FERNANDA CRISTINA JULIATTI

SOYBEAN DISEASES INTEGRATED CONTROL WITH BIOLOGICAL ADND CHEMICAL FUNGICIDES IN DIFFERENTS RESISTANCE LEVEL OF GENOTYPES

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Orientadora: Profª. Drª. Ana Paula Nogueira Oliveira

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Aos meus irmãos Breno e Bruno, meus amigos em especial minha amada esposa Andrea pelo amor incondicional

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ABSTRACT

JULIATTI, FERNANDA CRISTINA. **Soybean diseases integrated control with biological and chemical fungicides in differents resistance level of genotypes.** 2021. Uberlândia: UFU, 93 p. Tese (Doutorado em Agronomia/Genética) – Universidade Federal de Uberlândia, Uberlândia - MG.

For "4.0 Agriculture strategies", the development of integrated crop solutions joining new resistant cultivars, chemical and biological control against diseases, are primordial steps in the preservation of plant yield potential. The objective of this thesis was to evaluate the soybean genotypes response with qualitative (monogenic heritage) and quantitative (polygenic heritage) resistance level for Asian Soybean Rust (ASR) in association with chemical and biological fungicides for Area Under Disease Progress AUDPC) and Grain Yeld. The genotypes studied, consisted of four promising soybean lines (UFU L266, UFU L216, UFU L154, UFU L218) developed by Germplasm Laboratory of Uberlândia Federal University (LAGER / UFU) with partial resistance against *Phakopsora pachyrhizi* and six cultivars with complete resistance as TMG 7062 IPRO, TMG 7063 IPRO and susceptible to rust, the BMX DESAFIO 8473RSF, BMX FLECHA IPRO, NA 5909 IPRO, MONSOY 7739 IPRO. The study was conducted in two different seasons in field conditions (2017/2018 and 2018/2019). The variables consisted of 40 different management combinations, varying the genetic, chemical (trifloxystrobin + prothioconazole) and biological *(Penicillium* spp.) controls. The fungicides were sprayed alone (or chemical or biological), or associated (chemical + biological), with an interval of 14 days, in four applications, during the crop development. It was evaluated severity for ASR, powdery mildew and Septoria brown spot and calculated AUDPC (area under disease progress curve), for the genotypes with and without association with fungicides. Grain yield was also evaluated. In the present study was observed different interaction between the genotype, chemical and biological fungicides, for AUDPC and Grain Yeld. Considering only genetic control, genotypes with complete resistance for soybean as TMG 7062 IPRO AND TMG 7063 IPRO with dominant resistance genes performed with the lowest AUDPC levels independent trial season for ASR but didn't corresponded on higher Grain Yeld levels. Followed, the partial resistance genotypes UFU L266 and UFU L216 performed as second lowest AUDPC level. The cultivars BMX FLECHA IPRO performed as the highest AUDPC levels in both seasons for ASR. According to AUDPC levels on the two seasons evaluated, the diseases pressure influenced the resistance expression. According to data from both season, for genotypes with partial or non-resistance level, was higher influence and interaction with fungicides management. The use of trifloxystrobin + prothioconazole solo and mixture of trifloxystrobin + prothioconazole + biological fungicide (*Penicillium* sp.) in mostly genotypes tested had thelowest AUDPC levels and highest values for Grain Yield. In both season, genotypes with partialor without resistance to ASR had higher synergic interaction with chemical and biological fungicides.

Keywords: Asian soybean rust, partial resistance, fungicides, biological, management.

RESUMO

JULIATTI, FERNANDA CRISTINA. **Controle integrado de doenças em soja com fungicidas químicos e biológicos em diferentes níveis de resistência**. 2021. Uberlândia: UFU, 2021. 93 p. Tese (Doutorado em Agronomia/Genética) – Universidade Federal de Uberlândia, Uberlândia - MG.

Para as "Estratégias de Agricultura 4.0", o desenvolvimento de soluções de cultivo integradas unindo novas cultivares resistentes, controle químico e biológico contra doenças, são etapas primordiais na preservação do potencial produtivo das plantas. O objetivo desta tese foi avaliar a resposta de genótipos de soja com resistência qualitativa (herança monogênica) e quantitativa (herança poligênica) de resistência à ferrugem asiática da soja em associação com fungicidas químicos e biológicos para Área abaixo da curva de progresso da doença (AUDPC) e produtividade. Os genótipos estudados, consistiram de quatro promissores genótipos de soja (UFU L266, UFU L216, UFU L154, UFU L218) desenvolvidos pelo Laboratório de Germoplasma da Universidade Federal de Uberlândia (LAGER / UFU) com resistência parcial contra *Phakopsora pachyrhizi* baseado na Fazenda Gloria na cidade de Uberlândia, estado de Minas Gerais e mais seis cultivares com resistência completa como TMG 7062 IPRO, TMG 7063 IPRO e suscetíveis à ferrugem, a BMX DESAFIO 8473RSF, BMX FLECHA IPRO, NA 5909 IPRO, MONSOY 7739 IPRO. O estudo foi realizado em duas safras de ano em condições de campo (2017/2018 e 2018/2019. As variáveis consistiram em 40 diferentes combinações de manejo, variando os controles genético, químico (trifloxistrobina + prothioconazole) e biológico (*Penicillium* spp.). Os fungicidas foram aplicados de forma isolada (ou químico ou biológico) ou associada (químico + biológico) com intervalo de 14 dias, em quatro aplicações, durante o desenvolvimento do cultivo. Foram realizadas avaliações de severidade para ferrugem asiática, Oídio e Mancha de Septoria e calculado a AACPD (área abaixo da curva de progresso da doença) e produtividade. No presente trabalho foram observadas interações entre os genótipos estudados, e os manejos com fungicida químico e biológico para AACPD e produtividade. Cultivares TMG 7062 IPRO E TMG 7063 IPRO com genes de resistência dominantes apresentaram quando avaliado o efeito genético solo, menores níveis de AACPD em ambas as safras para ferrugem, apesar de não representar em maior índice de produtividade. Genótipos com resistência parcial UFU L266 e UFU L216 apresentaram em seguida às cultivares anteriormente citadas os menores níveis. BMX FLECHA IPRO apresentou os maiores níveis de AACPD em ambas as safras para ferrugem. De acordo com os níveis de AACPD nos dois anos avaliados, a pressão das doenças influenciou a expressão da resistência. Foi observado que as combinações geraram diferenças significativas. O uso de genes dominantes para a ferrugem da soja e trifloxistrobina + prothioconazole solo e/ou associado ao *Penicillium* spp. reduziu significativamente o AACPD. Em ambas as safras, genótipos com resistência parcial e sem presença de genes de resistência tiveram maior interação com o manejo de fungicidas e melhores repostas em produtividade.

Keywords: Ferrugem Asiática da Soja, resistência parcial, fungicidas, controle, manejo.

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SUMMARY

INTRODUCTION

Soybean is one of the main items inside the Brazilian agricultural production. SINCE 2018, Brazil is the largest soybean producer around the world (UNITED STATES DEPARTMENT OF AGRICULTURE 2019). Among the various biotic constraints, plant diseases are detrimental to soybean production, negatively impacting Grain Yield and quality. Cultivar selection, environmental conditions, previous disease history, previous crop, and crop management practices are some of the factors that influence the occurrence of soybean diseases (MUELLER et al., 2016). Globally, more than 100 diseases are listed in the soybean crop, of which approximately 50 have already been identified in Brazil (SINCLAIR, BACKMAN, 1989).

With the increase and expansion of the productive areas of a single crop, the importance of the diseases increases, raising annually the risk of considerable economic impacts, together with the fluctuation of the climatic conditions. Pre-harvest losses due to diseases, animal pests, weeds, and abiotic stresses and harvest destroy yearly amount to about 35% of the total possible biological product of 3.153 mt, with 1051.5 mt being lost before harvest (MUELLER et al., 2016).

Asian soybean rust caused by *P. pachyrhizi H. Sidow & P. Sidow* has been a serious disease in Asia for many decades. Asian soybean rust (*P. pachyrhizi*) is a very destructive disease that undermines the current soybean production system in Brazil and can cause yield losses of up to 90%. The disease was first reported in Brazil in open field areas in 2001. Until now, due to limited availability of soybean resistant varieties, fungicide spray is the most used strategy for controlling ASR, although some populations of the pathogen have shown increased tolerance to certain active ingredients (GODOY et al., 2016).

Brown spot caused by the *Septoria glycines* is another secondary disease and that occurs from the beginning to end of the soybean cycle, which is disseminated to all the Brazilian soybean producing regions, differing in importance from one region to another. In field experimental studies in 2018 season, observed correlation until 64% for brown spot and yield (JULIATTI et al., 2019).

Powdery mildew caused by *Microsphaera diffusa* is an obligate plant parasite that is very common in cultivated crops such as soybean, sunflower, and bean, and on weeds growing in or around cropped fields. Soybean powdery mildew was first observed in Germany in 1921 (WAHL, 1921). In Brazil, the disease was considered of secondary

importance (FERREIRA et al., 1979). Under severe infection conditions, a control method is necessary because the fungus causes direct damage to the leaf tissue and prevents photosynthesis, resulting in prematurely falling dry leaves, which may cause yield losses ranging from 26 to 50%, depending on the phenological stage at which infection occurs (IGARASHI et. al., 2010).

The specific active ingredients registered to control mostly diseases are in three major groups of systemic fungicides, formed by the triazoles (demethylation inhibitors - DMI), strobilurins (quinone outside inhibitors - QoI), and carboxamides (inhibition of succinate dehydrogenase - SDHI) (BUTZEN et al., 2005). From the 2007/2008 crop season, it was observed that *P. pachyrhizi* populations in Brazilian fields were reported to being less sensitive to this fungicide groups. (FUNGICIDE RESISTANCE ACTION COMMITTEE 2017).

Plant resistance can be defined as the ability of the host to prevent the growth and development of the pathogen (PARLEVLIET, 1997). The pyramiding of resistance genes in a single soybean cultivar was theorized to bring more durable resistance against *P. pachyrhizi* populations in the field (ARIAS et al., 2008).

According to Parlevliet (1978), selection for partial resistance in the presence of larger genes may be undesirable, since the effect of larger genes may suppress the effect of the smaller genes under certain experimental conditions. One way to avoid erroneous selections is to use a breed with the widest possible virulence spectrum.

Martins and Juliatti (2014) aimed to perform the soybean genotypes characterization for ASR resistance to facilitate the work of breeders in the selection of promising genotypes for the use in breeding programs. It was quantifyed rust severity through some partial resistant genotypes, estimated that rust resistance is a characteristic controlled by 2 to 23 genes that are predominantly dominant.

In integrated disease management, the use of biological fungicides is gaining more and more space, as it is believed that sustainability is in making chemical and biological controls allied, allowing their use in rotation, or associated.

Considering the importance of soybean cultivation in the Brazilian economy, and the lack of information on integrated strategies to control diseases such as Asian Soybean Rust, combining genetic, chemical, and biological control, this research proposes to obtain results that will be useful for further use in soybean genetic improvement programs and productive system management programs in a practical way.

OBJECTIVES

For Integrated control, the adoption of good cultural, biological, and chemical practices is critical for resistance management successful. On this background, the study general objective was to evaluate the capacity of combining soybean genotypes that show or not different levels of resistance to Asian Soybean Rust with chemical and biological fungicide associated on the management.

The specific objectives were:

Assess the benefits associating chemical and biological fungicides with level of different soybean genotypes in the LAGER/UFU program, with partial resistance to Asian Soybean Rust and secondary diseases if they occur in field conditions;

Assess the benefits associating chemical and biological fungicides on soybean commercial materials with stainless steel technology and other commercial materials regarding to Asian Soybean Rust and secondary diseases if they occur in field conditions;

Correlate the capacity of combining genetic, chemical, and biological control aiming Grain Yeld potential;

Correlate the capacity of combining rust-tolerant soybean genotypes with chemical and biological fungicides aiming at possible synergistic / antagonistic or other effect.

1. THEORICAL REFERENCE

1.1 Crop history and agronomic aspects (*Glycine max* L. Merrill)

Soybean is commonly considered one of the oldest cultivated crops, native to North and Central China (HYMOWITZ, 1970). The first recording of soybeans was in a series of books known as Pen Ts'ao Kong Mu written by the emperor Sheng Nung in the year 2838 B.C., in which the various plants of China are described. Historical and geographical evidence suggests that soybeans were first domesticated in the eastern half of China between the 17th and 11th century B.C. (HYMOWITZ, 1970).

The soybean of five millennia ago, differs a lot from what we know today: they were ground plants that developed along rivers and lakes a kind of wild soybeans. The process of "domestication" of this culture occurred in the eleventh century a. C., from natural crosses made by Chinese scientists (ZHENG-YI; RAVEN, 2004).

The *Glycine max* species probably has the *Glycine* soybean species as an ancestral plant: Both are tetraploid but cultivated soybean has been considered a stable tetraploid with diploidized genomes (SKORUPSKA et al., 1989). Commercial cultivation begins in the early twentieth century in the United States, and in the second decade of the twentieth century, the oil and protein content of the grain attracted the attention of the world's industries. The subgenus Soja, to which *G. max* belongs, also includes *G. soja* Sieb. and Zucc. (2n=40) and *G. gracilis* Skvortz. (2n=40), wild and semi-wild annual soybean relatives from Asia. *Glycine soja* (2n=40) is a wild viny annual with small and narrow trifoliate leaves, purple flowers, and small round brown-black seeds. It grows wild in Korea, Taiwan, Japan, Yangtze Valley, N.E. China, and areas around the border of the former USSR. *Glycine gracilis*, an intermediate in form between *G. soja* and *G. max*, has been observed in Northeast China (SKVORTZOW, 1927). Interspecific, fertile hybrids between *G. max*. and *G. soja* (SIEB AND ZUCC.) and between *G. max* and *G. gracilis* (KARASAWA, 1952) have been easily obtained.

Soybeans were first introduced into the United States, in 1765 (HYMOWITZ; HARLAN, 1983). Cultivated soybean, *G. max* (L.) Merrill., is in the family Leguminosae, the subfamily *Papilionoideae,* the tribe *Phaseoleae,* the genus *Glycine Wild*. and the subgenus Soja (Moench). It is an erect, bushy herbaceous annual that can reach a height of 1.5 meters.

Soybean can be classified, according to its growth habit, as determinate or indeterminate. In cultivars of determinate growth habit, the apical meristem stops the differentiation of new leaves after floral induction, while in cultivars of indeterminate growth habit, the differentiation

of leaves occurs over some time after the floral induction (TANAKA; SHIRAIWA, 2009). In the last years, the search for soybean cultivars with indeterminate growth significantly increased in Brazil (PERINI et al., 2012).

Determinate genotypes are primarily grown in the southern United States (Maturity Groups V to X). Indeterminate genotypes continue vegetative activity throughout the flowering period and are grown primarily in central and northern regions of North America (Maturity Groups 000 to IV). Semi-determinate types have indeterminate stems that terminate vegetative growth abruptly after the flowering period. None of the soybean varieties are frost tolerant, and they do not survive. It is used in food, especially in the edible oils industry, because it is considered a protein source (AZEVEDO et al., 2010).

Soybean was introduced in Latin America sometime between 1565 and 1815, through the "Chinatown" that existed in Acapulco at the time. The earliest known reference in Brazil is from 1882, when Professor Gustavo Dutra of the School of Agronomy of Bahia wrote a four-page article on "Soy" in the Producers Journal. Soy was introduced into the country that year, and in 1892 it was being propagated as a forage crop. With the help of Japanese immigrants who had been arriving in Brazil since 1908, culture was introduced and expanded in different Brazilian regions (SHURTLEFF; AOYAGI, 1980).

Soybean arrived in Brazil, introduced from the USA, in 1882. It entered via Salvador, Bahia State (Northeast Region), where latitude is low (around 12°S to 13°S) and the climate is tropical. The American cultivars tested were adapted to temperate climate (latitudes near or higher than 30°), so they bloomed early, did not develop satisfactorily, resulting in low yields. Because of that, until 1980, soybean was restricted to the south of the country (temperate and subtropical region) (DALL'AGNOL, 2016).

Only after more than half a century of its arrival in Brazil, American soybean genotypes were screened for the conditions of the Brazilian South Region. Initially, the adapted germoplasm was used to produce biomass for feeding cattle, and the few grains produced were used for feeding pigs on farm. After the 1940s, soybean progressively toggled from biomass production to grain-producing crop. During that decade, production increased from a mere 457 t in 1941 to 25 881 t in 1949, the year Brazil first figured in international statistics as a producer of the oilseed (DALL'AGNOL, 2016).

The fast expansion began in the 1960s, when a government program to boost Brazilian wheat production was launched in the southernmost state of Brazil, Rio Grande do Sul. This program also benefited soybean as the crop entered the summer season in succession with wheat in the winter (leguminous and grass succession) to optimize the use of land as well as of

agricultural machinery. From the South Region, it expanded to the Midwest (tropical region) in the 1980s. That was possible thanks to the development of cultivars well adapted to the low latitude of the region since the new cultivars were less sensitive to photoperiodic variations (daylight span).

In the 1990s, soybean advanced towards the center north of the country (around 10°S to 12°S) and in the 2000s, it expanded farther to the north (latitudes near 0° to 5°S or 5°N). The importance of soybean for the Brazilian agricultural development has been so significant that it is possible to divide the expansion of the sector into two periods: before and after soybean. Also, it is possible to divide soybean development into two stages: development in the subtropics (up to 1980) and expansion in the tropics (from the 1980s on) (DALL'AGNOL, 2017).

The boom of the soybean production was accompanied by the production of other crops, with special emphasis on maize. Both crops together account for more than 80% of the total area and 85% of the production of grains in the country, whose growth in the period 1990–2017 was expressive (313% against only 76% in the increase of area), indicating that the increase in productivity was also significant. The soybean revolution, accompanied by the development of other crops, transformed Brazil from a food importer in the 1960's to one of the major exporters in the 2000's (DALL'AGNOL, 2017).

Cerrado soils are naturally too acidic and too poor in nutrients, the first step to make them suitable for soybean and agriculture in general was to lime and amend them with macro and micronutrients, following indications of soil analysis. The second step was to develop the tropical soybean, adapted to those conditions. These cultivars are characterized by a long juvenile period, which inhibits the early flowering of soybean under short daylight conditions (low latitude), because they are much less sensitive to photoperiodic variations. This allows soybean to be established successfully in any latitude of the Brazilian territory (CATTELAN; DALL'AGNOL, 2018). The plant is characterized to morphological feature the presence of root nodules. These can perform the biological nitrogen fixation (BNF) from the symbiotic interaction with nitrogen fixing bacteria species of the *Bradyrhizobium* genus. Another turning point was the widespread use of inoculation with nitrogen fixing bacteria (rhizobia), dispensing the use of mineral nitrogen fertilizer. That generates annual savings over than US $ 7.0 billion (NOGUEIRA; HUNGRIA, 2013), and contributes to the reduction of environmental contamination with greenhouse gases and of groundwater with nitrites and nitrates.

Technological solutions enabled the cultivation of soybean in the Cerrado Region, and it allowed the dedicated entrepreneurs who left the south in the quest for success to prosper and become the largest producers of soybean in the country. The social rise of these migrants occurred with little governmental support, and the most efficient ones became modern enterprisers, enjoying, today, a high standard of social and economic life.

According to Norman Borlaug, Nobel Peace Prize of 1970, the conquest of Cerrado should be considered one of the major achievements of the 20th century due to the amount of area incorporated into the process of food production (SILVA, 2012). It is noteworthy that, although there are big soybean farms in the Cerrado Region nowadays, some with areas over 10 000 ha, most farms are still small or medium size.

The largest Brazilian producers are Mato Grosso, Paraná and Rio Grande do Sul - respectively, they are estimating a production of 31.49, 19.16 and 16.63 million tons in 2017/2018 (CONAB, 2018). In this context, Brazilian exports of the soybean complex (grain, bran, and oil) increased from US $ 4.2 billion in 2000 to US $ 17.2 billion in 2009, which indicates the main increase of a product in agricultural exports of the period (World Soybean Production, 2014).

