BRENO CEZAR MARINHO JULIATTI

BIOCHEMICAL, PHYSIOLOGICAL AND EPIDEMIOLOGICAL CHARACTERIZATION OF SOYBEAN GENOTYPES (*Glycine max*) WITH PARTIAL RESISTANCE AGAINST SOYBEAN RUST (*Phakopsora pachyrhizi* Sydow & P.Sydow)

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fitopatologia, área de concentração em Fitopatologia, para a obtenção do título de Doutor.

Prof. Edson Ampélio Pozza
Orientador

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Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Juliatti, Breno Cezar Marinho.
Biochemical, physiological and epidemiological characterization of soybean genotypes (*glycine max*) with partial resistance against soybean rust (*Phakopsora pachyrhizi* Sydow & P.Sydow) / Breno Cezar Marinho Juliatti. - 2018.
132 p.

Orientador(a): Edson Ampelio Pozza.
.
Tese (doutorado) - Universidade Federal de Lavras, 2018.
Bibliografia.

1. Asian soybean rust. 2. Partial resistance. 3. Epidemiology. I. Pozza, Edson Ampelio. . II. Título.

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APROVADA em 29 de agosto de 2018.

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LAVRAS - MG
2018

A todos aqueles que de alguma forma estiveram e estão comigo.

Pela amizade oferecida, pelo companheirismo e pela amizade verdadeira, características as quais não me trariam até aqui.

Em especial ao Juliatti, meu pai: e dona Mirtaceae, minha mãe, pelo dom sublime que me deram "a vida".

Aos meus irmãos, Fernanda e Bruno, meus primos Thales, Felipe, Hiago e Mardem, e todos os meus amigos em especial Pedro Ivo, Alexandre, Edgard, Igor e Leandro pela amizade em seu pleno significado.

E a minha namorada Lorena, pelo amor em sua continuidade.

DEDICO

AGRADECIMENTOS

Foram 3 anos de experiências, e agradeço por todos os tipos que vivi, mesmas as ruins. E que apesar de terem sido obstáculos, me fizeram mais forte. Agradeço a dedicação demonstrada pela excelente equipe que trabalhou comigo neste tempo, e que resultou no trabalho a seguir, informações dignas do rompimento das barreiras do conhecimento. Nuca fui bom com palavras, e farei uma síntese do que sinto, mas quero deixar bem claro para as pessoas aqui mencionadas, caso leiam este agradecimento, deixo o meu "Muito Obrigado".

Primeiramente aos colegas de trabalho da Universidade Federal de Lavras e Departamento de Fitopatologia (UFLA-DFP)– Nathan Carvalho, Alan Carlos, Manoel Batista, Gustavo Silveira, Bruno Marques, Ana Cristina, Stephanny Araújo, Deila Magna, Bruno Costa, Dalyse Toledo, Milene Alves e ao meu para sempre querido amigo e colega de trabalho Stélio Jorge Castro Gadaga (In memoriam) –, por me apoiarem no que diz respeito à instalação, condução, avaliação dos experimentos e elaboração dos resultados.

Aos membros das gestões 2015 e 2016 do Núcleo de Estudos em Fitopatologia (NEFIT), além do coordenador Dr. Flávio Medeiros.

Agradeço também aos técnicos, Ariane, Sâmara, Kássio, Ralf, Carzim, Sílvio e Roberto pelo apoio na coordenação, nas análises, na avaliação e na coleta dos dados e materiais cedidos; ao Prof. Dr. Fernando Cezar Juliatti da UFU, pelo apoio na pesquisa no que tange às sementes dos genótipos testados, à estrutura, à descrição dos dados obtidos e às interpretações técnicas; e pôr fim ao Prof. Edson Ampélio Pozza da Universidade Federal de Lavras, por ter me orientado, pelos ensinamentos, confiança e dado suporte para a finalização deste trabalho.

Um especial agradecimento a Universidade Federal de Uberlândia e Universidade Federal de Lavras os quais estarão para sempre registradas na minha formação profissional, além da empresa JuliAgro B, G & P e a empresa Cerrado View pelo mapeamento por sensoriamento através de drones. Agradeço mais uma vez a todos pelo apoio neste trabalho. Sem ele, a realização da presente pesquisa não seria possível.

"Resistance is not black and white, but various shades of gray."

R.R Nelson 1981

ABSTRACT

Climatic conditions, inoculum concentration, and plant susceptibility directly affect the manifestation of Asian rust in soybean also plant resistance is characterized by an alteration in pathogen survival skills and perpetuation process. The development of new resistant cultivars against diseases is a primordial step in the preservation of plant yield since the chemical control is losing efficacy in field. The objective of this work was to analyze a diversity of parameters, interacting with a commercial cultivar and soybean genotypes with partial resistance in Asian rust management, under different conditions. In the first study we identified the reaction and interaction of a single fungicide spray with 6 soybean genotypes and one susceptible standard (Desafio RR 8473 RSF) of the soybean breeding and improvement program developed by (LAGER / UFU), after inoculation of *Phakopsora pachyrhizi*. The variables evaluated made before and after inoculation, consisted on agronomic traits and disease progress. The study was conducted in two different seasons inside a greenhouse. The results indicated significant difference between season and genotype and interaction between split-plots ($p<0.05$). The Genotypes 2 (F8 BRSGO Luziânia X Potenza), 3 (F8 BRSGO Caiapônia X Potenza), 6 (F8 BRSGO Luziânia X Potenza) performed partial resistance, so we can conclude that exist genes of interest in the Brazilian germplasm, cultivar Potenza. In the second paper, the objective was to evaluate the activity of enzymes (POX and PAL), formation of horizontal barriers and photosynthetic parameters in plants infected with Asian rust. The genotypes that stood out with the higher overall enzyme activity (PAL, POX), lignin content and epidermis cell wall thickness was the 2 (F8 BRSGO Luziânia X Potenza) and 3 (F8 BRSGO Caiapônia X Potenza), these genotypes showed chemical traits for horizontal resistance composition. In the third experiment, the influence of temperature (18, 21, 24, 27 and 30 °C) and leaf wetness duration (0, 6, 12, 24 and 48 hours) on the penetration of the causal agent of soybean Asian rust (*Phakopsora pachyrhizi*) was quantified under controlled environment. There is a relationship between temperature, leaf wetness with AUDPC and incubation period. The highest AUDPC occurred at 24 °C and leaf wetting period of 24 hours, however the highest AUDPC were in the range of 21 to 24 °C of temperature and after 12 hours of leaf wetting duration. The genotypes 2 (F8 BRSGO Luziânia X Potenza) and 3 (F8 BRSGO Caiapônia X Potenza), showed partial resistance traits against rust progression, during the monocycle experiment.

Keywords: Asian soybean rust, partial resistance, epidemiology, enzymes, epicuticular wax

SUMMARY

FIRST PART

1 INTRODUCTION

Soybean is the most important agricultural item in Brazilian agriculture. During the 2016/2017 crop, 113.923 million tons were harvested. Brazil is the second largest soybean producer around the world (USDA, 2017; CONAB, 2017). According to Juliatti et al. (2005), diseases are one of the major factor that limits yield, profitability and the success of your production. Globally, more than 100 diseases are listed in the soybean crop (SINCLAIR AND BACKMAN, 1989), of which approximately 50 have already been identified in Brazil. 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. It is estimated that the annual production losses due to diseases will be 15 to 20%, however some can reach up to 100% (EMBRAPA, 2007; JULIATTI et al., 2005).

The Asian rust of soybean (ASR), caused by the fungus *Phakopsora pachyrhizi* Syd., is one of the main phytosanitary problems of the crop (JULIATTI et al., 2003). The damage caused by ASR is the reduction in the number of pods, and grains, and your weight, due to premature plant defoliation (OGLE, BYTH AND MCLEAN, 1979). Disease control has required a combination of cultural practices to minimize damage and loss. 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). The active ingredients registered to control this disease 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; FARIAS, ABDELNOOR and GODOY, 2014). 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 (FARIAS, ABDELNOOR and GODOY, 2014; FRAC, 2017). With this new scenario, control strategies, such as the use of resistance are desirable for the efficient management of the crop.

Plant resistance can be defined as the ability of the host to prevent the growth and development of the pathogen (PARLEVLIET, 1997). Growers and breeders of soybean want rapidity and immediate results to lower the infection caused by rust, so they adopted the use

of materials with complete resistance using RPP genes. So far six single dominant resistance loci genes have been mapped (Rpp1 to Rpp6) and are described in soybean resistant reaction against *P. pachyrhizi* infection with the appearance of reddish-brown (RB) lesions, while susceptible plants react with the formation of tan lesions (BROMFIELD and HARTWIG, 1980; MCLEAN AND BYTH, 1980; BROMFIELD AND MELCHING, 1982; HARTWIG AND BROMFIELD, 1983; HARTWIG, 1986; GARCIA et al., 2008; LI et al., 2012). Some authors reported that these dominant genes isolated are not effective when faced with different isolates in the population of *P. pachyrhizi* (BONDE et al., 2006; YAMANAKA et al., 2008; MILES et al., 2011). 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). But the "vertifolia effect "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 (VAN DER PLANK 1963). One of the main objectives of most amateur plant breeders will be to restore the horizontal resistances that were lost to the "vertifolia effect".

The partial resistance is characterized by the reduction of the epidemic rate, the decrease in the number and size of the lesions, the decrease in the production of urediniospores, and the increase in the latent period. This reduces the amount of inoculum and disease intensity during the crop cycle (WANG AND HARTMAN, 1992). This type of resistance becomes visible after the non-durable or monogenic resistance has been overcome by a new breed of pathogen (PARLEVLIET, 1997). According to Parlevliet (1978), selection for partial resistance in the presence of major 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) quantifying rust severity through partial resistant genotypes, estimated that rust resistance is a characteristic controlled by 2 to 23 genes that are predominantly dominant. This job 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.

Based on the above, the objective was to study the biochemical, physiological and epidemiological characterization of soybean genotypes with partial resistance of a germplasm

source program developed by the Germplasm Laboratory in the Federal University of Uberlandia (LAGER/UFU).

2 THEORETICAL REFERENCE

2.1 General aspects of soybean (*Glycine max* (L.) Merrill)

The soybean belongs to the family Fabaceae (legume), as well as beans, lentils and peas. It is used in food, especially in the edible oils industry, because it is considered a protein source, (AZEVEDO et al., 2010). 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. In this context, 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 and 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). The center of origin of this culture lies in the region of Manchuria, where winter wheat was grown in the time of the great Chinese dynasties. In the West, the grain appears in the late fifteenth and early sixteenth century, the time of so-called great European navigation period. 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. After the first World War in 1919, soybean became an important commodity. In 1921, the American Soybean Association (ASA) was founded, as and helped to consolidate the soybean production chain in the world (APROSOJA, 2017). Currently, the world's largest soybean producers, according to data from 2017, are the United States (34%), followed by Brazil (32%), Argentina (16%), China (6%) and India (4%). World soybean production in 2017 was 340.07 million tons (FAO, 2017).

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 and AOYAGI, 1980).

In 1900 and 1901, the Agronomic Institute of Campinas (IAC), in São Paulo, promoted the first distribution of soybeans for São Paulo farmers, and during this period, the first soybean crop was registered in Rio Grande do Sul (RS), where the crop found effective conditions to develop and expand, given the climatic similarities of the southern US origin ecosystem of existing genetic materials with the prevailing climatic conditions in the extreme south of Brazil. Incentives create by the Brazilian government for wheat production in the mid-1950s, also stimulated farmers to produce soybean, since it was technically (legume succeeding) and economic (better utilization of land, machinery / implements, infrastructure and manpower), the most interesting crop as an alternative (summer season) to succeed the wheat (winter season). However, the first major expansion of soybeans in Brazil occurred in the 1970s, with a 25.9% increase in production - from 1.3 to 8.8 million hectares planted (DIAS et al., 2007).

The success of soybeans in the United States, together with the rise of the poultry sector in the south of that country, has raised the interest of studies in Brazil for the development of soybeans that could be grown at lower latitudes. Researchers have quickly developed adapted late cycle varieties for warmer climates, focusing on photoperiod in the growth and development of soybeans. The new varieties became the opening for Brazilians: for this, the researchers took the technology of low latitude and developed a germplasm that could be implanted in the three southern states of Brazil - Rio Grande do Sul, Santa Catarina and Paraná -, regions with a similar climate to the south of the USA (SCHNEPF, DOHLMAN and BOLLING, 2001). In the 1980s, the Brazilian Agricultural Research Corporation (EMBRAPA) had advanced in the search for adequate photoperiod, successfully adapting soybeans at even lower latitudes. The development of such technology has opened the western and northern regions of the country, which lie between 15° south latitudes and 5° north latitudes, to produce soybeans. In this way, the region that showed the greatest potential was the Brazilian Savanah (Cerrado), which encompasses more than 200 million hectares of low forest, easy to explore and with predictable rainfall levels. The development of lower latitude varieties begins the real history of the Brazilian soybean complex. It should be noted that, in 2017/2018, the country is estimating to produce 114.97 thousand tons, which corresponded to 26.8% of the world production. In the 2014/2015 harvest, production was 90.1 million tons, surpassed only by the American, 97 million. The largest Brazilian

producers are Mato Grosso, Paraná 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 (WSB, 2014). Even so, the Cerrado agriculture presents major challenges. Infrastructure is underdeveloped, markets are distant, soils are relatively poor and there are environmental concerns, but with continued use of the right technology and proper management, it will continue to be an important window of global soybeans.

2.2 Major crop diseases

Wrather et al. (2001) estimated a loss of 15 million tons in production due to diseases in the ten largest soybean producing countries in the world, which generated an estimated economic loss of US $ 3.31 billion. Even though productivity per high area, production losses in soybean cultivation occur mainly due to the attack of pathogens of varied etiology. In Brazil, 45 diseases occur, of which 28 are caused by fungi; eight, per virus; bacteria and nematodes add up to three diseases each; and there are three other diseases of unknown etiology (ALMEIDA, 1997; YORINORI, 1997; EMBRAPA, 2011). In the case of soybeans, the annual production losses due to diseases are estimated at around 20%, but some may cause losses of almost 100% (EMBRAPA, 2007). There are reports from producers about the reduction of approximately 30% in soybean yield in areas with high nematode populations (DIAS et al., 2007) and up to 100% with Asian rust (*Phakopsora pachyrhizi*) (EMBRAPA, 2005), which favored the creation of a sanitary emptiness during the off-season of the culture, for the reduction of this last illness. The expansion of the crop allowed, for example, the cultivation of soybeans in irrigated areas in the Cerrado region, in the autumn and winter seasons for seed production. In the meantime, the proximity of large-growing regions with extensive soybean areas in Mato Grosso and Paraná favored the survival and dispersion of fungi causing anthracnosis (*Colletotrichum truncatum*), rust (*Phakopsora pachyrhizi*), stem canker (*Diaporthe phaseolorum,* white mold (*Sclerotinia sclerotiorum*), root red rot (*Fusarium solani* f.s *glycines*), downy mildew (*Peronospora manshurica*), root gall nematodes (*Meloidogyne* spp.) and cyst nematode (*Heterodera glycines*) (EMBRAPA, 2011).

