all growing season. On the other hand, at Pedra Preta EDSV was lower than MDSV for all assessed period (Figure 4.6).

A comparative analysis of MDSV and EDSV is presented in Table 4.14. By analyzing statistical errors it was possible to notice changes between sowing dates, growing seasons and sites. Lower errors of disease severity values (EDSV) associated with LWD estimated by NHRH ≥ 90 % occurred in Ponta Grossa, with ME = 0.070, MAE = 0.192 and RMSE = 0.502 (Table 4.14).

The DSV for Campo Verde and Pedra Preta were underestimated. However, Pedra Preta had higher DSV errors when compared with Campo Verde (ME = -0.017; MAE = 0.372; RMSE = 0.677), mainly due to the errors of LWD estimated by NHRH ≥ 90 % (Table 4.14).

Fungicide spray timing was determined based on two thresholds of cumulative DSV for recommending sprays (6 and 9). For the more conservative threshold, similar numbers of sprays per season were recommended for all experimental sites. In average, both ASR disease warning systems, using measured and estimated LWD, showed the same number of required sprays, corresponding roughly to 5 sprays (Table 4.15 and 4.16).

By adopting the less conservative spray threshold, differences in the number of recommended sprays per season were observed for all sites. The number of sprays recommended for Campo Verde and Pedra Preta was around 5 per season for both EDSV and MDSV; whereas for Ponta Grossa, the ASR warning system recommended around 4 sprays when using both measured and estimated LWD.

In all simulations for the experimental sites a considerable frequency of true negatives (N) were observed. By taking into consideration a more conservative spray threshold, false negatives (F) were observed in Campo Verde (2014/2015 soybean growing season) and Pedra Preta (2015/2016 soybean growing season) (Table 4.15). High values of F imply unreliability in an ASR warning system use, once at this situation ASR warning system with estimated LWD did not recommend sprays when it is required (Table 4.15). A high number of true positives (H) was observed in Ponta Grossa, with H = 12 (Table 4.15).

Table 4.14. Statistical errors for the comparison between daily disease severity values considering measured (MDSV) and estimated (EDSV) LWD for each sowing date throughout two soybean growing seasons in Campo Verde, MT, Ponta Grossa, PR, and Pedra Preta, MT.

Sites	Growing season	Sowing date	ME	MAE	RMSE
			Units of severity value		
Campo Verde	2014/2015	1st	-0.044	0.378	0.632
		2nd	-0.078	0.411	0.675
		3rd	-0.033	0.433	0.691
		4th	-0.033	0.411	0.691
		5th	-0.078	0.389	0.675
	2015/2016	1st	0.022	0.400	0.775
		2nd	0.000	0.378	0.745
		3rd	0.078	0.322	0.658
		4th	0.044	0.333	0.667
		5th	-0.044	0.267	0.558
Ponta Grossa	2014/2015	1st	0.033	0.167	0.459
		2nd	0.033	0.167	0.459
		3rd	0.044	0.156	0.447
		4th	0.067	0.156	0.447
		5th	0.067	0.156	0.447
	2015/2016	1st	0.078	0.211	0.506
		2nd	0.078	0.256	0.587
		3rd	0.100	0.233	0.568
		4th	0.111	0.222	0.558
		5th	0.089	0.200	0.537
Pedra Preta	2015/2016	1st	-0.178	0.333	0.632
		2nd	-0.178	0.333	0.632
		3rd	-0.189	0.344	0.658
		4th	-0.244	0.378	0.683
		5th	-0.244	0.378	0.683

Table 4.15.Contingency table for spray timing determined by ASR warning system for a more conservative threshold, using measured and estimated LWD, taking into account NHRH ≥ 90 % as LWD estimator.

Site	Growing Season	Sowing date	Number of spray		Contingency Table			
			Measured LWD	Estimated LWD	H	N	M	F
Campo Verde	2014/2015	1	5	5	0	7	0	5
		2	5	5	1	11	0	4
		3	6	5	0	5	0	6
		4	5	5	1	11	0	4
		5	5	5	1	5	0	5
	2015/2016	1	5	5	0	9	0	5
		2	6	6	1	5	5	0
		3	6	6	3	8	3	0
		4	5	6	0	2	6	0
		5	6	6	5	11	1	0
Ponta Grossa	2014/2015	1	5	5	4	26	1	0
		2	5	5	2	15	2	1
		3	5	5	4	23	1	0
		4	5	5	1	10	4	0
		5	5	5	1	13	2	2
	2015/2016	1	4	6	1	6	4	1
		2	5	5	1	12	1	3
		3	5	5	3	25	1	1
		4	5	5	3	14	1	1
		5	5	5	4	24	1	0
Pedra Preta	2015/2016	1	5	5	0	12	0	5
		2	5	5	1	9	0	4
		3	5	5	1	10	0	4
		4	6	5	0	4	0	6
		5	6	5	0	6	0	6

H and N denote hits by the method. H = sprays were recommended by the estimated inputs when necessary according to the measured inputs (true positive outcome); N = no spray was recommended using the estimated weather inputs, and the MDSV threshold using measured weather inputs did not recommend a spray (true negative). M and F represent errors by the method. M means sprays that were recommended by estimated LWD inputs when they were not required by using measured LWD inputs (false positive); F signifies when using estimated-LWD inputs did not result in a spray recommendation, whereas using the measured-LWD inputs recommended spraying (false negative).

Table 4.16.Contingency table for spray timing determined by ASR warning system for a less conservative threshold, using measured and estimated LWD, considering $NHRH \geq 90\%$ as LWD estimator.

Site	Growing Season	Sowing date	Number of spray		Contingency Table			
			Measured LWD	Estimated LWD	H	N	M	F
Campo Verde	2014/2015	1	5	4	2	16	2	1
		2	5	5	1	17	1	3
		3	5	4	1	13	0	4
		4	5	5	0	12	1	4
		5	5	4	1	9	0	4
	2015/2016	1	5	5	3	19	0	2
		2	5	5	1	9	4	0
		3	5	5	3	9	1	1
		4	5	5	0	4	5	0
		5	5	5	0	6	0	5
Ponta Grossa	2014/2015	1	4	4	2	34	1	1
		2	5	4	1	25	1	3
		3	4	4	0	30	1	3
		4	5	5	2	25	1	2
		5	4	5	0	11	5	0
	2015/2016	1	4	4	3	28	1	0
		2	5	5	2	22	0	3
		3	4	4	3	29	0	1
		4	5	5	3	21	0	2
		5	4	4	3	26	0	1
Pedra Preta	2015/2016	1	5	5	0	17	0	5
		2	5	5	0	19	0	5
		3	5	5	5	18	0	0
		4	5	5	0	17	0	5
		5	5	5	1	13	0	4

H and N denote hits by the method. H = sprays were recommended by the estimated inputs when necessary according to the measured inputs (true positive outcome); N = no spray was recommended using the estimated weather inputs, and the MDSV threshold using measured weather inputs did not recommend a spray (true negative). M and F represent errors by the method. M means sprays that were recommended by estimated LWD inputs when they were not required by using measured LWD inputs (false positive); F signifies when using estimated-LWD inputs did not result in a spray recommendation, whereas using the measured-LWD inputs recommended spraying (false negative).

By using a less conservative threshold for five sowing dates in Campo Verde and Pedra Preta, it was possible to observe a lower number of false negatives (F) compared with a more conservative threshold. The opposite occurred in Ponta Grossa, where the number of false negatives increased by almost three times as opposed to a more conservative threshold (Table 4.16). A worse method performance based on spray timing was also noticed in Ponta Grossa, whilst the number of true positives compared to a more conservative threshold was reduced by 20 % (Table 4.16).

When considering less and more conservative thresholds, higher values of fraction of correct estimates (θ_1) for a $\text{NHRH} \geq 90\%$ method were obtained for Ponta Grossa in comparison to both sites in the State of Mato Grosso.

Ponta Grossa showed, for the more conservative threshold, θ_1 values of 0.884 and 0.869 for 2014/2015 and 2015/2016, respectively. In Campo Verde and Pedra Preta lower values of θ_1 were found for the two growing seasons. In Campo Verde, for both growing seasons, average θ_1 was 0.662. On the other hand, θ_1 in Pedra Preta was 0.632. Reductions of θ_1 for both Mato Grosso sites are related with a high number of false positive and false negative alarms in comparison to Ponta Grossa.

For all locations and growing seasons, higher values of θ_1 were obtained for a less conservative threshold compared to the most conservative one. Such increases of θ_1 reflected a better performance of LWD method to estimate the fraction of correct estimates; moreover higher θ_1 is a consequence of low errors (M and F).

At Campo Verde, a less conservative threshold resulted in $\theta_1 = 0.783$ and 0.750 , respectively, for 2014/2015 and 2015/2016 growing seasons. At Pedra Preta, a clear improvement of θ_1 for a less conservative threshold ($\theta_1 = 0.826$) was envisioned in relation to the more conservative threshold ($\theta_1 = 0.632$). In Ponta Grossa, θ_1 values were 0.878 and 0.946 for 2014/2015 and 2015/2016 growing seasons, respectively. According to Dietterich (2000), the θ_1 could be used to measure the agreement between measured and estimated variables. In this case, the closer θ_1 is to 1 the more reliable is the necessity of spray using the LWD estimated by $\text{NHRH} \geq 90\%$. Kim et al. (2010) indicated that θ_1 values greater than 0.8 indicate acceptable accuracy of the method used to estimate a given variable.