1.2 Asian Soybean Rust (*Phakopsora pachyrhizi*): economic impact, etiology, Symptomatology

Given the tropical climate and the large, cropped area, Brazil faces a huge challenge to control the pests and diseases that affect its production fields (DALL'AGNOL, 2017). Many diseases can affect and reduce soybean yield at a commercial scale; however, since 2001 Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* H. Sydow & P. Sydow, has affected soybean crop at economical levels, with yield reductions of up to 70% (HARTMAN et al., 2015).

Asian rust,a disease extremely aggressive under tropical conditions and, therefore, responsible for the consumption of the major part of the fungicides sprayed in Brazil. To reduce the amount of inoculum of the fungus responsible for the disease (*P. pachyrhizi*), Brazil adopts the host-free period (vazio sanitário), a period of 60 to 90 days in which the farmer is prohibited to sow or keep live soybean plants in the field to decrease the inoculum of the fungus. In addition soybean found on highways sides or elsewhere are subject to eradication (DALL'AGNOL, 2017).

P. pachyrhizi was first reported in Japan in 1903. In the early decades of that century, soybean rust was described throughout the Eastern Hemisphere, but with records of severe

epidemics only in the tropical and subtropical regions of Asia and Australia (HARTMAN; WANG; SHANMUGASUNDARAM, 1991; SINCLAIR; HARTMAN, 1999).

ASR has been a serious disease in Asia for many decades. It appeared in Africa in 1997 and appeared in the Americas fields in 2001. In the USA, it was first found in the continent, in late 2004, probably brought in by a hurricane; it was considered such a threat that it was listed as a possible weapon of bioterrorism. ASR cannot overwinter in areas with freezing temperatures, but it can spread by wind rapidly over such large distances, its development can be so explosive, and it can cause such rapid loss of leaves that it is now one of the most feared diseases in the world's soybean-growing areas (JULIATTI et al., 2017).

The greatest damage was registered in Uganda, Kenya, Rwanda, Zambia, Zimbabwe, Mozambique, and South Africa (KAWUKI; TUKAMUHABWA; ADIPALA, 2004). Deslandes in 1979 described inside soybean test fields, the presence of rust in the city of Lavras (MG), being a matter of concern for a decade by the high potential for damages in Asian countries (JULIATTI, 2003).

The non-confirmation of its Potential for damages, over the years, has reduced the priority of research on this disease, reaching the total deactivation. In the 1990/91 crop, rust epithets in São Gotardo and Presidente Olegário, in Minas Gerais and in the Federal District. These sporadic outbreaks, mainly in susceptible cultivars, indicated the destructive potential of the disease. In experimental areas of the Federal University of Uberlândia, there were severe rust in susceptible cultivars, such as MG / BR 46 (Conquista) (JULIATTI, 2003).

In the 2001/02 season, the rust reached all the soy between Encarnación and Catuetê, in Paraguay, however, the drought in the second half of the cycle, and the use of fungicides avoided greater losses. In Brazil, until 04/27/02, the disease was found in the states of RS, PR, SP, GO, MS, and MT reaching in 250 municipalities spread throughout Brazil (YORINORI et al., 2002; YORINORI et al., 2004). The greatest losses occurred in Chapadão do Sul, Chapadão do Céu and Alto Taquari, being estimated at 30-50%. At harvest time, soybean rust caused grain losses estimated at 569.2 thousand tons, equivalent to 125.5 million dollars. In this harvest the producers were totally unprepared against rust and most of the spray of fungicides was delayed (YORINORI et al., 2004b).

In the 2002/2003 season, again the producers were not prepared for the control of rust. In many crops, the use of fungicides was late due to lack of product and / or excessive rainfall, which prevented the spraying. In this crop, the losses caused by the disease were estimated at 3.4 million tons of grain. Given the occurrence of rust in 80% of the Brazilian area cultivated with soybean and the average of an additional spray of fungicide throughout this area, the

expenditures on chemical control reached an estimated US $ 426.6 million (YORINORI et al., 2004).

Rust damage cost from the 2002/2003 to the 2016/2017, reached the amount of US $ 15 billion ((EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA (EMBRAPA), 2017)). In Cerrado, the evolution of the Asian soybean rust (ASR) in relation to the Septoria brown spot was observed, which was previously the prevailing disease. The reproductive stages R2 to R5, mainly in susceptible cultivars such as MG / BR 46 (Conquista), had problems with rust epidemics. The crops in central pivots were marked as the beginning of the epicenter of rust to rainfall areas and responsible for increased inoculum in the 2003/2004 harvest (JULIATTI; POLIZEL; JULIATTI, 2004).

The losses of Brazilian soybeans, in this harvest, due to rust Asia, were estimated at 4.6 million tons, and the cost of rust, at level of producer and government, was $ 2.2 billion. During the 2004/2005 crop season, there was a drought situation in the region in the middle of the crop. The drought, accompanied by high temperatures (35 °C – 40 °C), development of rust. Loss of soybeans attributed to drought were estimated at more than 11 million tons. Despite the climatic conditions not favorable to the development of the disease, there were still where rust has developed, but in the great majority the disease has not reached the level of economic damage. Despite this unfavorable situation, there were on average, more than one application of fungicides (YORINORI, 2005).

Until 1992 *P. pachyrhizi* was recognized as the only species that causes soybean rust, but ONO, BUTIRICA & HENNEN (1992) developed a detailed study of comparison between American and Asian isolates. They demonstrated that the isolates from Asia and Australia were morphologically distinct and pathogenesis of the American isolates, being proposed the separation of the causal agent of the ASR in two species. Then, gave the name of *Phakopsora pachyrhizi* from the Eastern Hemisphere (Asia and Australia) and *Phakopsora meibomiae* from the Western Hemisphere.

Carvalho Júnior; Figueiredo (2000), related the history of crop damages in Brazil. They proposed to be *P. meibomiae* and not *P. pachyrhizi* the agent etiological analysis of the Deslandes report in 1979. The authors then suggested that in 2000, occurred in Brazil, only *P. meibomiae*. As the morphological distinction between the two species is difficult due to the formation of telia serum, which rarely form in a tropical climate, have been developed primers specific for the two species of *Phakopsora* which, through the polymerase chain reaction (PCR), quickly allow the identification of the species (FREDERICK et al., 2002). By means of

molecular analysis, the authors demonstrated the morphology of teliospores, which two species caused rust on soybeans.

Isolates of *P. meibomiae* and *P. pachyrhizi* showed only 80% similarity in the nucleotide sequence. The PCR on rust field material collected in Minas Gerais in 1979 and 1983, detected mixed infections of the two species of *Phakopsora* (AKAMATSU, FIGUEIREDO; HARAKAVA, 2004). The detection of *P. pachyrhizi* in these samples was surprising, since there was no severe attack in those years, as it is expected that occurs in the presence of *P. pachyrhizi*. through real time PCR, it was found that the DNA concentration was 100 times higher for rust American than for Asian rust. The finding that the species *P. pachyrhizi* was already present in Brazil, suggests that an aggressive race arrived in the American continent in the 2001, probably from Africa.

Despite the finding of *P. pachyrhizi* in samples the first report of the disease (YORINORI et al., 2002) is considered the initial milestone of Asian rust in the American continent, once that from that date the disease was rapidly spread throughout the Western Hemisphere and began to occur at epidemic levels in the main Brazilian soybean producing states, causing damage from 10 to 80% of production.

The symptoms or signals of ASR can appear at any time in the phenological cycle of the crop, but it has appeared more frequently in plants close to flowering. Symptoms are most frequently observed on the leaflets. The symptoms caused by ASR differ from American rust only by the predominance of the reddish-brown (RB) coloration of the lesions. In Asian rust, the lesions of the susceptible cultivars are predominantly light brown (TAN), but when in high incidence, it can cause foliar stature, resembling the foliar *Cercospora* (JULIATTI, 2018).

In resistant or tolerant cultivars, the lesions are predominantly reddish brown (RB). The initial symptoms of rust are characterized by tiny dots (1-2 mm in diameter), darker than leaf tissue, from greenish-to-greenish gray. Due to the biotrophic habit of the fungus, in susceptible cultivars, infected cells die only after abundant sporulation has occurred. Because of this, the lesions are not easily visible at the beginning of the infection (JULIATTI, POLIZEL and JULIATTI, 2004).

As the infected tissues die, the spots increase in size (1-4 mm), becoming a reddish-brown color. Progressively, the uredines, also called pustules, become light brown to dark brown, open in a tiny pore, expelling the urediniospores. The urediniospores, initially hyaline colored, become beige and accumulate around the pores or are carried by the wind. The number of uredinias / lesions can vary from one to six.

Three types of lesions may occur when different soybean cultivars are inoculated with different *P. pachyrhizi* isolates: “tan” lesions, “RB” type lesions, or type 0 lesions. is characterized by lesions of 0.4 mm^2, usually with 2 to 5 udders on the underside of the leaf, 2 weeks after inoculation and is considered a symptom of host susceptibility. In the RB-type symptom, reddish brown lesions of 0.4 mm^2 are formed, generally with 0 to 2 uredinia on the abaxial side of the leaf, 2 weeks after inoculation and is a symptom indicating the associated resistance with host hypersensitivity (JULIATTI, 2018).

Type 0 is the absence of evidence that is visible macroscopically, indicating immunity or proximity to immunity (BROMFIELD; MELCHING; KINGSOLVER, 1980). The uredinia that cease to sporulate, usually shows the pustules with their pores clearly open. *P. pachyrhizi* infection causes rapid yellowing and premature fall of leaves preventing full grain formation. The earlier defoliation occurs, the smaller the grain size and, consequently, the greater the loss of yield and quality (GODOY; KOGA; CANTERI, 2006).

1.3 Septoria Brown Spot (*Septoria glycines*): economic impact, etiology, Symptomatology

The brown spot, caused by *Septoria glycines* Hemmi is probably the most widespread soybean disease worldwide (FAO, 1995). The disease is a highly prevalent foliar disease in the United States (ALLEN; BRADLEY, 2017) and other soybean production areas, such as Argentina, Brazil, and China (HARTMAN, 2015). In 2006, the losses estimated in Brazil was over 340.000 tons (WRATHER, A. et al., 2010).

This disease has been studied epidemiologically since 1915 (WOLF; LEHMAN, 1926; LIM, 1979; LIM, 1980; PATAKY; LIM, 1981; KAMICKER; LIM, 1985), however until the 1980s there were no records of resistant soybean varieties.

There are some controversies arose about the first report. WOLF; LEHMAN (1926) reported its occurrence for the first time in the United States, in North Carolina. ATHOW (1973); LIM (1989) mention that the brown spot was registered in the United States in 1922 and for the first time described in Japan in 1951. BENEDICT (1964) records that the disease was first observed around 1934. However, it soon became evident that the disease under study was identical to that described by HEMMI (1915) in Japan (WOLF; LEHMAN, 1926).

From the last years, the disease occurrence has been evaluated worldwide and in 2006 was considered the most prevalent soybean disease during vegetative stages of crop development. It was also important in late reproductive stages, causing premature senescence.

When the symptoms of the disease reach 30% vertical progress of the plant at the R6 physiological state there is a 10% yield loss, but if the symptoms reach 80% vertical progress, then the there is a 27% yield loss (LIN et al., 2020). It can also infect pods and seeds, but the pathogen is rarely seed-borne (HARTMAN, 2015). In the field, symptoms on leaves can be observed as early as V2 to V3 stage (MUELLER, 2016) and the disease gradually develops to the upper canopy throughout the growing season (LIN etal., 2020). The incubation period (the time between infection to showing visible symptoms) of *S. glycines* has been reported to vary depending on host maturity (LIM, 1979).

Septoria Brown Spot (SBS) was first described in Brazil at 1972 (LUZZARDI et al., 1972). First experimental studies proved this disease can cause damages until 30% (ALMEIDA, 1980). Brown spot occurs from the beginning to end of the soybean cycle, which is disseminated to all the Brazilian soybean producing regions, differing in importance from one region to another. In recent studies in field in 2018 season, observed correlation until 64% for brown spot damage and yield losses in foliar severity at 80% (JULIATTI et al., 2019).

The fungus *Septoria glycines* has a teleomorphic form of *Mycosphaerella unspenkajae* Mashk. & Tomil, not yet identified in Brazil. After the death of the infected tissue, the fungus produces globose-shaped pycnids (60-125μm in diameter) inside, with openings in the lower and upper surfaces of the leaves. Inside the pycnids, the hyaline, filiform, curved conidia are formed, with 1-3 septa, and measuring 21-50 μm x 1.4-2.0 μm. Under abundant humidity, a mass of conidia is expelled through the pores, forming cirrus circles (ATHOW, 1973; LIM, 1989; MCGEE, 1992).

The primary inoculum can originate in the crop residues and infected seeds (ITO; TANAKA, 1993). From the cotyledons, or the remains of previous culture, the fungus can infect the primary leaves (ATHOW, 1973; LIM, 1989; MCGEE, 1992). The dispersion of the conidia only occurs through the action of rain that suspends the conidia in droplets that are carried and deposited on the surfaces to be infected (ALMEIDA et al., 2005).

Regarding the variability of the pathogen, despite few studies, no pathogenic variability was detected among 25 isolates of *S. glycines* from different parts of the USA (KAMICKER; LIM, 1985). MMBAGA (1980) studied the morphological variability in culture medium of *S. glycines* isolated from different locations, Wisconsin, Iowa, Indiana, Illinois, Minnesota, Michigan, and Brazil. After the evaluations were carried out, it was not evident to the author morphological variability among the isolates in the soybean culture, although the latter observed differences in the growth and sporulation of the pathogen in response to different temperatures.

The disease can appear at the seedling stage shortly after planting, but it becomes more severe when it oceurs near maturity. This disease is one of the most important of the so-called end-of-cycle complex, causing considerable damage at the late soybean growth stages. It must be considered, however, that its presence in the initial stages determines its severity during the final stages of the soybean crop. Therefore, the higher the disease incidence and severity at early vegetative stages, the higher is the disease incidence and damage at maturity. Damages can include premature defoliation, a shortened life cycle, and yield loss (MANTECÓN, 2008).

It also may occur on stems and pods as plants approach maturity Infection, usually initiated by conidia from pycnidia that overwinter on diseased plant debris in the soil, is most prevalent in fields planted to soybeans in consecutive years. Warm, moist weather and poor drainage favor the spread of the disease (WOLF, 1926). The severity of the disease increases with an increase in the wetting period from 6 to 36 hours and the optimal temperature for the development of the disease is 25°C, with symptoms developing between 15 to 30°C (ALMEIDA et al., 2005).

The fungus survives on infected leaf and stem residue. Warm, wet weather favors disease development. Disease usually stops developing during hot, dry weather but may become active again near maturity or when conditions are more favorable. Spores developed on cotyledons and unifoliate leaves are the inoculum for later infections of trifoliate leaves, stems, pods, and surrounding plants (MANTECÓN, 2008).

1.4 Powdery mildew (*Microsphaera diffusa*): Economic Impact, Etiology, Symptomatology

Powdery mildew is one of the most important and well-studied groups of plant pathogenic fungi. The term "powdery mildew" is used to denote both the disease and the group of fungi Ascomycetes, of the order *Erysiphale*s, family *Erysiphaceae*. Powdery mildew is an obligate plant parasite that is very common in cultivated crops such as soybean, sunflower, and bean, and on weeds growing in or around cropped fields. Some species are host-specific, and others can infect a wide range of plant species (GLAWE, 2008).

Soybean powdery mildew was first observed in Germany in 1921 (WAHL, 1921). The levels of damage caused by soy mildew have been different, depending on the locality and climate of the cultivation region, disease management and genotypes used. The first reports of losses in production date from 1972 and 1973 in Georgia, United States, as well as from 1975 in Iowa, when there was widespread disease and significant economic losses (SARTORATO; YORINORI, 1997). Yield loss due to infection of *M. diffusa* in some countries has been

reported to reach 30% when *M. diffusa* infects at the beginning of plant growth in some susceptible varieties (PHILLIPS, 1984). Powdery mildew disease was also reported in Brazil where the disease occurred throughout the planting area of soybean with yield losses up to 40% in recent studies (ALAMEIDA et al., 2008).

According to AGRIOS (2004), when severely affected by the disease, soybean crops can estimate yield between 30 and 40%. The first year of the powdery epidemic in Brazil occurred in the 1996/1997 harvests, when it reached soybean production areas from the central Cerrado to Rio Grande do Sul, causing average losses between 15 and 20%, with extremes of 50 to 60% (YORINORI, 1997). Since then, changes in the climate in subsequent harvests, with predominance of droughts and high temperatures, made the South region and the Cerrado over 800 m suffer from greater severity of the disease (SARTORATO; YORINORI, 2001). In a more recent study, IGARASHI et al., (2010) also observed productivity losses varying between 26 and 50%.

M. diffusa is a mandatory disease and has capacity to infect the aerial part of the soybean plant, including stems, petiole, and pods, however, its occurrence is mor common in leaves (SILVA, et al., 2013; YULIA et al., 2017). Its pathogenesis begins with deposition of its conidia on the leaf, resulting in a layer of white and powdery mycelium. With the advance of colonization and the colony aging, the color of fungal structures changes from white to gray, brown (YULIA et al., 2017). The fungus survives in voluntary soybean plants and is easily spread by the wind, which makes more difficult management practices.

These fungi are characterized as whitish colonies with a powdery appearance on the surface of the entire aerial part of living plants, especially the leaves. They are fungi highly evolved and specialized, with a very restricted range of hosts, generally not exceeding the limits of a single host family, most of them being restricted to only a few species of a single genus (STADNIK, 2001). They are presented in anamorphic forms, corresponding to the asexual phase, and teleomorphic forms, corresponding to the sexual phase. They occur in all regions of the planet and in most cultures. Although they rarely cause the death of the plant, they deplete its nutritional reserves, thus dramatically decreasing the productive potential of the crop (STADNIK; RIVERA, 2001).

Soybean powdery mildew, in its anamorphic form, is identified as *Oidium* sp., which is the most observed form in the field and in a greenhouse. According to ALEXOPOULOS AND MIMS (1979), this fungus belongs to the class Deuteromycetes, subclasse *Hyphomycetidae*, order *Moniliales* and family *Moniliaceae*.

Oidium sp. presents hyaline, septate mycelium with thin walls that superficially penetrate the host tissue. It is limited to the epidermis of the leaf and does not passes through the stomatal chamber. Has pressure gauges for fixing the mycelium on the surface leaf and haustoria initiation, which, in turn, are formed inside the cells epidermal, absorbing nutrients from the host. Unbranched conidiophores are short and thin, formed from one or more cells, giving rise to an upright chain of conidiospores (GLAWE, 2008).

These present a maturation from the most distal towards the base of the chair and are cylindrical, hyaline, single-celled, uninucleated, vacuolated, thin-walled, containing oil drops and various granules; its dispersion occurs mainly by the wind (MENEZES; OLIVEIRA, 1993; MIGNUCCI; CHAMBERLAIN, 1978; STADNIK, 2001; YORINORI, 1997).

This anamorphic form being the most found in culture, its mycelial development occurs at temperatures between 18 and 30 ° C (YORINORI, 1997a) and in relative humidity between 50 and 70% (AGRIOS, 2004), but it can also occur with low relative air humidity (GHINI; HAMADA; BETTIOL, 2011).

This form predominates in the autumn / winter period, and infection can occur at any stage plant development; however, it is more common in stages between early flowering and full filling of pods (YORINORI, 1997). The fungus, in its teleomorphic form, is classified as *Erysiphe diffusa* (Cooke and Peck) U. Braun and S. Takam. Although powdery mildew disease was initially attributed to the fungus *Erysiphe polygoni* DC. Merat, which in fact can infect soy, the fungus *Erysiphe diffusa* is now recognized as the main cause of the disease and has the synonym *Microsphaera diffusa* Cooke and Peck (SARTORATO; YORINORI, 2001; TANAKA et al., 1993).