2.3 Rust resurgence in Brazil and economic impact

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 (BROMFIELD, 1984; HARTMAN, WANG and SHANMUGASUNDARAM, 1997; SINCLAIR and HARTMAN, 1999). It was only in the 1990s that the disease was recorded on the African continent, the countries bordering Asia, and later southern Africa in 2001. The greatest damage was registered in Uganda, Kenya, Rwanda, Zambia, Zimbabwe, Mozambique and South Africa (KAWUKI, TUKAMUHABWA and ADIPALA, 2004). Deslandes in 1979 described inside soybean test fields, the presence of rust in the city of Lavras (MG), this issue raised a potential risk for Asian countries production. The non-confirmation of potential damages, over the years, reduced the priority of disease research. In the 1990/91 crop, rust developed epidemics 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%. The 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., 2004). 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. The rust damage cost from the 2002/2003 to the 2016/2017, reached the amount of US $ 15 billion (EMBRAPA, 2017). In the 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 and 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).

2.4 Etiology

Until 1992 *P. pachyrhizi* was recognized as the only species that causes soybean rust, but Ono, Butirica and 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 soybean rust 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 and 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 and 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.

2.5 Symptomatology

The symptoms or signals of Asian rust 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 Asian rust 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*. 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 uredinias on the abaxial side of the leaf, 2 weeks after inoculation and is a symptom indicating the associated resistance with host hypersensitivity. Type 0 is the absence of evidence that is visible macroscopically, indicating immunity or proximity to immunity (BROMFIELD, MELCHING and KINGSOLVER, 1980). The uredinias 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 and CANTERI, 2006). In several geographic regions where, Asian rust has been reported at epidemic levels, damage varies from 10 to 90% of production (HARTMAN, WANG and SHANMUGASUNDARAM, 1997; YORINORI et al., 2002).

2.6 Effects of temperature and moisture in the disease epidemiology

Several epidemiological studies have been developed to correlate disease components with climatic variables and productivity, to provide ancillary information to prediction and damage models (YANG et al., 1991; HARTMAN, WANG, TSCHANZ, 1991; REIS, SARTORI and CAMARA, 2004; ZAMBENEDETTI et al., 2007; BONDE, NESTER and BERNER, 2012). The environment can directly affect the plant diseases intensity, acting on host and pathogen. The main environmental factors are temperature, rainfall, relative air humidity, light intensity, fertility and soil pH, as well as atmospheric CO_2 concentration, among others (LUCK et al., 2011). Temperature and moisture are the two most important environmental factors to determine the rate of the polycyclic stages from germination to the colonization of a disease agent like the soybean rust (MELCHING et al., 1989; BONDE, NESTER and BERNER, 2012). Moisture is described as the driving force to increase disease intensity, even though rain can provide the necessary moisture, longer periods of day with dew, is probably the more important source of free water for fungi penetration and development on hosts (BROMFIELD, 1984; MELCHING et al., 1989; BONDE, NESTER and BERNER, 2012). The temperature in this binomial interaction, also has directly influence in the pathogen metabolic stages of the life cycle, since when an optimal temperature is present the pathogen can perform their metabolic functions with minimal stress during the infection (BONDE et al., 2007; DIAS et al., 2005). The rust is a biotrophic parasite and survives in dry period months and under unfavorable conditions, in alternative hosts. It also does not require open stomata to penetrate and can frequently penetrates directly through the

cuticle and epidermis, making infection quicker and easier. The processes of spore germination, infection, latent period, lesion expansion and sporulation are influenced by meteorological like temperatures between averages of 12 to 25 °C with air relative humidity between 70 and 80%, and high temperatures reached during daylight do not inhibit fungus development (MARCHETTI, MELCHING and BROMFIELD, 1976; MELCHING et al., 1989). Uredospores collected of the Wills cultivar had the germination significantly reduced when exposed to temperatures ranging from 28.5 to 42.5 °C and exposure to temperatures lower than 24.5 °C for 8 h can initially retard germination (KOCHMAN, 1979). Also, a favorable range of temperature, heavily influences different levels of disease intensity over time on different cultivars or susceptible plants like one of the alternative host of soybean rust the kudzu of the *Pueraria* genera (SLAMINKO et al., 2008). Other authors also described that optimal temperature averages ranges between 20-25 °C, followed always by greater periods of leaf wetting produced lower latent and incubation periods in different cultivars and genotypes (KOCHMAN, 1979; CASEY, 1980; Vale, 1985; SINCLAIR and BACKMAN, 1989; ALVES et al., 2007; BONDE, NESTER and BERNER, 2012).The interaction between pathogen and host will lead to the appearance of the typical lesion of the disease and the various events that occur between deposition and the formation of new urediniospores constitute a single cycle of infection, or the monocycle.

Author of various sources of works with the combined effect of leaf wetness and temperature, usually describe that the optimal leaf wetting period and temperature range were 6 to 24 hours and 20-23 °C respectively. Marchetti, Uecker and Bromfield (1975) determined once the infection was established within the optimum range of temperature and leaf wetness, the pathogen was able to colonize the tissue, even under a temperature of 30 °C, lethal to the infectious process. However, the temperature of 30 °C the incubation and latency periods were 6 and 12 days, respectively, against 4 and 9 days when colonization occurred under the optimal temperature of 23 °C. Melching et al., 1989 studying duration, frequency and temperature of different wetting periods regimes determined that no rust lesions were formed on leaves of the soybean cultivar Wayne in temperatures lower than 9 °C and above 28.5 °C even with a 16 hours of dew period. They also stated that a period of 6 to 7 hours of continuous leaf wetting were required for rust lesions to develop between 18 to 26.5 °C. Del Ponte et al. in 2006, observed that greater leaf wetness found in periods inside the raining season in Brazil, improves ASR disease severity. At least six hours of moisture is required for infection to occur at 24 °C in japan (KITANI, INOUE and NATSUME, 1960), from experiments in South Africa, no rust infection was found in soybean plants incubated at

temperatures below 15 or greater than 30 °C (CALDWELL et al., 2005) and in Australia a 17 to 27 ºC temperature regime was clearly more favorable for rust infection during 16 hours of dew (KOCHMAN, 1979). In greenhouse and growing chambers, it was determined that the minimum period of leaf wetness for greater infection in soybean leaflets is 6 hours at the temperature range between 15 °C and 27.5 °C for the cultivars Conquista, Savana and Suprema (ALVES et al., 2007). Temperatures above 30 °C and below 15 °C had reduced disease progress levels also the minimum latent period was 6 days for the cultivar Conquista, and 9 days for the cultivars Savana and Suprema at a range of 15 to 25 °C. Bonde, Nester and Berner (2012) studying two scenarios of environment combined effects, determined that temperatures higher than 35 °C and subsequent dew period had significant reduction of rust lesions on the susceptible American cultivar Williams 82. They also concluded that extreme lower temperatures below 18°C for short periods can account for observed absence or delay of soybean rust development in the southeastern United States. Vale, Zambolim and Chaves, (1990) studying the effect of temperature and duration of leaf wetness on *P. pachyrhizi* infection in the Paraná cultivar, obtained no infection on all wetting regimes of the temperatures of 12 °C and 28 °C. They also observed the maximum number of lesions.cm^{-2} on the inoculated leaves under a temperature of 20 ºC and at least 16 hours of leaf wetness, they also concluded this to be the optimal conditions to *P. pachyrhizi* infection. Souza and Fernandes (2008) evaluating the influence of leaf wetness in rust progression during 30 days on two soybean cultivars the Monsoy 8008 RR and Conquista under greenhouse conditions, found that that there was no significant interaction between period of leaf wetting and genotypes and reported that 30 hours of wetting period provided the highest number of pustules and lesions. Danelli and Reis (2016) determined at 10 °C and 30 °C, no rust leaf infection was detected for soybean cultivars BRSGO 7560 and BRS 246 RR. They also related the occurrence of ASR in temperatures between 22 °C and 25 °C, in this range was detected in the cultivars the largest number of spores, lesions and uredinias per lesion. Also, the statistical analysis showed differences when both cultivars were compared regarding spore number and lesions, with the BRS 246 RR showing the highest values, while 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, this explained the lower values of disease intensity from the material derivative from American ascensions.

Most of the authors that related the binomial (temperature x wetting period) interacting with different cultivars and genotypes in relation to Asian soybean rust progression, proposed the hypothesis that the differences in disease intensity, incubation and latent periods may be

associated to host characteristics. This hypothesis was proposed with a small amount of data, and in a manner of qualitative in the presence or not of the disease instead of quantitative. So further works with more empirical data need to be done to get a deeper understanding on the relation of resistance and or partial resistance in lower levels of disease intensity and rates interacting with different conditions of temperature and leaf wetness period. The entry of the pathogen in the United States, was discussed by Pivonia and Yang (2006), they pointed out that in the United States, the occurrence of lower temperatures in the months of May through June limit the development of the disease in the South, which causes delay in the establishment of the epidemic in the North. In Brazil, the main soybean producing regions present favorable climatic conditions for the survival and the infectious process of the pathogen year-round. Since Brazil is one of the biggest soybean producer in the world and for its inherent tropical morphoclimatic characteristic, diseases of fungi etiology like the Asian rust, have the perfect environment to survive and maintain its life cycle all year around. Therefore, the studies of weather effects and its relationship with plant resistance, is important to estimate the potential disease occurrence and formulate strategies to control before the epidemics begins in vulnerable geographic regions. Also, the investigation of the incubation period and latent infection potential in major producing regions throughout the months of the year with different soybeans variety is a crucial step to model the epidemics and thus assist the fungicides spray with decay of efficacy in the integrated management of soybean diseases (SINCLAIR and BACKMAN, 1989; PIVONIA and YANG, 2004).

2.7 Plant responses against biotic stress

To defend themselves against the attack of pathogens and insects, the plants have structural and biochemical constituent barriers, as well as an inducible defense system (JONES and DANGL, 2006; MONTESANO, BRADER and PALVA, 2003). The perception of pathogens by plants involves the recognition of pathogenic elicitor molecules or originated from direct plant injury (DURRANT and DONG, 2004). As a result, numerous cellular changes and the activation of signaling cascades in the plasma membrane lead to the induction of interconnected networks of signaling molecules, such as salicylic acid (AS), jasmonic acid, ethylene and nitric oxide, for the rapid formation of defense responses with the aim of limiting or inhibiting the dispersion of pathogen in the plant (HARRISON, BALDWIN, 2004). Partial resistance also is related to acts as a localized defense system characterized by rapid cell necrosis near the pathogen invasion site. Necrotic plant cells are

not the only ones responsible for containing the development of the pathogen. In these cells, occurs the accumulation of toxic metabolites, such as phytoalexins that is also an antimicrobial compound (DIXON, 1986). In addition, necrotic cells provide secondary elicitors, which stimulate defense mechanisms in plant cells near the infection site, such as increased callose production, deposition of phenolic compounds and lignification of the cell wall in the infected cell. The lignification and cross-linking between proteins, makes the cell wall strengthened (BOWLES, 1990; KEEN et al.,1990). After the recognition of the pathogen, the first defense response of the plant is the oxidative explosion, which corresponds to the generation of reactive oxygen species (ROS) (H_2O_2, O_2-, OH-). ROS usually occur in cell metabolism, but when accumulated, they become toxic to the cell. Stressed plants usually use a complex defense system made up of antioxidants and a diverse range of enzymes, such as phenylalanine ammonia liase (PAL), catalase (CAT), peroxidase (POX), glutathione reductase and ascorbate peroxidase (APX). These enzymes are used to stabilize the ROS and protect the cells from oxidative damage (HOSSAIN, UDDIN, 2011). Increased levels of ROS activate the synthesis of salicylic acid. At the same time, salicylic acid enhances the production of ROS that is important for local macroscopic HR the oxidative burst, which, in turn, is an initial event in the plant response against pathogen invasion (GRANT and LAMB, 2006; POZO, LOON and PIETERSE, 2004). Although there are exceptions, it can be stated that biotrophic or hemibiotrophic pathogens are more sensitive to salicylic acid-mediated induced defenses, whereas necrotrophic pathogens and insects are more sensitive to acid-mediated defenses jasmonic and ethylene (GLAZEBROOK, 2005; PARK et al., 2007). Peroxidases (POX) are another important class of PR protein, which belong to the PR-9 family and participate in several important physiological processes. Catalyze the oxidation and eventual polymerization of alcohol hydroxycinnamic acid in the presence of hydrogen peroxide, resulting in lignin (LOON and STRIEN, 1999; MONTEIRO et al., 2016). These enzymes are also involved in the cleaning of ROS and the oxidation of various substrates using hydrogen peroxide (KAWAOKA et al., 2003). In addition, this enzyme has been associated with lignification, since it forms cross-links between phenolic groups and wall, pectin’s and other polymers (MENDONÇA and GUERRA, 2003). In nature, plants cope with simultaneous or subsequent invasion by different pathogens or insects, which may influence the host's induced defense response (STOUT, THALER and THOMMA, 2006). Thus, plants need regulatory mechanisms to adapt effectively to changes in their environment. This regulatory mechanism is known as cross talk between defense signaling pathways, which allows the plant to adjust its defense response to the invaders. Signaling interactions may be

mutually antagonistic or synergistic, resulting in negative or positive functional outcomes (KOORNNEEF and PIETERSE, 2008; BARI and JONES, 2009).

Also, the formation of lignin and phenolic compounds, are linked to the phenylpropanoid metabolism. This metabolism includes a complex series of biochemical pathways that provide plants with various combinations (BOATRIGHT et al., 2004). Lignin biosynthesis involves several enzymes, among them phenylalanine ammonia liase (PAL), which catalyzes the conversion of phenylalanine into trans-Cinnamic acid, resulting in phytoalexins, phenolic compounds and lignin, which may confer greater resistance to the cell wall of plants to pathogens (NAKAZAWA, NOZUE and YASUDA, 2001). Several simple phenylpropanoids are produced from 2,3-trans cyclic acid by a series of hydroxylation, methylation and dehydration reactions, such as p-coumaric, caffeic, ferulic, synergic and simple coumarins, in addition to the benzoic, β-hydroxybenzoic and salicylic acids (VLOT, DEMPSEY and KLESSIG, 2009). The conversion of trans-Cinnamic acid, to benzoic acid may involve β-oxidation, synthesizing intermediates prior to forming the benzoic acid. Benzoic acid, through the enzyme benzoic acid-2-hydroxylase, is converted to salicylic acid, which can be conjugated to glucose by the action of salicylate glucosyl transferase, forming the glycosylated salicylic acid (VLOT, DEMPSEY and KLESSIG, 2009). It was observed, by means of the microarray, that partial resistant plants differ in the gene expression profile before even from the insect attack, indicating that the defense is already activated as a priming mechanism (CARDOSO et al., 2014). Van de Mortel et al. (2007) described differences of mRNAs between the soybean Rpp2-resistant genotype PI230970 and the highly susceptible genotype Embrapa-48 after rust infection using also the microarray analysis, and an overrepresentation of genes associated of the phenylpropanoid pathway occurred 1 to 2 days earlier in PI230970 than in the susceptible plants.