The k agreement index of correct spray recommendations based on measured and estimated LWD ranged from 0.249 to 0.545 for a less conservative threshold. For the most conservative threshold, k values varied between 0.09 and 0.567. In both situations, higher k values were calculated for Ponta Grossa and lower ones were determined for both sites in

Mato Grosso. The k agreement index closer to 1 represents a more reliable prediction of a given variable (KIM et al., 2010; DIETTERICH, 2000).

4.4. Discussion

Many researchers reported the importance of LWD for plant diseases occurrence and the majority of disease-warning systems has LWD as an input variable (IGARASHI et al., 2016; BONDALAPATI et al., 2011; BATZER et al., 2008; DUTTWEILER et al., 2008; GLEASON et al., 2008; DALLA MARTA; MAGAREY; ORLANDINI, 2005). However, relatively few studies have examined applications of LWD estimation methods within the context of simulated operations of warning systems (MONTONE et al., 2016). Plenty of studies focused on LWD determination and estimation in Southeastern Brazil (SENTELHAS et al., 2008; SENTELHAS; GILLESPIE, 2008; SENTELHAS et al., 2006). More recently, Alvares et al. (2015) used a geographical information system, geostatistics, and multivariate procedures based on the $NHRH \geq 90\%$ approach to characterize both temporal and spatial variability of LWD across Brazil, and developed a monthly LWD method to predict LWD all over the country.

The current study is the first one that evaluates the performance of method-based LWD estimates for operating a disease warning system in Brazil. The use of empirical methods to estimate LWD is an important way to make warning systems available where LWD is not measured. Therefore, the results of this study show how different empirical methods for estimating LWD impact disease-warning systems in different Brazilian locations, not only for ASR but also for other pathosystems of economic importance.

In compliance with recommendations reported by Gleason (1994) and Sentelhas et al. (2008), mean absolute errors (MAE) < 2.0 h per day were classified as acceptable for operational use of LWD estimates in disease-warning systems. A justification for such criterion is that variation in measured LWD per day within the canopy of a single crop often exceeds the aforementioned threshold (SENTELHAS et al., 2005). A slightly higher value of mean MAE was obtained in Campo Verde, but remained very close to the considered limit. At the other two sites, MAE ranged from 0.95 to 1.63 h for all LWD methods.

According to Sentelhas et al. (2008), under field conditions with no irrigation systems, LWD is caused mainly by rain or dew. Different sources of wetness have a capacity of changing patterns of LWD, and as a result bring about significant effects on the

performance of LWD estimating methods. For the conditions of the present study, rainfall was the most prevailing source of wetness for all sites (Figure 4.2).

Effects of rainfall events on the performance of LWD methods were evidenced in Campo Verde, where higher LWD errors were observed in relation to Ponta Grossa and Pedra Preta. Throughout both crop growing seasons at Campo Verde, a higher number of rainfall events were recorded with higher volumes than at other sites, including many events of >100 mm per day. According to Magarey et al. (2005), rainfall events, especially with high volumes, often change patterns of LWD. Increases of LWD values as a consequence of rainfall events were also found by Sentelhas et al. (2005).

Heavy rains have influence on LWD patterns due to the increase in soil moisture, leading to higher RH and longer dew periods. Based on that, rainfall amount and duration may be a reasonable key point to explain the highest errors of estimated LWD during the rainy days compared to dry days (Tables 4.8, 4.9 and 4.10).

Fluctuations in accuracy of LWD estimated by the assessed methods herein reinforce the necessity of calibration to account for variations that may occur under different climates (KIM; WANG; YANG, 2005). In almost all cases, however, the NHRH method estimated LWD most accurately. For all sites, the RH threshold stayed around $90\% \pm 2\%$. Based on that, $\text{NHRH} \geq 90\%$ method was adopted as the reference for LWD estimation and it was used as an input in the ASR warning system to predict disease severity values in soybean production fields and then recommend sprays for ASR control.

The decision of choosing $\text{NHRH} \geq 90\%$ as an estimator of LWD was based on the following main aspects: a) according to Sentelhas et al. (2008) $\text{NHRH} \geq 90\%$ method resulted in the most accurate estimates even under situations where more complex physical methods were used; b) $\text{NHRH} \geq 90\%$ approach has been considered to be an easy way to determine LWD in operational systems, producing therefore reliable data in different climatic regions (ROWLANDSON et al., 2014; DURIGON; LIER, 2013; BONDALAPATI et al., 2011; GLEASON et al., 2008).

Sentelhas et al. (2008) evaluated three empirical methods based on RH and concluded that the 90% RH threshold was the best one to represent a wide range of conditions. Another aspect that reinforces the practical usefulness of a single RH threshold for LWD estimation deals with errors inherent to RH measurements. For instance, commercial RH sensors usually have an error range of $\pm 3\%$ with a 1% change in measured RH (VAISALA, 2012). Therefore, there is little to be gained in LWD estimation accuracy by varying the threshold by $\pm 3\%$ (ROSLI et al., 2017).

Using a single RH threshold also makes LWD estimation simpler for growers and agricultural advisers (GLEASON et al., 2008). Campbell and Madden (1990) identified several barriers to growers to adopt disease-warning systems in a large scale, and one of these problems is the difficulty to use it and obtain the weather data required. Gleason et al. (2008) mentioned that one way to reduce the distance between growers and disease-warning systems is to make it more straightforward to understand and a user-friendly.

Errors in estimating disease severity values (EDSV), by using estimated LWD based on $NHRH \geq 90\%$ as inputs, exhibited different patterns among field sites. In Campo Verde, EDSVs led off and on to both overestimation (at the beginning of the growing season) and underestimation (at the end of the growing season) (Figure 4.6). Such variations increased the number of misses and false alarms for both ASR warning system thresholds (Table 4.15 and 4.16).

The consequence of false positives (M) would lead to an application of unnecessary fungicide sprays, resulting in a rising of production costs in the farm whenever the weather happens to be unfavorable to ASR. On the other hand, false negatives (F) should be considered a main error due to not recommend sprays in a correct time whenever diseases occur in the field, leaving soybean plants susceptible to be infected by ASR and suffer considerable reductions in yield and grade.

As in Campo Verde, many false negatives were also calculated in Pedra Preta city. In this area, MDSV was underestimated by ASR warning system that used estimated LWD as an input. As mentioned previously, incorrect timing, especially owing to false negatives might compromise the disease-warning system performance. In contrast, Ponta Grossa showed significant values of true positives (H) and true negatives (N), and both reflected a good performance of $NHRH \geq 90\%$ as an input for ASR warning system, especially considering correct positives, because sprays timing occurred on the same day that MDSV and EDSV were taken into account.

Igarashi et al. (2016) tested an ASR warning system proposed by Reis; Sartori; Câmara (2004) near Ponta Grossa, and observed that the warning system provided reliable recommendations for spray timing to control this disease. In this way and considering a lower difference between MDSV and EDSV calculated in Ponta Grossa using ASR disease warning system, the outcomes reported by the aforementioned authors confirm the feasibility of $NHRH \geq 90\%$ as a LWD estimator.

Discrepancies in disease estimation and sprays timing to control ASR might be ascribed to different climatic conditions (Table 4.1). Similar climatic conditions were

identified for Campo Verde and Pedra Preta, both in the state of Mato Grosso, in the Center-Western region of the country (Figure 4.1). Such sites were characterized by high rainfall and high air temperature regimes (Table 4.1), evidencing environmental conditions that are more conducive to ASR epidemics (DEL PONTE; ESKER, 2008; DEL PONTE et al., 2006a). Ponta Grossa, on the other hand, is located at the Southern region (Figure 4.1), where during the soybean growing season air temperature and rainfall were lower than its regime in Mato Grosso, which in turn reduced MDSV (Table 4.1).

4.5. Conclusions

LWD estimated by empirical RH-based methods revealed acceptable accuracy and precision for its use as input in the ASR warning system at three experimental sites in Brazil. The LWD estimation method of $NHRH \geq X\%$ having as threshold RH of $90\% \pm 2\%$ had the highest accuracy for estimating LWD. Considerable errors to predict ASR using estimated LWD were obtained in Campo Verde and Pedra Preta. However, a good performance of $NHRH \geq 90\%$ as an input of ASR warning system was observed in Ponta Grossa, which was basically governed by the better performance of $NHRH \geq 90\%$ for estimating LWD at this site.