This fungus belonging to the Ascomycetes class, subclass Hymenoascomycetidae (Pyrenomycetes) order Erysiphales and family Erysiphaceae (ALEXOPOULOS; MIMS, 1979). *Erysiphe diffusa* presents differentiated gametangiums, the anteriad being smaller than the ascogon, and performs sexual reproduction through plasmogamy to, thus, to form the cleistothecium (SARTORATO; YORINORI, 2001). These have coloring ranging from white, yellow, pink, brown, dark brown or reddish, when immature, and rust-brown to black, when mature, with an average number of twenty mycelial appendages with simple apex of indefinite size (MENEZES; OLIVEIRA, 1993; SINCLAIR, 1999).

The ascocarps are formed in a superficial mycelium, with no stroma formation. They have several walls with thick walls that surround the ascospores, being fixed in the hymenium and having the mycelium septated, branched and with generally uninucleate cells (MENEZES;

OLIVEIRA, 1993). With the maturation of ascos, the ascospores are released, which, like the conidiospores, they are also dispersed by the wind (LOPEZ; RIVERA, 2001).

Formation of cleistothecium is very rare, requiring low temperatures; however, so far, its presence in soy in Brazil has been reported (MENEZES; OLIVEIRA, 1993; SARTORATO; YORINORI, 2001).

Symptoms of the disease are in the form of white patches of mycelium and conidia of fungi growing on plants, especially on the upper surface of the leaves that are then enlarged and covered the entire surface of the leaf. Other symptom is the emergence of patches of green and yellow islands on leaves. Powdery mildew disease can lead to high yield losses. Disease that occurs can lead to the decline in the quantity, weight and physical quality of seeds and reduce germination of seeds.

1.5 Genetic resistance: Partial and Dominant resistance as soybean diseases management strategies

The genetic resistance to diseases can be defined as a host ability to prevent the growth and development of the pathogen (PARLEVLIET, 1997). Partial resistance is a characterization of the reduction in epidemic rates, by reduction of number and size of lesions, decrease in spore production and increase on latent period. This causes the population of the pathogen to be reduced, and consequently a decline in the amount of inoculum and intensity of the disease (WANG; HARTMAN, 1992). This type of resistance became evident and important when a monogenic resistance is overcome by a new breed of pathogen (PARLEVLIET, 1997).

Seven “major” genes for resistance (*Rpp*1, *Rpp*2, *Rpp*3, *Rpp*4, *Rpp*5, *Rpp*6 and *Rpp*7) to *P. pachyrhizi* have already been identified in plants of the genus *Glycines* (BROMFIELD; HARTWIG, 1980; HARTWIG, 1986; CALVO et al., 2008; LI et al., 2012; LIU et al., 2016). However, these genes confer resistance to a limited number of rust isolates, these specific resistance genes are quickly overcome, since the pathogen presents high genetic variability and although the occurrence of pathotypes denounces this characteristic, little is known about this variability (BROMFIELD; MELCHING; KINGSOLVER, 1980; BONDE et al., 2006; CARNEIRO et al., 2007).

The presence of multiple virulence genes in the pathogen and the absence of multiple resistance genes in the host confers a major competitive advantage to rust, reducing the expectation of using gene rotation or pyramiding as a measure for disease control, since the

pathogen generally retains virulence genes that may or not be expressed in their life cycle (HARTMAN; WANG; SHANMUGASUNDARAM, 1997).

Marchetti; Uecker; Bromfield (1975) comparatively analyzed the development of uredinia in tissues of Lee 68 and PI 200492, and concluded that slower uredinial development, shorter period during which new uredinial form, and earlier senescence of uredinia, variables used to quantify partial resistance, contribute to the reduction in the amount of secondary inoculum, thus diminishing the potential for pathogen spread in the field.

According to Vello; Brogin; Arias (2002), numerous genotypes vertical resistance has not been stable in different regions of the world. Bromfield (1975) reported that the introductions of PI 200499 and PI 200492 (*Rpp1*), with resistance to soybean rust, were used as sources of resistance in breeding programs in Taiwan and Australia. Singh et al. (1974) described the magnitude of resistance in this plant introductions PI 200465, PI 200466, PI 200477, PI 200490, PI 220492 (*Rpp1*) and PI 200468.

Sinclair and Shurtleff (1975) considered three sources of vertical resistance: PI 200490 and PI 200492 (*Rpp1*) and PI 230970 (*Rpp2*), in addition to the cultivar Ankur (PI 462312, with the *Rpp3* gene). BERNARD et al. (1991) released three genotypes derivate of William 82 with resistance to rust L85-2378 (*Rpp1*), L86-1752 (*Rpp2*) and L87-0482 (*Rpp4*). Hartwig (1996) identified as a source of resistance the lineage D86-8286 (PI 518782), and a second lineage, which had as donor *Rpp4* gene, to PI 459025.

The *Rpp*6 gene was mapped on the PI 567102B to a third *Rpp* locus on the chromosome 18 approximately 40 cM from the *Rpp*4 locus and about 66 cM from the *Rpp1* locus (LI et al., 2012; LIU et al., 2015). Until now six loci were reported in the literature and patent claimed at least 10 other loci associated with soybean rust resistance, and recessive resistance genes are present in different loci on the PI 200456 (*Rpp*5) (GARCIA et al., 2008; BAILEY et al., 2014). Childs et al., (2017), reported a new resistance gene (*Rpp7*), this gene was mapped to a 154-kb interval on chromosome 19 on a different genomic location and not related to any previously reported *Rpp* genes.

In the United States, resistance to ASR was evaluated in more than 16,000 genotypes with a mixture of rust isolates about 3000 genotypes were selected based on low visual severity and presence of RB lesions. Afterwards, about 800 genotypes were selected and among them, the authors believe that resistance genes could be incorporated into the commercial cultivars (MILES; FREDERICK; HARTMAN, 2006). But the ability to develop cultivars with the pyramiding of *Rpp* genes is limited by some factors like the presence of various germplasm accessions with the same *Rpp3* locus, the limitation of recombination by genotypes with closer

genetic background (*Rpp1* and *Rpp4* loci are only about 30 cM apart) gene not providing resistance to native populations of *P. pachyrhizi* and poor agronomical characteristics (yield) in the occurrence of gene introgression (WALKER et al., 2014; KING et al., 2015; HARRIS et al., 2015).

Lesion color is known to be controlled by resistance genes of *Rpp*, and usually this reaction is considered when selecting resistant genotypes, but screening on soybean germplasm for additional sources of resistance has not revealed genes that, individually, confer stable resistance in the modern agronomic setting (YAMANAKA et al.,, 2010; YAMANAKA et al., 2013; KAWASHIMA et al., 2016).

Also, the variation among genotypes makes difficulty to group and phenotype when a limited number of lesion types, such as RB (Resistant) and TAN (Susceptible) and their mixture could result in variation of lesion color when a higher number of pustules in present (MILES; FREDERICK; HARTMAN, 2006; INAYATI; YUSNAWAN, 2016). Given the rapid breakdown of *Rpp*s gene from 1 to 6, there is a concern that the fungi are adapting and may have developed new specific resistance genes during the field season (PAUL et al., 2013; AKAMATSU, 2013; KAWASHIMA et al., 2016).

The “vertifolia effect” hypothesis or products derived by gene pyramiding, were related with loss of horizontal resistance which occurs during breeding for vertical resistance. Its meaning was later extended to include the loss of horizontal resistance that occurs during breeding under the protection of pesticides and in the appearance of virulent pathogens race who break the resistance (VANDERPLANK, 1963). Also, the resistance of soybean genotypes to rust can vary temporally and geographically (KATO; YORINORI, 2008; AKAMATSU et al., 2013; PAUL et al., 2013; TWIZEYIMANA; HARTMAN, 2012; WALKER et al., 2014).

Lately, rust samples collected in Brazil have been tested for sensitivity to these fungicides since 2007 by FRAC. These fungicides performance was still good, however for the first time in the 2015-16 and particularly in the 2016-17 crop, areas under intensive use of SDHIs and with conditions of high disease pressure, these fungicides presented a loss of performance. Samples of ASR (ASIAN SOYBEAN RUST) populations collected at these sites, indicated a mutation in the C subunit at position I86F (FRAC, 2017). Also, the higher severity of rust in the Brazilian savannah (Cerrado) in 2003-2004; 2015-2017, and the fact that resistant cultivars with *Rpp* genes are susceptible to *P. pachyrhizi* isolates from the Brazilian savannah, are a clear indication of the genetic variability of the fungus (JULIATTI et al., 2003; YORINORI, 2004; JULIATTI et al., 2017). An issue still unsolved is the possible occurrence

of a new pathotype in regions where there was practically no record of the ASR in the previous year of production.

Test reactions carried out in EMBRAPA and Paraguay, with Brazilian isolates from the 2002-2003 season interacting with germplasm resistant to *P. pachyrhizi* (*Rpp*1, *Rpp*2, *Rpp*3 and *Rpp*4), showed that their response was very similar, with several germplasms behaving as resistant. However, when these were inoculated with Cerrado isolates, they were compared to the tests performed in the United States, with a Zimbabwe isolate, and the Cerrado isolate was concluded to being practically identical to that of Zimbabwe (JULIATTI et al., 2005; MARTINS et al., 2007).

Due to the variability of the pathogen, especially in the Brazilian savannah (YORINORI, 2004), studies for the identification of resistant cultivars should be carried out, especially of commercial cultivars that present partial resistance. Hartman; Miles; Frederick (2005) pointed out that fungus variability is the main factor in the breakdown of vertical resistance genes. After reports and confirmation cases of ASR resistance to the *Rpp* genes and groups of chemical fungicides, the alternative of partial resistant cultivars, is gaining importance in this scenario of uncertainties.

In the actual agricultural scenario, where higher costs and more time are involved in the development of new molecules to control ASR, we can raise the life span of fungicides on the market by reducing the rates of efficacy drop with the adoption of partial resistance in disease management (JULIATTI, 2018)

Since none of the known *Rpp* genes provides resistance against all isolates of *P. pachyrhizi* (HARTMAN et al., 2005) and the ability of ASR to overcome single-gene qualitative resistance has been reported (HARTMAN; MILES; FREDERICK, 2005), development of durable ‘‘less-rusting” and ‘‘slow-rusting” cultivars is one of the options for breeding for resistance to soybean rust (LI; YOUNG, 2009).

For cultivar development and yield improvement a durable and stable resistance like partial resistance can provide an economic and environmentally friendly way to protect soybean crops from the majority *P. pachyrhizi* pathotypes on different geographical regions. When durable resistance is the goal in a breeding program it is a necessary to realize that there is no guarantee that the resistance selected for is indeed durable. Only time and exposure of these genes on a large-scale production can give us the definitive answer (PARLEVLIET, 1997). Is possible to increase the probability of durable resistance considerably by concentrating on resistance that have lasted for a considerable time, with components of partial resistance that

can be expressed with “major” or/and “minor” genes and avoiding genes that showed notorious short lifespan (PARVLIEVET, 1980).

When a plant breeder, seeks resistance to a given pathogen, he often crosses a cultivar with a resistance gene or line with a locally adapted that is not extremely susceptible to the pathogen. Is common to this parent possess variable levels of partial resistance, in fact cultivars or lines without any partial resistance are rare (PARLEVLIET; KUIPER, 1977; NIKS, 1983; PARLEVLIET, 1978; PARLEVLIET et al., 1979). Parlevliet; Van Ommeren in 1975 related that different stages of the soybean cultivars, at the inoculation and evaluation, may result in incorrect analysis to quantify resistance.

The partial resistance expression of late cycle genotypes is different from those of early cycle genotypes, also differences in the amount of inoculum applied may result in underestimation of disease levels (PARLEVLIET, 1981). Under such conditions major gene resistance shows up very well, but partial resistance expression, however, could disappear. Especially with wind-borne pathogens like the rust, the level of partial resistance in this case, can be seriously underestimated or overestimated. In an earlier period, the differences between genotypes could not reach the maximum values, at a later period they tend to disappear (VAN DER PLANK, 1968).

Martins and Juliatti (2014) studying the partial resistance in the control of Asian rust, quantified the severity of the disease through the parents and their respective F2 and F3 generations (Caiapônia x IAC-100 and Luziânia x Potenza crosses). From these data, they estimated the mean and variance of the genetic components to obtain the number of genes also the broad- and narrow-sense heritability’s. They concluded that rust resistance is a characteristic controlled by 2 to 23 genes that are predominantly dominant, and the estimate of narrow-sense heritability was greater than 70% for the Caiapônia x IAC-100 cross, and the wide-sense heritability was greater than 60% for the Luziânia x Potenza cross, leading to a conclusion that is possible to successfully select resistant individuals in early generations.

The parental variety’s IAC100, Luziânia, Caiapônia and Potenza, also are the base for several crossings to obtain some of the genotypes. The cultivar IAC100 is reported to have resistance against the complex of stink bugs (MCPHERSON, 2007), the parental IAC100 also was related to have partial resistance against soybean rust infection sharing this trait with the cultivar Potenza (SILVA; JULIATTI; SILVA, 2007). Carneiro (2007) studying rust epidemics in the Tianá e E-313 cultivars, obtained asymptotical stabilization of disease on severity levels much smaller than 1, and the author considered an evidence of partial resistance on those cultivars.

The greatest difficulty in the development of partial resistance cultivars is the evaluation of segregated population lines and distinct maturation periods. Besides this physiological difference, there is also a difference in environmental conditions influencing maturation. A series of field trials were conducted in 1985 by Tschanz and Wang to obtain disease progress curves under different environmental conditions. The authors concluded that ASR resistance was influenced by environmental factors or physiological effects. This fact was confirmed in other rust severity assessment, when instability of the rust severity was displayed by some parental soybean lines (PIEROZZI et al., 2008).

The influence of plant age and defoliation caused by *P. pachyrhizi* infection was studied by MELCHING et al. (1989). Plants with 15 to 20 days after sowing were more susceptible than plants with 50 days after sowing. The older leaves were more susceptible than the younger ones because they produced larger lesions, more spores per lesion, more lesions per cm^2 and earlier latent period. Furtado (2007) also observed that the disease is more severe in the older trefoil of soybean plants. According to Piovesan et al., (2009) resistance stability is evaluated by inspecting the points near the plant origin, which correspond to more stable environments and genotypes.

Martins and Juliatti in 2011 emphasized that genealogical analysis of genotypes, provided by crossings of parental cultivars BRSMG Liderança, may have contributed to the stability of these genotypes for rust resistance. Also learning the metabolic pathways involved in response to *Phakopsora pachyrhizi* on partial resistant soybean and quantify metabolic differences between infected plants with different susceptibilities levels is important on the development of improved cultivars that produce more stable yields under different environmental conditions.

Resistant soybean to rust would present responses like those found in the defense routes activated in drought periods, like formation and distribution of epicuticular wax, increase of lignin content on cell wall and enzyme activity. Therefore, the assessed of the cultivars performance at different environments aiming cultivars more specific and suitable cultivars for any environment on the rust occurrence, and the best combination of tactics to control ASR will help producers and researchers in their crop planning decisions and breeding programs (JULIATTI, 2018).

The search to find soybean cultivars resistant to *S. glycines* has been going on for decades. Athow (1973) detected differences in susceptibility between genotypes. Lim (Unpublished data) and Lim (1979) evaluated more than 7000 strains in a germplasm bank in the United States, but no source of resistance was found. According to Young and Ross (1979),

a high level of resistance was not found in the 626 strains tested in the seedling stage in a greenhouse and full pod stage in the field.

To find alternatives to locate some source of resistance to *S. glycines*, Lim (1979; 1983); Young and Ross (1978) observed in the evaluation of experiments for resistance to *S. glycines* there are two distinct types of brown spot lesions in the infected soy. Reddish brown angular spots surrounded by a yellow (chlorotic) area that are associated with plants from yellow seeds, and dark brown angular spots with no yellowish surrounding area (non-chlorotic) that are associated with plants from green seeds.

Lesions surrounded by yellowish area over time coalesce and produce extensive yellow areas, the leaves that have these lesions fall prematurely and possibly lead to a decrease of productivity. Non-chlorotic lesions, on the other hand, would limit the loss of the photosynthetic area to the disease (YOUNG; ROSS, 1978).

To prove this theory, LIM (1979 and 1983) studied the effect of both types of lesions on the development of the disease in the field and found that the percentage of diseased leaf area of non-chlorotic lesions was lower than that of chlorotic lesions in the soybean R1 stage. However, at stage R7, all plants were severely ill and there was no difference in the severity of the brown spot between the two types of lesion. There were no significant differences in severity, apparent infection rate, productivity, or number of defoliated nodes between chlorotic and non-chlorotic lesions.

In Brazil, Almeida (1980) studying the reaction of soybean genotypes to *S. glycines* observed, in a greenhouse, that certain soybean genotypes showed differences regarding the incubation period and the intensity of leaf symptoms and levels of yellowing of the limbus leaf. Almeida (2001) found the occurrence of different levels of susceptibility among soybean genotypes for the pathogen S. glycines.

Juliatti et al., (2006), revealed that it would be possible to find resistance of cultivars to *S. glycines*. Evaluating the reaction of soybean to leaf diseases, the authors found field resistance in some strains to brown spot.

Studies on the inheritance of soybean resistance to powdery mildew show that two genes are responsible for resistance. A dominant gene, called *Rmd*-c, keeps the plant resistant throughout the soybean cycle (LOHNES; BERNARD, 1992). Another gene, also dominant and called *Rmd*, is responsible for conferring adult plant resistance (MIGNUCCI; LIM, 1980). In this case, the plants have susceptibility in the initial stage of development, however, acquire resistance to the measure reaching the adult stage. Under conditions less favorable to soy mildew, plants with the *Rmd* gene have little or no powdery mildew in the field.

Apparently, the pathogenic variability of the fungus has overcome the resistance of some cultivars, demanding continuous work of genetic improvement and studies of the diversity of genes resistant in the available germplasms. The presence of larger genes in the inheritance of resistance to powdery mildew is also reported by MIGNUCCI; LIM (1980).

Complete resistance is controlled by a genedominant *Rmd*-c, allelic to the gene that confers resistance to the adult plant (*Rmd*). *Rmd-c* gene originates from the CSN cultivar and is associated with the Rps2 gene that confer resistance to *Phytophtora* root rot (*Phytophtora megasperma* var. *oyae*) (LOHNES; BERNARD, 1992).

In Brazil, inheritance studies have also been performed and indicating the presence of a single gene controlling resistance to powdery mildew soy (GONÇALVES et al., 2002; UNÊDA-TREVISOLI et al., 2002; ARIAS et al., 2004).

The *Rmd* resistance gene, present in the Monsoy cultivar, is linked to the E3 gene which gives the characteristic of flowering delay when the natural length day is prolonged for 20 hours using fluorescent light (BUZZELL; PALMER, 1989). The *Rmd* gene, in turn, is linked to the Rj2 gene, which confers the characteristic of non-nodulation with certain strains of *Bradyrhizobium japonicum* and the *Rps*2 gene, which provides resistance to *Phytophothora* rot.

The order of these genes on the map genetic value of soy is *Rj2RmdRps2* (LOHNES et al., 1993). This sequence has been confirmed with mapping in the linkage group J of the soybean, thus identifying genes linked to resistance (Resistance-Like Gene - RLGs) and correlated with the genetic map of soy obtained by the USDA-ARS (POLZIN et al., 1994; GRAHM et al., 2002).

There is an indication of that the same *Rmd* gene is linked with the locus for resistance to brown rot of the soybean stem (*Phialophora gregata*), therefore it is recommended that these genes be used as markers in soy improvement programs for resistance to these diseases (LOHNES; NICKELL, 1994).

2.6 Diseases chemical control, fungicides resistance risk and management strategies

According to Hewitt (1998); Azevedo (2007), the importance and use of fungicides in agriculture has increased rapidly in recent years due to the combination of a series of biological qualities, among them: high fungitoxicity to several pathogens that cause major diseases such as rusts, powdery mildew, and leaf spots, especially in cereals, quick penetration and translocation in plant tissues with uniform distribution; eradicative/curative action on infections already begun, being used based on preestablished control levels, avoiding costs with

preventive applications, often unnecessary and with prolonged residual effect, enabling the use of lower doses and/or longer intervals between applications, thereby reducing the number of sprays.