Previous studies of soybean plant–pathogen interactions showed that soybean resistance to fungal diseases were correlated with the presence of higher levels of the phytoalexin glyceollin that is formed by the PAL pathway also was correlated with cell wall lignification. PAL also is related to be higher in inoculated resistant genotypes or cultivars in comparison with the susceptible plants, indicating a possible protective role of lignin in rust infection development. The synthesis of antimicrobial phytoalexins and cell wall reinforcement is part of a plant's innate or basal resistance that helps partial resistant plants to prevent the initial infection and colonization by most fungi (DAKORA and PHILLIPS, 1996; Dixon, 2001; Lozovaya et al., 2004; Lozovaya et al., 2007; Lygin et al., 2009). Lozovaya et al., 2004 studying infection of the fungi *Fusarium solani* f. sp. *Glycines* on soybean genotypes

reported that, after inoculation, glyceollin accumulated to much lower concentrations in roots of the susceptible cv. Spencer than in the partially resistant genotype PI567374. Learn 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. To date, there are few soybean cultivars with resistance to rust or partial resistance, however, it is verified that biotrophic pathogens and insects are more sensitive to defenses mediated by salicylic acid, it is believed that, possibly, resistant soybean 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.

2.8 Partial resistance and pathogen variability

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 And HARTMAN, 1992). This type of resistance became evident and important when a monogenic resistance is overcome by a new breed of pathogen (PARLEVLIET, 1997). Sevenmajo genes for resistance (Rpp1, Rpp2, Rpp3, Rpp4, Rpp5, Rpp6 and Rpp7) to *P. pachyrhizi* have already been identified in plants of the genus *Glycines* (BROMFIELD and HARTWIG, 1980; HARTWIG, 1986; CALVO et al., 2008; LI et al.,2012; LIU et al., 2016; CHILDS et al., 2017). 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 and 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 and 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 and Arias (2002), numerous genotypes vertical resistance have 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 Rpp6 gene was mapped on the PI 567102B to a third Rpp locus on the chromosome 18 approximately 40 cM from the Rpp4 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 (rpp5) (GARCIA et al., 2008; BAILEY et al. 2014). In 2017, Childs et al. 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 *P. pachyrhizi* 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 and 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; CHILDS et al., 2017). 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 and HARTMAN 2006; INAYATI and YUSNAWAN, 2016). Given the rapid breakdown of Rpps 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 (VAN DER PLANK 1963). Also, the resistance of soybean genotypes 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).

Lately, rust samples collected in Brazil have been tested for sensitivity to these fungicides since 2007 by FRAC. These fungicides still have good performance, 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 and 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* (Rpp1, Rpp2, Rpp3 and Rpp4), 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 and 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. 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, and FREDERICK, 2005), development of durable ‘‘less-rusting” and ‘‘slow-rusting” cultivars is one of the options for breeding for resistance to soybean rust (LI and 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, 1983). 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 (CLIFFORD, 1974; 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 and KUIPER, 1977; NIKS, 1983; Parlevliet, 1978; PARLEVLIET et al., 1979). Parlevliet and 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 may 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 and SILVA, 2007; MARTINS et al., 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.

REFERENCES

AKAMATSU, M. A.; FIGUEIREDO, M. B.; HARAKAVA, R. Detecção e distinção de *Phakopsora pachyrhizi* e *P. meibomiae* em Amostras do Herbário Uredinológico do Instituto Biológico. **Fitopatologia Brasileira, Brasília**, v.29, supl., p. 277-278, 2004. (Resumo).

ALMEIDA, A.M.R. Doenças da soja (*Glycine max* L.). In: KIMATI, H. **Manual de fitopatologia (doenças das plantas cultivadas)**. São Paulo: Ed. Agronômica Ceres, 1997.

ALVES, M. D.E C.; POZZA, E. A.; FERREIRA, J. B.; ARAÚJO, D. V.; COSTA, J. C. B.; DEUNER, C. C.; MUNIZ, M. F. S.; ZAMBENEDETTI, E. B.; MACHADO, J. C. Intensidade da ferrugem asiática (*Phakopsora pachyrizi* H. Sydow & P. Sydow) da soja (*Glycine max* L.) Merr.) nas cultivares Conquista, Savana e Suprema sob diferentes temperaturas e períodos de molhamento foliar. **Summa Phytopathologica**. v. 33, p. 239-244, 2007.

APROSOJA. Monitoring results and use recommendations available at [http://www.aprosoja.com.br/sobre-a-soja/a-historia-da-soja] Accessed 10 December 2017.

ARIAS, C.A.A.; TOLEDO, J.F.F.; ALMEIDA, L.A., PIPOLO, A.E.; CARNEIRO, G.E.S, ABDELNOOR, R.V.; RACHID, B.F.; RIBEIRO, A. Asian rust in Brazil: **Varietal resistance**. In KUDO, H.; SUENAGA, K.; SOARES, R.M.; TOLEDO, A. (eds.) Facing the challenge of soybean rust in South America. JIRCAS, Tsukuba, p. 29-30, 2008.

AZEVEDO, E. Riscos e controvérsias na construção social do conceito de alimento saudável: o caso da soja. **Revista Saúde Pública**. [s.l.], v. 4, n. 45, p. 781-788, 2010.

BARI, R.; JONES, J. D. G. Role of plant hormones in plant defence responses. **Plant Molecular Biology**. Dordrecht, v. 69, n. 4, p. 473-488, 2009.

BOATRIGHT, J.; NEGRE, F.; CHEN, X.; KISH, C.M.; WOOD, B.; PEEL, G.; ORLOVA, I.; GANG, D., RHODES, D.; DUDSREVA, N. Understanding *in vivo* benzenoid metabolism in petunia petal tissue. **Plant Physiology**, Bethesda. v. 135, n. 4, p. 1993-2011, 2004.

BONDE, M.R.; NESTER, S.E.; AUSTIN, C.N.; STONE, C.L.; FREDERICK, R.D.; HARTMAN, G.L.; MILES, M.R. Evaluation of virulence of *Phakopsora pachyrhizi* and *P. meibomiae* isolates. **Plant Disease.** v.90, p.708-716, 2006.

BONDE, M.R.; BERNER, D.K.; NESTER, S.E.; FREDERICK, R.D. Effects of temperature on urediniospore germination, germ tube growth, and initiation of infection in soybean by *Phakopsora* isolates. **Phytopathology**, v.97, p.997-1003, 2007.

BONDE, M. R.; NESTER, S. E.; BERNER, D. K. Effects of soybean leaf and plant age on susceptibility to initiation of infection by Phakopsora pachyrhizi. **Plant Health Progress. Online publication. doi**: 10.1094/PHP-2012-0227-01-RS. v. 10, 2012.

BOWLES, D.J. Defense-related proteins in higher plants. **Annu Rev Biochem** 59: 873-907, 1990.

BROMFIELD, K.R. Soybean Rust - **Monograph No. 11**. Saint Paul MN. APS Press, 1984.

BROMFIELD, K.R.; HARTWIG, E.E. Resistance to soybean rust and mode of inheritance. **Crop Science.** v. 20, p. 254-255, 1980.

BROMFIELD, K.R.; MELCHING, J.S. Sources of specific resistance to soybean rust. **Phytopatology.** v. 72, p. 706, 1982.

BROMFIELD, K.R.; MELCHING, J.S.; KINGSOLVER, C.H. Virulence and aggressiveness of *Phakopsora pachyrhizi* isolates causing soybean rust. **Phythopathology**, Lancaster. v. 70, p. 17- 21, 1980.

BUTZEN, S.; MARCON, A.; MCINNES, B.; SCHUH, W. Asian soybean rust: fungicide application technology. **Crop Insights**, Johnston. v. 15, n. 1, p. 1-6, 2005.

CALVO, E.S.; KIIHL, R. A. S.; GARCIA, A.; HARADA, A.; HIROMOTO, D. M. Two major recessive soybean genes conferring soybean rust resistance. **Crop Science**, Madison, v. 48, n.4, p.1350-1354, 2008

CALDWELL, P.M.; NUNKUMAR, A.; PRETORIUS, Z.A.; FERNANDES, J.M.C. Development of Phakopsora pachyrhizi at different temperatures, relative humidities and leaf wetness duration. In**: International workshop on plant disease epidemiology**, 9., Landerneau, Proceedings. Bordeaux: Societé Francaise de Phytopathologie, 2005.

CARDOSO, D. C.; MARTINATI, J. C.; GIACHETTO, P. F.; VIDAL, R. O.; CARAZZOLLE, M. F.; PADILHA, L.; GUERREIRO-FILHO, O.; MALUF, M. P. Large-scale analysis of differential gene expression in coffee genotypes resistant and susceptible to leaf miner-toward the identification of candidate genes for marker assisted-selection. **BMC Genomics**, London. v. 15, 2014.

CARNEIRO, L.C. Caracterização epidemiológica da resistência parcial e análise de tolerância de genótipos de soja à ferrugem asiática. 2007. 75f. **Tese (Doutorado em Agronomia)** – Curso de Pós-graduação em Agronomia, Universidade de São Paulo - Escola Superior de Agricultura Luiz de Queiroz, SP.
CARVALHO JUNIOR, A.A.; FIGUEREDO, M.B. A verdadeira identidade da ferrugem da soja no Brasil. **Summa Phytopathologica**, Jaboticabal, v. 26, p.197-200, 2000.

CASEY, P.S. The epidemiology of soybean rust *Phakopsora pachyrhizi* Syd. **Soybean Rust Newsletter**, Shanhua, v. 4, n. 1, p.3-5, 1980.

CHILDS, S. P., KING, Z. R., WALKER, D. R., HARRIS, D. K., PEDLEY, K. F., BUCK, J. W., LI, Z. Discovery of a seventh Rpp soybean rust resistance locus in soybean accession PI 605823. **Theoretical and Applied Genetics**, v. 131, p. 27–41, 2017.

CONAB. Acompanhamento da safra brasileira: safra 2016/2017 available at [http://wwwconabgovbr/OlalaCMS/uploads/arquivos/17_09_12_10_14_36_boletim_graos_setembro_2017pdf] Accessed 20 December 2017.

CONAB. Acompanhamento da safra brasileira: safra 2017/2018 available at [http://www.conab.gov.br/conabweb/download/safra/estudo_safra.pd] Accessed 15 April 2018.

DANELLI, A. L. D.; REIS, E. M. Quantification of incubation, latent and infection periods of *Phakopsora pachyrhizi* in soybean, according to chronological time and degree-days. **Summa Phytopathologica**, v. 42, n. 1, p. 11-17, 2016.

DAKORA, F.D and PHILLIPS, D.A. Diverse functions of isoflavonoids in legumes transcend anti- microbial definitions of phytoalexins. **Physiol. Mol. Plant Pathol**.v. 49, p.1-20, 1996.

DEL PONTE, E..; GODOY, C.V.; LI, X.; YANG, X.B. Predicting severity of Asian soybean rust epidemics with empirical rainfall models. Phytopathology, v. 96, p.797-80, 2006.

DESLANDES, J.A. Ferrugem da soja e de outras leguminosas causadas por *Phakopsora pachyrhiz*i no Estado de Minas Gerais. **Fitopatologia Brasileira**, Brasília. v. 4, p. 337-339, 1979.

DIAS, M. B.; POZZA, E. A.; ABREU, M. S.; MIRANDA, E. O. Efeito da temperatura no crescimento micelial, produção e germinação de conídios de *Colletotrichum* spp. isolados de *Coffea arabica* L. **Ciência e Agrotecnologia**. v. 29, p. 545-552, 2005.

DIAS, W.P.; SILVA, J.F.V.; GARCIA, A.; CARNEIRO, G.ES. Nematoides de importância para a soja no Brasil. In: **Boletim de Pesquisa de Soja 2007**. Rondonópolis: Fundação MT, 2007.

DIXON, R.A. The phytoalexin response: elicitation, signaling, and control of host gene expression. **BiolRev**, Camb Philos Soc 61: 239-292, 1986.

DIXON, R.A. Natural products and disease resistance. **Nature**, v. 411, p. 843- 847, 2001.

DURRANT, W. E.; DONG, X. Systemic acquired resistance. **Annual Review of Phytopathology**, Palo Alto. v. 42, p. 185-209, 2004.

EMBRAPA. Centro Nacional de Pesquisa de Soja. Tecnologia de produção de soja na região central do Brasil – **Sistema de Produção**, Londrina, n. 11, 2007.

EMBRAPA. Tecnologias de produção de soja Região Central do Brasil 2005. Empresa Brasileira de Pesquisa Agropecuária available at [http://www.cnpso.embrapa.br] Accessed 15 August 2017.

EMBRAPA. Tecnologias de produção de soja Região Central do Brasil 2017. Empresa Brasileira de Pesquisa Agropecuária available at [http://www.cnpso.embrapa.br] Accessed 15 August 2017.

EMBRAPA. Tecnologias de produção de soja: região central do Brasil – 2012 e 2013. **Sistema de Produção**, Londrina, n. 15, p. 1-261, 2011.

FAO. World production in 2017 available at [http://www.fao.org/faostat/en/] Accessed 15 December 2017.

FARIAS, J.R.B.; ABDELNOOR, RV.; GODOY, CV. **Risco do surgimento e do aumento de populações resistentes a fungicidas do fungo causador da Ferrugem-Asiática da soja**. Londrina: EMBRAPA, Nota técnica, 2p, 2014.

FRAC. Monitoring results and use recommendations available at [http://www.frac.info/working-group/sdhi-fungicides/general-use-recommendations] Accessed 04 September 2017.

FREDERICK, R.D.; SNYDER, C.L.; PETERSON, G.L.; BONDE, M.R. Polimerase chain reaction assays for the detection and discrimination of the soybean rust pathogens *Phakopsora pachyrhizi* and *P. meibomiae*. **Phytopathology**, Lancaster, v. 92, p. 217-227, 2002.

FURTADO, G.Q. **Ferrugem asiática da soja:** métodos de preservação dos urediniósporos e fatores relacionados à infecção do hospedeiro. 2007. 80p. Tese (Doutorado) - Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba.

GARCIA, A.; CALVO, E.S.; KIIHL, R.A.S.; HARADA, A.; HIROMOTO, D.M VIEIRA, L.G.E. Molecular mapping of soybean rust (*Phakopsora pachyrhizi*) resistance genes: discovery of a novel locus and alleles. **Theor Appl Genet**. v. 117, p. 545-553, 2008.

GLAZEBROOK, J. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. **Annual Review of Phytopathology**, Palo Alto. v. 43, p. 205-227, 2005.

GRANT, M.; LAMB, C. Systemic immunity. **Current opinion in plant biology**, v. 9, n. 4, p. 414-420, 2006.

GODOY, C.V.; KOGA, L.; CANTERI, M.G. Diagrammatic scale for assessment of soybean rust severity. Fitopatologia Brasileira, Brasília, v.31, p. 63-68, 2006.

GODOY, Claudia Vieira et al. Ferrugem-asiática da soja no Brasil: passado, presente e futuro. **Pesquisa Agropecuária Brasileira**, v. 51, n. 5, p. 407-421, 2016.

HARRIS, D.K.; KENDRICK, M.D.; KING, Z.R.; PEDLEY, K.F.; WALKER, D.R.; CREGAN, P.B.; BUCK, J.W.; PHILLIPS, D.V.; LI, Z.; BOERMA, H.R. Identification of unique genetic sources of soybean rust resistance from the USDA soybean germplasm collection. **Crop Science**, v.55, p.2161-2176, 2015.