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5. RAINFALL-BASED ASIAN SOYBEAN RUST WARNING SYSTEM EFFECT ON CROP PROFITABILITY IN DIFFERENT BRAZILIAN REGIONS

ABSTRACT

Phakopsora pachyrhizi is a fungus that causes Asian soybean rust (ASR). To control ASR fungicides sprays must be applied at a correct time, however, ASR is normally controlled following a calendar-based approach. Such approach considers only aspects of the crop, disregarding the influence of local weather conditions on disease progress. The aim of the current study was to evaluate the profitability of a rainfall based warning systems to recommend sprays for ASR control at three states in Brazil. Field trials were conducted at Piracicaba, SP, Ponta Grossa, PR, Campo Verde, MT, and Pedra Preta, MT. For each site, sowing dates ranged from October to December during 2014/2015 and 2015/2016 growing seasons. The experiment was conducted in a randomized block design with three treatments and four repetitions for each site. The treatments were: 1) CALEND - calendar-based sprays in a 14-day interval from the R1 stage; T2) PREC_1 system threshold 1 (80% severity cut-off) and PREC_2 system threshold 2 (50% severity cut-off). The sprays were done with a commercial mixture (Azoxystrobin + Benzovindiflupir, 150 g per ha). Weather data were measured at an automatic weather station, located in the experimental sites. The variables analyzed were: yield (kg ha^{-1}), the mass of thousand grains (g^{-1}) and profitability (kg ha^{-1}). In Piracicaba, PREC_1 presented an effective performance during the sowing dates performed in October and November of 2015, where the yields were 4868.5 and 4920.9 kg ha^{-1} , respectively. For PREC_2, soybean yield was 33 kg ha^{-1} lower than CALEND, however, using the ASR warning system it saved three fungicide sprays. In Campo Verde and Pedra Preta, PREC_1 and PREC_2 presented good performance to ASR control, which was related to higher total rainfall over the soybean growing seasons. In Ponta Grossa, PREC_1 and PREC_2 were not effective to control ASR, showing a relative yield lower than CALEND for the most of the soybean growing season. The performance of the ASR warning systems was directly related to the rainfall occurrence in the studied sites. Therefore, where the warning system did not performed well, the inclusion of other weather variables, such as leaf wetness duration, into the ASR warning system could improve the accuracy for spray recommendation.

Keywords: *Phakopsora pachyrhizi*; *Glycine max*; Spray timing; Preventive sprays; Integrated Disease Management

5.1. Introduction

Soybean (*Glycine max*) area has increased all over the world, due to a high demand for protein and oil (MURITHI et al., 2016). Actually, the main soybean producers are the United States and Brazil, representing around 60% of the global soybean production (FAOSTAT, 2017). With Brazilian soybean fields expanding to different regions of the country, production is becoming each day more dependent on chemicals, mainly for controlling Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi*, which was firstly reported in commercial areas of the country in the 2001/2002 growing season (YORINORI et al., 2005)

P. pachyrhizi is a biotrophic basidiomycete fungus which is the main pathogen that causes ASR. The power of destruction provided by ASR in the Brazilian soybean fields is ascribed to the very favorable weather conditions for the pathogen to infect the plants and also due to the lack of soybean cultivars resistant to the disease (GODOY et al., 2016). Therefore, reduction in soybean yield may be up to 70% under favorable weather conditions and inefficient disease control (HARTMAN et al., 2016; DALLA LANA et al., 2014; YORINORI et al., 2005).

Among the weather variables that affect ASR, air temperature and leaf wetness duration (IGARASHI et al., 2014; BONDE; NESTER; BERNER, 2013, 2012; ALVES et al., 2007; MELCHING et al., 1989; MARCHETTI; MELCHING; BROMFIELD, 1976), sunlight (DIAS; LI; YANG, 2014; DIAS et al., 2010) and rainfall (MINCHIO et al., 2016; STEFANELLO et al., 2016; DEL PONTE et al., 2006a, 2006b; TAN; YU; YANG, 1996) are the most important, which make them potential inputs for developing ASR warning systems to recommend fungicide sprays and to assess the risk of outbreaks.

Disease-warning systems (DWS) are decision tools, based on the relationships between pathogen, host and weather conditions, that aim to define a better time for spraying the crops in order to diseases (GLEASON et al., 2008). As a consequence, DWS promotes rationalization of fungicides use to manage plant diseases, normally reducing the number of sprays (ABULEY; NIELSEN, 2017; ROSLI et al., 2017; PAVAN; FRAISSE; PERES, 2011; GLEASON et al., 1995; MADDEN; PENNYPACKER; MACNAB, 1978). Even DWS being a useful tool for growers, most of them prefer to use the calendar-based spray system, since pre-scheduled makes their life easier.

Although the calendar-based approach is more adopted by growers, such procedure disregards the influence of weather conditions on disease development. Thus, the number of

sprays and their timing are normally inappropriate, reducing the control efficiency (MEGETO et al., 2014). Evidences that fungicides sprays has being applied in incorrect times for ASR control has resulting in a higher frequently of events of pathogen resistance to different chemical groups used in their control (GODOY et al., 2016; LIU et al., 2016; YAMANAKA et al., 2015; YU et al., 2015).

According to Campbell and Madden (1990), several barriers are responsible for avoiding the growers to use a DWS to recommend their sprays in a large scale, but the main one is the difficulties to obtain reliable weather data required for running the system. Furthermore, most of the growers believe that disease models are too complex and can increase the crop risks. Therefore, they are only concerned with avoiding disease, and applying control practices without considering the influence of weather conditions on disease progress. One way to promote the use of a DWS by growers is to make it more straightforward to understand and user-friendly (GLEASON et al., 2008).

A wise strategy to make DWS for disease control more acceptable by growers is by using single weather variables and running simple models, which must be site specific validated. In this context, rainfall may be considered a potential variable to be used as DWS input, since it is easily recorded in the agricultural fields and can indirectly represent the influence of other weather variables, such as leaf wetness duration.

The positive effect of rainfall on ASR occurrence and development was observed by Del Ponte et al. (2006a) in Brazil, where cumulative rainfall and number of days with rain above 1 mm presented a strong correlation with ASR severity, with a Pearson coefficient of correlation of 0.95 and 0.93, respectively. Similar results were found by Megeto et al. (2014) and Minchio et al. (2016) in Brazil and by Young et al. (2011) in the USA. DWSs for ASR control, based on rainfall data, have been tested under field conditions in several parts of the world. Kelly et al. (2015) evaluated the performance of a rainfall-based DWS, considering the study of Del Ponte al. (2006a) to control ASR in Florida, US, and observed that this system allowed to save one spray when compared with the calendar-based spray schedule, for which two sprays were always applied. Such authors also observed that spray reduction plots had 300 kg ha⁻¹ more than the plots where calendar system was used. Similar results were found for two field trials in south and southeast Brazilian regions by Sentelhas et al. (2012).

According to Twizeyimana and Hartman (2016) the efficiency of fungicides to control ASR depends largely on the correct sprays timing. Mueller et al. (2009) verified that the definition of the appropriate time to sprays soybean against ASR is the major factor to define the success of control, moreover, they confirm that ASR predictive models are crucial

for fungicide recommendation ensuring yield protection without excessive fungicide applications, promoting more profitability for soybean growers.

Considering that fungicide spray is the most efficient method to control ASR and that this is normally defined by the calendar, there is a necessity of tools to improve the spray recommendations, since control should be applied in the right time to be more efficient. Based on that, the hypothesis of this study is that DWSs when well-adjusted can promote better ASR control, by defining correctly the time for fungicide sprays, providing higher profitability for the growers and lower environmental impacts. Thus, the objective of this study was to evaluate the profitability of soybean production when using ASR disease-warning systems, based on rainfall data in relation to the calendar-based spray system.

5.2. Material and Methods

For this study, the performance of a DWS for ASR control, based on rainfall data, was compared with a calendar-based approach. The DWS was based on the study of Del Ponte et al. (2006a) and more details about this system can be obtained in Chapter 2. The choice of the rainfall based DWS to assess soybean production profitability was made according to the good performance of this system detailed in Chapter 2.

To evaluate the performance of different thresholds at the rainfall based DWS to recommend sprays to control ASR, field trials were carried out in four locations: Piracicaba, SP; Ponta Grossa, PR; Campo Verde and Pedra Preta, both in MT (Table 5.1).

In Piracicaba and Ponta Grossa, the experimental units comprised five rows of 4-m length and spaced in 0.45 m, totaling 266,666 plants ha⁻¹. While in both field trials in Mato Grosso experimental plots had five rows of 7-m length, with the same row spacing and plant population. Before sowing, soybean seeds were treated with a mixture of fungicide and insecticide of protecting action (Pyraclostrobin), systemic (Thiophanate-methyl), contact and ingestion (Fipronil) at a dosage of 200 mL for each 100 kg of seeds. Moreover, seeds were inoculated with N-fixing bacteria using 2 ml of *Bradyrhizobium japonicum* inoculums (lineages SEMIA 5079 and 5080) at a concentration of 5 x 10⁹ units forming colonies (UFC ml⁻¹). In all sites weeds, pests and disease, excluding ASR, were managed with specific chemicals, infestation and infection degrees in order to guarantee no external interference on the results. Soil fertilization for Piracicaba and Ponta Grossa was based on soil chemical analysis. In Campo Verde and Pedra Preta, soil fertilization was performed by a chemical fertilizer with a nitrogen, phosphorus, and potassium (NPK) formulation corresponding to 12-

46-00 at a dose of 152 kg ha⁻¹, which was applied to the sowing row. Still, 20 kg ha⁻¹ of ammonium sulfate was applied to the sowing row while the addition of chlorate of potassium was done at two distinct moments: in the sowing and 30 days after sowing.