The success of a phytosanitary treatment program for the control of several diseases primarily depends on the use of a fungicide of proven efficiency and of a technology developed for its application. The influence of uncontrollable meteorological, biological, and agronomic factors should also be considered (AZEVEDO, 2007; BOLLER, 2007).

Fungicides manufactured to control diseases are effective, but the success will largely depend on proper application. Proper application starts with selecting the right equipment, specifically nozzles, and spraying the right amount of fungicide uniformly across the field before the disease is detected. Pesticide manufacturers have invested heavily to determine the most effective as well as economical application rate for the fungicides labeled for soybean rust (AZEVEDO, 2007; BOLLER, 2007).

The control of Asian soybean rust is a major concern for soybean producers in Brazil. Considering the plant development stage at the time of applications, often with complete closure and large leaf area, it is generally agreed that the application techniques need to provide droplets with good penetration and coverage of leaves, even for fungicides with systemic action (GODOY et al., 2007).

Fungicides from chemical groups of triazoles, strobilurins, carboxamides, and, from the last five seasons, the protectants are the mostly used to control the disease, with difference in the preventive and curative efficiency between the active ingredients within each group (AZEVEDO, 2005; ALMEIDA et al., 2005; JULIATTI et al., 2006).

Fungicides known as QoIs (quinoline outside inhibitors) are broad-spectrum fungicides and include three families of strobilurin fungicides and two others represented by compounds fenamidone and famoxadone (BUZZERIO, 2007). The mechanism of action of strobilurins occurs through inhibition of the mitochondrial respiration, which blocks the electron transfer between cytochrome b and c_1 at the Qo site, interfering with the ATP production. Strobilurins are referred to as “QoI” or Group II fungicides, which is simply a reference to their unique mode of action. The fungitoxicity of strobilurins has been one of the reasons for using these compounds in programs aimed to control diseases in plants. In the specific case of soybean rust, these fungicides have been widely used in mixtures with triazoles (AZEVEDO, 2007).

The use of simple formulations for this disease is unusual, although there are registered products. From the Products tested and registered for soybean rust, pure, or in mixtures with

triazoles, it could be observed that the dosages of these compounds range from 0.20 to 0.5 L of cyproconazole. The translocation of strobilurins certainly is not one of the most important features of this chemical group of fungicides. The fungitoxicity, the action spectrum and the effective residual period are peculiar characteristics of this group of products, but there are differences in the translocation of these compounds when applied to control soybean rust. The main strobilurins registered in Brazil to control soybean rust are: azoxystrobin, pyraclostrobin, trifloxystrobin, and picoxystrobin (JULIATTI et al., 2017).

Triazoles are versatile organic fungicides of broad spectrum, with apoplastic preferential systemicity, eradicative/curative action and long residual effect. Chemically, they are formed by the addition of different radicals to a basic molecule of 1,2,4-triazole. They are classified as (a) triazoles with keto: triadimefon radicals; (b) triazole with ketal: propiconazole radicals and etaconazole; (c) triazoles with hydroxy: triadimenol radicals, bitertanol, and dichlobutrazole; (d) triazoles without other functional groups: fluotrimazol (LYR, 1995).

The systemicity for specific fungicides (strobilurin and triazole) to control soybean rust has been demonstrated in an experiment conducted under controlled conditions. Analyzing the behavior of strobilurins, it could be observed that azoxystrobin has a mild redistribution throughout the leaf, moving through the xylem, following the transpiration stream, thus proving its systemic effect. Pyraclostrobin is only visible in nervures and at low concentrations; not spreading to the rest of the leaf, showing no significant systemic effect (JULIATTI et al., 2017).

The main triazoles registered in Brazil to control soybean rust are cyproconazole, difenoconazole, epoxiconazole, fluquinconazole, flutriafol, tebuconazole, tetraconazole, metconazole, and prothioconazole. There are some triazoles + triazole mixture registered propiconazole + cyproconazole, cyproconazole + difenoconazole. The main of triazoles and strobilurin mixtures registered for the control of soybean rust are: azoxystrobin + cyproconazole, azoxystrobin + tebuconazole, azoxystrobin + flutriafol, epoxiconazole + pyraclostrobin, trifloxystrobin + cyproconazole, trifloxystrobin + prothioconazole, trifloxystrobin + tebuconazole, picoxystrobin + cyproconazole, and picoxystrobin + tebuconazole. As main triazoles and benzimidazoles mixtures to control soybean rust are methyl-thiofanate + flutriafol, epoxiconazole + methyl-thiofanate, carbendazim + flutriafol and tebuconazole + carbendazim. In 2016 was launched the first triple ready mix with triazole + carboxamide and strubilurin (JULIATTI et al., 2017).

Fungicides known as SDHIs include eight different chemical groups of carboxamides repre- sented by phenyl-benzamides, phenyl-oxo-ethyl thiofene amide, pyridinyl-ethyl-benzamide, furan-carboxamides, oxathiin-carboxamides, thiazole-carboxamides, pyrazol-

carboxamides, and pyridine-carboxamides. The mechanism of action of carboxamides occurs on the target enzyme succinate dehydrogenase (SDH, so called complex II in the mitochondrial respiration chain), which is a functional part of the tricarboxylic cycle and linked to the mitochondrial electron transport chain. SDH consists of four subunits (A–D) and the binding site of the SDHIs (the ubiquinone binding site) is formed by the subunits B–D (JULIATTI et al., 2017).

Carboxamides are broad- spectrum fungicides that inhibit fungal cell respiration, which prevents energy production and leads to rapid cell death. While it may not be critical to know how carboxamides work, it is important to recognize the SDHI designation and be aware that all carboxamides have the same mode of action. The new broad-spectrum fungicides class has been quickly adopted by the market, which may lead to a high selection pressure on various pathogens. All the 17 marketed SDHI fungicides bind to the same ubiquinone-binding site of the SDH enzyme. Their primary biochemical mode of action is the blockage of the TCA cycle at the level of succinate to fumarate oxidation, leading to an inhibition of respiration (SIEROTZKI, 2013).

The protective fungicides are intended to ensure the protection of plants before pathogen attack. They must be applied before pathogens infect, forming a protective barrier toxic for fungi and bacteria in plants. When applied to the surface of plant organs, exert a toxic barrier preventing the penetration of fungi by inhibiting the spore's germination process. The characteristic of the contact protective fungicides as not to penetrate the plant is essential that they do not become phytotoxic to plants (JULIATTI et al., 2017).

Recently, after problems in efficacy with the two most used fungicides group DMI's, QoI's and SDHI's due to sensitivity reduction of Asian rust in soybeans, some multisite groups as copper-based, dithiocarbamates, and chloronitriles products have been tested in combination with more specific systemic products to the disease to improve the effectiveness and resistance management (JULIATTI et al., 2017).

Fungicides copper base are contact products and are characterized by forming a toxic barrier that prevents the germination of spores on the surface of the sheet, as altered metabolism and inhibits proteinic and enzymatic action over 20 mechanisms impeding the penetration of the fungus in the tissue leaf. A low risk of resistance due to the large number of work sites in the pathogen. It is necessary to caution in the preparation and application of fungicides, because in some situations can cause phytotoxicity or burning the plants. Other care and constant hustle to keep the product in suspension evenly and avoid settling in the application tank bottom is fundamental (JULIATTI et al., 2017).

Phthalonitriles (chlorothalonil) are characterized by benzene ring formed only by carbon. In cyclic structures from group lying one nitrogen atom and may also be a sulfur atom depending on the formulation and active ingredient. These fungicides are rapidly metabolized in plants and become constituent proteins. The mode of action of the heterocyclic nitrogen is of the interference of DNA and RNA synthesis exhibits good protective action depending on the concentration used (JULIATTI et al., 2017).

The dithiocarbamates fungicides mark the beginning of the use of organic fungicides. They are derivatives of carbamic acid compounds and generally have a broad action being one of the most used fungicides consumption. Dithiocarbamates were originally used in the rubber production process. The first dithiocarbamate fungicide known was patented in 1934. Since then, new generations of dithiocarbamates base metal salts (ferbam) showed good control levels in diseases in ornamentals. This group is currently performing as a very important tool in resistance management in various pathosystems. The dithiocarbamates act primarily through inhibition of enzymes of the power production cycle of the pathogen cells and makes them unavailable for the body of metal ions such as copper and iron (JULIATTI et al., 2017).

Main multisites under development and registered for soybean rust as the protectants research and use in Brazil to control soybean rust is quite new, the number of registered is small (mancozeb, mancozeb + azoxystrobin, chlorothalonil + tebuconazole and copper oxyclhoride). But regarding the field trial tests by antirust consortium diverse mixtures with protectants and systemic compounds are under development as important soybean diseases management tool (JULIATTI et al., 2017).

Among fungicides, there are differences in efficacy, residual period, metabolic stability, and transportation rate, demanding from producer, researcher, and technical assistance, criteria in choosing the product to be used in each situation (JULIATTI et al., 2017).

Another every important point: in addition to rust, it is necessary to consider the occurrence of other diseases such as anthracnose, late season diseases (target leaf spot, Septoria leaf spot, and powdery mildew), which may require a combination of different active ingredients (JULIATTI et al., 2017).

Resistance is a stable and heritable change in a fungal population in response to the application of a fungicide, resulting in a reduction of sensitivity to the product (EPPO 1988). With the intro- duction of systemic fungicides with specific mechanism of action, the problem worsened and since then, several plant pathogens of economically important crops have shown resistance to a variety of groups of fungicides (JULIATTI et al., 2017).

The inherent risk of resistance depends on several factors that may be associated with the product (persistence in the plant, mechanism of action, mono- genic resistance, among others) and with the target (life cycle, genetic variability, mutation potential, existence of cross-resistance, adaptability, or fitness, among others). These factors do not necessarily operate alone and do not apply in all cases. The agronomic risks should also be considered, i.e., crops over large areas with short rotation, monoculture, use of transgenic plants with genes expressing pesticide activity, geographic isolation of populations, and high population densities (JULIATTI et al., 2017).

The strategies of chemical control for diseases should be based on five main points: (1) disease monitoring, (2) phenological stages of the culture, (3) choice of the fungicide, (4) application timing, and (5) application technology (AZEVEDO, 2007).

The disease monitoring and its identification in the early stages are essential for the efficient use of the chemical control and the frequent inspection of the tillage should be carried out. The protecting of plants must occur before the appearance of the first lesions (preventive) or at the beginning when the inoculum potential is still low. The spraying should reach maximum leaf area, and fungicides with longer residual period and systemicity should be selected (AZEVEDO, 2007; BALARDIN et al., 2007).

According to AZEVEDO (2007), the spraying programs based on phenological stages can also be used for major crops such as soybeans, corn, bean, and rice. The most illustrative and practical example is the soybean culture. For diseases of the aerial parts, there is what is called the critical period of protection. This period runs from the end of the vegetative period until R6 stadium. It changes between cultivars, and a difference of 15 days between early and intermediate cycles is common. Fungicide applications should be made within this period, especially respecting the critical stage of each disease and the residual period of several products. The protection of the culture against rust will always require the observation of the phenological stadium, and stage from the beginning of flowering until full flowering is currently considered as critical period for the first spraying of fungicides (JULIATTI et al., 2017).

Monitoring methods have been described in various publications (CHIN, 1987; DENHOLM et al., 1992; DEKKER et al., 1982). In an attempt to standardize the testing internationally, FAO (1982) and FRAC (1991), show in detail the recommended methods for the major groups of fungicides.

2.7 Diseases biological control and management strategies

The practice of biological control is growing every day in agriculture, because consists of an alternative to chemical control and promotes, as advantages, the absence of residues in food and the environment, the reduction of exposure to pesticide workers and the low risk of resistance development by pathogens. This condition, coupled with greater awareness in society, has generated great interest in the search for alternative pathogen control systems. In integrated disease management, the use of bio fungicides is gaining more and more space, as it is believed that sustainability is in making chemical and biological controls allied, allowing their use in rotation, or associated.

In this type of control, microorganisms, insects, plants or even animals that contribute in some way to the control of pathogens or pests. In the case of the use of microorganisms, the control occurs as the result of the interaction between host, pathogen, and a variety of nonpathogenic microorganism to the host that interacts with the potential to limit pathogen activity or induce resistance in the host (BETTIOL; GHINI, 1995).

Examples of biocontrol agents are *Bacillus subtilis*, *Clonostachys rosea* and *Trichoderma* spp., used in different cultures for the control of pathogens such as *Fusarium*, *Phytophthora, Pythium, Rhizoctonia, Sclerotium, Sclerotinia, Macrophomina, Botrytis* and *Crinipellis* (BETTIOL, 2011; BETTIOL et al., 2012; MORANDI; BETTIOL, 2009). Another biological agent that has been studied is *Penicillium* spp., which has shown relative success in controlling white spot in corn. In this case the metabolites resulting from their fermentation (JULIATI et al., 2012).

Although there has been interest during the past 30 years in identifying microorganisms that are antagonistic to rust fungi, there are relatively few reports of such associations with *P. pachyrhizi*. However, mycoparasitic interactions between *Verticillium psalliotae* (*Lecanicillium psalliotae* (Treschew) Zare & W. Gams) and *P. pachyrhizi* were reported in which *V. psalliotae* formed appressoria-like structures at possible infection sites on urediniospores. The primary mode of parasitism was reported to be degradation of urediniospores by β-glucanase, chitinase, and protease (SAKSIRIRAT etet al., (1991), SAKSIRIRAT AND HOPPE, (1991). Other *Lecanicillium* spp. were reported as pathogens of aphids, scale insects, ticks, and whiteflies (AREVALO et al., (2009); CUTHBERTSON et al., (2008); LIU et al., (2009), PIRALI et al., (2007). A sister taxon, *Simplicillium,* was associated with ticks, nematodes, and scale insects as well as rusts, such as *Hemileia vastatrix* (coffee rust) and *Uromyces pencanus* (BISCHOFF; WHITE (2004); POLAR et al., (2005). *Lecanicillium*

and *Simplicillium* (both formerly *Verticillium* spp.) are included in the family *Cordycipitaceae,* which also includes the anamorphic genera *Beauveria* and *Isaria* (SUNG et al., (2007); ZARE AND GAMS (2001).

This family consists of entomopathogenic and mycoparasitic ascomycetes. Teleomorphs of *Simplicillium* are *Torrubiella* spp. (Cordycipitaceae), which are pathogens of spiders and scale insects BISCHOFF AND WHITE (2004). Although not reported as an entomopathogen or mycoparasite, *S. lanosoniveum* (J.F.H. Beyma) ZARE; GAMS, 2001 was recovered from the coffee rust pathogen, *Hemileia vastatrix*, and from scale insects on coffee.

Until recently, there were no documented cases in which *S. lanosoniveum* had been associated with *P. pachyrhizi*. However, *S. lanosoniveum* was recently reported to be the causal agent of brown spot on the aquatic ferns *Salvinia auriculata* and *Salvinia molesta* in Taiwan (CHEN, 2008). *S. lanosoniveum* did not cause lesions or necrosis on soybean in either coinoculated treatments or *Simplicillium* sp. only controls (unpublished data). In 2007, we observed the mycophile fungus *S. lanosoniveum* intertwined within, around, and suspended above uredinia (pustules) of *P. phakopsora*.

Fungal growth was clearly associated with uredinia but absent on healthy leaf surfaces (WARD, 2011). The objectives of this study were to examine *S. lanosoniveum* as a colonizer of uredinia of *P. pachyrhizi* and its effects on uredinial development, urediniospore production, and viability of urediniospores and to assess its potential as a biological control agent. This study examines the in-situ interactions between *P. pachyrhizi* and *S. lanosoniveum*, and for this purpose we utilized field-grown naturally infected leaves rather than leaves from greenhouse-grown plants to more closely approximate host–pathogen–mycophilic fungus interactions as they would occur in the field.

Furthermore, was evaluated the effects of *S. lanosoniveum* on soybean rust by inoculating plants with this fungus under field conditions. Colonization of soybean leaves by *S. lanosoniveum* and the rust pathogen were monitored using quantitative real-time PCR (qPCR) as well as visual disease ratings (WARD, 2011).

In 2010 at the Laboratory of Mycology (LAMIP) of the Federal University of Uberlândia, a strain of the genus *Penicillium* sp. contaminating petri dishes with the isolate of the fungus *Sclerotinia sclerotiorum*. An antagonistic effect to the growth of this pathogen was observed, as well as other soil and *Microsphaera diffusa* pathogens. The initial application for patent and protection has been filed with the Intellectual Agency (Innovation Agency) of this institution. To verify a dose scale and to ratify this potential fungicidal effect, a second step of laboratory, greenhouse and field studies with suspensions fermented in BDA (potato dextrose-

agar) in different concentrations emerged, on the main pathogens of some annual crops. Between 2012 and 2013, field and laboratory trials were conducted to evaluate the potential as bio fungicide in soybean, in seed treatment application modalities aiming at the control of seed-borne phytopathogens, and in foliar spraying to evaluate the control potential of *P. pachyrhizi* and *S. sclerotiorum*. Bio fungicide has been shown to have good potential for pathogens evaluated in field foliar application. Regarding seed treatment, potential for some pathogens was also observed: *Cladosporium* sp. and *Cercospora kikuchii* (unpublished data).

MATERIAL & METHODS

The experiments were conducted in field conditions for two consecutive seasons. They were established on Juliagro Research Station, located on BR 365 – KM 640, at Uberlândia city, MG State, under coordinates 18° 53’ 47’ latitude (South) e 48° 25’ 8’’ longitude (West), at 838 meters over the sea. The climate of the region is defined as Cwa according to the classification of Köppen (1923), presenting hot and rainy summer and dry winter, with two

well-defined seasons, one hot and rainy and the other cold and dry being the temperature of the hottest month above 22 °C. The soil present median texture.

The experimental design was in factorial (Split-plot). A randomized blocks design (10 x 4) with 4 replicates was adopted. Each plot size was composed by 6 soybean lines with 5 meters length spaced by 0,5m (meters) each other (3x5m) = 15 m². The factor genotypes was composed by: UFU L266, UFU L216, UFU L154, UFU L218 (Crosses on Table 1), BMX DESAFIO 8473RSF, TMG 7062 IPRO, TMG 7063 IPRO, Brasmax FLECHA IPRO, Nidera 5909 IPRO, Monsoy 7739 IPRO; followed by the factor fungicides: 1) Untreated check; 2) Chemical fungicide with active ingredients trifloxystrobin + prothioconazole / vegetal oil (commercial rate 0,4 / 0,4 $L.ha^{-1}$); Bio fungicide (*Penicillium* sp.)/ vegetal oil (2,0 / 0,4 $L.ha^{-1}$); 4) trifloxystrobin + prothioconazole + Bio fungicide (*Penicillium* sp.)/ vegetal oil (0,4 + 2,0 / 0,4 $L.ha^{-1}$).

Table 1- Lines and crosses Soybean Breeding Program (LAGER/UFU) used in the experiment.

LINE	CROSSING
UFU L266	Luziânia x Potenza F7
UFU L216	Luziânia x Potenza F7
UFU L154	Luziânia x PotenzaF7
UFU L218	Luziânia x Impacta F7

Source: MARTINS, J.A.S.; JULIATTI, F.C.; SANTOS, V.A.; POLIZEL, A.C.; JULIATTI, F.C. Latent period and the use of principal components analysis for partial resistance to soybean rust. Summa Phytopathologica, v.33, p.364-371, 2007.

Table 2- Soybean genotypes and chemical/biological treatments used in the experiment. Uberlândia – MG, 2020.