HARRISON, M. J.; BALDWIN, I. T. Biotic interactions: ploy and conter-ploy in the biotic interactions of plants. **Current Opinion in Plant Biology**, London, v. 7, n. 4, p. 353-355, 2004.

HARTMAN, G.L.; WANG, T.C.; TSCHANZ, A.T. Soybean rust development and the quantitative relationship between rust severity and soybean yield. **Plant Disease**, Saint Paul. v.75, p. 596-600, 1991.

HARTMAN, G.L.; WANG, T.C.; SHANMUGASUNDARAM, S. Soybean rust research: Progress and future prospects. In: **World soybean research conference**, 5., 1997, Bangkok. Proceedings. Bangkok: Kasetsart University Press. p. 180-186, 1997.

HARTMAN, G. L., MILES, M. R., FREDERICK, R. D. Breeding for resistance to soybean rust. **Plant Disease**. v.89, p. 664–666, 2005.

HARTWIG E.E. Resistance to soybean rust. In **Proceedings of the Soybean Rust Workshop, 9–11 August 1995**, eds SINCLAIR, J.B.; HARTMAN, G.L. pp. 65–66. College of Agricultural, Consumer and Environmental Sciences, National Research Laboratory, Urbana, Illinois, 1996.

HARTWIG, E.E. Identification of a 4th major gene conferring resistance to soybean rust. **Crop Science**. v. 26, p. 1135-113, 1986.

HARTWIG, E.E.; BROMFIELD, K.R. Relationships among three genes conferring specific resistance to rust in soybeans. **Crop Science**. v. 23, p. 237-239, 1983.

HOSSAIN, M. A.; UDDIN, S. N. Mechanisms of waterlogging tolerance in wheat: morphological and metabolic adaptations under hypoxia or anoxia. **Australian Journal of Crop Science**, Melbourne. v. 5, n. 9, p. 1094-1101, 2011.

INAYATI, A; YUSNAWAN, E. Characteristics of superior soybean breeding lines tolerance to rust (*Phakopsora pachyrhizi* Syd.). **Biosaintifika: Journal of Biology & Biology Education**, v. 8, n. 1, p. 47-55, 2016.

JONES, J. D. G.; DANGL, J. L. The plant immune system. **Nature**, London, v. 444, n. 7117, p. 323-329, 2006.

JULIATTI, F.C.; BORGES, E.M.; PASSOS, R.R.; CALDEIRA JÚNIOR, J.C.; BRANDÃO, A.M. Doenças da soja. **Cultivar**, Uberlândia. n.47, p. 13, 2003.

JULIATTI, F. C., POLIZEL, A. C., JULIATTI, F.C. **Manejo integrado de doenças da soja**. Uberlândia: Composer. P. 327, 2004.

JULIATTI, F.C.; POLIZEL, A.C.; BALARDIN, R.S.; VALE, F.X.R. Ferrugem da soja – Epidemiologia e manejo para uma doença reemergente. In: LUZ, W.C.; FERNANDES, J.M.C.; PRESTES, A.M.; PICININI, E.C. **Revisão anual de patologia de plantas** Passo Fundo. v. 13, p. 351-395, 2005.

JÚNIOR, H.B.M.; FERNANDES, J.J. Período de molhamento foliar para ocorrência de ferrugem asiática (*Phakopsora pachyrhizi*) em dois cultivares de soja (*Glycine max*). **Enciclop biosfera**, Centro Científico Conhecer - Goiânia, vol.6, N.11, p. 11, 2010.

KATO, M.; YORINORI, J.T. A study on a race composition of *Phakopsora pachyrhizi* in Brazil: a difficulty of race identification. **JIRCAS Working Report**, v. 58, p. 94-98, 2008.

KAWAOKA, A.; MATSUNAGA, E.; ENDO, S.; KONDO, S.; YOSHIDA, K.; SHINMYO, A.; EBINUMA, H. Ectopic expression of a horseradish peroxidase enhances growth rate and increases oxidative stress resistance in hybrid aspen. **Plant Physiology**, Rockville. v. 132, n. 3, p. 1177-1185, 2003.

KAWASHIMA, C.G., GUIMARAES, G.A., NOGUEIRA, S.R., MACLEAN, D., COOK, D.R., STEUERNAGEL, B. A pigeonpea gene confers resistance to Asian soybean rust in soybean. **Nat. Biotechnol**. v.34, p. 661-665, 2016.

KAWUKI, R.S.; TUKAMUHABWA, P.; ADIPALA, E. Soybean rust severity, rate of rust development, and tolerance as influenced by maturity period and season. **Crop Protection**, Guildford. v. 23, p. 447-455, 2004.

KEEN, N. T. Phytoalexins and their elicitors. In: HOAGLAND, R. E. (Ed.). **Microbes and microbial products as herbicides**. Washington: American Chemical Society. p. 114-129, 1990.

KITANI, K.; INOUE, Y.; NATSUME, T. Studies on soybean rust and its control. II. Studies on the control of soybean rust. Shikoku Nogyo Shikenjo Hokoku, **Bulletin of the Shikoku Agricultural** Experiment Station, 5, 343-58, 1960.

KING, Z.R.; HARRIS, D.K.; PEDLEY, K.F.; SONG, Q.; WANG, D.; WEN, Z.; BUCK, J.W.; LI Z.; BOERMA, H.R. A novel Phakopsora pachyrhizi resistance allele (Rpp) contributed by PI 567068A. **Theor Appl Genet**. v. 129, p,517–534, 2015.

KOCHMAN, J. K. The effect of temperature on development of soybean rust (*Phakopsora pachyrhizi*). **Australian Journal of Agricultural Research**, v. 30, n. 2, p. 273-277, 1979.

KOORNNEEF, A.; PIETERSE, C. M. Cross talk in defense signaling. **Plant Physiology**, Rockville. v. 146, n. 3, p. 839-844, 2008.

LI, S.; YOUNG, L. D. "Evaluation of selected genotypes of soybean for resistance to *Phakopsora pachyrhizi*." *Plant Health Progress* doi: 10.1094/PHP-2009-0615-01-RS, 2009.

LI, S.; SMITH, J.R.; RAY, J.D.; FREDERICK, R.D. Identification of a new soybean rust resistance gene in PI567102B. **Theor Appl Genet** 125: 133-142, 2012.

LIU, M., LI, S., SWAMINATHAN, S., SAHU, B. B., LEANDRO, L. F., CARDINAL, A. J., CIANZIO, S. R. Identification of a soybean rust resistance gene in PI 567104B. **Theoretical and Applied Genetics**, v. 129, p. 863–877, 2016.

LOON, L. C. VAN; STRIEN, E. A. VAN. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. **Physiological and Molecular Plant Pathology**, London, v. 55, n. 2, p. 85-97, Aug. 1999.

LOZOVAYA, V.V., LYGIN, A.V., LI, S., HARTMAN, G.L. and WIDHOLM, J.M. Biochemical response of soybean roots to *Fusarium solani* f. sp. glycines infection. **Crop Sci**. v. 44, p.819-826, 2004.

LOZOVAYA, V.V.; LYGIN, A.V.; ZERNOVA, O.V.; ULANOV, A.V.; LI, S.X.; HARTMAN G.L. Modification of phenolic metabolism in soybean hairy roots through down regulation of chalcone synthase or isoflavone synthase. **Planta**. v. 225, p. 665-679, 2007.

LUCK, J.; SPACKMAN, M.; FREEMAN, A.; TREBICKI, P.; GRIFFITHS, W.; FINLAY, K.; CHAKRABORTY, S. Climate change and diseases of food crops. **Plant Pathology**. v. 60, p.113-121, 2011. doi: 10.1111/j.1365-3059.2010.02414.x.

LYGIN, A.V., LI, S., VITTAL, R., WIDHOLM, J.M., HARTMAN, G.L., LOZOVAYA, V.V. The importance of phenolic metabolism to limit the growth of *Phakopsora pachyrhizi*. **Genet. Resist**. v. 99, p. 1412-1420, 2009.

MARCHETTI, M.A.; MELCHING, J. S.; BROMFIELD, K. R. The effects of temperature and dew period on germination and infection by urediniospores of *Phakopsora pachyrhizi*. **Phytopathology**, St. Paul. v.66, p.461-463, 1976.

MARCHETTI, M.A.; UECKER, F.A.; BROMFIELD, K.R. Uredinial development of *Phakopsora pachyrhizi* in soybeans. **Journal of Phytopathology**, Lancaster, v.65, p.822-823, 1975.

MARTINS, J.A.S.; JULIATTI, F.C. Genetic control of partial resistance to Asian soybean rust. **Acta Sci Agron**. Maringá. v. 36(1), p. 11-17, 2014. doi:10.4025/actasciagron. v36i1.16919.

MCLEAN, R.J.; BYTH, D.E. Inheritance of resistance to rust (*Phakopsora pachyrhizi*) in soybeans. **Australian Journal of Agricultural Research**. v.31 p.951-956, 1980.

MELCHING, J.S.; DOWLER, W.M.; KOOGLE D.L.; ROYER, M. Effect of duration, frequency, and temperature of leaf wetness period on soybean rust. **Plant Disease**. v. 73, p.117-122, 1989.

MENDONÇA, S. C.; GUERRA, N. B. Métodos físicos e químicos empregados no controle do escurecimento enzimático de vegetais. **Boletim SBCTA**, Campinas, v. 37, n. 2, p. 113-116, 2003.

MILES, M.R.; BONDE, M.R.; NESTER, S.E.; BERNER, D.K.; FREDERICK, R.D.; HARTMAN, G.L. Characterizing resistance to *Phakopsora pachyrhizi* in soybean. **Plant Disease.** v. 95, p. 577-581, 2011.

MILES, M.R.; FREDERICK, R.D.; HARTMAN, G.L. Evaluation of the soybean germplasm for resistance to *Phakopsora pachyrhizi*. **Plant Health Progress**. 2006. doi 10.1094/PHP-0104-01-RS. Available at [http://www.frac.info/working-group/sdhi-fungicides/general-use-recommendations] Accessed 010 december 2017.

MONTEIRO, A.C.A.; RESENDE, M.L.V.; VALENTE, T.C.N.; JUNIOR, P.M.R.; PEREIRA, V.F.; COSTA, J.R.; SILVA, J.A.G. Manganese Phosphite in Coffee Defence against *Hemileia vastatrix*, the Coffee Rust Fungus: Biochemical and Molecular Analyses **Journal of Phytopathology**., v 164, p 1043-1053, 2016.

MONTESANO, M.; BRADER, G.; PALVA, E. Pathogen derived elicitors: searching for receptors in plants. **Molecular Plant Pathology**, London. v. 4, n. 1, p. 73-79, 2003.

NAKAZAWA, A.; NOZUE, M.; YASUDA, H. Expression pattern and gene structure of phenylalanine ammonia-lyase in *Pharbitis nil.* **Journal of Plant Research**, Tokyo. v. 114, n. 2, p. 323-328, 2001.

NIKS, R.E. Comparative histology of partial resistance and the nonhost reaction to leaf rust pathogens in barley and wheat seedlings. **Phytopathology**, v.73, p. 60-64, 1983.

OGLE, H.J.; BYTH, D.E. and MCLEAN, R. Effect of rust (*Phakopsora pachyrhizi*) on soybean yield and quality in south-eastern Queensland. Aust. **J. Agric**. Res. v. 30, p. 883-893, 1979.

ONO, Y.; BURITICÁ, P.; HENNEN, J.F. Delimitation of *Phakopsora*, *Physopella* and *Cerotelium* and their species on Leguminosae. **Mycological Research**, Cambridge, v.96, p.825- 850, 1992.

PARK, S. W.; KAIMOYO, E.; KUMAR, D.; MOSHER, S.; KLESSIG, D. F. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. **Science**, New York. v. 318, n. 5847, p. 113-116, 2007.

PARLEVLIET, J.E. T.; VAN OMMEREN A. "Partial resistance of barley to leaf rust, *Puccinia hordei*. II. Relationship between field trials, micro plot tests and latent period." **Euphytica**, v. 2, n. 2, p. 293-303, 1975.

PARLEVLIET, J.E. Further evidence of polygenic inheritance of partial resistance in barley to leaf rust, *Puccinia hordei*. **Euphytica.** v. 27, p. 369-379, 1978.

PARLEVLIET, J.E. Present concepts in breeding for disease resistance. In: **Brazilian Phytopathology Congress.** Fitopatologia Brasileira, Brasília. v. 22, p. 7-15, 1997.

PAUL, C.; HARTMAN, G.L.; MAROIS, J.J.; WRIGHT, D.L.; WALKER, D.R. First report of *Phakopsora pachyrhizi* overcoming soybean genotypes with Rpp1 or Rpp6 rust resistance genes in field plots in the United States. Plant Dis. v. 97, p. 1379, 2013.

PIEROZZI, P.H.B.; RIBEIRO, A.S.; MOREIRA, J.U.V.; LAPERUTA, L. di C.; RACHID, B. F.; LIMA, W.F.; ARIAS, C.A.A.; OLIVEIRA, M.F. de; TOLEDO, J.F.F. de. New soybean (Glycine max Fabales, Fabaceae) sources of qualitative genetic resistance to Asian soybean rust caused by *Phakopsora pachyrhizi* (Uredinales, Phakopsoraceae). **Genetics and Molecular Biology**, v.31, p.505-511, 2008.

PIVONIA, S., YANG, X.B. Assessment of potential year-round establishment of soybean rust throughout the world. **Plant Disease**, v.88, p.523-529, 2004.

PIVONIA, S.; YANG, X.B. Relating epidemic progress from a general disease model to seasonal appearance time of rusts in the United States: Implications for soybean rust. **Journal of Phytopathology**, Lancaster, v.96, p. 400-407, 2006.

POZO, M. J.; VAN LOON, L. C.; PIETERSE, CORNÉ MJ. Jasmonates-signals in plant-microbe interactions. **Journal of Plant Growth Regulation**, v. 23, n. 3, p. 211-222, 2004.

REIS, E.M.; SARTORI, A.F.; CÂMARA, R.K. Modelo climático para a previsão da ferrugem da soja. **Summa Phytopathologica**, Jaboticabal. v.30, n.2, p.290-292, 2004.
SCHNEPF, RD.; DOHLMAN, E.; BOLLING, C. **Agriculture in Brazil and Argentina**, WRS-01-3. Washington: Economic Research Service, USDA, 2001.

SHURTLEFF, W.; AOYAGI, A. **History of fermented soymilk and its products: history of soybeans and soyfoods**: 1100 B. C. to the 1980s. Soyfoods Center, Lafayette, [s.n.], p. 10, 1980.

SILVA, V. A. S.; JULIATTI, F. C.; SILVA, L. A. S. Interação entre resistência genética parcial e fungicidas no controle da ferrugem asiática da soja. **Pesquisa Agropecuária Brasileira**, Brasília, v.42, n.9, p.1261-1268, 2007.

SINCLAIR, J.B.; BACKMAN, P.A. **Compendium of soybean diseases**. 3rd St. Paul: American Phytopathological Society, p. 24-27, 1989.