The sowing dates considered in this study were within the sowing window recommended for soybean crop in Brazil (CONAB, 2017), which respect the phytosanitary free-host period for soybean cultivation (GODOY et al., 2016) and had a lower climatic risk to induce yield gaps (BATTISTI; SENDELHAS, 2014).

Table 5.1. Field trials location, their geographical coordinates, Köppen climate classification (ALVARES et al., 2013), soybean season, cultivar sowed with respective sowing date adopted to assess the performance of different thresholds in the rainfall based disease warning system for soybean Asian rust in Brazil.

Site	Lat	Long	Alt	Climate*	Season	Cultivar	Sowing
Piracicaba	22°42'S	47°37'W	567m	Cwa	2014/2015	Brasmax®	18/Nov
						Potência RR	12/Dec
					2015/2016	MonSoy-	22/Oct
						6410® Ipro	19/Nov 18/Dec
Ponta Grossa	25°05'S	50°09'W	969m	Cfb	2014/2015	Brasmax®	16/Oct
						Potência RR	11/Nov 18/Dec
					2015/2016	MonSoy-	08/Oct/
						6410® Ipro	21/Nov 11/Dec
Campo Verde	15°23'S	55°03'W	667m	Aw	2014/2015	TMG® 132	21/Oct
						RR	13/Nov 12/Dec
					2015/2016	TMG® 132	23/Oct
						RR	07/Nov 09/Dec
Pedra Preta	16°51'S	54°01'W	745m	Aw	2015/2016	TMG® 132	06/Nov
						RR	03/Dec

*Cwa - humid subtropical, with dry winter and hot summer, Cfb - humid subtropical, without dry season and temperate summer, and Aw - tropical with dry winter.

For each site, field trials were conducted under a randomized block design allotted in split plots with four replications. At the different experimental sites, the main factor

comprised the different sowing dates and the secondary factor referred to the thresholds used in the DWS for ASR control. The treatments adopted in the field trials followed the phenological scale proposed by Fehr et al. (1971) and are described below:

- 1- Calendar-based system (CALEND) – with the sprays following market strategy. In this method, fungicide spray starts when soybean plants reach the R1 phenological stage. After the first spray, the new sprays will be applied every 14 days until the plants reach R7 phenological stage;
- 2- Disease-warning system based on rainfall data (PREC_1) – with sprays recommended in accordance with the prediction equations of final severity proposed by Del Ponte et al. (2006a), and daily values of severity assessment starting when soybean plants reach V3 phenological stage. This treatment considered as cut-off the estimated severity of 80%, which represents a less conservative threshold.
- 3- Disease-warning system based on rainfall data (PREC_2) – follows the same procedure presented for PREC_1; however, taking into account for spray decision an estimated severity cut-off of 50%, representing a more conservative threshold.

In all treatments, ASR control was performed with the fungicide composed by a mixture of QoI and SDHI (Azoxystrobin + Benzovindiflupyr, respectively) in a dosage of 150 g ha⁻¹. After each fungicide application, a 14-day period with no fungicide application was considered under the assumption that fungicide residues on leaves would continue to suppress ASR infection effectively during this period. The fungicide sprays were applied with a pressurized costal sprayer of carbon dioxide (CO₂), with an application bar containing four sprayers (TEEJET XR/11002, VP) spaced 0.45 m. Sprayers were regulated at a pressure from 2 to 2.5 bar, with applications performed in a speed of 4.5 km h⁻¹ for distributing approximately 200 L of fungicide ha⁻¹.

To enable the use of prediction algorithms for ASR control by the rainfall based DWS, daily meteorological data were collected by automatic weather stations (AWS) deployed at approximately 10 m away from each experiment. The AWSs had electronic sensors to measure global solar radiation (Pyranometer CM3, Kipp & Zonen trademark), air temperature and relative humidity (HMP35C, Vaisala trademark), wind speed and direction (03001, Young trademark), rainfall (TR-565M model), and leaf wetness duration (cylinder sensors, Weather Innovation trademark). All sensors were coupled to Campbell Scientific Inc.

data acquisition systems (CR-10x and CR-1000 models), which were programmed to record variables at each minute and store averages at 15-min intervals

Soybean plants were harvested by a mechanized process in Piracicaba, Campo Verde, and Pedra Preta, and with a semi-mechanized process in Ponta Grossa. In all field trials, the procedure happened when soybean plants reached the physiological maturity. For each plot, three rows with two meters length were harvested to determine the yield, mass of thousand grains (MTG) and profitability.

Soybean yield was obtained from the weight of all soybean grains harvested from each useful plot. To determine the MTG samples of a hundred seeds were weighed at each plot by means of a scale with a precision of centesimal grams. For both variables data were corrected by a water content of 130 g kg^{-1} (13%).

To determine the profitability of each area and treatment, the spray cost was considered. According to Mato-Grossense Institution of Agricultural Economy (IMEA, 2017), the spray cost for ASR control during the 2014/2015 and 2015/2016 were R\$ 156.40 and R\$ 224.60 per hectare, respectively. Based on that, R\$ 200.00 ha^{-1} was assumed as the average for the analysis. To be compared with the soybean yield, the spray cost was converted into kg ha^{-1} based on the average sale price of the soybean bag of 60 kg from both soybean seasons, which was of R\$ 62.43 (CEPEA, 2017). Correlating the average of the sale price of the soybean with spray cost the fungicide spray cost was determined as 3.2 soybean bags, resulting in 192 kg of soybean grains for each application ha^{-1} .

In order to determine the relative yield gain, it was established the calendar based approach to recommend spraying as standard treatment, and because of that profitability obtained in this treatment was established as zero. In a sequence profitability obtained in PREC_1 and PREC_2 were compared to that. The procedure was performed for each sowing date and field trial, and afterward, a general average was obtained encompassing all sources of variation.

Variables obtained throughout the field trials in each site for different sowing dates were submitted to the analysis of variance with the application of the F test. The means were compared by Tukey test at 5% of significance.

5.3. Results and Discussion

Analyzing the performance of disease-warning systems to control ASR in Piracicaba, SP, PREC_1 did not present effective disease control in three sowing dates due to no sprays

recommendation. In the absence of fungicide sprays yield reductions of at least 1000 kg ha⁻¹ was observed in PREC_1 compared to CALEND and PREC_2. Furthermore, reduction in mass of thousand grains (MTG) was observed in PREC_1 as a consequence of a higher ASR severity (Table 5.2).

Yield and MTG reductions in soybean plants infected by *P. pachyrhizi* are associated with chlorosis around leaf infections, premature defoliation which promote early maturation (GODOY et al., 2016; HARTMAN et al., 2016). The consequence of the ASR symptoms, especially defoliation, is the translocation reduction of the photo-assimilates destined for formation and filling of grains. In this way, yield reductions of 80 % were reported in Asia (HARTMAN; WANG; TSCHANZ, 1991; YANG et al., 1990), 60 % in South America (YORINORI et al., 2005), 27 % in southern United States (SIKORA et al., 2010; MUELLER et al., 2009; WRATHER; KOENNING, 2009) and 80 % in Africa (LEVY, 2005).

Thus, the ASR control using fungicides is essential for maximizing yields; however, it is necessary to determine the better time for applying fungicides. In this study, for each threshold used to control ASR, they recommend different number and time of sprays, which varied according to the sowing date. In Piracicaba, five sprays were recommended by CALEND during all sowing dates, while PREC_1 and PREC_2 recommend a maximum of three to five sprays, respectively. For experiments where PREC_1 recommend fungicide sprays, no yield differences ($P \leq 0.05$) were observed between treatments even with less fungicide spray (Table 5.2).

For PREC_1, during the sowing dates of October and November of 2015, the yields reached 4868.5 and 4920.9 kg ha⁻¹, respectively, very similar to the results obtained with CALEND. However, PREC_1 recommended respectively three and two fungicide sprays less than CALEND, having as a consequence a more profitable condition in PREC_1 (Table 5.2).

When comparing PREC_2 with CALEND it was observed that sprays recommended by the warning system ranged from three to five, while CALEND always recommended five sprays. Considering all sowing dates no significant differences in yield, MTG and profitability were observed. In average soybean yield obtained in CALEND was 4614.0 kg ha⁻¹, whereas yield in PREC_2 was only 33.0 kg ha⁻¹ lower, but with one spray less. Therefore, the yield gain obtained by CALEND did not cover the cost of a single fungicide spray for ASR control

As mentioned before, the ASR management is performed mainly by fungicide sprays (SCHERM et al., 2009; TWIZEYIMANA; HARTMAN, 2016), which has been done through a calendar-based approach, with sprays starting at R1 phenological stage (beginning of bloom) (FEHR et al., 1971), with reapplications every 14 days. Many authors mentioned that

this procedure is conservative, since it does not consider the weather favorability for disease progress. However, the main concern about not using a DWS is in relation to spray timing, once an inappropriate spray can compromise ASR control and, consequently, crop yield (ABULEY; NIELSEN, 2017; KELLY et al., 2015; MEGETO et al., 2014; DEL PONTE et al., 2006a).

Table 5.2. Number of fungicide sprays, crop yield, mass of a thousand grains and soybean profitability as a function of different sowing dates and different disease-warning systems for controlling Asian soybean rust in Piracicaba, SP, Brazil.