Treatments					
Genotype	Genotype/Name	Product name	Concentration kg or L	Commercial Rate (kg or L. ha^{-1})	Spray
1	UFU L266	Untreated check	-	-	ABCD*
2	UFU L266	tryfloxistrobyn + prothioconazole	150 + 175	0,4[1]	ABCD*
3	UFU L266	Biofac	-	2,0[1]	ABCD*
4	UFU L266	tryfloxistrobyn + prothioconazole + Biofac	150 + 175 -	0,4+ 2,0[1]	ABCD*
5	UFU L216	Untreated check	-	-	ABCD*
6	UFU L216	tryfloxistrobyn + prothioconazole	150 + 175	0,4[1]	ABCD*
7	UFU L216	Biofac	-	2,0[1]	ABCD*
8	UFU L216	tryfloxistrobyn + prothioconazole + Biofac	150 + 175 -	0,4+ 2,0[1]	ABCD*
9	UFU L154	Untreated check	-	-	ABCD*
10	UFU L154	tryfloxistrobyn + prothioconazole	150 + 175	0,4[1]	ABCD*
11	UFU L154	Biofac	-	2,0[1]	ABCD*
12	UFU L154	tryfloxistrobyn + prothioconazole + Biofac	150 + 175 -	0,4+ 2,0[1]	ABCD*
13	UFU L218	Untreated check	-	-	ABCD*
14	UFU L218	tryfloxistrobyn + prothioconazole	150 + 175	0,4[1]	ABCD*

15	UFU L218	Biofac	-	2,0[1]	ABCD*
16	UFU L218	tryfloxistrobyn + prothioconazole + Biofac	150 + 175 -	0,4+ 2,0[1]	ABCD*
17	BMX DESAFIO 8473RSF	Untreated check	-	-	ABCD*
18	BMX DESAFIO 8473RSF	tryfloxistrobyn + prothioconazole	150 + 175	0,4[1]	ABCD*
19	BMX DESAFIO 8473RSF	Biofac	-	2,0[1]	ABCD*
20	BMX DESAFIO 8473RSF	tryfloxistrobyn + prothioconazole + Biofac	150 + 175 -	0,4+ 2,0[1]	ABCD*
21	TMG 7062 IPRO	Untreated check	-	-	ABCD*
22	TMG 7062 IPRO	tryfloxistrobyn + prothioconazole	150 + 175	0,4[1]	ABCD*
23	TMG 7062 IPRO	Biofac	-	2,0[1]	ABCD*
24	TMG 7062 IPRO	tryfloxistrobyn + prothioconazole + Biofac	150 + 175 -	0,4+ 2,0[1]	ABCD*
25	TMG 7063 IPRO	Untreated check	-	-	ABCD*
26	TMG 7063 IPRO	tryfloxistrobyn + prothioconazole	150 + 175	0,4[1]	ABCD*
27	TMG 7063 IPRO	Biofac	-	2,0[1]	ABCD*
28	TMG 7063 IPRO	tryfloxistrobyn + prothioconazole + Biofac	150 + 175 -	0,4+ 2,0[1]	ABCD*
29	BMX FLECHA IPRO	Untreated check	-	-	ABCD*

30	BMX FLECHA IPRO	tryfloxistrobyn + prothioconazole	150 + 175	0,4[1]	ABCD*
31	BMX FLECHA IPRO	Biofac	-	2,0[1]	ABCD*
32	BMX FLECHA IPRO	tryfloxistrobyn + prothioconazole + Biofac	150 + 175 -	0,4+ 2,0[1]	ABCD*
33	NA 5909 IPRO	Untreated check	-	-	ABCD*
34	NA 5909 IPRO	tryfloxistrobyn + prothioconazole	150 + 175	0,4[1]	ABCD*
35	NA 5909 IPRO	Biofac	-	2,0[1]	ABCD*
36	NA 5909 IPRO	tryfloxistrobyn + prothioconazole + Biofac	150 + 175 -	0,4+ 2,0[1]	ABCD*
37	MSOY 7739 IPRO	Untreated check	-	-	ABCD*
38	MSOY 7739 IPRO	tryfloxistrobyn + prothioconazole	150 + 175	0,4[1]	ABCD*
39	MSOY 7739 IPRO	Biofac	-	2,0[1]	ABCD*
40	MSOY 7739 IPRO	tryfloxistrobyn + prothioconazole + Biofac	150 + 175 -	0,4+ 2,0[1]	ABCD*

* Spray time ABCD – 4 sprays with 14 days interval. – A – V7 soybean stage spray; B – 14 days after A; C – 14 days after B; D – 14 days after C.

[1] Aureo oil adjuvant – 0,4 $L.ha^{-1}$

The genotypes candidates for these study from Uberlândia Federal University Soybean program, were consisted of four promising soybean genotypes developed by the LAGER / UFU improvement program with partial resistance against *Phakopsora pachyrhizi* (Table 1), and six more commercial variety considered resistant TMG 7062 IPRO, TMG 7063 IPRO and commercial genotypes considered as checks and without known genes to rust, the BMX DESAFIO 8473RSF, BMX FLECHA IPRO, NA 5909 IPRO, MONSOY 7739 IPRO. The UFU genotypes had partial resistance traits in field trials for *P. pachyrhizi* (Martins et al., 2007; Silva et al., 2007 and Martins; Juliatti 2014) and in greenhouse conditions for *Heterodera glycines* (JULIATTI et al., 2017).

Martins and Juliatti (2014) studying the partial resistance in the management of Asian rust, quantified the severity of the disease through the parents and their respective F2 and F3 generations (Luziânia x Potenza and Luziânia x Impacta crosses). From these data, they estimated the mean and variance of the genetic components to obtain the number of genes also the broad- and narrow-sense heritability's. They concluded that rust resistance is a characteristic controlled by 2 to 23 genes that are predominantly dominant, and the estimate of narrow-sense heritability was greater than 60% for the Luziânia x Potenza cross, leading to a conclusion that is possible to successfully select resistant individuals in early generations. The parental variety's IAC100, Luziânia, Caiapônia and Potenza, also are the base for several crossings to obtain some of the genotypes.

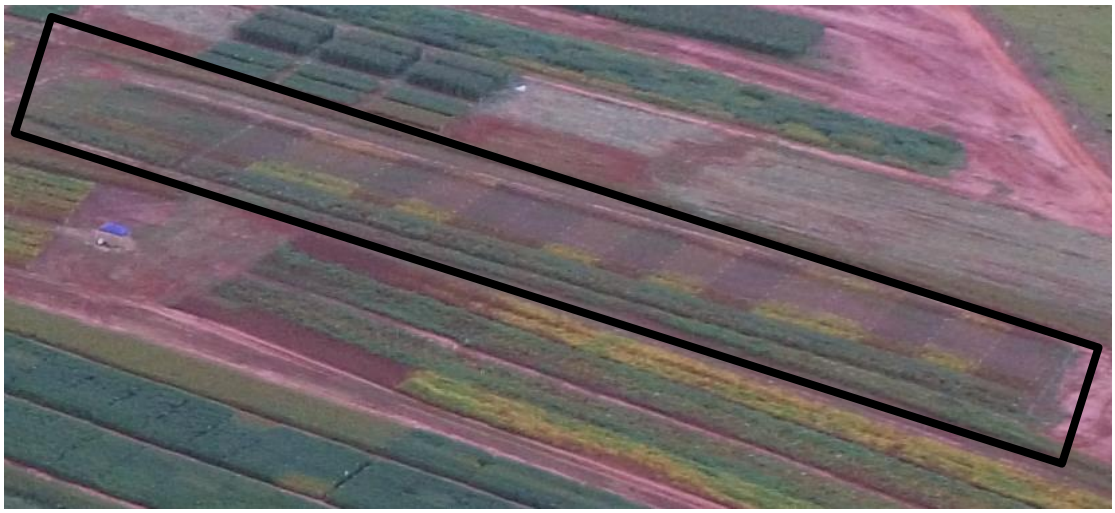
The soil used on both season was a tillage soil prepared by plow and infestans desiccation before sowing. The first season experiment was sowed on 11/23/2017 and the second-year experiment was sowed 11/27/2018 (Figure 1). The seeds number used per meter for all genotypes tested were fixed on 18 seeds per meter. At sowing time was adopted formulated fertilizer (NPK) – 7-35-17 at rate of 233 kg. ha^{-1} and 30 days after emergence on the two years experiments.

Source: Juliatti, F. Ca.

1.1. Crop management

For the first and second seasons study sowing time (11/23//2017 and 11/27/2018 respectively), the seeds emergence started in the last five days after and between the genotypes stabilized until ten days after emergence. The plant stand in field was considered 1,66 plants per linear meter and between the lines spacing at 0,5 meters, corresponding about 300.000 plants per hectare (Figure 2).

Figure 2. Closed View from trial season 2018/19. Uberlândia – MG. 2019.



Source: Juliatti, F.Ca.

1.2 Fungicides spray technologies

The equipment used for foliar sprays were pressurized CO_2 backpack with 6 droplets spaced 30 cm each other. The first spray was at V7 (Fehr; Caviness, 1977), soybean phenological stage and the others were sprayed at 14 days interval. The weather conditions during each spray were collected and described on the Table 3 and 4 below, even the spray technology adopted, and further information related to the sprays for each experiment.

Table 3- Spray data and technology adopted in the first season experiment. Uberlândia – MG, 2021.

Spray information	Spray A	Spray B	Spray C	Spray D
Spray Method:	Leaf	Leaf	Leaf	Leaf
Spray data	04/01/18	18/01/18	10/02/18	24/02/18
Spray Technology:	MAG 2 – Empty Cone	MAG 2 – Empty Cone	MAG 2 – Empty Cone	MAG 2 – Empty Cone
Droplets number:	6	6	6	6
Spray bar size:	3 m	3 m	3 m	3 m
Volume (L/ha):	150	150	150	150
Target stage:	Preventive	Preventive	With Symptoms	With Symptoms
Crop Stage:	V7	R1	R3	R5.1
Temperature at spray time (ºC):	25	27	26	28
Air Humidity (%):	60	61	59	66
Wind velocity (km/h):	6	7	8	10

Table 4- Spray data and technology adopted in the second season experiment. Uberlândia – MG, 2021.

Spray information	Spray A	Spray B	Spray C	Spray D
Spray Method:	Leaf	Leaf	Leaf	Leaf
Spray data	09/01/19	23/01/19	06/02/19	20/02/19
Spray Technology:	MAG 2 – Empty Cone	MAG 2 – Empty Cone	MAG 2 – Empty Cone	MAG 2 – Empty Cone
Droplets number:	6	6	6	6
Spray bar size:	3 m	3 m	3 m	3 m
Volume ($L.ha^{-1}$):	150	150	150	150
Target stage:	Preventive	Preventive	With Symptoms	With Symptoms
Crop Stage:	V7	R1	R3	R5.1
Temperature at spray time (ºC):	21	22	25	23
Air Humidity (%):	61	66	62	61
Wind velocity ($km.h^{-1}$):	7	4	3	6

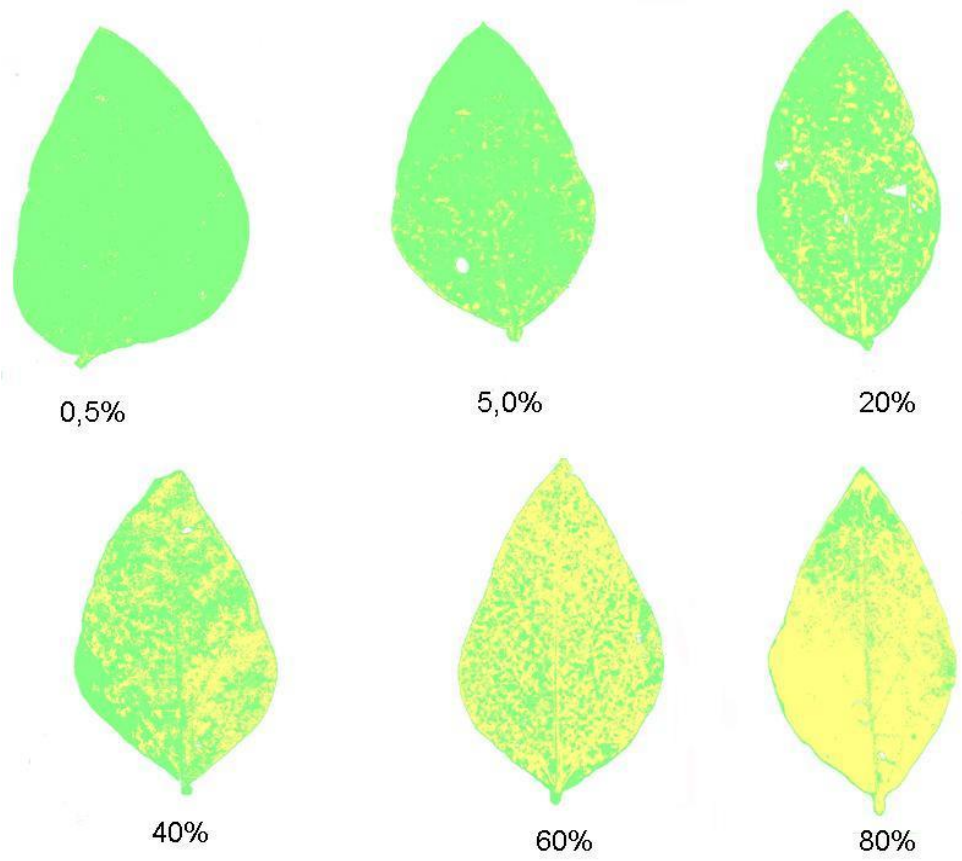
1.3 Assessment methodology

During the two seasons experiments development, were obtained data for diseases incidence and severity (rust and other diseases), fungitoxicity and grain yield (kg ha^{-1}).

1.3.1 Asian Soybean Rust Assessment

Incidence and severity data were collected on the total of 5 assessments at the first symptons on the soybean genotypes in the 3 lines of the 5 lines of each plot. Each plot was assessed on all plant canopy (30 leaflets aleatory collected in the middle of the plant canopy) to determinate the diseases severity. In each assessment, were used diseases scales range from 0 to 100% of the disease severity, on 0 related to 0 disease symptoms on the leaves and 100% means all collected leaflet were covered by diseases symptoms. For rust assessment in specific were used diagrammatic scale developed por POLIZEL; JULIATTI (2010), as observed on Figure 3.

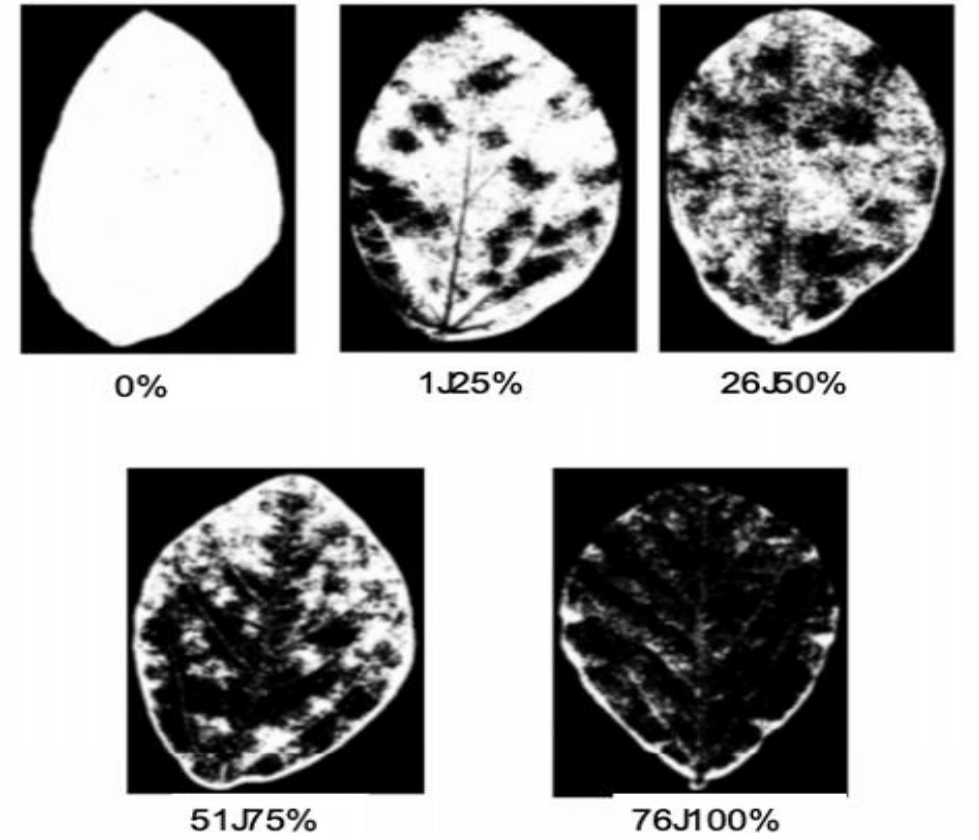
Figure 3. Diagrammatic scale to access rust severity on soybean Polizel & Juliatti (2010).



1.3.2 Powdery mildew Assessment

Incidence and severity data were collected on the total of 5 assessments at the first symptons on the soybean genotypes in the 3 lines of the 5 lines of each plot. Each plot was assessed on all plant canopy (30 leaflets aleatory collected on over plants canopy) to determinate the diseases severity. In each assessment, were used diseases scales range from 0 to 100% of the disease severity, on 0 related to 0 disease symptoms on the leaves and 100% means all collected leaflet were covered by diseases symptoms. For powdery mildew assessment in specific were used diagrammatic scale developed by POLIZEL; JULIATTI (2010) observed on Figure 4.

Figure 4. Diagrammatic scale for powdery mildew severity on soybean (Polizel and Juliatti, 2010).

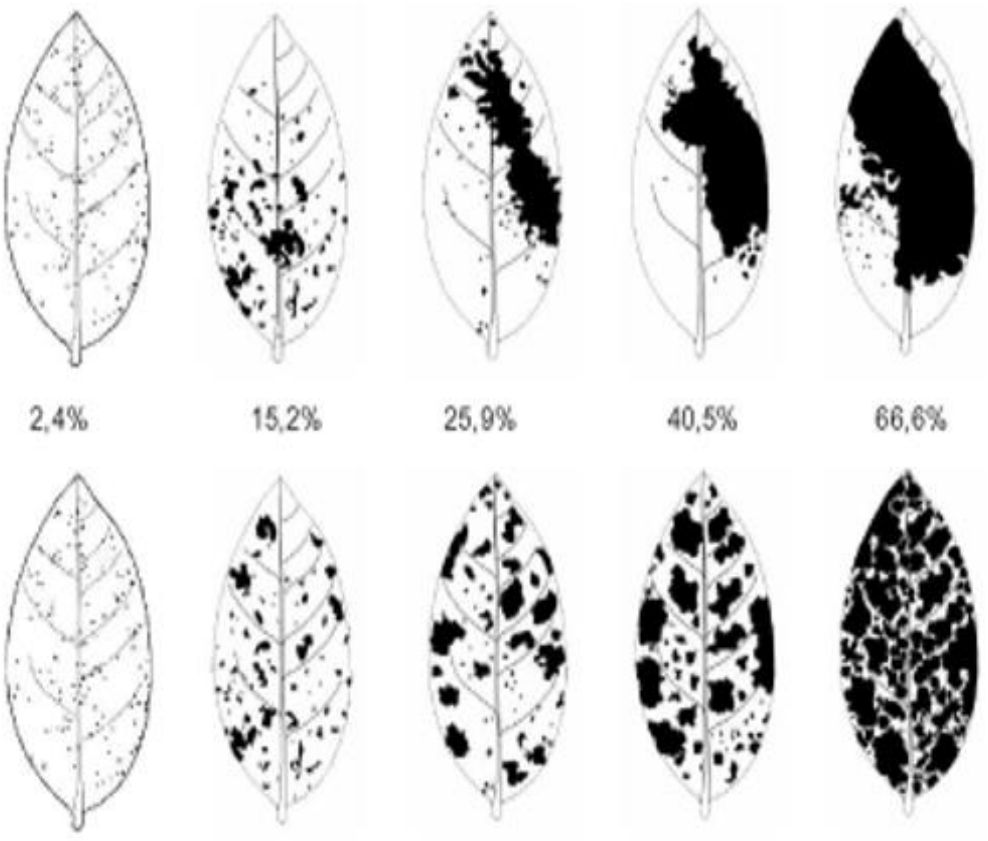


1.3.3 Septoria Brown Spot Assessment

Incidence and severity data were collected on the total of 5 assessments at the first symptoms on the soybean genotypes in the 3 lines of the 5 lines of each plot. Each plot was

assessed on all plant canopy (30 leaflets aleatory collected on over plants canopy) to determinate the diseases severity. In each assessment, were used disease scale range from 0 to 100% of the disease severity, on 0 related to 0 disease symptoms on the leaves and 100% means all collected leaflet were covered by diseases symptoms for Septoria Brown spot assessment in specific were used diagrammatic scale developed by Martins et al., (2004) observed on Figure 5.