SINCLAIR, J.B.; HARTMAN, G.L. Soybean rust. In: HARTMAN, G.L.; SINCLAIR, J.B.; RUPE, J.C. (Ed.). **Compendium of soybean diseases**. 4 th.ed. St Paul: American Phytopathology Society. p. 25-26, 1999.

SINCLAIR, J.B.; SHURTLEFF, M.C. **Compendium of soybean diseases**. Am. Phytopathol Soc., p. 68, 1975.

SINGH BB.; GUPTA SC.; SINGH BD. Sources of field resistance to rust and yellow mosaic diseases of soybean. **Indian J Genet Plant Breed**. v. 34, p. 400–404, 1974.

SKORUPSKA, H.; ALBERTSEN, MC.; LANGHOLZ, KD, PALMER, RG. Detection of ribosomal-RNA genes in soybean, (*Glycine max* (L) Merr., by in situ hybridization. **Genome** v. 32, p. 1091-1095, 1989.

SLAMINKO, T. L., MILES, M. R., FREDERICK, R. D., BONDE, M. R., and HARTMAN, G. L. New legume hosts of *Phakopsora pachyrhizi* based on greenhouse evaluations. **Plant Disease**. 92:767-771, 2008.

SOUZA, A. R.; FERNANDES, J. J. Efeito do período de molhamento foliar na ocorrência da ferrugem asiática (*Phakopsora pachyrhizi*) na soja (*Glycine max*). In: SEMANA ACADÊMICA, 5. **Anais…** Uberlândia. Universidade Federal de Uberlândia, 2008.

STOUT, M. J.; THALER, J. S.; THOMMA, B. P. H. J. Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. **Annual Review of Entomology**, Stanford. v. 51, p. 663-689, 2006.

TSCHANZ, A.T. and WANG, T.C. Interrelationship between soybean development, resistance, and *Phakopsora pachyrhizi*. Anais, **Fifth International Congress of the Society for the Advanced of Breeding Research in Asia and Oceania**, Bangkok, Thailand. P. 14-20, 1985.

TWIZEYIMANA, M.; HARTMAN, G. L. Pathogenic variation of *Phakopsora pachyrhizi* isolates on soybean in the United States from 2006 to 2009. **Plant disease**, v. 96, n. 1, p. 75-81, 2012.

USDA (2017) Overview United States Department of Agriculture available at [http://wwwersusdagov/topics/crops/soybeans-oil-cropshtml] Accessed 20 October 2017.

VAN DER PLANK, J. E. **Plant diseases**. Elsevier Science, 1963.

VAN DE MORTEL, M.; RECKNOR, J.C.; GRAHAM, M.A.; NETTLETON, D.; DITTMAN, J.D.; NELSON, R.T.; GODOY, C.V.; ABDELNOOR, R.V.; ALMEIDA, A.M.R.; BAUM, T.J. and WHITHAM, S.A. Distinct biphasic mRNA changes in response to Asian soybean rust infection. **Molecular Plant-Microbe Interactions, v.** 20, p. 887-899, 2007.

VALE, F. X. R.; ZAMBOLIM, L.; CHAVES, G. M. Efeito do binômio temperatura duração do molhamento foliar sobre a infecção por *Phakopsora pachyrhizi* em soja. **Fitopatologia Brasileira**, Brasília DF, v. 15, n. 3, p. 200-202, 1990.

VALE, F.X.R. **Aspectos epidemiológicos da ferrugem (*Phakopsora pachyrhizi* Sydow) da soja (*Glycine max* L. Merrill)**. 104p. Tese (Doutorado em Fitopatologia) Universidade Federal de Viçosa, Viçosa, MG, 1985.

VELLO, N. A.; BROGIN, R. L.; ARIAS, C. A. A. Estratégias de melhoramento para o controle da ferrugem da soja. **Anais do II Congresso**. Brasileiro de Soja e Merco soja. Foz do. Iguazú. PR. Brasil. pp. 188-196, 2002.

VLOT, A. C.; DEMPSEY, D. M. A.; KLESSIG, D. F. Salicylic acid, a multifaceted hormone to combat disease. **Annual Review Phytopathology**, Palo Alto. v. 47, p. 177-206, 2009.

WANG T.C.; HARTMAN G.L. Epidemiology of soybean rust and breeding for host resistence. **PI. Prot Bull**. v. 34, p. 109-149, 1992.

WALKER, D. R., HARRIS, D. K., KING, Z. R., LI, Z., BOERMA, H. R., BUCKLEY, J. B., WEAVER, D. B., SIKORA, E. J., SHIPE, E. R., MUELLER, J. D., BUCK, J. W., SCHNEIDER, R. W., MAROIS, J. J., WRIGHT, D. L.; NELSON, R. L. Evaluation of soybean germplasm accessions for resistance to *Phakopsora pachyrhizi* populations in the southeastern United States, 2009-2012. **Crop Sci.** v. 54, p.1673-1689, 2014.

WRATHER, J. A.; ANDERSON, T. R.; ARSYAD, D. M.; TAN, Y.; PLOPER, L. D.; PORTA-PUGLIA, A.; YORINORI, J. T. Soybean disease loss estimates for the top ten soybean producing countries in 1998. **Canadian Journal of Plant Pathology**, Ontario. v. 23, n. 2, p. 115-121, 2001.

WSB. World Soybean Production in 2014. Estimative available at [http://www.wsp.com] Accessed 8 January 2018.

YAMANAKA, N.; SILVA, DCG.; PASSIANOTO, ALL.; NOGUEIRA, LM.; POLIZEL, AM.; PEREIRA, R.M.; SANTOS, JVM.; BROGIN, RL.; ARIAS, CAA.; HOFFMANN-CAMPO, CB.; NEPOMUCENO, AL.; ABDELNOOR, RV. Identification of DNA markers and characterization of the genes for resistance against Asian soybean rust. **JIRCAS Working Report**. v.58, p. 99-107, 2008.

YAMANAKA, N, YAMAOKA, Y, KATO, M, LEMOS, NG, PASSIANOTTO, ALL, SANTOS, JVM, BENITEZ, ER, ABDELNOOR, RV, SOARES, RM; SUENAGA K. Development of classification criteria for resistance to soybean rust and differences in virulence among Japanese and Brazilian rust populations. **Tropical Plant Pathology**. v. 35, p. 153-162, 2010.

YAMANAKA N., LEMOS N. G., UNO M., AKAMATSU H., YAMAOKA Y., ABDELNOOR R. V., ET AL. Resistance to Asian soybean rust in soybean lines with the pyramided three Rpp genes. **Crop Breed. Appl. Biotechnol**. v. 13, p. 75–82, 2013.

YANG, X.B.; TSCHANZ, A.T.; DOWLER, W. M.; WANG, T.C. Development of yield loss models in relation to reductions of components of soybean infected with *Phakopsora pachyrhizi.* **Journal of Phytopathology**, Berlin, v.81, p.1420-1426, 1991.

YORINORI, J. T. A ferrugem “asiática” da soja no continente americano: evolução, importância econômica e estratégias de controle. In: JULIATTI, F.C., POLIZEL, A.C., HAMAWAKI, O.T. (Org.) In: **Workshop brasileiro sobre a ferrugem asiática**, 1., Uberlândia. Coletânea. Uberlândia: EDUFU, 2005. p. 21-37, 2005.

YORINORI, J.T. Epidemia da ferrugem da soja (*Phakopsora pachyrhizi*) no Brazil e no Paraguai, em 2001 e 2002. **Fitopatologia Brasileira**, Jaboticabal, v.27 (Supl.), p. 178, 2002.

YORINORI, J.T. **Situação da ferrugem asiática da soja no Brasil e na América do Sul**. In: YORINORI, J.T. Lazzaroto, J.J. Londrina: EMBRAPA , p. 27, 2004.

YORINORI, JT. Soja [*Glycine max* (L.) Merril] – Controle de doenças. In: VALE, FXR.; ZAMBOLIM, L. (Eds.). **Controle de doenças de plantas: grandes culturas**. Viçosa: Editora da UFV; Brasília: Ministério da Agricultura e do Abastecimento, 1997.

ZAMBENEDETTI, E. B., E.; ALVES, E. A.; POZZA, E D. V.; ARAÚJO, E C.; GODOY, V. Avaliação de parâmetros monocíclicos e da intensidade da ferrugem asiática (*Phakopsora pachyrhizi*) em diferentes genótipos de soja e posições de copa. **Summa Phytopathologica**. v. 33(2), p. 178-181, 2007.

ZHENG-YI, W.; RAVEN, PH. (Eds.). **Flora of China.** vol 5, 2003.

ARTICLE 1

COMPARISON OF GENOTYPES WITH PARTIAL RESISTANCE AGAINST SOYBEAN RUST IN RELATION TO THE BINOMIAL TEMPERATURE AND LEAF WETNESS DURATION

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ABSTRACT

The objective of the study was to determine the influence of temperature (18, 21, 24, 27 and 30 °C) and leaf wetness duration (0, 6, 12, 24 and 48 hours) on the penetration and temporal progress of Asian soybean rust (*Phakopsora pachyrhizi*) was studied using

genotypes/cultivar with different levels of partial resistance. The area under disease progress curve (AUDPC) was measured under the interaction and influence of temperature and leaf wetness duration. The incubation period was measured only by the influence of the temperature. There were significant differences (P=0.001) in AUDPC between genotypes under the interaction of the binomial (temperature x leaf wetness duration - LWD) and for incubation period at different temperatures the different temperatures. No signals or symptoms of rust appeared at the temperatures 18 °C and above 30 °C. The relationship between temperature and leaf wetness duration (LWD) affect the AUDPC and temperature affects incubation period. The highest AUDPC values occurred at 24 °C and leaf wetting period of 24 hours, and the lower values were achieved at temperatures higher than 27 ºC. Genotype 1 or cultivar Desafio RR 8473 RSF, showed susceptibility to the monocycle parameters of ASR.ASR lesions started to appear in average at least 15 days to 20 DAI in partial resistant genotypes: Genotypes 2 - F8 BRSGO Luziânia X Potenza, 3 - F8 BRSGO Caiapônia X Potenza and Genotype 4 - F8 BRSGO Caiapônia X IAC100 who also had lower AUDPC and severity values, during the monocycle experiment.

Key words: *Glycine max*, incubation, rust, latency

INTRODUCTION

Asian soybean rust (*Phakopsora pachyrhizi*) is considered the most severe and destructive disease of soybean plants (*Glycine max*) in Brazil agriculture system. The symptoms of Asian soybean rust (ASR) can appear at any time in the phenological cycle of the crop, but generally happens more frequently in plants in the flowering stage (Yorinori, 2005). The success of the pathogen infection depends on the sequence of events determined by spore germination, appressorium formation and penetration. Each of these events and the subsequent colonization and sporulation are influenced by biotic factors/interaction and abiotic factors (Vale, Zambolim and Chaves, 1990). The abiotic factors, like foliar dew period and temperature (binomial), influence heavilythe pathogen temporal progression (Juliatti et al., 2005). We know that hours of dew period can contribute to the initial fungal infection. But regions with weeks of longer dew periods, higher humidity and deposition of the water film on leaves, limits the germination and penetration of fungal diseases in the soybean plants. This happens because of the excess of water, is no longer necessary to continue the infection process of the host, since the fungus can obtain water from the host itself in the colonization

stage (Huber and Itier 1990). Moreover, in the case of Asian rust, genetic mutations may occur, with individuals capable of adapting to the most diverse climatic conditions, chemical fungicides and technological levels of agriculture production (Juliatti et al., 2017). The infection in field occurs when the right amount of leaf dew is the driving force to increase disease intensity. Even with constant periods of rain, the necessary moisture for rust uredinospores germination and spread could not be achieved. So, the right periods of time in a day with dew, is probably the most important source of free water for fungi penetration and development on hosts tissue (Bromfield, 1984; Melching et al., 1989; Bonde, Nester and Berner, 2012). Del Ponte et al. in 2006, observed that greater leaf wetness period improves ASR disease severity. *Phakopsora pachyrhizi* is a biotrophic parasite, it can survive in dry period months with unfavorable conditions, in alternative hosts like the kudzu (*Pueraria* genera) and can penetrates directly through the cuticle and epidermis, making infection quicker and easier (Zambedetti et al., 2007; Bradley et al., 2010).

The temperature in this binomial interaction, also has directly influence in the pathogen metabolic stages of the life cycle, since when an optimal temperature is present the pathogen can perform their metabolic functions with minimal stress during the infection (Bonde et al., 2007; Dias et al., 2005). Favorable range of temperature heavily influences different levels of disease intensity over time on different cultivars or susceptible plants like one of the alternative host of soybean rust the kudzu of the *Pueraria* genera (Bonde et al., 2009). But for soybean, the processes of spore germination, infection, latent period, lesion expansion and sporulation are related to a range of temperatures between 12 to 25 °C with air relative humidity between 70 and 80%. Also, high temperatures reached during daylight do not inhibit fungus development (Marchetti, Melching and Bromfield, 1976; Melching et al., 1989). Marchetti, Uecker and Bromfield (1975) determined once the infection was established within the optimum range of temperature, the pathogen was able to colonize leaf tissue, even at a temperature of 30 °C, that is usually reported to be lethal to the infectious process. However, in this temperature the incubation and latency periods were 6 and 12 days, respectively, against 4 and 9 days when colonization occurred under the optimal temperature of 23°C.

Other authors describe this binomial interaction (temperature x wetting period) with *P. pachyrhizi* infection, reporting optimal temperature averages ranges between 20-25 °C, followed always by greater periods of leaf wetting produced lower latent and incubation periods in different cultivars and genotypes (Kochman, 1979; Casey, 1980; Vale, 1985; Sinclair and Backman, 1989; Alves et al., 2007; Bonde, Nester and Berner, 2012). The

interaction between pathogen and host will lead to the appearance of the typical lesion of the disease and the various events that occur between deposition and the formation of new urediniospores constitute a single cycle of infection, or the monocycle. Melching et al., 1989 studying duration, frequency and temperature of different wetting periods regimes determined that no rust lesions were formed on leaves of the soybean cultivar Wayne in temperatures lower than 9° C and above 28.5°C even with a 16 hours of dew period. They also stated that a period of 6 to 7 hours of continuous leaf wetting were required for rust lesions to develop between 18 to 26.5 °C. At least six hours of moisture is required for infection to occur at 24 ºC in Japan (Kitani, Inoue and Natsume 1960), from experiments in South Africa, no rust infection was found when incubating inoculated plants at temperatures below 15 or greater than 30ºC (Caldwell et al., 2005) and Kochman (1979) found that a 17 to 27 ºC temperature regime was clearly more favorable for rust infection during 16 hours of dew. The entry of the pathogen in the United States, was discussed by Pivonia and Yang (2006), they pointed out that in the United States, the occurrence of lower temperatures in the months of May through June limit the development of the disease in the South, which causes delay in the establishment of the epidemic in the North. In Brazil, the main soybean producing regions present favorable climatic conditions for the survival and the infectious process of the pathogen year-round. Since Brazil is one of the biggest soybean producers in the world and for its inherent tropical morphoclimatic characteristic, diseases of fungi etiology like the Asian rust, have the perfect environment to survive and maintain its life cycle all year round.