Sowing date	Treatment	Spray	Yield		MTG		Profitability	
			kg ha ⁻¹		g ⁻¹		kg ha ⁻¹	
18/Nov/2014	UNS	0	3111.1	c	143.3	b	3111.1	b
	CALEND	5	5032.4	a	190.0	a	4072.4	a
	PREC_1	0	3819.5	bc	158.8	b	3819.4	ab
	PREC_2	5	4930.6	ab	178.3	a	3970.6	a
12/Dec/2014	UNS	0	2724.5	b	125.0	b	2724.5	b
	CALEND	5	4291.7	a	163.3	a	3331.7	a
	PREC_1	0	2587.9	b	125.0	b	2587.9	b
	PREC_2	3	3810.2	a	157.3	a	3234.2	a
22/Oct/2015	UNS	0	3973.8	a	133.3	b	3973.8	a
	CALEND	5	5031.8	a	175.0	a	4071.8	a
	PREC_1	2	4868.5	a	154.5	b	4484.5	a
	PREC_2	5	5267.6	a	175.5	a	4307.6	a
19/Nov/2015	UNS	0	3556.3	b	127.5	b	3556.3	b
	CALEND	5	4693.9	a	165.0	a	3733.9	ab
	PREC_1	3	4920.9	a	160.5	a	4344.9	a
	PREC_2	4	4677.1	a	155.5	a	3909.1	ab
18/Dec/2015	UNS	0	2505.7	b	109.3	b	2505.7	b
	CALEND	5	4023.8	a	149.3	a	3063.8	a
	PREC_1	0	2695.3	b	110.8	b	2695.3	b
	PREC_2	5	4220.1	a	150.5	a	3260.1	a

Yield, mass of thousand grains and profitability data followed by different letters are statistically different by the Tukey's test ($\alpha = 0.05$)

The effects of the spray timing to control ASR can be observed in the plots sowed on 18/Nov/2014, 22/Oct/2015, and 18/Dec/2015, where five sprays were recommended by CALEND and PREC_2. For the sowing dates cited above fungicide sprays were applied as a preventive way, always earlier than those recommended by CALEND. The PREC_2 determined that sprays should be applied ten, one and eleven days before than CALEND, respectively, on 18/Nov/2014, 22/Oct/2015, and 18/Dec/2015 sowing dates, a fact that promoted yield increments of 110.0 kg ha⁻¹.

According to Mueller et al. (2009), the major factor that defines the success or failure in the ASR control is the timing of fungicide sprays, because when sprays are applied in a curative way a higher number of applications are required. Furthermore, after the infection the foliar tissues affected by the fungus will no longer have functionality for the plant. Twizeyimana and Hartman (2016) and Preez and Diane (2005) also observed that fungicide sprays applied in a preventive way reduced the *P. pachyrhizi* sporulation compared with curative applications.

Good performance of the rainfall disease warning system also was obtained in Campo Verde, MT (Table 5.3). In contrast to Piracicaba, the disease-warning system in Campo Verde recommended sprays to control ASR for all sowing dates, which was a consequence of higher rainfall occurrence all over the two soybean growing seasons.

In Campo Verde no yield differences were identified between treatments used to control ASR ($P \leq 0.05$); however, divergent spray numbers were required to control ASR for each treatment and sowing dates. In general, sprays applied in CALEND and PREC_2 ranged from three to five, while in PREC_1 the variation was from one to five sprays. Considering all sowing dates, CALEND and PREC_2 recommended in average 4.3 sprays, whereas PREC_1 indicated 2.5 sprays, being such a treatment more profitable than other treatments adopted, since no difference was observed among treatments.

PREC_1 was more profitable to control ASR in Campo Verde, raising profitability by 105 and 101 kg ha⁻¹ compared to CALEND and PREC_2, respectively. Furthermore, the profitability in PREC_1 was also a consequence of the better time to make sprays, which demonstrated different behavior throughout the 2014/2015 and 2015/2016 growing seasons. Throughout the first growing season, fungicide sprays were applied in a preventive way or at the same moment of the spraying carried out in CALEND; the opposite was identified during 2015/2016, where PREC_1 determined the necessity of the first sprays on the same day or a bit delayed in relation to CALEND.

Table 5.3. Number of fungicide sprays, crop yield, mass of a thousand grains and soybean profitability as a function of different sowing dates and different disease-warning systems for controlling Asian soybean rust in Campo Verde, MT, Brazil.

Sowing date	Treatment	Spray	Yield		MTG		Profitability	
				kg ha ⁻¹		g ⁻¹		kg ha ⁻¹
21/Oct/2014	UNS	0	3187.5	b	114.8	b	3187.5	a
	CALEND	5	4184.0	a	150.6	a	3224.0	a
	PREC_1	4	4045.2	a	145.6	a	3277.1	a
	PREC_2	5	4041.7	a	145.5	a	3081.7	a
13/Nov/2014	UNS	0	1213.0	b	65.5	b	1213.0	b
	CALEND	5	2606.5	a	140.8	a	1646.5	a
	PREC_1	5	2706.0	a	146.1	a	1746.0	a
	PREC_2	5	2916.7	a	157.5	a	1956.7	a
12/Dec/2014	UNS	0	351.9	b	19.0	c	351.9	a
	CALEND	3	511.6	a	27.6	b	-64.4	b
	PREC_1	3	513.9	a	27.8	b	-62.1	b
	PREC_2	5	870.4	a	47.0	a	-89.6	b
23/Oct/2015	UNS	0	2805.2	a	109.6	b	2805.2	a
	CALEND	5	3205.1	a	114.1	a	2245.1	ab
	PREC_1	2	3030.9	a	112.2	a	2646.9	a
	PREC_2	4	2878.5	a	115.3	a	2110.5	b
07/Nov/2015	UNS	0	1929.9	b	78.8	b	1929.9	a
	CALEND	4	2578.8	a	88.5	a	1810.8	a
	PREC_1	3	2534.9	a	90.3	a	1958.9	a
	PREC_2	4	2669.1	a	90.9	a	1901.1	a
09/Dec/2015	UNS	0	668.6	b	63.3	b	668.6	a
	CALEND	4	1475.9	a	92.9	a	707.9	a
	PREC_1	1	1398.6	a	85.8	a	630.6	a
	PREC_2	3	1400.4	a	83.1	a	632.4	a

Yield, mass of thousand grains and profitability data followed by different letters are statistically different by the Tukey's test ($\alpha = 0.05$)

The opposite rainfall pattern at both growing seasons was the main trigger to determine fungicide sprays recommended by PREC_1. The first fungicide spray in soybean sowed on 21/Oct/2014 and 13/Nov/2014 was anticipated by three days compared to

CALEND. For both sowing dates, the period between sowing and the first spray was 53 and 30 days, respectively. For both periods aforementioned a high frequency of heavy rainfall was recorded; for the first sowing date an overall of 711 mm distributed within 18 rainfall events was registered, while for the second growing season there were 24 rainfall events, which totaled 871 mm (Figure 2.3).

By analyzing the performance of PREC_1 faced with sowing dates of October and November of 2015 in Campo Verde, it was observed that the DWS with the proposed threshold did not recommend the first sprays at the same time that CALEND did. The delay in those fungicide applications could be related to irregular rainfall regime at the beginning of the 2015/2016 growing season, as observed in Figure 2.3.

Recommendations for early or late spraying by PREC_1 in Campo Verde confirm a good performance of the disease-warning system to control ASR because spraying was required at the time when environmental conditions were favoring ASR progress and vice-versa. The consequence for both periods was the reduction in fungicide sprays and the increase of profitability (Table 5.3).

According to Rowlandson et al. (2014), disease-warning systems will consider the weather conditions to recommend sprays and therefore it will be lower under unfavorable conditions to the disease and higher under favorable conditions for disease outbreak. Furthermore, the use of rainfall warning system, using the less conservative threshold (PREC_1), proved to be a useful tool for managing ASR in Campo Verde, which could be incorporated into the soybean integrate disease management.

In Campo Verde, the PREC_2 did not promote a reduction in the number of sprays in comparison with CALEND (Table 5.3). As a consequence of that similar mean yields were obtained considering all sowing dates, with values of 2426.9 and 2462.8 kg ha⁻¹ for CALEND and PREC_2, respectively. The positive aspect of PREC_2 was that all sprayings were recommended in a preventive way, which is important to control ASR appropriately (TWIZEYIMANA; HARTMAN, 2016; GODOY et al., 2009; MUELLER et al., 2009; PREEZ; DIANE, 2005).

In Pedra Preta, the PREC_1 presented satisfactory performance to control ASR for both sowing dates, recommending four fungicide sprays, the same number applied according to CALEND. Comparing PREC_1 with CALEND on the first sowing date similar values of yield, MTG and profitability were attained ($P \leq 0.05$). For the second sowing date, soybean yield garnered in PREC_1 was 991.0 kg ha⁻¹ higher than that recorded by CALEND (Table 5.4).

The treatment PREC_2 showed a good performance to control ASR only in soybean plots sowed on 03/Dec/2015, resulting in a higher yield compared to CALEND ($P \leq 0.05$). Although in PREC_2 was applied an extra fungicide spray than in CALEND and in PREC_1, the profitability was not compromised (Table 5.4).