Figure 5. Diagrammatic scale for Septoria Brown Spot severity on soybean (Martins et al., 2004).



1.3.4 Area Under Disease Progress Curve (AUDPC)

After severity assessment during the trial's development, was calculated the AUDPC, area under disease progress curve (SHANER; FINNEY, 1990). The AUDPC formula was described on the Figure 2 below.

$$*AACPD = \Sigma\ [(y_i + y_{i+1})/2]\ x\ (t_{i+1} - t_i)$$

Where:

y_i = initial disease severity

y_{i+1} = final disease severity

$t_{i+1} - t_i$ – assessment interval

1.3.5 Fungitoxicity

Fungitoxicity for fungicides were assessed for each genotype 7 days after each spray. For the data collect were used a scale from 0 a 100% according to FRANS et al., (1986) described on figure 6.

Figure 6. Fungitoxicity scale by Frans et al., 1986.

Rating	Weed Control	Crop Damage	Precision (%)
0	No weed control	No crop reduction or injury	2
10	Very poor weed control	Slight crop discoloration or stunting	5
20	Poor weed control	Some crop discoloration, stunting, or stunt loss	5
30	Poor to deficient weed control	Crop injury more pronounced, but not lasting	10
40	Deficient weed control	Moderate injury, crop usually recovers	10
50	Deficient to moderate weed control	Crop injury more lasting, recovery doubtful	10
60	Moderate weed control	Lasting crop injury, no recovery	10
70	Weed control somewhat less than satisfactory	Heavy crop injury and stand loss	10
80	Satisfactory to good weed control	Crop nearly destroyed - A few surviving plants	5
90	Very good to excellent weed control	Only occasional live crop plants left	5
100	Complete weed destruction	Complete crop destruction	2

1.3.6 Grain Yield

At harvest time, was collected on each plot separately, 50 plants to determinate Grain Yield. After harvest, the plants were trail, and the grain weighing was determinated. Grain humidity was corrected to 13% at formula below and the data was converged to kilos per hectare.

$$\frac{PF = PUx\ (100\text{-}UI)}{100\text{- }UF}$$

Where:
PF – final sample corrected
PI – inical sample weight

UI – initial humidity
UF: final humidity (13%)

1.4 Statistical analysis

For data to adhere to specific assumptions about the underlying data collection, is important the evaluation and validation of proposed and fitted models to ensure reliability of the models before ANOVA analysis (Supplementary materials). By this we assume data with proper high level of reliability, we need to assume the data to be **normally distributed** with a mean of zero and a constant (yet unknown) variance (σ, **homogeneity of variance**) and residuals (and thus observations) are also assumed to all be **independent**.

About normal distribution or null hypothesis, if the p value is less than the chosen alpha level, then the null hypothesis is rejected and there is evidence that the data tested are not normally distributed. On the other hand, if the p value is greater than the chosen alpha level, then the null hypothesis (that the data came from a normally distributed population) cannot be rejected (e.g., for an alpha level of .05, a data set with a p value of less than .05 rejects the null hypothesis that the data are from a normally distributed population (RAZALI et al., 2017).

Homogeneity of variance and independence are encapsulated within the single symbol for variance (σ^2). In assuming equal variances and independence, we are assuming about the variance-covariance structure of the populations (and thus residuals).

The Bartlett's test (is used to test if k samples are from populations with equal variances (SNEDECOR et al., 1989). For the Durbin–Watson or independence analysis, the test statistic is used to detect the presence of autocorrelation at lag 1 in the residuals (prediction errors) from a regression analysis. The results when the assumptions are attended is bounded tests for the null hypothesis that the errors are serially uncorrelated against the alternative hypothesis (DURBIN; WATSON, 1950).

The data that obtained a normal distribution, were submitted to analysis of variance (F test). All analysis of variance, Yield grain and AUDPC data were performed using the R software (R Core Team, 2017) with the add-on packages gregmisc (Warnes, 2015) and ExpDes for ranking (Ferreira et al., 2003) and agricolae for phytopathometry (MENDIBURU, 2005).

For weather data, the weather variables were collected by a Vantage Pro2 – Davis Station installed at JulioAgro (id: 00: 1D:0A:01:02:5C). This dispositive has wireless transmission up to 300 mts and it is powered outside with solar energy. It has a programmable data logger each 60 minutes. The vantage pro 2 make measurements of ambient variables such as temperature:

from -40º to + 65ºC (± 0.5º); Humidity: from 0 to 100% (± 3%); Pressure: from 540 to 1100 (± 1.0 hPa); Windspeed: from 3 to 241 km / h (± 5%); Direction: from 0º to 360º (± 4º); Rainfall: from 0 to 9999 mm/d.

RESULTS AND DISCUSSION

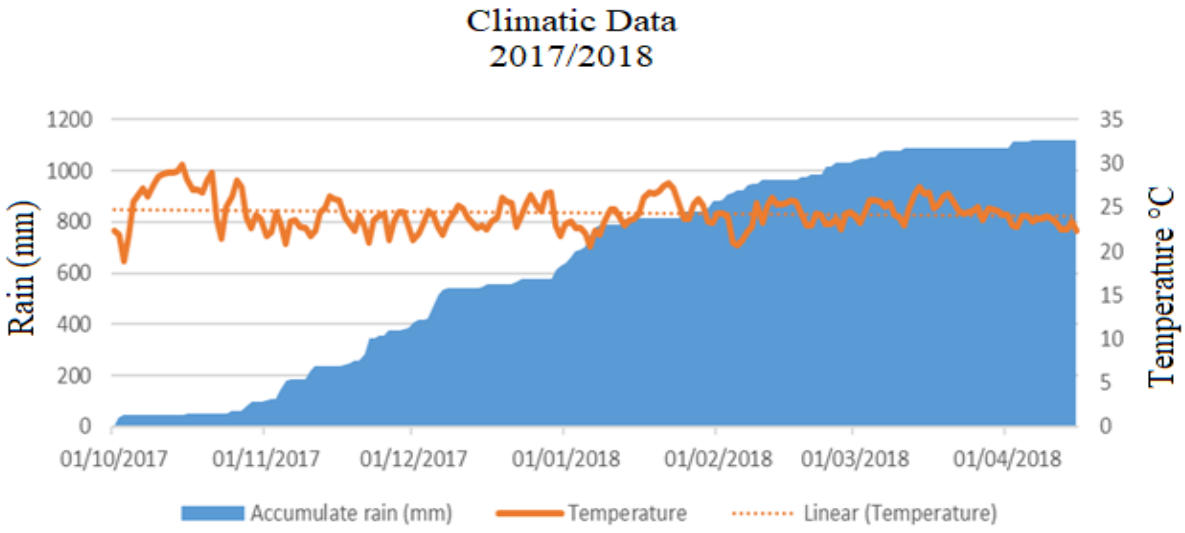
2.1 Asian Soybean Rust progression (*Phakopsora pachyrhizi*)

For better results discussion it was necessary to observe weather conditions data collected for the experiments at sowing time until harvest in both seasons (Figure 7 and 8).

The weather conditions during first season experiment had an average of 25 ºC and only after 151 days the area of experiment topped the 1000 mm mark of accumulate rain. Meanwhile the second season (Figure 8) presented lower average in temperature 23.5 °C and need at least 166 days to pass the 1000 mm mark of accumulate rain.

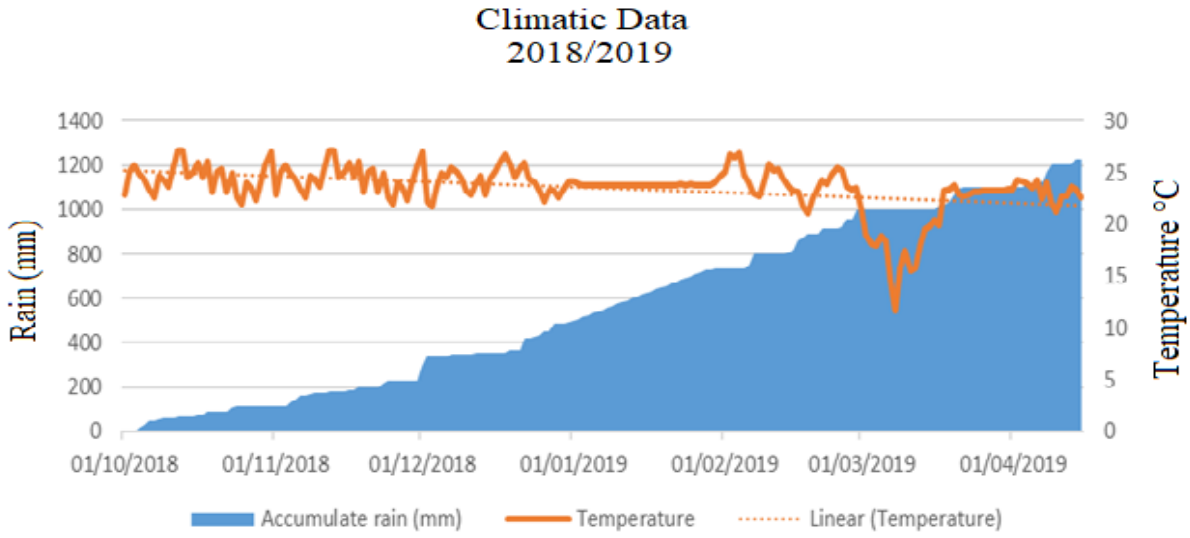
The first season ealier achievement of optimal conditions for rust dissemination and incubation contributed to the reflex of higher AUDPC levels for rust progression.

Figure 7. Weather data conditions from the season 2017/2018 during experiment conduction.



Source: Davis Vantage pro 2

Figure 8. Weather data conditions from the season 2018/2019 during experiment conduction.



Source: Davis Vantage pro 2

According to interaction analysis for both seasons they were independent each other. The data collected for rust AUDPC in both seasons' (2017/2018 and 2018/2019) was not possible to agrupate because there was difference between repeated experiments over. Then, the results of the assays were presented separately after ANOVA analysis (Supplementary materials - S6).

Zambenedetti et al., (2007) obtained in its monocycle study that the cultivar BRS 134 and BRS 231 with an early period of rain and favorable conditions he obtained higher AUDPC values in plants. Vale; Zambolim and Chaves, (1990) studying the effect of temperature and duration of leaf wetness on *P. pachyrhizi* infection observed the maximum number of lesions cm^{-2} on the inoculated leaves after at least 16 hours of leaf wetness, they also concluded this to be the optimal conditions to *P. pachyrhizi* infection.

These variables were also used to model, simulate, and predict Asian rust by other authors using cultivars adapted to their countries. The authors concluded that ASR resistance was influenced by environmental factors or physiological effects (TSCHANZ and WANG, 1985; ZAMBOLIM and CHAVES, 1990; PIVONIA and YANG, 2004; REIS et al., 2004; PIEROZZI et al., 2008; VALE and JULIATTI, 2018).

In the Table 5, was presented AUDPC levels for each genotype and fungicide managements (chemical solo / trifloxistrobin + prothioconazole; biological solo /*Penicillium* sp.; and association of chemical with biological/ trifloxistrobin + prothioconazole; biological solo /*Penicillium* sp in season 2017/2018.

The first season data presented significant AUDPC difference ($p<0.001$) between the factors (genotypes and fungicides). Between genotypes at the split-plot no spray (without fungicide), the treatments were grouped in 6 different groups. It'spossible to highlight a group composed with higher levels of AUDPC (BMX FLECHA IPRO and BMX DESAFIO 8473 RSF susceptible pattern) and a second group with lowest AUDPC values (TMG 7062 abd TMG 7063 both contains *Rpps* genes).

Table 5 – Area under disease progress curve for Asian Soybean Rust in season 2017/18 for genotypes and fungicides management.

Genotypes	No Spray	Trifloxys.+ prothio.	*Penicillium* sp.	Trifloxys.+ prothio. + *Penicillium* sp.
BMX DESAFIO 8473RSF	1559 bA	51 bC	1404 bB	36 aC
BMX FLECHA IPRO	1836 aA	102 aB	1800 aA	71 aB
MO 7739 IPRO	1365 cA	162 bC	1145 cB	136 aC
NA 5909 RR	1632 bA	13 bC	1435 bB	6 aC
TMG 7062 IPRO	466 fA	7 cB	566 fA	7 aB
TMG 7063 IPRO	820 eA	13 cB	735 eA	5 aB
UFU L218 Conv.[10]	1366 cA	143 bC	997 dB	80 aC
UFU L154 Conv.[10]	1330 dA	258 aC	1138 cB	103 aD
UFU L216 Conv.[10]	1051 dA	123 bB	928 dA	53 aB
UFU L266 Conv.[10]	1155 dA	281 aC	956 dB	168 aB
S-W			0,89	
Bartlett			0,002	
Durbin-Watson			0,93	
VC-Plot (%)			13,80	
VC-SplitPlot (%)			16,32	

1- Analysis by four Split-plots (no spray treatment, trifloxistrobin + prothioconazole/Aureo Chemical Fungicide, Biofac Biological Fungicide, and association of the previous two).

2- ns = non-significant interaction among genotypes;

3- Original Means.

4- Means followed by the same lowercase letter in the column belongs to same group by the Scott-Knott test at 10% probability.

5- Means followed by the same uppercase letter in the line belongs to same group by the Scott-Knott test at 10% probability.

6- Values of S-W in bold mean normal distribution by Shapiro-Wilk test at the .05 significance level.

7- VC = Variance coefficient.

8- Residue independency attendance when in bold (non-rejected H_0) by Durbin Whatson test at the .05 significance level.

9- Values attendance of homoscedasticity in bold mean by Bartlett test at the .05 significance level.

10 – Conventional genotype from LAGER/UFU.

In general, the data composition between fungicides factors, showed that fungicide and genetic management associated by chemical fungicide solo (trifloxystrobin + prothioconazole) and mixture of chemical and biological (trifloxystrobin + prothioconazole + *Penicillium* sp. – Table 5) presented the lowest AUDPC values ranging 51 for trifloxystrobin + prothioconazole and 36 value for trifloxystrobin + prothioconazole + *Penicillium* sp. at BMX DESAFIO 8473 RSF cultivar in comparison with the 1559 AUDPC of the no spray split plot (Table 5).

Danelli and Reis, (2016) determined difference in levels of severity and AUDPC in a trial with two genotypes. The first being BRS 246 R, who shoed the highest values, meanwhile BRSGO 7560 showed the lowest values. The authors concluded that the cultivar BRSGO 7560 carries a *Rpp* gene that confers vertical resistance to soybean rust. Zambenedetti et al., (2007) also correlated in its study that the BRS 134 and BRS 231 with an early cycle had higher AUDPC than the PI 459025 an America cultivar with the *Rpp4* resistance gene.

The genotypes UFU L218, UFU L154, UFU L216 and UFU L266 from LAGER/UFU program demonstrated variable data for AUDPC values and differences in statistical group (ranging values from 1051 to 1366). In the multiple comparison procedure by Scott-Knott, the data was segregated in different groups for 2 of the four split-plots (except of trifloxystrobin + prothioconazole + *Penicillium* sp. mix), the genotypes L216 and L266 presented lower AUDPC and contrasted positively against other UFU genotypes.

Some of these genotypes and their parental presented evidence of partial resistance in other authors works, increasing latency period, lowering AUDPC in general and promoting longer period of incubation during field trials even in optimal conditions with 22 °C and increased dew period (SILVA et al., 2007 and JULIATTI, 2018). The longer a fungus takes to incubate inside a host tissue, slower is their rate of growth and fewer cycles of reproduction are developed in a crop season (MADDEN; HUGHES and BOSCH, 2007).

Parlevliet, (1983), Martins et al., (2007) and Vallavielle-Pope et al., (2000) stated that cultivars with incubation periods longer than 14 days could be classified as having partial resistance. Previous authors combinating partial genetic resistance and fungicide in controlling ASR, observed significant effects of the cultivars, fungicide, and interaction between these two factors for AUDPC and productivity, standing out the cultivars IAC–100, Potenza and UFUS-Impacta as partially resistant to the ASR.

Martins et al., (2018), described that resistance of soybean o ASR and white mold, conducted in greenhouse conditions the resistance response for lines UFU L 254, L266, L216

and L218 for Asian soybean rust by artificial inoculation. They observed in line UFU L 266 and UFU 218 lower AUDPC levels with significant differences to the other lines studied in both experiments. In the present experiment in field conditions, the genotypes UFU L266 and UFU L216 presented lower severity and AUDPC data in comparison with UFU L218 and L254 (Table 5).

After reports and confirmation cases of ASR resistance to the *Rpp* genes and groups of chemical fungicides, the alternative of partial resistant cultivars, is gaining importance in this scenario of uncertainties. Also, the producer's environment is under constant change with weather conditions changing hourly and daily. In this presumable agricultural scenario, where higher costs, more time are involved in the development of new fungicide molecules to control ASR, and Rpp genes being break down we need start use tools like partial resistance in the management of ASR.

There were difference levels on commercial genotypes considers susceptible to soybean rust as Monsoy 7739 IPRO with lowest AUDPC levels besides the non-known resistance genes for soybean rust.

Considering maturity group influence in rust progression, with longer cycle genotypes sustaining higher chance of increase levels of AUDPC levels, the experiment didn't show positive data in correlation for this variable. The adjustment of the model to the data must be done by replacing the variable time (DAI) for a relative value that considers the time needed for the genotype to complete its "Relative Lifetime" (RLT) cycle (TSCHANZ and TSAI, 1983). But since different early cycle genotypes as NA 5909 (5.9 maturity) presented higher AUDPC in the no spray split-plot than Monsoy 7739 IPRO (7.7 maturity group) (Table 5).

Cultivars with vertical resistance as TMG 7062 IPRO and TMG 7063 IPRO, differentiate positively ($p<0.10$) and were segregated in the ranking test in different groupmate with lower disease levels (AUDPC and severity) in all split-plots treatments.

All genotypes including the example of UFU L 154, the lowest AUDPC values were observed in the split-plot trifloxystrobin + prothioconazole and the mix with *Penicillium* sp. management. The increment of biological treatment with *Penicillium* sp. applied presented significant difference ($p<0.10$) against trifloxystrobin + prothioconazole only in this case.

This could be attributed to level of partial resistance response in combination of biological increment, could be proved with a higger expression of partial resistance as the resistance of soybean cultivars that contains major genes to rust can vary temporally and geographically (KATO and YORINORI, 2008; AKAMATSU et al., 2013; PAUL et al., 2013; TWIZEYIMANA and HARTMAN, 2012; WALKER et al., 2014).

In tropical climatic scenario with higher selection of rust pathotypes that are under intense exposition of fungicides a biological compound in this trial could be an evidence of genes that confers horizontal resistance, to be inserted on disease management to help diminished the survival and progression of the ASR agent (PARLEVLIET, 1997).

ASR damage in influenced by weather conditions, genotypes aspects, pathogen population, crop management and aspects related with chemical and biological control (BALARDIN; NAVARINI; ALLAGNOLL, 2005). Precipitation is a critical factor for rust development. Severity data obtained in different regions in Brazil with distinct pluviometry regime, shown high correlation (CARNEIRO, 2007), and can explain uncommon characteristics of *P. pachyrhizi* urediniospores keeping together united, not been east spread by wind (MELCHING; BROMFIELD; KINGSOLVER, 1979).

The data from 2018/2019 trial (Table 6), also presented significant AUDPC differences and interaction ($p<0.10$) between genotypes and fungicides management. In these conditions of average disease pressure, usually materials with partial genetic resistance can highlight the effects of horizontal resistance.

In general, the lowest AUDPC values were observed on the spli-plot with bio/chemichal fungicides mix (trifloxystrobin + prothioconazole and trifloxystrobin + prothioconazole + *Penicillium* sp.). Considering genotypes effects, TMG 7062 and TMG 7063, showed lower AUDPC values in all split plots, this data is correlated with the first trial in the previous season.