Most of the authors that related the binomial interacting with different cultivars and genotypes in relation to Asian soybean rust progression, proposed the hypothesis that the differences in disease intensity, incubation and latent periods may be associated to host characteristics. The greatest difficulty in the development of partial resistance, is to determine these differences on several environmental conditions. These factors are determinant for the success of the wide geographical distribution, directly influencing the severity of the disease, causing great economic losses (Wrather et al., 2001). 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). In greenhouse and growing chambers, it was determined that the minimum period of leaf wetness for greater infection in soybean leaflets is 6 hours at the temperature range between 15 °C and 27.5 °C for the cultivars Conquista, Savana and Suprema (Alves et al.,

2007). Temperatures above 30 ºC and below 15 ºC had reduced disease progress levels also the minimum latent period was 6 days for the cultivar Conquista, and 9 days for the cultivars Savana and Suprema at a range of 15 to 25 °C. Bonde, Nester and Berner (2012) studying two scenarios of environment combined effects, determined that temperatures higher than 35 °C and subsequent dew period had significant reduction of rust lesions on the susceptible American cultivar Williams 82. They also concluded that extreme lower temperatures below 18°C for short periods can account for observed absence or delay of soybean rust development in the southeastern United States. Souza and Fernandes (2008) evaluating the influence of leaf wetness in rust progression during 30 days on two soybean cultivars the Monsoy 8008 RR and Conquista under greenhouse conditions, found that that there was no significant interaction between period of leaf wetting and genotypes and reported that 30 hours of wetting period provided the highest number of pustules and lesions. Danelli and Reis (2016) determined at 10 and 30 °C, no rust leaf infection for soybean cultivars BRSGO 7560 and BRS 246 RR and related the occurrence in temperatures between 22 and 25 °C the largest number of spores, lesions and uredinias per lesion. Also, the statistical analysis showed differences when both cultivars were compared regarding spore number and lesions, with the BRS 246 RR showing the highest values, while 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, this explained the lower values of disease intensity from the material derivative from American ascensions. Zambenedetti et al. (2007) obtained in its monocycle 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. Vale, Zambolim and Chaves (1990) studying the effect of temperature and duration of leaf wetness on *P. pachyrhizi* infection in the Paraná cultivar, obtained no infection on all wetting regimes of the temperatures of 12 and 28 °C. They also observed the maximum number of lesions.cm^{-2} on the inoculated leaves under a temperature of 20 ºC and at least 16 hours of leaf wetness, they also concluded this to be the optimal conditions to *P. pachyrhizi* infection. The climate conditions reached in their experiment had an average of 24.4º C and relative air humidity of 69%. These variables were also used to model, simulate and predict Asian rust by other authors using cultivars adapted to their countries (Pivonia and Yang, 2004; Reis et al., 2004). These monocycle studies of weather effects and plant resistance expression are important to estimate the potential disease occurrence and formulate strategies to control before the epidemics begins in vulnerable geographic regions and help farmers increase yield (Bromfield, 1976; Parlevliet, 1997; Leite and Amorim, 2002; Uchôa et al., 2012). In view of

the above, this study aimed to verify the behavior of genotypes with partial resistance against Asian soybean rust (ASR) in relation to different temperatures and leaf wetness duration.

MATERIAL AND METHODS

Experimental information and genotypes

The experiment was conducted in foam trays (60 cells) inside air-conditioned chambers with controlled temperature and humidity during a 50-day period. The experiment design consisted in three-way factorial classification with 7 genotypes interacting with two fixed effects, five temperatures. (18, 21, 24, 27 and 30 °C) and four-leaf wetting periods (0, 6, 12 and 24 hour). Each replicate was composed of one plant per cell on the tray. The genetic material, consisted of six promising soybean genotypes developed by the LAGER / UFU improvement program (Table 1), based in Gloria Farm localized in Uberlândia City, Minas Gerais state and one more variety considered susceptible to rust, the DESAFIO RR - 8473RSF. These genotypes had partial resistance traits in field trials for *P. pachyrhizi* (Martins et al., 2007, Silva et al., 2007 and Martins and Juliatti 2014) and in greenhouse conditions for *Heterodera glycines* (Juliatti et al., 2017). The seeds of the F8 generation were taken from pods of their respective genotypes, during the season 2014/2015, in an experimental field describe above. Each lineage was characterized depending on its field rust resistance response and classified according to their cycle (days) and degree of relative maturity (early maturity groups between 6 and 7).

Table 1 Soybean genotypes of the breeding program LAGER / UFU, used in the assays.

		Factors			
Genotype Code	**Lager-UFU Code**	**Variety and Field Crossings**	**Temperature °C**	**Leaf Wetting (hours)**	**Cycle (days)**
1	L200	Desafio 8473SRF Susceptible Check			120*
2	L224	F8 BRSGO Luziânia X Potenza			120*
3	L266	F8 BRSGO Caiapônia X Potenza	18 21	0 6	120*
4	L144	F8 BRSGO Caiapônia X IAC100	24 27	12 24	120*
5	L279	F8 BRSGO Caiapônia X Potenza	30		120*
6	L254	F8 BRSGO Luziânia X Potenza			120*
7	L218	F8 BRSGO Luziânia X			120*

* Approximated values

Seeds were sowed in traysthat contained 1.6 kg of a substrate composed of sphagnum (70%), roasted rice straw (20%) and perlite (10%) with balanced soluble macro and micro nutrients. Roughing was carried out 10 days after sowing them, leaving only one plant per cell, which consisted the experimental plot. The plants were kept in a greenhouse until the vegetative stage V3, then were transferred to their respective constant temperatures chambers of 18, 21, 24, 27 and 30 °C with photoperiod of 12 h until the end of the experiment.

Inoculation and leaf wetness

P. pachyrhizi urediniospores present in fresh leafy uredinia serum, were collected from a field nursery plot with the cultivar Desafio 8473SRF.The suspension was prepared with distilled water containing 0.01% Tween 20 (v:v) and calibrated in a Neubauer chamber for a final concentration of 8.0 x 10^4 urediniospores.ml^{-1}. After 7 days of plant acclimatization inside their respective temperature's chambers, the inoculum suspension was uniformly sprayed on both sides of the leaves. Immediately after inoculation, the plants were incubated in dark inside the climatic chamber during the duration of the leaf wetting period. All the tested leaf wetness periods (6, 12 and 24 hours), except the 0-hour treatment, had the moist chamber (~100% of air humidity) simulated by a sealed environment. This simulation consisted in the placement of trays containing inoculated plants, under transparent plastic bags previously moistened to create an atmosphere rich in water vapor. The irrigations were carried out by depositing water directly on the base of a plant's stem. The plants were then submitted to different leaf wetness periods of 0, 6, 12 and 24 hours. After the removal of the last plastic bags of the 24-hour moisture period, the humidity in the air-conditioned chambers were maintained in 85% ± 10 with fog nebulizers.

Disease assessment

Four severity assessments were performed on the central leaflet of all the trifoliate leaves in each plant. The severity was measured after training with a diagrammatic scale developed by Godoy et al. (2006), until the last day of evaluation (45 days). These data were integrated into the area under the disease progress curve (AUDPC), that was calculated in according to Shanner and Finey (1977). This AUDPC data was then subjected to analysis of

variance between three factors, seven genotypes/cultivar, temperature and Leaf Wetness duration (LWD). When significative differences were obtained, surface response graphs were generated between the evolution of AUDPC in the binomial response for each genotype/cultivar. The incubation curve was calculated as a regression in function of the elapsed time, in days, between inoculation the and evolution of severity at the 5 temperatures regimes (18, 21, 24, 27 and 30 °C) for each genotype/cultivar. Also, to explain the appearance of the first signs of disease in the predicted incubation curves. One last assessment was made, it consisted in the registration of the day that the first signal of disease incidence appeared in any of the genotypes inoculated leaves submitted at different temperatures. This data was performed from the inoculation and continued every 5 days until the end of the experiment (9 evaluations in total).

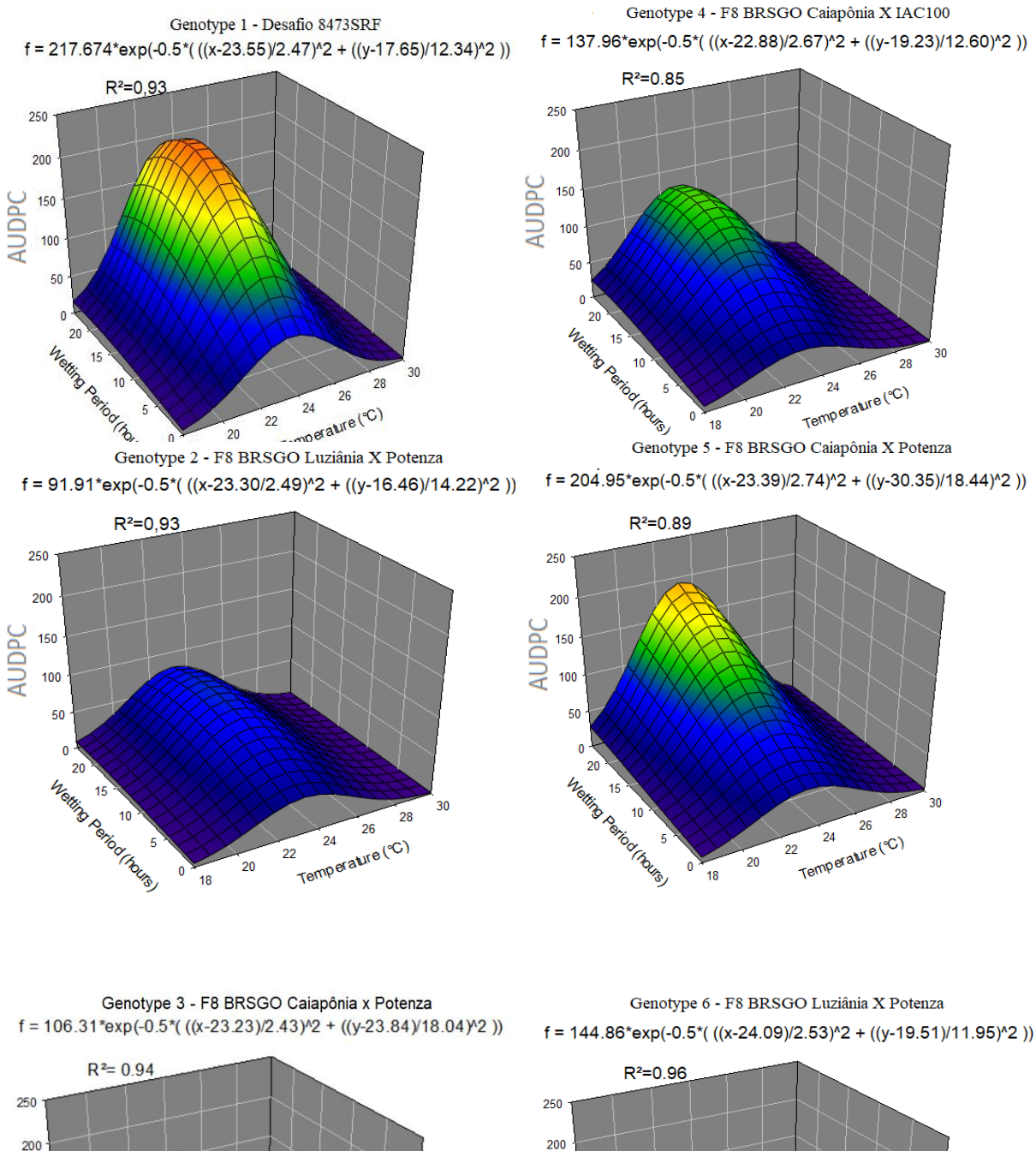
Statistical analysis

The data obtained at each assay were submitted to the Shapiro-Wilk test to evaluate the assumptions of the analysis of variance (Supplementary Materials). The data that obtained a normal distribution, were submitted to analysis of variance (F test). If F test was significative, the results of the surface response with AUDPC in the binomial and incubation period with severity at different temperatures were used to generate a response surface for the first and polynomial regression curves for the second. For the surface response, AUDPC data in the binomial interaction, was fitted to 3D regressions (Plane, Paraboloid, Gaussian and Lorentzian). For incubation, disease severity data at different temperatures, were fitted to the polynomial regressions (linear, quadratic and cubic). The model that gave the highest coefficient of determination (R^2), low residual means square (RMS) and best fit residual plot distribution, was selected as the most appropriate model for describing the temporal pattern of the disease (Madden, 2007). All analysis of variance, regression and AUDPC data were performed using the R software (R Core Team, 2017) with the add-on packages ExpDes (Ferreira et al., 2003) and agricolae (Mendiburu, 2005). The surface response graph was generated by the Sigma Plot® 12.0 program

RESULTS

Binomial and surface response of AUDPC

There was significant AUDPC differences and interaction ($p<0.001$) between temperature, leaf wetness period and tested genotypes (Figure 1 and Table S1). The genotypes 5, 7 and the susceptible check, had the highest values for AUDPC, genotypes 4 and 6 had relative lower values, but still presented susceptibility in comparison with the genotypes 2 and 3, who showed the lowest values of disease, these traits could be an indicative of partial resistance. In general, the highest AUDPC values (60 to 200) were showed in the temperature range of 22 to 26 °C with above 10 hours of leaf wetting. At all temperatures, except 18 and 30 °C, it was possible to observe symptoms or signals, however, the highest AUDPC was observed at the temperature of 24 °C for all genotypes. At temperatures of 21 and 27 °C the AUDPC between 10 to 60, lower values in comparison with the range of 22 to 26 °C. The period without prolonged leaf wetting (0 hours), showed the lowest AUDPC (0 to 50) for all temperature ranges, also were the conditions with dimmed symptoms and signals appearance.