Just like in Campo Verde, in Pedra Preta considerable rainfall variations were also observed during the first sowing dates, especially in October and November. As a consequence, rainfall based ASR warning system recommended later sprays (PREC_1 = +16 days and PREC_2 = +10 days) than CALEND, considering that there was a low risk of ASR occurrence.

Table 5.4. Number of fungicide sprays, crop yield, mass of a thousand grains and soybean profitability as a function of different sowing dates and different disease-warning systems for controlling Asian soybean rust in Pedra Preta, MT, Brazil.

Sowing date	Treatment	Spray	Yield		MTG		Profitability	
				kg ha ⁻¹		g ⁻¹		kg ha ⁻¹
06/Nov/2015	UNS	0	2349.6	b	100.6	b	2349.6	b
	CALEND	4	3643.3	a	114.1	a	2875.3	a
	PREC_1	4	3551.4	a	112.2	a	2783.4	a
	PREC_2	4	2855.6	b	115.3	a	2087.6	b
03/Dec/2015	UNS	0	1880.3	b	71.4	b	1880.3	b
	CALEND	4	1902.7	b	72.8	b	1134.7	c
	PREC_1	4	2893.9	a	99.2	a	2125.9	a
	PREC_2	5	2941.9	a	100.5	a	1981.9	b

Yield, mass of thousand grains and profitability data followed by different letters are statistically different by the Tukey's test ($\alpha = 0.05$)

In the beginning of the soybean season in Pedra Preta, the irregular rainfall might be the main factor that promoted yield and profitability reductions under PREC_2. Considering the 10-day periods between November and December, only in the second 10-day period of December was recorded considerable rainfall (100 mm in five rainfall events), being this able to make PREC_2 to recommend a spray to ASR control (Figure 2.4). Thus, PREC_2, due to a more conservative threshold, recommend spray when it was not required. Over the action period of the fungicide (14 days) 385 mm in 11 days of rainfall was recorded shortly after fungicide spray, a fact that might promote a reduction in the fungicide performance to control ASR. Moreover, after such a wet period, seven days of drought were noticed in such a way as

to reduce the risk of ASR. Under this low disease risk environment the next spray following PREC_2 was recommended for January 29 of 2016, 18 days after the first spray; thus the soybean plants remained four days unprotected and under risk of ASR infection.

The occurrence of a heavy rainfall reduces the fungicide efficiency, due to interference on fungicide absorption rates by leaves and washing of the fungicides from the soybean tissues, reducing the action period of the chemical (WANG; LIU, 2007; FIFE; NOKES, 2002). The effect of simulated rainfall after fungicide spray to control ASR was observed by Stefanello et al. (2016) in Southern Brazilian region, where the ASR control was negatively affected by simulated rainfall 120 minutes after spraying. Thus, in Pedra Preta, the occurrence of rainfalls after spraying, in addition to drought period that reduced ASR risk can affect ASR control provided by PREC_2 (Table 5.4).

With the normality of the rainfall in Pedra Preta, the risk of ASR occurrence raised and four and five sprays were applied in PREC_1 and PREC_2, respectively. For both treatments, applications were done with at least three days earlier than CALEND, which resulted in a yield increment of 1,000 kg ha⁻¹ when compared to CALEND ($P \leq 0.05$) (Table 5.4). The first fungicide spray in PREC_2 occurred on 12/Jan/2016, 17 days before that recommended by PREC_1. Due to the more conservative threshold that promoted precocity in the first spray associated with a high ASR risk, an additional spray was necessary in PREC_2.

In spite of more sprays, PREC_2 profitability was higher than CALEND ($P \leq 0.05$). Similar results were found by Kelly et al. (2015) in Florida, USA, where the rainfall totaled 1107 mm over the soybean growing season promoting an increase of ASR risk, as a result the rainfall based warning system recommended four sprays against two of the calendar based approach. Nevertheless, in Florida, more fungicide sprays did not provide significant increases in soybean yield, which was justified by the authors as a consequence of the low ASR pressure of inoculum in the experimental area.

In Ponta Grossa, the disease-warning systems presented higher variability in the sprays recommendations when compared to other locations, as a consequence of a rainfall variation throughout the two crop growing seasons (Figure 2.2). PREC_1 showed a good performance only in October sowing dates, when saved two and three sprays during 2014 and 2015, respectively, and culminated in similar yield, MTG, and profitability in relation to CALEND ($P \leq 0.05$) (Table 5.5). For the sowing dates aforementioned fungicide applications occurred 19 and 30 days after CALEND, respectively, in 2014 and 2015. Throughout both growing seasons, the first spray was applied at the first 10-day period of January, as a result of high rainfall events recorded between the beginning of December and January, reaching the

threshold of 80 % of estimated severity by PREC_1. Reduction in spraying number associated with equal yield and MTG values compared to CALEND was also obtained by Sentelhas et al. (2012) in Ponta Grossa, using the same disease-warning system with a similar threshold to control ASR.

For the remained sowing dates the number of fungicide sprays recommended by PREC_1 ranged from zero to four. On the sowing dates that PREC_1 determined less spray than CALEND, the ASR was not controlled effectively by the disease-warning system, which resulted in lower yields compared to CALEND. With exception of Piracicaba during 2014/2015 growing season, Ponta Grossa presented less rainfall compared with other locations. This possibly is the main reason for the low performance of PREC_1 for the remaining sowing dates. Del Ponte et al. (2006a) correlating ASR severity values with rainfall also verified that the low frequency of heavy rains in Southern Brazil affected the ASR final severity.

On 11/Nov/2014 sowing date, four sprays were recommended by PREC_1 and CALEND, and the use of the disease-warning system did not generate increment in soybean production components analyzed herein. Although no increments of yield was noticed, the first spray following PREC_1 was made two days before CALEND and as mentioned previously, preventive sprays are crucial to ensure an efficient ASR control.

PREC_2 conditioned yield gain with less sprays than CALEND only in soybean plots sowed on 18/Dec/2014 ($P \leq 0.05$) (Table 5.5). For the period in analysis PREC_2 saved one spray and promote yield gain of 944.0 kg ha⁻¹ compared to CALEND. For 16/Oct/2014, 08/Oct/2015, and 21/Nov/2015 sowing dates, PREC_2 showed a similar performance as CALEND, indicating the same number of sprays and similar values of yield, MTG, and profitability (Table 5.5).

For 11/Nov/2014 and 11/Dec/2015, PREC_2 was not efficient to control ASR in Ponta Grossa. Throughout the 11/Nov/2014 sowing date, the disease-warning system recommended five fungicide sprays, one more than other treatments. Even with more sprayings, the use of PREC_2 did not provide an increase in yield, therefore reducing the profitability. The opposite was reported over the sowing of 11/Dec/2015 where PREC_2 determined one spray less compared to CALEND, but with yield and profitability lower than CALEND ($P \leq 0.05$).

Table 5.5. Number of fungicide sprays, crop yield, mass of a thousand grains and soybean profitability as a function of different sowing dates and different disease-warning systems for controlling Asian soybean rust in Ponta Grossa, PR, Brazil.

Sowing date	Treatment	Spray	Yield		MTG		Profitability	
				kg ha ⁻¹		g ⁻¹		kg ha ⁻¹
16/Oct/2014	UNS	0	2231.5	a	163.0	a	2231.5	a
	CALEND	5	2828.7	a	173.5	a	1868.7	a
	PREC_1	3	2486.1	a	162.0	a	1910.1	a
	PREC_2	5	3222.2	a	164.5	a	2262.2	a
11/Nov/2014	UNS	0	1416.7	b	160.8	a	1416.7	b
	CALEND	4	3377.4	a	176.5	a	2609.3	a
	PREC_1	4	2682.9	a	180.0	a	1914.9	ab
	PREC_2	5	2476.9	a	162.5	a	1516.9	b
18/Dec/2014	UNS	0	1212.9	b	114.5	b	1212.9	c
	CALEND	4	2509.3	a	136.3	a	1741.3	b
	PREC_1	0	1800.9	b	119.5	b	1800.9	b
	PREC_2	3	3453.7	a	135.0	a	2877.7	a
08/Oct/2015	UNS	0	2045.1	b	111.5	b	2045.1	c
	CALEND	4	4513.9	a	168.5	a	3745.8	ab
	PREC_1	1	4368.5	a	153.0	a	4176.5	a
	PREC_2	4	3666.6	a	153.3	a	2898.6	b
21/Nov/2015	UNS	0	1285.3	b	96.0	b	1285.3	b
	CALEND	5	3556.3	a	130.8	a	2596.3	a
	PREC_1	2	1109.3	b	92.8	b	725.3	b
	PREC_2	5	3181.5	a	127.8	a	2221.5	a
11/Dec/2015	UNS	0	760.4	c	93.5	b	760.4	c
	CALEND	4	2157.0	a	111.8	a	1581.0	a
	PREC_1	1	734.9	c	98.0	b	542.9	c
	PREC_2	3	1899.8	b	115.3	a	1323.8	b

Yield, mass of thousand grains and profitability data followed by different letters are statistically different by the Tukey's test ($\alpha = 0.05$)

As the disease-warning systems used herein consider just rainfall data, the variability of such a weather variable interferes into their performance. Errors for predicting final ASR

severity also was observed by Igarashi et al. (2016), by evaluating ASR models during the 2011/2012 and 2012/2013 seasons in Londrina, PR.