The genotype BMX Flecha IPRO presented the highest AUDPC data (1225), reflecting its susceptibility traits. The UFU L216 and UFU L266 from LAGER/UFU program presented in general average AUDPC levels (849 and 823) and presented significant difference ($p<0.10$) against other genotypes like UFU L218 and UFU L154 (983 and 1094).

The Rpps cultivars from TMG presented the lowest AUDPC values considering all split plots. At the second season, was not observed difference levels on commercial genotypes considers susceptible to soybean rust as Monsoy 7739 IPRO and NA 5909 (5.9 maturity), supposing the disease pressure can influence in the genotype response for resistance.

Marchetti; Uecker and Bromfield, (1975) determined once the infection was established the incubation and latency periods could not be reflected in AUDPC values only on severity, so severity data evolution over time should be further examined for detection of variations of incubation latency periods.

Table 6 – Area under disease progress curve for Asian Soybean Rust in season 2018/19 for genotypes and fungicides management.

Genotypes	No Spray	Trifloxys.+ prothio.	*Penicillium* sp.	Trifloxys.+ prothio. + *Penicillium* sp.
BMX DESAFIO 8473RSF	894 dA	174 bB	255 gB	173 bB
BMX FLECHA IPRO	1225 aA	46 cB	159 aA	49 cB
MO 7739 IPRO	785 eA	267 aB	775 dA	211 bB
NA 5909 RR	778 eA	23 cC	560 eB	24 cC
TMG 7062 IPRO	481 fA	7 cB	402 fA	4 cB
TMG 7063 IPRO	376 gA	3 cB	308 gA	5 cB
UFU L218 Conv.[10]	983 cA	321 aB	929 cA	308 aB
UFU L154 Conv.[10]	1094 bA	287 aB	1039 bA	277 aB
UFU L216 Conv.[10]	849 eA	333 aB	805 dA	315 aB
UFU L266 Conv.[10]	823 eA	267 aB	798 dA	272 aB
S-W			**0,92**	
Bartlett			0,001	
Durbin-Watson			**0,95**	
VC-Plot (%)			14,54	
VC-SplitPlot (%)			14,69	

1- Analysis by four Split-plots (no spray treatment, trifloxistrobin + prothioconazole/Aureo Chemical Fungicide, Biofac Biological Fungicide, and association of the previous two).

2- ns = non-significant interaction among genotypes;

3- Original Means.

4- Means followed by the same lowercase letter in the column belongs to same group by the Scott-Knott test at 10% probability.

5- Means followed by the same uppercase letter in the line belongs to same group by the Scott-Knott test at 10% probability.

6- Values of S-W in bold mean normal distribution by Shapiro-Wilk test at the .05 significance level.

7- VC = Variance coefficient.

8- Residue independency attendance when in bold (non-rejected H_0) by Durbin Whatson test at the .05 significance level.

9- Values attendance of homoscedasticity in bold mean by Bartlett test at the .05 significance level.

10 – Conventional genotype from LAGER/UFU

About the contribution of live organisms or biological control, makes possible to use microorganisms for disease management. In the past decades, many bacteria including *Bacillus spp and fungis like Penicillium sp.*, have been studied and have shown potential for biocontrol (MA et al., 2017; TIAN et al., 2014; ZHAO et al., 2018).

In general, there was interaction between the genotypes and the fungicide management in the AUDPC levels. According to data from both season, for genotypes with partial or susceptibility trait, the influence, and the combo/mix of chemical and biological products, influenced positively in the overall reduction of AUDPC levels.

The genetic or resistance characterization against rust needs the correspondence as well to AUDPC impacting in Yield parameters. Genotypes with dominant genes resistance for rust

as TMG 7062 IPRO and TMG 70 63 IPRO provided lower AUDPC levels and higher Yield considering only the genetic control.

In both seasons the values of S-W, mean normal distribution by Shapiro-Wilk test at the .05 significance level. The Values attendance of homoscedasticity in bold mean by Bartlett test at the .05 significance level.

2.2 Septoria Brown spot *(Septoria glycines*)

The weather conditions data obtained (Figure 7), during the first season, could explain the lower AUDPC levels (Table 7). Jung et al., 2002; Li; Yang, 2019, suggested that yield losses caused by a disease complex at the end of the soybean crop cycle have worsened recently. These diseases are disseminated in all Brazilian and US soybean cropping regions, which differ only in disease severity.

The brown spot, caused by the *Septoria glycines*, is perhaps the main end-of-cycle disease and is disseminated throughout the regions. Brown spot needs in its life cycle lower humidity period (dew in contrast of rust), but as well higher frequency of droplets infection could attribute general infection at temperatures closer to 25 °C.

There was difference between repeated experiments over. Then, the results of the assays were presented separately after ANOVA analysis (Supplementary materials – S5).

During 2017/2018 season, there was significant AUDPC differences and interaction for ranking test Scott-Knott ($p<0.10$) between genotypes and split-plot fungicide management. For genotypes MONSOY 7739 IPRO, TMG 7062 IPRO, TMG 7063 IPRO and UFU L 154 had correlation associating genetic control and fungicide management where the lowest AUDPC values were observed on fungicide and genetic management with trifloxystrobin + prothioconazole and the trifloxystrobin + prothioconazole + *Penicillium sp*.

Considering only genetic control, the genotypes UFU L266, UFU L 218, UFU L 154, NA5909 RR, BMX FLECHA and BMX DESAFIO 8473RSF had the lowest values for AUDPC. Genotypes TMG 7062 IPRO, TMG 7063 IPRO, MONSOY 7739 IPRO and UFU L 216 IPRO had the highest values.

ALMEIDA, (2001) performed under greenhouse conditions, the screening of soybean (*Glycines max*) genotypes resistant to brown spot, by inoculating one-month-old plants with a suspension of spores, calibrated to 10^6 spores/ml. Evidence of resistance in any cultivar against brown spot infection was not observed during trials, only management effect against pathogen progress. However, cultivars CTS-40, IAS-2, IAS-5, PI 230 975, and PI 204 332 exhibited less infected leaf area and a longer period to reach 5% disease severity. Severity established based on the number of days (latency) to is a good indicator for screening soybean genotypes with resistance to *S. glycines*.

Table 7 – Area under disease progress curve for Septoria Brown Spot in season 2017/18 for genotypes and fungicides management.

Genotypes	No Spray	Trifloxys.+ prothio.	*Penicillium* sp.	Trifloxys.+ prothio. + *Penicillium* sp.
BMX DESAFIO 8473RSF	141bA	60 bA	103 bA	61 bA
BMX FLECHA IPRO	243 bA	164 bA	196 bA	152 bA
MO 7739 IPRO	364 aA	81 bB	294 aA	111 bB
NA 5909 RR	202 bA	131 bA	168 bA	162 bA
TMG 7062 IPRO	369 aA	132 bB	422 aA	197 aB
TMG 7063 IPRO	346 aA	107 bB	299 aA	142 bB
UFU L218 Conv.[10]	137 bA	123 bA	174 bA	94 bA
UFU L154 Conv.[10]	231 bA	136 bB	220 bA	108bB
UFU L216 Conv.[10]	386 aA	294 aB	343 aA	275 aA
UFU L266 Conv.[10]	136 bA	109 bA	143 bA	99 bA
S-W			**0,47**	
Bartlett			0,004	
Durbin-Watson			**1,82**	
VC-Plot (%)			53,23	
VC-SplitPlot (%)			41,10	

1- Analysis by four Split-plots (no spray treatment, trifloxistrobin + prothioconazole/Aureo Chemical Fungicide, Biofac Biological Fungicide, and association of the previous two).

2- ns = non-significant interaction among genotypes;

3- Original Means.

4- Means followed by the same lowercase letter in the column belongs to same group by the Scott-Knott test at 10% probability.

5- Means followed by the same uppercase letter in the line belongs to same group by the Scott-Knott test at 10% probability.

6- Values of S-W in bold mean normal distribution by Shapiro-Wilk test at the .05 significance level.

7- VC = Variance coefficient.

8- Residue independency attendance when in bold (non-rejected H_0) by Durbin Whatson test at the .05 significance level.

9- Values attendance of homoscedasticity in bold mean by Bartlett test at the .05 significance level.

10 – Conventional genotype from LAGER/UFU.

At the second season (Figure 8), an inversion occurred in weather conditions, and higher levels of AUDPC were obtained (Table 8). These higher levels could not be attributed to sowing period, but to to rain fall occurrence. Also, a second factor being inoculum in field from previous year accumulated together with higher frequency in rains in the first days of cycle (rain droplets with conidia infecting initial trifoliate leaves. During the 2018/2019 crop season, higher AUDPC levels of septoria rot were obtained in plants through different genotypes and fungicides management (Table 8).

There was significant AUDPC differences and interaction ($p<0.10$) between genotypes and fungicides management. In general, the lowest AUDPC values were observed on fungicide and genetic management with trifloxystrobin + prothioconazole and trifloxystrobin + prothioconazole + *Penicillium sp*. for all genotypes tested. Considering only genetic control, Genotype TMG 7063 IPRO had the highest value. As in the second season disease pressure higher than the first season, the interaction with genetic control and fungicide management was clearer. There was some interaction response for *Penicillium sp.* management solo and the genotypes testes meaning lowest AUDPC levels observed on genotypes FLECHA IPRO, MONSOY 7739 IPRO, NA 5909 IPRO, TMG 7062 IPRO and TMG 7063 IPRO.

About maturity group influence in brown lead spot progression, with longer cycle genotypes sustaining higher chance of increase levels of AUDPC levels, the experiment didn't show positive data in correlation for this variable for these disease also. But since different early cycle genotypes as NA 5909 (5.9 maturity) presented same AUDPC in the no spray split-plot than Monsoy 7739 IPRO (7.7 maturity group) (Table 8).

Considering only genetic control, the genotypes UFU L216 and UFU L 266, had the lowest values for AUDPC. Genotype TMG 7063 IPRO had the highest value (same observed on season 2017/2018).

Table 8 – Area under disease progress curve for Septoria Brown Spot in season 2018/19 for genotypes and fungicides management.

Genotypes	No Spray	Trifloxys.+ prothio.	*Penicillium* sp.	Trifloxys.+ prothio. + *Penicillium* sp.
BMX DESAFIO 8473RSF	849 bA	190 bD	319 dC	564 aB
BMX FLECHA IPRO	601 cA	300 aC	431 dB	313 cC
MO 7739 IPRO	639 cA	164 bC	380 dB	151 dC
NA 5909 RR	681 cA	179 bC	569 cB	221 cC
TMG 7062 IPRO	753 cA	237 bC	539 cB	227 cC
TMG 7063 IPRO	1063 aA	353 aC	866 aB	386 bC
UFU L218 Conv.[10]	663 cA	308 aB	617 cA	304 cB
UFU L154 Conv.[10]	678 cA	326 aB	711 bA	265 cB
UFU L216 Conv.[10]	527 dA	194 bB	532 cA	129 dB
UFU L266 Conv.[10]	428 dA	115 bB	326 dA	83 dB

S-W	**0,95**
Bartlett	**0,08**
Durbin-Watson	**1,07**
VC-Plot (%)	23,21
VC-SplitPlot (%)	19,16

1- Analysis by four Split-plots (no spray treatment, trifloxistrobin + prothioconazole/Aureo Chemical Fungicide, Biofac Biological Fungicide, and association of the previous two).

2- ns = non-significant interaction among genotypes;

3- Original Means.

4- Means followed by the same lowercase letter in the column belongs to same group by the Scott-Knott test at 10% probability.

5- Means followed by the same uppercase letter in the line belongs to same group by the Scott-Knott test at 10% probability.

6- Values of S-W in bold mean normal distribution by Shapiro-Wilk test at the .05 significance level.

7- VC = Variance coefficient.

8- Residue independency attendance when in bold (non-rejected H_0) by Durbin Whatson test at the .05 significance level.

9- Values attendance of homoscedasticity in bold mean by Bartlett test at the .05 significance level.

10 – Conventional genotype from LAGER/UFU.

2.3 Powdery mildew *(Microsphaera diffusa*)

There was difference between repeated experiments over. Then, the results of the assays were presented separately after ANOVA analysis (Supplementary materials – S4).

About climatic conditions (Figure 7 and 8) the data observed during the first season could explain the higher AUDPC levels since rust and the *Microsphaera* fungus occur simultaneously in soybean crops after the reproductive stage with higher frequency.

The data presented (Table 9) at the first season trial (2017/2018), there was significant AUDPC differences and interaction ($p<0.10$) for genotypes and fungicides management at ranking test (Scott-Knott).

For genotypes mostly tested, had correlation associating genetic control and fungicide management where the lowest AUDPC values were observed on fungicide and genetic management with trifloxystrobin + prothioconazole and the trifloxystrobin + prothioconazole + *Penicillium sp*. in both seasons (Table 9).

UFU L154 and UFU L216 had no significance differences on AUDPC levels independent of fungicide management.

Table 9 – Area under disease progress curve for Powdery mildew in season 2017/18 for genotypes and fungicides management.

Genotypes	No Spray	Trifloxys.+ prothio.	*Penicillium* sp.	Trifloxys.+ prothio. + *Penicillium* sp.
BMX DESAFIO 8473RSF	741 bA	34 aB	747 bA	42 aB
BMX FLECHA IPRO	963 aA	25 aC	672 bB	11 aC
MO 7739 IPRO	961 aA	131 aB	910 bA	84 aB
NA 5909 RR	1072 aA	7 aC	731 bB	3 aC
TMG 7062 IPRO	1018 aA	17 aC	1278 aB	21 aC
TMG 7063 IPRO	865 bA	7 aB	773 bA	14 aB
UFU L218 Conv.[10]	315 cA	0 aB	193 cB	11 aB
UFU L154 Conv.[10]	238 cA	19 aA	102 cA	8 aA
UFU L216 Conv.[10]	0 dA	7 aA	7 cA	0 aA
UFU L266 Conv.[10]	276 bA	67 aB	234 cB	38 aA
S-W			0,0003	
Bartlett			0,0001	
Durbin-Watson			**1,01**	

VC-Plot (%)	41,17
VC-SplitPlot (%)	49,21

1- Analysis by four Split-plots (no spray treatment, trifloxistrobin + prothioconazole/Aureo Chemical Fungicide, Biofac Biological Fungicide, and association of the previous two).

2- ns = non-significant interaction among genotypes;

3- Original Means.

4- Means followed by the same lowercase letter in the column belongs to same group by the Scott-Knott test at 10% probability.

5- Means followed by the same uppercase letter in the line belongs to same group by the Scott-Knott test at 10% probability.

6- Values of S-W in bold mean normal distribution by Shapiro-Wilk test at the .05 significance level.

7- VC = Variance coefficient.

8- Residue independency attendance when in bold (non-rejected H_0) by Durbin Whatson test at the .05 significance level.

9- Values attendance of homoscedasticity in bold mean by Bartlett test at the .05 significance level.

10 – Conventional genotype from LAGER/UFU.

In second season (2018/2019) data (Table 10), besides lower disease pressure, there was significant AUDPC differences and interaction ($p<0.10$) between genotypes and fungicides management. In general, the lowest AUDPC values were observed on fungicide and genetic management with trifloxystrobin + prothioconazole and the trifloxystrobin + prothioconazole + *Penicillium sp.* Considering only genetic control, genotypes TMG 7062 IPRO, NA 5909 RR had the highest values respectively. Besides the second season disease pressure was lower than the first season, the interaction with genetic control and fungicide management was clearer.

There was some interaction response for Biofac management solo and the genotypes genotypes UFU L216, UFU L 218, UFU L 154 and UFU L 266 with lower AUDPC. The genotypes from LAGER/UFU breeding program presented evidence of stable resistance level against powdery mildew in both seasons experiment (Table 10). Considering only genetic control, the genotypes UFU L266, UFU L216, UFU L218, UFU L154, lowest values for AUDPC. Genotypes TMG 7062 IPRO, NA 5909 RR, MONSOY 7739 IPRO, and BMX FLECHA IPRO had the highest values (Table 10).

Table 10 – Area under disease progress curve for Powdery mildew in season 2018/19 for genotypes and fungicides management.

Genotypes	No Spray	trifloxys.+ prothio.	*Penicillium* sp.	trifloxys.+ prothio. + *Penicillium* sp.
BMX DESAFIO 8473RSF	125 cA	0 aB	36 cB	45 aB
BMX FLECHA IPRO	144 cA	0 aB	81 bA	1 aB
MO 7739 IPRO	218 cA	68 aB	169 bA	75 aB

NA 5909 RR	343 bA	0 aB	325 aA	0 aB
TMG 7062 IPRO	473 aA	7 aC	310 aB	3 aC
TMG 7063 IPRO	123 cA	56 aB	137 bA	4 aB
UFU L218 Conv.[10]	5 dA	0 aA	5 cA	0 aA
UFU L154 Conv.[10]	50 dA	3 aA	34 cA	0 aA
UFU L216 Conv.[10]	0 dA	0 aA	0 cA	0 aA
UFU L266 Conv.[10]	0 dA	0 aA	0 cA	0 aA
S-W			0,0001	
Bartlett			0,0001	
Durbin-Watson			**1,43**	
VC-Plot (%)			123,45	
VC-SplitPlot (%)			92,59	

1- Analysis by four Split-plots (no spray treatment, trifloxistrobin + prothioconazole/Aureo Chemical Fungicide, Biofac Biological Fungicide, and association of the previous two).

2- ns = non-significant interaction among genotypes;

3- Original Means.

4- Means followed by the same lowercase letter in the column belongs to same group by the Scott-Knott test at 10% probability.

5- Means followed by the same uppercase letter in the line belongs to same group by the Scott-Knott test at 10% probability.

6- Values of S-W in bold mean normal distribution by Shapiro-Wilk test at the .05 significance level.

7- VC = Variance coefficient.

8- Residue independency attendance when in bold (non-rejected H_0) by Durbin Whatson test at the .05 significance level.

9- Values attendance of homoscedasticity in bold mean by Bartlett test at the .05 significance level.

10 – Conventional genotype from LAGER/UFU.

2.4 Fungitoxicity and Grain yield

There was difference between repeated experiments over. Then, the results of the assays were presented separately after ANOVA analysis (Supplementary materials – S7).

No fungitoxicity signs were observed during trials in season 2017/18 and 2018/19 (Tables 11 and 12 - Fungitoxicity data). Grain Yield data revealed that the first season experiment demonstrated lower values (2385 kg. ha^{-1} – Table 11) after overall average calculation of the split-plot without fungicide spray in contrast of the data obtained at the second season (4381 kg. ha^{-1} – Table 12). In first season the higher rust and powdery mildew severity contributed significantly to the increased impact of yield. There was significant yield performance difference ($p<0.10$) for genotypes and fungicides management (Tables 11 and 12).

In first season, considersing genetic effect solo, TMG 7062 IPRO and TMG 7063 IPRO resistant to rust and NA5909 RR (considered susceptible to rust) had higher Yield (Kg. ha^{-1}) in comparison to other genotypes. LAGER/UFU program showed no significant differences in compare to commercial genotypes without fungicide management (Table 11).

For genotypes mostly tested, had higher Grain Yield values associating genetic control and fungicide management, in significance for management with trifloxystrobin + prothioconazole and the trifloxystrobin + prothioconazole + *Penicillium sp* in both seasons, except for TMG 7062 IPRO and TMG 7063 IPRO, same genotypes with *Rpp* genes, supposing lower Grain Yield protection response in use of fungicides. Higher Grain yield could be related to defoliation by disease evolution, since when earlier defoliation occurs, the smaller the grain size and, consequently, the greater the loss of yield and quality (GODOY; KOGA AND CANTERI, 2006).

UFU L154 and UFU L266 didn't show evidence of Grain Yield increment considering partial resistance effect solo, but, show Grain Yield increment with management trifloxystrobin + prothioconazole and the trifloxystrobin + prothioconazole + *Penicillium sp*. Providing higher Grain Yield with significance for TMG 7062 IPRO and TMG 7063 IPRO (Table 11).

The greatest difficulty in the development of partial resistance is determine these differences on several environmental conditions. But his problem could be solved with new crossing of materials with higher genetic potential for yield with partial resistant genotypes.