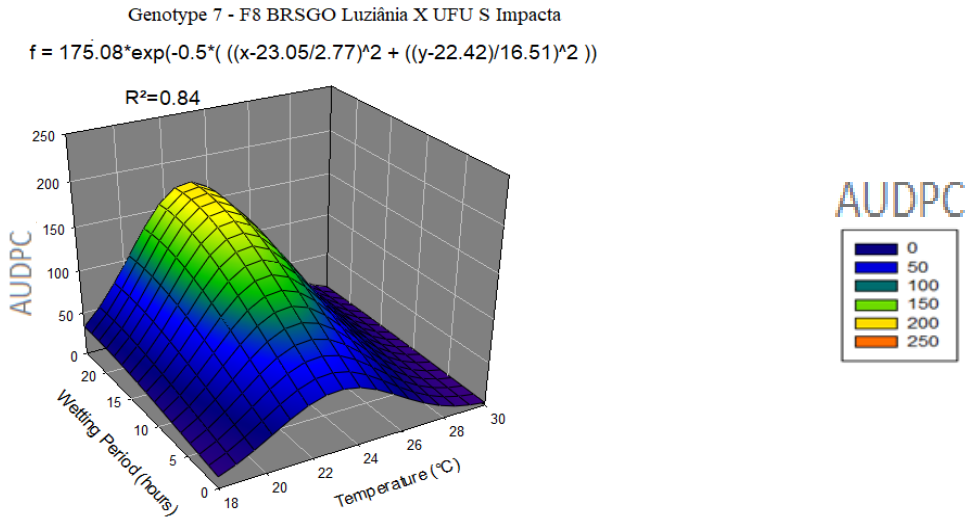
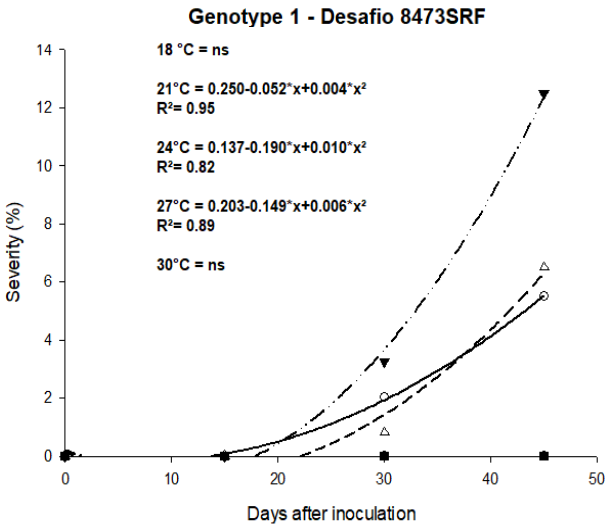


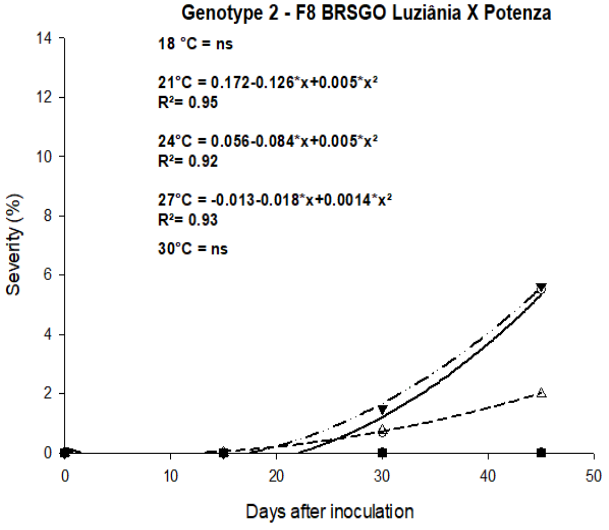
Figure 1 Gaussian models of response surface of *P pachyrhizi* AUDPC in soybean genotypes, as a function of the temperature and duration of the leaf wetting period, described by the function, where Z is the AUDPC, x is the temperature and y is the duration of the leaf wetting period.

Incubation period

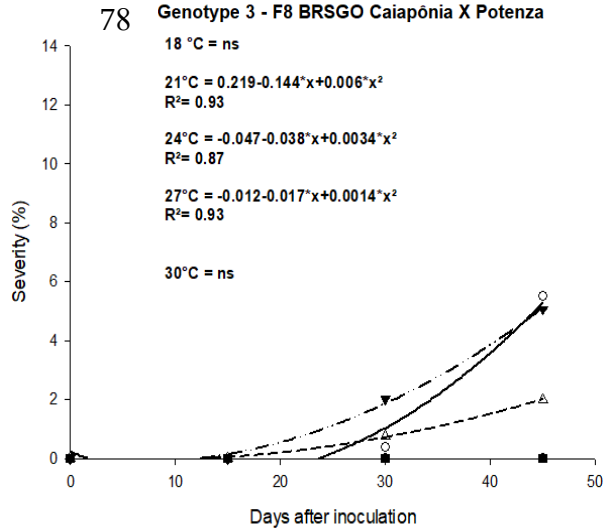
During the 50 days of experiment (02/04/2017 - 04/05/2017) the first signals of rust appeared at, 15 DAI in the susceptible cultivar Desafio 8473SRF, 10 DAI in genotypes 4, 5 and 7, 20 DAI in genotypes 2 and 6 and 25 s in genotype 3. The ANOVAs results (Table S2), indicated significant severity differences ($p<0.001$) between inoculated genotypes and interaction when those were submitted at different temperature regimes, during the period of experimental assessment. After this analysis, we modeled an estimation of disease incubation period with severity in function of temperature (Figure 2) and compared to the day that the

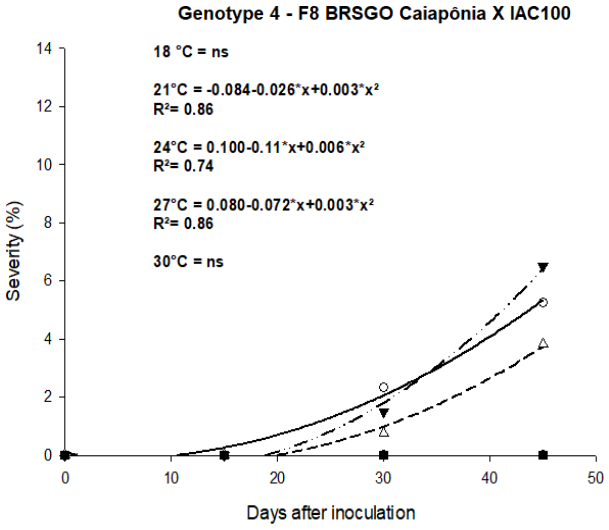
first incidence of ASR was observed. After 10 days of ASR uredinospores inoculation, at the temperature regime of 21 °C the first symptoms started to appear on genotypes 4, 5 and 7 at 15 days on the susceptible cultivar (genotype 1) and 20 days in genotypes 2 and 6 and 25 days in genotype 3. At 15 days of inoculation in the temperature regime of 24 °C, genotypes 2, 3 and 7 showed the first signals of rust, the other genotypes only presented the first signals after 20 days of inoculation. At 27 °C the signals of rust, appeared later in the inoculated leaves in comparison with other temperature regimes. Only after 25 days of inoculation, the signals appeared on genotypes 1, 5 and 7 and in 20 days on genotypes 2, 3, 4 and 6. No signals/symptoms of rust were observed in the genotypes leaves submitted at constant temperatures of 18 and 30 °C during the 50 days of assay. In general, when the inoculated genotypes were submitted to higher temperatures like 24 and 27 °C, the signals started to appear at least 5 days later in comparison with the lowest temperature employed who showed results (21 °C). But for genotypes 2 and 3 the signals on the lower temperature (21 °C) only appeared at a later period in comparison with the temperatures of 24 °C and 27 °C, resulting in an inversion of results. About the severity levels, in general the highest values (%) were obtained when the inoculated genotypes were submitted at a constant temperature of 24°C being later followed by 21 °C and for last at the constant temperature of 27 °C. We can observe in the quadratic regressions, the inoculated check Desafio 8473SRF obtained the highest average value of severity (12%) at the temperature of 24 °C, it also showed the highest curve slope growth during the 50 days of experiment. The genotypes 2, 3 and 4, could be highlighted and related to have partial resistance, since after the inoculation of these genotypes plants, they showed lower average severity levels during the 50 days of experiment. Genotypes 2 and 3 at temperature of 27 °C reached only 2% of average severity between plants and genotype 4 obtained the double (4%) but still a lower value in comparison with other genotypes. At temperature of 21 °C and 24 °C the tree genotypes obtained similar the maximum average severity level (~6%). We can conclude that when the inoculated genotypes were submitted to conditions with temperatures of 21 and 27 ° C, they presented lower average values of disease severity and later appearance of symptoms or signals 20 DAI). The minimal average severity value was 2 % for genotypes 2 and 3 at a constant temperature of 27 °C and the maximum of 12% was resulted on the inoculated check plants submitted to a constant temperature of 24 °C. As related to the previous data, the *P. pachyrhizi* provoked minor infection and growth when influence under of 21 and 27 ºC in comparison with the constant temperature of 24 °C.

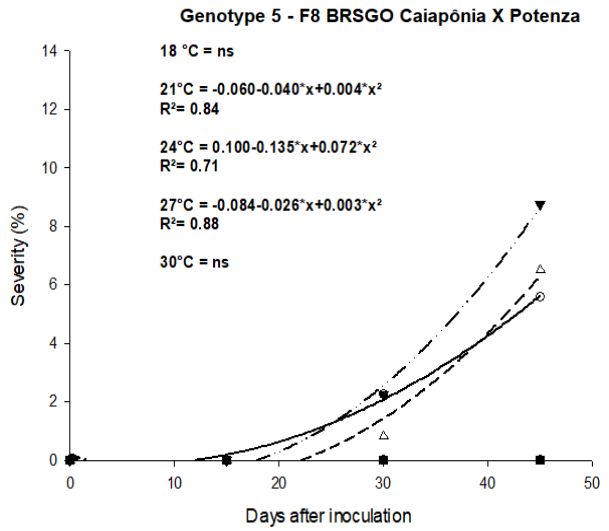

Genotype 1 - Desafio 8473SRF
18 °C = ns
21°C = 0.250-0.052*x+0.004*x²
R²= 0.95
24°C = 0.137-0.190*x+0.010*x²
R²= 0.82
27°C = 0.203-0.149*x+0.006*x²
R²= 0.89
30°C = ns
Severity (%)
Days after inoculation

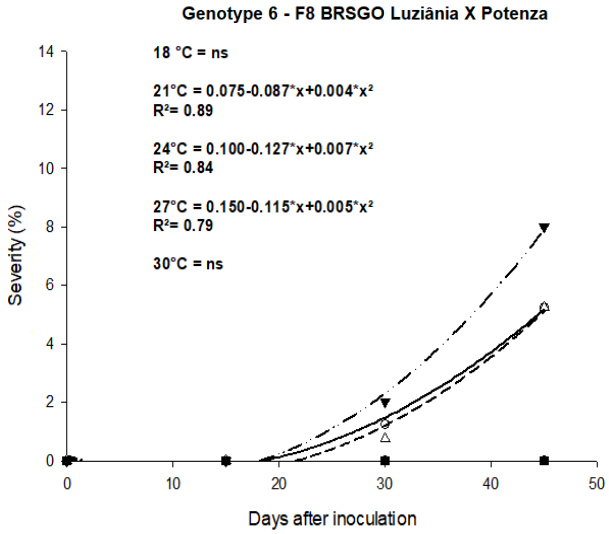

Genotype 2 - F8 BRSGO Luziânia X Potenza
18 °C = ns
21°C = 0.172-0.126*x+0.005*x²
R²= 0.95
24°C = 0.056-0.084*x+0.005*x²
R²= 0.92
27°C = -0.013-0.018*x+0.0014*x²
R²= 0.93
30°C = ns
Severity (%)
Days after inoculation

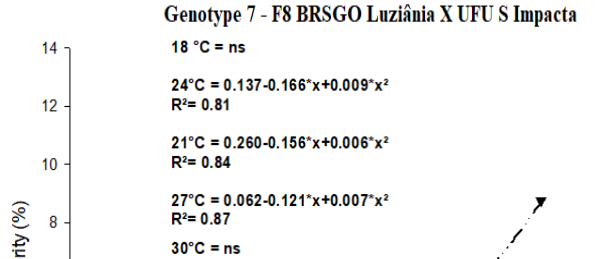
78


Genotype 3 - F8 BRSGO Caiapônia X Potenza
18 °C = ns
21°C = 0.219-0.144*x+0.006*x²
R²= 0.93
24°C = -0.047-0.038*x+0.0034*x²
R²= 0.87
27°C = -0.012-0.017*x+0.0014*x²
R²= 0.93
30°C = ns
Severity (%)
Days after inoculation


Genotype 4 - F8 BRSGO Caiapônia X IAC100
18 °C = ns
21°C = -0.084-0.026*x+0.003*x²
R²= 0.86
24°C = 0.100-0.11*x+0.006*x²
R²= 0.74
27°C = 0.080-0.072*x+0.003*x²
R²= 0.86
30°C = ns
Severity (%)
Days after inoculation


Genotype 5 - F8 BRSGO Caiapônia X Potenza
18 °C = ns
21°C = -0.060-0.040*x+0.004*x²
R²= 0.84
24°C = 0.100-0.135*x+0.072*x²
R²= 0.71
27°C = -0.084-0.026*x+0.003*x²
R²= 0.88
30°C = ns
Severity (%)
Days after inoculation


Genotype 6 - F8 BRSGO Luziânia X Potenza
18 °C = ns
21°C = 0.075-0.087*x+0.004*x²
R²= 0.89
24°C = 0.100-0.127*x+0.007*x²
R²= 0.84
27°C = 0.150-0.115*x+0.005*x²
R²= 0.79
30°C = ns
Severity (%)
Days after inoculation


Genotype 7 - F8 BRSGO Luziânia X UFU S Impacta
18 °C = ns
24°C = 0.137-0.166*x+0.009*x²
R²= 0.81
21°C = 0.260-0.156*x+0.006*x²
R²= 0.84
27°C = 0.062-0.121*x+0.007*x²
R²= 0.87
30°C = ns

7

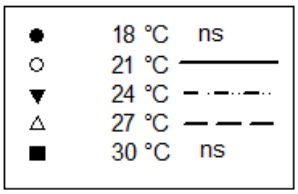


Figure 2 *P. pachyrhizi* incubation period in soybean genotypes at the temperatures of 18°C, 21ºC, 24°C, 27°C e 30°C.

DISCUSSION

Binomial and surface response of AUDPC

Several epidemiological studies have been developed to correlate disease components with climatic variables and productivity, to provide ancillary information to prediction and damage models. Most of the authors that related the binomial interacting with different cultivars and genotypes in relation to Asian soybean rust progression, proposed the hypothesis that the differences in disease intensity, incubation and latent periods may be associated to host characteristics. But, few of them proved and added up the influence of genetic resistance in these equations (Kochman, 1979; Casey, 1980; Vale, 1985; Sinclair and Backman, 1989; Yang et al, 1991; Hartman; Wang; Tschanz, 1991; Reis, Sartori, Camara, 2004, Juliatti et al., 2005; Del Ponte et al. in 2006; Zambenedetti, 2007; Alves et al., 2007; Pierozzi et al., 2008; Bonde, Nester and Berner, 2012;).

Our study focused in the characterization of genetic resistance in detriment of changes in a binomial composed by leaf wetness duration and temperature. The highest AUDPC values were presented in the temperature range of 22 to 26 °C with above 10 hours of leaf wetting, reaching the highest value (185) on the susceptible cultivar Desafio 8473SRF at the temperature of 24 °C. When the genotypes were submitted at 18 and 30 °C no signals or symptoms were observed. The genotypes plants without prolonged leaf wetting (0 hours), had the lowest AUDPC (0 to 50) and higher latent periods within all temperature ranges. In the literature, most of the results with this binomial influencing *P. pachyrhizi* infection matched to ours. Most of these studies related temperature averages between 20 to 25 °C always

followed by greater periods of leaf wetting reproducing higher severity levels, lower latent and incubation periods in different cultivars and genotypes (Kochman, 1979; Casey, 1980; Vale, 1985; Sinclair and Backman, 1989; Alves et al., 2007; Bonde, Nester and Berner, 2012). Marchetti et al. (1976) and Melching et al. (1989) when studying the effect of the temperature and leaf wetness duration (LWD), on germination and infection by urediniospores of *Phakopsora pachyrhizi,* the fungus had an optimal germinate range of 15 to 26.5 ºC and maximum infection rate; severity occurred between 10 to 12 hours of leaf wetting period. They also related that susceptible soybean varieties, when inoculated didn't showed symptoms of rust under LWD than six hours and higher temperatures of 27 °C.