In a comparative analysis of the relative yield gain between the rainfall based ASR warning system with both thresholds and the calendar based approach, considering all the sowing dates at each site, it was noticed that in Piracicaba, PREC_1 was also not efficient to ASR control, reflecting in a lower relative yield gain compared to CALEND. On the other hand, PREC_2 pointed out an increased relative yield gain compared to CALEND. In Ponta Grossa the disease-warning system was not effective for the ASR control, generating losses of relative yield gain compared to CALEND. In contrast to both locations in Mato Grosso, relative yield gains were observed using the PREC_1 and PREC_2 systems compared to CALEND (Figure 5.1).

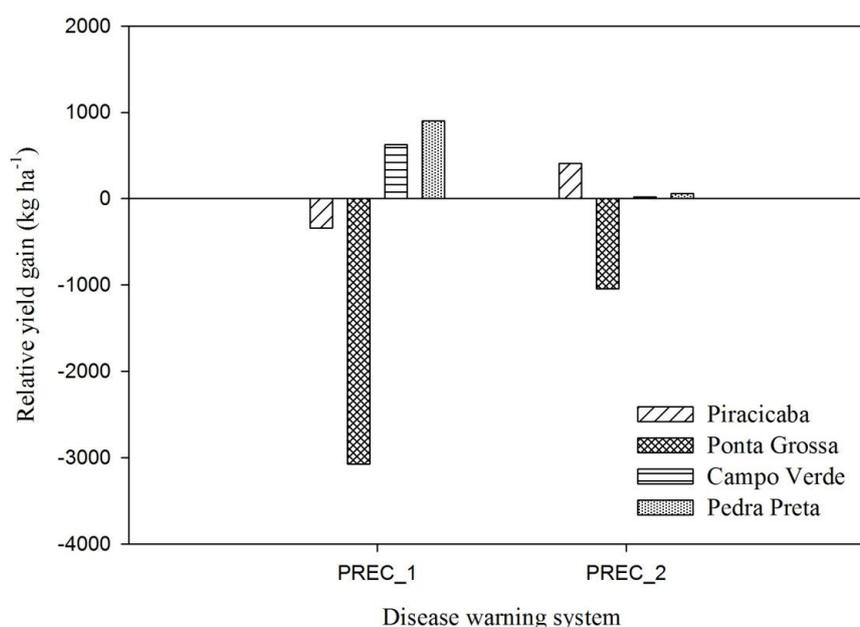


Figure 5.1. Relative yield gain of disease-warning systems based on rainfall data, with different thresholds (PREC_1 and PREC_2), compared with the calendar based approach (CALEND) for different sowing dates and field trials in Brazil.

Based on the rainfall variability in Piracicaba, PREC_2 provided an increase of 407.8 kg ha⁻¹ compared to CALEND. The opposite was found for PREC_1, which resulted in a reduction of 341.4 kg ha⁻¹. The low performance of PREC_1 was the absence of heavy rainfalls during 2014/2015 growing season and for sowing in December 2015 (Figure 5.2A). Under these conditions, leaf wetness duration, caused by dew, was the source of water for the disease, which was not reflected by the rainfall data.

Since Ponta Grossa was the site with the highest rainfall variability, in general, the disease-warning system based on rainfall data, for both thresholds, did not perform well to control ASR. Considering all sowing dates the cumulative value of relative yield reduction in relation to CALEND was 3071.8 kg ha⁻¹ for PREC_1 and 1041.7 kg ha⁻¹ for PREC_2. As can be visualized in Figure 5.2B during inefficient ASR control by the disease warning systems, an intense damage was identified promoting relative yield losses up to 1871.0 kg ha⁻¹ for PREC_1 during 21/Nov/2015 sowing date. Only PREC_2 during 18/Dec/2014 sowing date presented significant increments in relative yield, with a value of 1136.4 kg ha⁻¹ compared to CALEND.

As demonstrated by many authors, the rainfall regime is considered to be an epidemic triggering factor of ASR (MEGETO et al., 2014; DEL PONTE; ESKER, 2008; DEL PONTE et al., 2006a; TAN; YU; YANG, 1996). However, other weather variables can interfere into ASR progress in the field, and depending on the local climatic condition, rather than rainfall, air temperature and leaf wetness duration are also important for ASR occurrence (DANELLI; REIS, 2016; IGARASHI et al., 2014; BONDE; NESTER; BERNER, 2012; ALVES et al., 2007; MELCHING et al., 1989).

The ASR warning system used in the current study was based on the work of Del Ponte et al. (2006a), which found a strong correlation between ASR severity and rainfall observed in the reproductive soybean phase, for sowing dates ranging from October to early January, all over the soybean Brazilian production regions. Although those authors reported that temperature in the south of Brazil might influence the ASR development, the incorporation of air temperature into the ASR warning system based on rainfall data may promote a more pronounced accuracy for disease prediction.

Fundamentally, disease-warning systems are proposed according to weather variables that positively affect the disease progress, which might be different from region to region due to differences in climate conditions. Therefore, the identification of the weather variables that affect the disease progress is fundamental to a good performance of the disease-warning system (MAGAREY; ISARD, 2017). Furthermore, the rainfall based warning system used in the current study is an empirical model which reveal a strong dependence of the local conditions, thus, empirical relations need to be validated passing through possible parameterizations to avoid errors at an operational scale (ROWLANDSON et al., 2014; GLEASON et al., 2008).

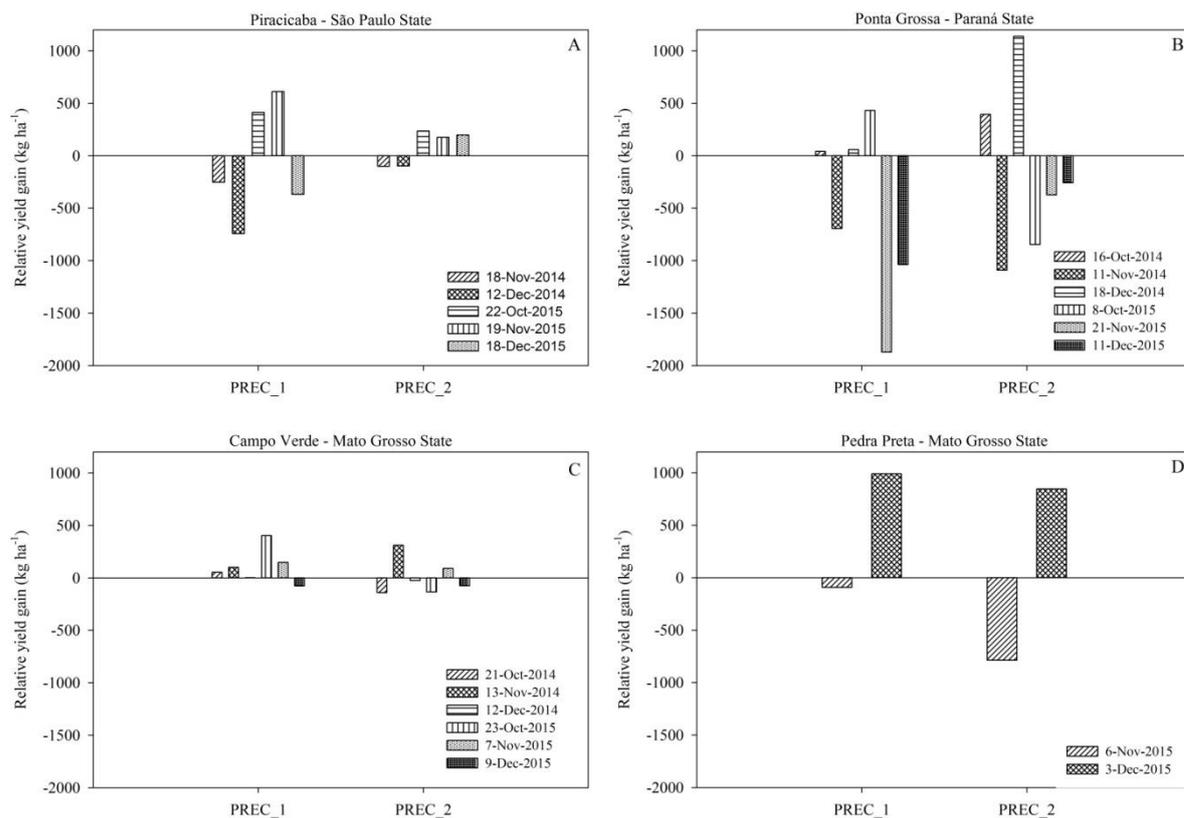


Figure 5.2. Relative yield gain of disease-warning systems based on rainfall data, with different thresholds (PREC_1 and PREC_2), compared with the calendar based approach (CALEND) for each sowing date for Piracicaba, SP (A), Ponta Grossa, PR (B), Campo Verde, MT (C), and Pedra Preta, MT (D).