Table 11 – Fungitoxicity and grain Yield (Kg. ha $^{-1}$) in season 2017/18 for genotypes and fungicides management.

Genotypes	No Spray	trifloxys.+ prothio.	*Penicillium* sp.	trifloxys.+ prothio. + *Penicillium* sp.	Fungitoxicity
BMX DESAFIO 8473RSF	2119 b B	3338 aA	2366 bB	3828 aA	0
BMX FLECHA IPRO	2034 bB	3347 aA	2334 bB	3401 bB	0
MO 7739 IPRO	2409 bB	3538 aA	2470 bB	4066 aA	0
NA 5909 RR	2691 aB	3706 aA	2787 aB	3821 aA	0
TMG 7062 IPRO	3388 aA	3741 aA	3347 aA	3369 bA	0
TMG 7063 IPRO	3016 aA	3353 aA	3115 aA	3186 bA	0
UFU L218 Conv.[10]	1978 bB	3169 aA	2500 bB	3491 bA	0
UFU L154 Conv.[10]	1634 bB	3553 aA	1934 bB	3609 aA	0
UFU L216 Conv.[10]	2178 bB	2703 bA	2370 bB	2967 bA	0
UFU L266 Conv.[10]	2409 bB	3538 aA	2494 bB	3816 aA	0
S-W			**0,05**		-
Bartlett			**0,50**		-
Durbin-Watson			**1,33**		**-**
VC-Plot (%)			13,60		-
VC-SplitPlot (%)			14,83		-

1- Analysis by four Split-plots (no spray treatment, trifloxistrobin + prothioconazole/Aureo Chemical Fungicide, Biofac Biological Fungicide, and association of the previous two).

2- ns = non-significant interaction among genotypes;

3- Original Means.

4- Means followed by the same lowercase letter in the column belongs to same group by the Scott-Knott test at 10% probability.

5- Means followed by the same uppercase letter in the line belongs to same group by the Scott-Knott test at 10% probability.

6- Values of S-W in bold mean normal distribution by Shapiro-Wilk test at the .05 significance level.

7- VC = Variance coefficient.

8- Residue independency attendance when in bold (non-rejected H_0) by Durbin Whatson test at the .05 significance level.

9- Values attendance of homoscedasticity in bold mean by Bartlett test at the .05 significance level.

10 – Conventional genotype from LAGER/UFU.

In second season the lower rust and powdery mildew severity contributed significantly to genetic inherent characteristic from genotypes provided higher Grain Yield. There was significant yield performance difference ($p<0.10$) for genotypes and fungicides management (Table 12).

Considersing genetic effect solo, TMG 7062 IPRO resistant to rust and BMX DESAFIO 8473RSF, FLECHA IPRO and NA5909 RR (considered susceptibles to rust) had higher Grain Yield (Kg. ha $^{-1}$) in comparison to other genotypes. UFU L 266 and MO7739 IPRO had lowest Grain Yield (Table 12).

UFU L218 had evidence of Grain Yield increment considering partial resistance effect solo with management of *Penicillium* sp. solo, trifloxystrobin + prothioconazole and the trifloxystrobin + prothioconazole + *Penicillium sp*. providing higher Grain Yield with significance (Table 12).

UFU L154, and UFU L266 had Grain Yield increment with management trifloxystrobin + prothioconazole and the trifloxystrobin + prothioconazole + *Penicillium sp*. providing higher Grain Yield with significance for TMG 7062 IPRO and TMG 7063 IPRO (Table 12).

Table 12 – Fungitoxicity and grain Yield (Kg. ha $^{-1}$) in season 2017/18 for genotypes and fungicides management.

Genotypes	No Spray	Trifloxys.+ prothio.	*Penicillium* sp.	Trifloxys.+ prothio. + *Penicillium* sp.	Fungitoxicity
BMX DESAFIO 8473RSF	5138 aA	5916 aA	5338 bA	6046 bA	0
BMX FLECHA IPRO	5429 aA	6292 aA	5780 aA	6526 bA	0
MO 7739 IPRO	4289 cB	5863 aA	4631 bB	6339 bA	0
NA 5909 RR	5973 aB	7063 aA	6235 aB	7778 aA	0
TMG 7062 IPRO	5816 aA	6312 aA	6123 aA	6816 aA	0
TMG 7063 IPRO	4956 bA	5417 bA	5287 bA	5646 bA	0
UFU L218 Conv.[10]	3342 dC	4896 bB	4756 bB	5721 bA	0
UFU L154 Conv.[10]	3058 dB	4409 cA	3318 cB	4917 cA	0
UFU L216 Conv.[10]	3150 dA	3598 dA	3371 cA	4266 dA	0
UFU L266 Conv.[10]	2668 eB	3571 dA	2724 dB	4141 dA	0
S-W			0,03		-
Bartlett			0,27		-
Durbin-Watson			**1,71**		**-**
VC-Plot (%)			8,27		-
VC-SplitPlot (%)			10,86		-

1- Analysis by four Split-plots (no spray treatment, trifloxistrobin + prothioconazole/Aureo Chemical Fungicide, Biofac Biological Fungicide, and association of the previous two).

2- ns = non-significant interaction among genotypes;

3- Original Means.

4- Means followed by the same lowercase letter in the column belongs to same group by the Scott-Knott test at 10% probability.

5- Means followed by the same uppercase letter in the line belongs to same group by the Scott-Knott test at 10% probability.

6- Values of S-W in bold mean normal distribution by Shapiro-Wilk test at the .05 significance level.

7- VC = Variance coefficient.

8- Residue independency attendance when in bold (non-rejected H_0) by Durbin Whatson test at the .05 significance level.

9- Values attendance of homoscedasticity in bold mean by Bartlett test at the .05 significance level.

10 – Conventional genotype from LAGER/UFU.

CONCLUSION

The strategy of use partial resistance associated with chemical and biological fungicides can be the best strategy considering the protection for resistance selection in field over the years, taking yield a drive for selection.

The lowest AUDPC level for ASR, Septoria brown spot and Powdery mildew and highest productivity values were observed on chemical and biological fungicide and genetic management associated in both seasons.

The are interaction of genetic, environment, chemical and biological tools. Those interaction suggest the assessment on genotypes and managements together for more assertive recommendation. The interaction between genotypes with partial or non-known resistance genes for rust had higher yield response when the associated management where adopted, suggesting to breeding programs include biological and chemical programs to select elite genotypes.

Materials with stainless steel technologies had lowest AUDPC levels considering only genetic control effect in both seasons. There was not correlation with materials with resistance to ASR to Septoria brown spot and Powdery mildew.

There are differences for diseases resistance and Grain Yield response, associating chemical and biological control in the genotypes studied providing synergic and no additive effects. TMG 7062 IPRO and TMG 7063 IPRO, same genotypes with *Rpp* genes, had lower Grain Yield protection response with use of fungicides.

Its fundamental considering in breeding programs resistance for secondary diseases as Septoria brown spot and powdery mildew, that can reduce grain yield potential and looking to guarantee the maximum grain yield potential.

For cultivar development and Grain Yield improvement a durable and stable resistance like partial resistance with good interaction to fungicides can provide an economic and environmentally friendly way to protect soybean crops from the majority *P. pachyrhizi* pathotypes on different geographical regions.

The probability to obtain superior genotypes its important the recombination with lines with some resistance genes for diseases and adapted lines with higher Grain Yield potential for region.

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SUPPLEMENTARY MATERIALS

Table S1 ANOVA of mean square and variance coefficient of: Area under disease progress c urve (AUDPC) in the assays with Split-plot design made in Uberlândia - MG, first assay 201 8.

		Mean Square Year 2018		
SV	**DF**	AUDPC Ferrugem	AUDPC Oídio	AUDPC Septoria
Genotypes	9	80.89 ***	38.53 ***	7.96 ***
Block	3	3.57 *	1.91 ns	0.48 ns
Residue a	27	7	2	3
Fungicides	3	1486.21 ***	185.58 ***	1.66 ***
Genotypes*Fungicides	27	18.44 ***	9.02 ns	2 *
Residue b	90	92	4	6
VC: Plot %	-	13.80	41.17	53.23
VC: Split-plot %	-	16.32	49.21	41.10

ns Not significant; *** Significant at 0.1% of probability; ** Significant at 1% of probability; * Significant at 5% of probability

DF: Degrees of freedom

Table S2 ANOVA of mean square and variance coefficient of: Area under disease progress c urve (AUDPC) in the assays with Split-plot design made in Uberlândia - MG, first assay 201 9.

		Mean Square Year 2019		
SV	**DF**	AUDPC Ferrugem	AUDPC Oídio	AUDPC Septoria
Genotypes	9	81.77 ***	129.464 ***	21.25 ***
Block	3	1.19 ns	0.883 ns	02.32 *
Residue a	27	5	3	7
Fungicides	3	1021.24 ***	46.02 ***	278.08 ***
Genotypes*Fungicides	27	20.13 ***	1.63 *	6.72 ***
Residue b	90	2	4	6
VC: Plot %	-	16.77	123.45	23.21
VC: Split-plot %	-	14.77	92.59	19.16

ns Not significant; *** Significant at 0.1% of probability; ** Significant at 1% of probability; * Significant at 5% of probability

DF: Degrees of freedom

Table S3 ANOVA of mean square and variance coefficient of: Yield (Produção) in the assays with Split-plot design made in Uberlândia – MG.

		Mean Square	
SV	**DF**	Yield 2018	Yield 2019
Genotypes	9	10.88 ***	30.80 ***
Block	3	2.13 ns	2.77 ns
Residue a	27	6	7
Fungicides	3	43.65 ***	21.51 ***
Genotypes*Fungicides	27	6.34 ***	0.86 ns
Residue b	90	5	6
VC: Plot %	-	123.45	15.94
VC: Split-plot %	-	92.59	14.98

ns Not significant; ** f probability; ** Signif ity; * Significant at 5% DF: Degrees of freedo

* Significant at 0.1% o icant at 1% of probabil of probability m

Table S4. Residual and joint analysis of AUDPC Oídio in function of the two-way factorial: genotypes x year (2018, 2019) in Uberlândia – MG.

Source of Variation	**Estimate**	**Std. Error**	**F value**	**P value**
(Intercept)	648.196	76.441	8.480	1.06e-15 ***
AnoB	-542.093	108.104	-5.015	9.14e-07 ***
AnoA:Bloco	-30.244	4.530	-6.676	1.20e-10 ***
AnoB:Bloco	-6.435	4.530	-1.421	0.1565 ns
GenFLECHA	26.469	93.387	0.283	0.7770 ns
GenMO7739	130.375	93.387	1.396	0.1637 ns
GenNA5909	61.906	93.387	0.663	0.5079 ns
GenTMG7062	192.062	93.387	2.057	0.0406 *
GenTMG7063	23.625	93.387	0.253	0.8005 ns
GenUFU218	-261.187	93.387	-2.797	0.0055 **
GenUFUL154	-299.250	93.387	-3.204	0.0015 **
GenUFUL216	-387.625	93.387	-4.151	4.33e-05 ***

GenUFUL266	-237.344	93.387	-2.541	0.0115 *
AnoB:GenFLECHA	-21.437	132.070	-0.162	0.8712 ns
AnoB:GenMO7739	-49.219	132.070	-0.373	0.7097 ns
AnoB:GenNA5909	53.594	132.070	0.406	0.6852 ns
AnoB:GenTMG7062	-45.500	132.070	-0.345	0.7307 ns
AnoB:GenTMG7063	4.887	132.070	0.037	0.9705 ns
AnoB:GenUFU218	212.406	132.070	1.608	0.1088 ns
AnoB:GenUFUL154	269.500	132.070	2.041	0.0422 *
AnoB:GenUFUL216	336.219	132.070	2.546	0.0114 *
AnoB:GenUFUL266	185.938	132.070	1.408	0.1602 ns

ns Not significant; *** Significant at 0.1% of probability; ** Significant at 1% of probability; * Significant at 5% of probability

DF: Degrees of freedom

Residual standard error: 264.1 on 298 degrees of freedom

Multiple R-squared: 0.4138, Adjusted R-squared: 0.3725

F-statistic: 10.02 on 21 and 298 DF, p-value: < 2.2e-16

Table S5. Residual and joint analysis of AUDPC Septoria in function of the two-way factorial: genotypes x year (2018, 2019) in Uberlândia – MG.

Source of Variation	Estimate	Std. Error	F value	P value
(Intercept)	143.513	45.675	3.142	0.001847 **
AnoB	537.470	64.595	8.321	3.18e-15 ***
AnoA:Bloco	-6.127	2.707	-2.263	0.024328 *
AnoB:Bloco	-23.601	2.707	-8.719	< 2e-16 ***
GenFLECHA	97.344	55.801	1.744	0.082107 *
GenMO7739	121.188	55.801	2.172	0.030661 *
GenNA5909	74.375	55.801	1.333	0.183597 ns
GenTMG7062	188.562	55.801	3.379	0.000824 ***
GenTMG7063	131.906	55.801	2.364	0.018727 ns
GenUFU218	40.250	55.801	0.721	0.471284 *
GenUFUL154	82.031	55.801	1.470	0.142600 ***
GenUFUL216	232.969	55.801	4.175	3.92e-05 ns
GenUFUL266	29.969	55.801	0.537	0.591625 ns
AnoB:GenFLECHA	-166.469	78.915	-2.109	0.035737 ns
AnoB:GenMO7739	-268.406	78.915	-3.401	0.000762 ns

AnoB:GenNA5909	-142.187	78.915	-1.802	0.072590 *
AnoB:GenTMG7062	-229.906	78.915	-2.913	0.003846 **
AnoB:GenTMG7063	54.688	78.915	0.693	0.488853 ns
AnoB:GenUFU218	-47.615	78.915	-0.603	0.546720 ns
AnoB:GenUFUL154	-67.375	78.915	-0.854	0.393919 ns
AnoB:GenUFUL216	-367.938	78.915	-4.662	4.72e-06 ***
AnoB:GenUFUL266	-272.344	78.915	-3.451	0.000639 ***

ns Not significant; *** Significant at 0.1% of probability; ** Significant at 1% of probability; * Significant at 5% of probability

DF: Degrees of freedom

Residual standard error: 157.8 on 298 degrees of freedom

Multiple R-squared: 0.5536, Adjusted R-squared: 0.5221

F-statistic: 17.6 on 21 and 298 DF, p-value: < 2.2e-16

Table S6. Residual and joint analysis of AUDPC Ferrugem in function of the two-way factorial: genotypes x year (2018, 2019) in Uberlândia – MG.

Source of Variation	**Estimate**	**Std. Error**	**F value**	**P value**
(Intercept)	1282.380	125.542	10.21	< 2e-16 ***
AnoB	-618.201	177.543	-3.482	0.000572 ***
AnoA:Bloco	-61.158	7.440	-8.220	6.30e-15 ***
AnoB:Bloco	-34.116	7.440	-4.586	6.67e-06 ***
GenFLECHA	189.537	153.373	1.236	0.217511 ns
GenMO7739	-60.583	153.373	-0.395	0.693125 ns
GenNA5909	8.992	153.373	0.059	0.953288 ns
GenTMG7062	-501.023	153.373	-3.267	0.001215 **
GenTMG7063	-369.414	153.373	-2.409	0.016621 *
GenUFU218	-115.959	153.373	-0.756	0.450211 ns
GenUFUL154	-55.268	153.373	-0.360	0.718844 ns
GenUFUL216	-223.716	153.373	-1.459	0.145718 ns
GenUFUL266	-122.566	153.373	-0.799	0.424848 ns
AnoB:GenFLECHA	55.999	216.903	0.258	0.796448 ns
AnoB:GenMO7739	196.033	216.903	0.904	0.366842 ns
AnoB:GenNA5909	-36.784	216.903	-0.170	0.865450 ns
AnoB:GenTMG7062	350.173	216.903	1.614	0.107494 ns
AnoB:GenTMG7063	168.151	216.903	0.775	0.438815 ns

AnoB:GenUFU218	377.016	216.903	1.738	0.083212 *
AnoB:GenUFUL154	355.399	216.903	1.639	0.102369 ns
AnoB:GenUFUL216	424.835	216.903	1.959	0.051087 ns
AnoB:GenUFUL266	288.160	216.903	1.329	0.185022 *

ns Not significant; *** Significant at 0.1% of probability; ** Significant at 1% of probability; * Significant at 5% of probability

DF: Degrees of freedom

Residual standard error: 433.8 on 298 degrees of freedom

Multiple R-squared: 0.3421, Adjusted R-squared: 0.2957

F-statistic: 7.378 on 21 and 298 DF, p-value: < 2.2e-16

Table S7. Residual and joint analysis of Yield (Produção) in function of the two-way factorial: genotypes x year (2018, 2019) in Uberlândia – MG.

Source of Variation	Estimate	Std. Error	F value	P value
(Intercept)	2378.40	216.76	10.97	< 2e-16 ***
AnoB	2196.55	306.54	7.166	6.11e-12 ***
AnoA:Bloco	62.84	12.85	4.892	1.64e-06 ***
AnoB:Bloco	80.48	12.85	6.266	1.30e-09 ***
GenFLECHA	-133.50	264.81	-0.504	0.61454 ns
GenMO7739	208.00	264.81	0.785	0.43281 ns
GenNA5909	338.91	264.81	1.280	0.20161 ns
GenTMG7062	548.44	264.81	2.071	0.03921 *
GenTMG7063	255.06	264.81	0.963	0.33624 ns
GenUFU218	-128.12	264.81	-0.484	0.62886 ns
GenUFUL154	-229.97	264.81	-0.868	0.38586 ns
GenUFUL216	-358.09	264.81	-1.352	0.17732 ns
GenUFUL266	151.53	264.81	0.572	0.56760 ns
AnoB:GenFLECHA	922.98	374.50	2.465	0.01428 *
AnoB:GenMO7739	-45.64	374.50	-0.122	0.90309 ns
AnoB:GenNA5909	976.66	374.50	2.608	0.00957 **
AnoB:GenTMG7062	520.56	374.50	1.390	0.16556 ns
AnoB:GenTMG7063	1.55	374.50	0.004	0.99670 ns
AnoB:GenUFU218	-143.51	374.50	-0.383	0.70184 ns
AnoB:GenUFUL154	-991.01	374.50	-2.646	0.00857 **
AnoB:GenUFUL216	-1420.68	374.50	-3.794	0.00018 ***

AnoB:GenUFUL266	-1997.48	374.50	-5.334	1.91e-07 ***

ns Not significant; *** Significant at 0.1% of probability; ** Significant at 1% of probability; * Significant at 5% of probability

DF: Degrees of freedom

Residual standard error: 749 on 298 degrees of freedom

Multiple R-squared: 0.7802, Adjusted R-squared: 0.7647

F-statistic: 50.37 on 21 and 298 DF, p-value: < 2.2e-16

UNIVERSIDADE FEDERAL DE UBERLÂNDIA
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ATA DE DEFESA - PÓS-GRADUAÇÃO

Programa de Pós-Graduação em:	Agronomia				
Defesa de:	Tese, 08/2021, PPGAGRO				
Data:	Sete de maio de dois mil e vinte e um	Hora de início:	08:00	Hora de encerramento:	12:12
Matrícula do Discente:	11713AGR011				
Nome do Discente:	Fernanda Cristina Juliatti				
Título do Trabalho:	Soybean diseases integrated control with biological and chemical fungicides in differents resistance levels of genotypes				
Área de concentração:	Fitotecnia				
Linha de pesquisa:	Melhoramento de Plantas				

Reuniu-se por videoconferência, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Agronomia, assim composta: Professores Doutores: Osvaldo Toshiyuki Hamawaki - UFU; José Magno Queiroz Luz - UFU; Juliana Araújo Santos Martins - IFTM; Edson Ampélio Pozza - UFLA; Ana Paula Oliveira Nogueira - UFU orientadora da candidata.

Iniciando os trabalhos o(a) presidente da mesa, Dra. Ana Paula Oliveira Nogueira, apresentou a Comissão Examinadora e o candidato(a), agradeceu a presença do público, e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as), que passaram a arguir o(a) candidato(a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

[A]provado(a).

Esta defesa faz parte dos requisitos necessários à obtenção do título de Doutor.

O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.

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