In Campo Verde and Pedra Preta a higher cumulated rainfall over the soybean growing seasons was identified, along with a considerable number of heavy rains recorded. As a consequence, rainfall based warning systems showed good performance for almost all sowing dates (Figure 5.2C and 5.2D), resulting in relative yield gains of 627.5 kg ha⁻¹ and 22.7 kg ha⁻¹, respectively, for PREC_1 and PREC_2 in Campo Verde. In Pedra Preta, relative yield gains were 889.3 kg ha⁻¹ for PREC_1 and 59.6 kg ha⁻¹ for PREC_2 (Figure 5.1).

The better performance of PREC_1 and PREC_2 in Campo Verde and Pedra Preta is related to the fact that rainfall intensity and distribution represents well the leaf wetness duration that takes place at both sites, which is basically associated to a more stable rainfall regime in Mato Grosso, when compared to Ponta Grossa and Piracicaba where rainfall inter-annual variability is higher.

5.4. Conclusions

The performance of the ASR disease warning system was conditioned by variations of the rainfall regime in all field trials assessed.

The rainfall-based warning system increased the profitability of soybean production in Campo Verde, Pedra Preta and Piracicaba, during sowing dates with high rainfall volume, evidencing to be an effective agricultural tool to promote ASR management at such sites.

In Ponta Grossa the ASR warning systems were not effective to ASR control. Thus, adjustments in the action thresholds of the ASR warning system, as well incorporation of other meteorological variables a part from rainfall into de disease-warning system in study might be necessary to improve their performance in further investigations.

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6. GENERAL CONCLUSIONS

Based on the results obtained in the current study the following conclusions were possible to be drawn:

The weather conditions in the locations studied were favorable for ASR, enabling the ASR occurrence with high destructive power without any disease control. Among the weather variables analyzed leaf wetness duration, cumulative rainfall and air temperature during leaf wetness duration were those more effectively correlated to ASR epidemics.

The disease-warning system based on rainfall data showed good performance to manage ASR, promoting an effective control associated with lower fungicide sprays in comparison to calendar-based approach. Considering all sowing dates throughout the two growing seasons rainfall based warning system with a more conservative threshold demonstrated better efficiency for controlling the ASR. On the other hand, on sowing dates varying from October to November, periods with higher rainfall, the rainfall-based warning system with less a conservative threshold provided a better performance for controlling ASR with fewer fungicide sprays.

The disease-warning systems based on leaf wetness duration and air temperature during the leaf wetness duration were able to predict ASR. However, compared to other methodologies to control ASR, the disease-warning systems overestimated ASR and, as a consequence, high spraying number was recommended to ASR control at all studied sites. Therefore, adjustments are required to make the methodology useful for ASR management.

LWD estimated by empirical RH-based methods presented acceptable accuracy and precision for its use as an input in the ASR warning system at three experimental sites in Brazil. The LWD estimation method of $NHRH \geq X\%$ having as threshold RH of $90\% \pm 2\%$ had the highest accuracy for estimating LWD. Considerable errors to predict ASR using estimated LWD were obtained in Campo Verde and Pedra Preta. Nevertheless, a good performance of $NHRH \geq 90\%$ as an input of ASR warning system was observed in Ponta Grossa, which was basically governed by the better performance of $NHRH \geq 90\%$ for estimating LWD at such a site.

The performance of rainfall-based warning system for recommending sprays to control ASR was conditioned by variations of the rainfall regime in all field trials assessed. Due to rainfall variations, the warning system recommended early and later fungicide sprays compared to calendar-based approach, providing a better timing to ASR control. Based on that, the warning system increased the profitability of soybean production in Campo Verde,

Pedra Preta and Piracicaba, during sowing dates with higher rainfall volume. On the other hand, at Ponta Grossa adjustments in the action thresholds of the ASR warning system or incorporation of other meteorological variables into the ASR warning system might be necessary to improve performance of such systems in order to guarantee sustainability in soybean production fields at a large scale.

ANNEX

Annex A - Epidemiological analysis and statistical coefficients for the exponential model to describe Asian soybean rust severity progress in Piracicaba and Ponta Grossa, Brazil.

Site	Growing Season	Sowing Date	ASR infection rate	Initial inoculum	R ²	p-value	ME	MAE	RMSE
Piracicaba	2014/2015	1st	0.073	0.025	0.945	0.030	0.053	0.144	0.209
		2nd	0.011	0.003	0.925	0.010	0.093	0.185	0.291
		3rd	0.109	0.002	0.913	0.001	0.039	0.118	0.198
		4th	0.109	0.006	0.994	0.010	-0.063	0.084	0.146
		5th	0.071	0.009	0.996	0.001	0.011	0.030	0.042
	2015/2016	1st	0.166	0.054	0.951	0.009	0.010	0.062	0.075
		2nd	0.103	0.001	0.936	0.000	6.046	6.048	15.794
		3rd	0.121	0.001	0.999	0.000	0.034	0.043	0.086
		4th	0.131	0.028	0.991	0.004	-0.019	0.047	0.063
		5th	0.056	0.008	0.662	0.047	0.000	0.003	0.003
Ponta Grossa	2014/2015	1st	0.074	0.019	0.827	0.004	0.047	0.149	0.230
		2nd	0.083	0.002	0.813	0.047	0.006	0.037	0.045
		3rd	0.078	0.006	0.893	0.021	-0.015	0.063	0.084
		4th	0.103	0.007	0.782	0.057	0.110	0.261	0.387
	2015/2016	1st	0.083	0.072	0.911	0.002	-0.021	0.069	0.098
		2nd	0.065	0.031	0.936	0.000	0.000	0.12	0.014
		3rd	0.046	0.032	0.763	0.001	-0.003	0.016	0.021
		4th	0.005	0.130	0.521	0.000	0.000	0.005	0.005

Annex B - Epidemiological analysis and statistical coefficients for the monomolecular model to describe Asian soybean rust severity progress in Piracicaba and Ponta Grossa, Brazil.

Site	Growing Season	Sowing Date	ASR infection rate	Initial inoculum	R ²	p-value	ME	MAE	RMSE
Piracicaba	2014/2015	1st	0.050	-0.412	0.729	0.298	-0.017	0.202	0.251
		2nd	0.060	-0.625	0.712	0.676	-0.051	0.283	0.390
		3rd	0.065	-0.944	0.492	0.674	-0.061	0.428	0.585
		4th	0.043	-0.523	0.403	0.519	0.017	0.334	0.369
		5th	0.041	-0.755	0.446	0.527	-0.015	0.396	0.472
	2015/2016	1st	0.061	-0.007	0.969	0.546	0.000	0.040	0.046
		2nd	0.050	-0.732	0.472	0.237	-0.032	0.353	0.435
		3rd	0.044	-0.564	0.558	0.527	0.018	0.285	0.351
		4th	0.172	-1.430	0.320	0.489	-0.131	0.608	0.814
		5th	0.000	0.009	0.772	0.281	0.000	0.002	0.002
Ponta Grossa	2014/2015	1st	0.019	-0.076	0.944	0.390	0.000	0.053	0.062
		2nd	0.003	0.001	0.999	0.437	0.000	0.001	0.000
		3rd	0.015	-0.178	0.435	0.635	0.023	0.196	0.211
		4th	0.031	-0.126	0.950	0.461	-0.004	0.077	0.084
	2015/2016	1st	0.045	-0.129	0.592	0.589	0.012	0.150	0.174
		2nd	0.005	0.022	0.958	0.184	0.000	0.009	0.010
		3rd	0.003	0.025	0.793	0.351	0.000	0.016	0.019
		4th	0.000	-5.715	0.522	0.003	-5.789	5.789	5.789

Annex C - Epidemiological analysis and statistical coefficients for the Gompertz model to describe Asian soybean rust severity progress in Piracicaba and Ponta Grossa, Brazil.

Site	Growing Season	Sowing Date	ASR infection rate	Initial inoculum	R ²	p-value	ME	MAE	RMSE
Piracicaba	2014/2015	1st	0.076	0.004	0.989	0.014	0.010	0.027	0.039
		2nd	0.100	0.000	0.838	0.161	0.097	0.110	0.207
		3rd	0.097	0.000	0.743	0.228	0.133	0.149	0.303
		4th	0.078	0.000	0.906	0.119	0.043	0.115	0.135
		5th	0.064	0.000	0.850	0.119	0.088	0.123	0.170
	2015/2016	1st	0.122	0.044	0.994	0.003	0.001	0.001	0.018
		2nd	0.081	0.000	0.862	0.001	0.073	0.085	0.145
		3rd	0.078	0.000	0.830	0.041	0.068	0.105	0.170
		4th	0.022	0.000	0.654	0.216	0.153	0.177	0.284
		5th	0.012	0.008	0.690	0.032	0.000	0.003	0.002
Ponta Grossa	2014/2015	1st	0.043	0.012	0.974	0.000	-0.005	0.027	0.040
		2nd	0.023	0.002	0.963	0.033	0.000	0.013	0.016
		3rd	0.037	0.002	0.647	0.050	0.006	0.130	0.152
		4th	0.063	0.004	0.996	0.008	-0.004	0.017	0.023
	2015/2016	1st	0.077	0.037	0.776	0.047	0.018	0.097	0.116
		2nd	0.025	0.029	0.966	0.000	-0.001	0.009	0.009
		3rd	0.017	0.031	0.791	0.001	-0.002	0.016	0.019
		4th	0.003	0.130	0.521	0.001	0.000	0.005	0.005