In the literature, several authors descripted different levels of ASR severity in different cultivars at similar environmental conditions. Kitani and Inoue in 1960, related least six hours of moisture is required for infection to occur at 24 ºC in a Japanese soybean cultivar. This temperature is inside the range of 17 to 27 °C, described in the experiment with ASR inoculation on the susceptible cultivar Wills made by Kochman in Australia at 1979. The author also reported that ASR infection was clearly more favorable when this temperature range was linked of 16 hours of continuous dew. More recently Vale, Zambolim and Chaves (1990) studying the effect of temperature and duration of leaf wetness on *P. pachyrhizi* infection in the cultivar Paraná (susceptible to ASR), obtained no infection on all LWD of the temperatures of 12 °C and 28 °C. They also observed the maximum number of lesions.cm^{-2} on the inoculated leaves under a temperature of 20 ºC and at least 16 hours of leaf wetness. In South Africa Caldwell et al. (2005) related no rust infection was found when inoculated plants were incubated at temperatures below 15 °C or greater than 30 ºC. Nunkumar, Caldwell and Pretorius studying the influence of temperature, relative humidity (RH) and moisture period (MP) in the number of pustules and lesions of ASR in the highly susceptible soybean cultivar LS5995.Determined that the highest number of pustules per lesion occurred at 21–24°C. The authors also stated that ASR infection can occur after at least 6 hours of moisture period but for a higher probability to a successful infection the plants need to be exposed at least to 16 h. Infection in they work did not occur on plants incubated at 15°C and 30°C in any moisture period. Alves et al. (2007) determined that the minimum period of leaf wetness for greater infection in soybean leaflets is 6 hours at the temperature range between 15 °C and 27.5 °C for the cultivars Conquista, Savana and Suprema. These authors also demonstrated a similar result to ours, demonstrating that temperatures above 30 ºC and below 15 ºC had reduced disease levels. The increasing LWD on the inoculated genotypes tested in our work, resulted in higher disease intensity and levels of AUDPC. Junior and Fernandes (2010) related that

longer leaf wetness periods increased the severity (%) of soybean rust, and number of necrotic lesions on cultivars MSOY 8008 RR and Conquista, both are considered with different level of susceptibility. Working with the previous cultivars Souza and Fernandes (2008), evaluating the influence of leaf wetness in AUDPC levels during 30 days of experiment under greenhouse conditions, found that no significant differences between both and leaf wetness. They also reported that 30 hours of wetting period provided the highest number of pustules and lesions. We can ensure that inoculated soybean plants, under longer leaf wetness periods, end up developing higher levels of ASR. Danelli and Reis (2016) determined at 10 and 30 °C, no rust leaf infection was detected. Also, the largest number of uredinospores, lesions and uredinias per lesion was obtained in temperatures between 22 and 25 °C for soybean cultivars BRSGO 7560 (resistant) and BRS 246 RR (susceptible). There was also significant differences for number of spores produced, where the resistant cultivar reached lower levels of uredinospores (3000 and 8000 cm^{2-1}) and uredinias per lesion (8 to 13). The authors concluded that the cultivar BRSGO 7560 carries a Rpp gene that confers vertical resistance to soybean rust, this explained the lower values of disease intensity from the material derivative from American ascensions.

When we contrast the influence of temperature in fungi development, there is a consensus among authors that temperatures below 15 °C are not suitable to rust development, but in our work, no signals or symptoms were observed when the inoculated plants were submitted to a constant temperature of 18 °C. Also, the lower disease levels results achieved at temperatures greater than 27 ºC matched with other results in the literature. The processes of spore germination, infection, latent period, lesion expansion and sporulation are related to a range of temperatures between 15 to 25 °C with air relative humidity between 70 and 80%. Some authors stated that high temperatures reached during daylight do not inhibit fungus development (Marchetti, Melching and Bromfield, 1975; Melching et al., 1989). The same authors discussed that once the infection was established within the optimum range of temperature, the pathogen was able to colonize leaf tissue, even at a temperature of 30 °C. This temperature is usually reported to be lethal to the infectious process. In the end we can infer that temperature in this binomial interaction, influences the transitions of pathogen metabolic stages in their life cycle. And when an optimal temperature is present on the environment, the pathogen can perform their metabolic functions with minimal stress during the infection (Bonde et al., 2007; Dias et al., 2005). Moreover, in the case of ASR, genetic mutations may occur, with individuals capable of adapting to different hosts biology, diverse climatic conditions, chemical products and technological levels of agriculture production

(Juliatti et al., 2017). *P. pachyrhizi* spores that encounters favorable environmental conditions with an optimum range of temperature, regular leaf wetness periods and susceptible host, rust has higher chances to survive and restart its polycycle (Bonde et al., 2009). The interaction between temperature and increasing leaf wetness duration, creates a synergism that will reflects in better condition for fungal infection and spread in host tissues and consequently will reflect in higher AUDPC values. If we add up a third factor associated to the host components like resistance genes that contributes to biochemical responses (enzymes like PAL, POX) and constitutive barriers (cell wall and lignin), the disease levels differences become greater and wider.

The entry of the pathogen in the United States, was discussed by Pivonia and Yang (2006), they pointed out that in the United States, the minimal temperature threshold to stress ASR and prejudice the survivor of a population between two infections, is the maintenance of temperatures below 11 °C trough 9 to 10 weeks. This limits the development of the disease in the South during the months of May through June, which causes delay in the establishment of the epidemic in the North. In an experiment made on highlands and mountains regions found in the Brazilian states of Rio Grande do Sul and Santa Catarina, Gallotti and Casa in 2012 described that soybean volunteer plants found on the field between harvest seasons, didn't host ASR. They explained that lower temperatures (0 to 5°C) on the on months of June and July in the North Plateau and the Serrano Plateau, kill or under develop most of the majority of volunteer soybean plants and dim the chances of uredinospore infection and survivor in leaf tissues. This situation could be related to the ones found by the lower risk of ASR epidemics in the beginning of the soybean season at the north states localized in US. But this region in Brazil are an exception, they are localized under temperate climatic conditions. The main soybean producing regions present tropical and favorable climatic conditions for the maintenance of the infectious process of the pathogen year-round.

In the experiment, genotypes 5, 7 and the cultivar Desafio 8473SRF obtained the highest values for AUDPC and were labeled as susceptible materials, genotypes 2 and 3 otherwise presented the lowest AUDPC values, in the same condition, and were labeled as indication of the presence of partial resistance. These genotypes also can be inserted as important sources of resistance to breed future soybean cultivars with good potential of yield, shorter cycle (120 days) and stable resistance. These variables are important to adopt the right management of the continuous ASR reproduction cycles (generating higher AUDPC) under favorable conditions of temperature and LWD found in the tropics during the year. Brazil is the second biggest soybean producer in the world and for its inherent tropical morphoclimatic

characteristic, diseases of fungi etiology like the Asian rust, have the perfect environment to survive and maintain its life cycle all year around. 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). So, in this tropical climatic scenario with higher selection of rust pathotypes that are under intense exposition of fungicides compounds and cultivars with genes that confers vertical resistance, the adoption of a more stable resistance, like the partial resistance is a necessary measure to be inserted on disease management to help diminished the survival and progression of the ASR agent (Parlevliet, 1997).

Incubation Period

After the determination of AUDPC differences and interaction between genotypes in the binomial, we established the necessary time the fungus needs to incubate in those inoculated genotypes at different temperatures. 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 (Jan and Gibson, 1998; 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. Meanwhile, the first signals of rust in our experiment, appeared in the susceptible cultivar Desafio 8473SRF at 15 DAI, at 10 days in genotypes 4, 5 and 7, at 20 DAI in genotypes 2 and 6 and 25 days in genotype 3. Between all temperature regimes adopted the first signals of rust started to appear sooner at the temperature of 21 °C (10 DAI) and in a later period on 27 °C (25 DAI). In general, when the inoculated genotypes were submitted to higher temperatures like 24 and 27 °C, the signals started to appear at least 5 days later in comparison with the lowest temperature employed who showed results (21 °C). Our results demonstrate that there is a wide range in values of disease and latent periods, when the inoculated genotypes and cultivars were submitted under different climatic conditions (binomial). Zambenedetti et al. (2007) working with monocycle and complete resistance related that cultivars BRS 134 and BRS 231 even with early cycle, had higher AUDPC than the PI 459025, an America cultivar with the Rpp4 resistance gene. In this work, the considered susceptible cultivars BRS 231 and FT-2 took nine days to suffer the first signals of rust and the PI 459025 with a complete resistance gene Rpp4, resulted in twelve days of latency. A small gap in days between the material results, but interesting for disease reduction whereas thinking reduction of pathogen

cycles of spore production. Melching et al. (1989), who studied the duration, frequency and temperature of different wetting periods regimes determined that no rust lesions were formed on leaves of the soybean cultivar Wayne in temperatures lower than 9 °C and above 28.5°C even with a 16 hours of dew period. They also stated that a period of 6 to 7 hours of continuous leaf wetting were required for rust lesions to develop between 18 to 26.5 °C. In the case of the cultivar Wayne, Melching, Bromfield and Kingsolver (1980), using different isolates from Taiwan, India, Australia, obtained after inoculation, lesions from 6 to 7 days in the cultivar. Also, an average of 8 to 14 uredinia per lesion, was showed after 7 weeks of experiment in 24.5 °C and 55 % of relative humidity. Marchetti, Uecker and Bromfield (1975) determined once the infection was established at a temperature of 30 °C, the incubation and latency periods were 6 and 12 days, respectively, against 4 and 9 days when colonization occurred under the optimal temperature of 23 °C. Bonde, Nester and Berner (2012) studying two scenarios of environment combined effects, determined that temperatures higher than 35 °C and subsequent dew period had significant reduction of rust lesions on the susceptible American cultivar Williams 82. They also concluded that extreme lower temperatures below 18°C for short periods can account for observed absence or delay of soybean rust development in the southeastern United States. These results corroborate to explain the motive that countries within temperate climatic conditions usually have smoother and localized epidemics of ASR, instead to the ones within tropical latitudes. Alves et al. in 2007 described the minimum rust latent period was 6 days for the cultivar Conquista, and 9 days for the cultivars Savana and Suprema in ta range he temperature of 15 to 25 °C. Vale, Zambolim and Chaves, (1990), evaluating the soybean Paraná cultivar, observed the first symptoms after 9 and 12 days of inoculation at 20 and 24 ºC respectively. In average most of the studies pointed that the signals and symptoms of rust started to appear at least 6 to 12 days after inoculation, but in our work the lesions started to appear in average at least 15 days to 20 days after inoculation. These differences at the results could probably be related to inoculum variation. Also, the highest severity levels obtained by monocycle experiments ranged between 12-20%, matching with the 12% showed by the cultivar Desafio RR 8473 RSF. Koga et al., 2008 quantifying resistance through latent period and severity progression, determined that genotypes BRS 124, BACURI, PI230970SH and PI224270-1 took in average 7.84 days to reach 50% of the severity in comparison with the 11.91 days reached by genotypes ER046881 trough ER062164. They also stated that the parameter incubation period ranged from 3 to 6 days and was not important to differentiate the genotypes, since some of them appearance of lesions in dates was not consistent. In our experiment the incubation period

resusts were consistent, and diverged from this authors. The appearance of lesions in our results influenced in diffent levels of severity, allowing grouping of genotypes with different partial resistance reaction.

The materials that showed desired partial resistance with higher latent period, lower severity and consequently lower AUDPC values are the results of the F8 crossings between the cultivars BRSGO Luziânia with Potenza (genotype 2), BRSGO Caiapônia with Potenza (genotype 3) and BRSGO Caiapônia X IAC100 (genotype 4). Juliatti et al. (2005), in a work with the cultivar UFUS Impacta, from hybridizations between the cultivars Cristalina and IAC 100, obtained partial resistance to *P. pachyrhizi* with low disease values. Oliveira and Juliatti (2017) obtained high yield and lower values of disease intensity from the materials derived from crossings between BRSGO Luziânia X UFUS Impacta, BRS Caiapônia X Potenza and BRSGO Caiapônia X IAC100. Santos et al. (2007) described the monocycle of genotypes derived by Cristalina and IAC 100 breeding. The results dmonstrated that both sources provided genotypes withhigher partial resistance to soybean rust, after 12 days of inoculation. Martins et al. (2007), when measuring the average incubation period in several soybean genotypes, verified that the genotypes provided by IAC 100 and Cristalina crosses, had a variation from 11.43 to 16.45 days. Parlevliet (1983) and Vallavielle-Pope et al. (2000) discussed that a single day increase in the establishment of parasitic relationships, is already a significant contribution to decrease the number of a fungus reproductive cycles in a crop (polycycle). Parlevliet (1997) defined genetic resistance as the as the host ability to prevent the growth and development of the pathogen and partial resistance is a characterization of the reduction in epidemic rates and increase on latent period. This reduction in rates causes a decrease in the population of the pathogen, and consequently a decline in the amount of inoculum and intensity of the disease (Wang and Hartman, 1992). This type of resistance became evident and important when a monogenic resistance is overcome by a new breed of pathogen (Parlevliet, 1997). Marchetti, Uecker and Bromfield (1975) concluded after analyzing the development of uredinia in tissues of Lee 68 and PI 200492,that slower lesion development, shorter period during the formatrion of new lesions and earlier senescence of rust lesions are variables related to partial resistance characterization and polygenic expression. These characteristics contribute to the reduction in the amount of secondary inoculum, thus diminishing the potential for pathogen spread in the field. Polygenic resistance is characterized in function of the expression of constitutive barriers of horizontal resistance like lignin and cell wall thickness, genes expression and molecular changes due to different mechanisms of resistance (Luck et al., 2011).

Monogenic resistance instead confer resistance to a limited number of rust isolates, the 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 (Bonde et al., 2006, Bromfield, Melching and Kingsolver, 1980,). Hartman et al in 2004, also pointed out that fungus variability is the main factor in the breakdown of vertical resistance genes. 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 (Tschanz, Wang, Tsai, 1983, Hartman, Wang, Shanmugasundaram, 1997). Given the rapid breakdown of Rpps 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). We can propose a future scenario of soybean with Rpp genes, cultivated during during all year on tropical conditions (Paraguay and Bolivia) having a serious risk to suffer the “vertifolia effect”, mostly because of pathogen variability under pression of selection by these major genes. 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). Attached to this, another issue is coming to the surface, breeding programs who focus on materials with vertical resistance to rust, end up losing genes who confers horizontal. This problem occurs when breeders select in field plants under the protection of pesticides and several characateristics expressed by minor genes, that can possible be interesting ended up disappearing by the pesticide effect. So in the face of a new virulent pathogen race, the plant resistance could be broken more easily if the plant doesn’t express the minor genes effect (Van der Plank 1963; Parlevliet, 1983).

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 and Kuiper, 1977; Parlevliet, 1978; Parlevliet et al., 1979; Niks, 1983). 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, and

Frederick, 2005), development of durable ‘‘less-rusting” and ‘‘slow-rusting” cultivars is one of the options for breeding for resistance to soybean rust (Li and Young 2009). 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). 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 producers enviroment 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.

The greatest difficulty in the development of partial resistance is determine these differences on several environmental conditions. But we can get around this using monocycle studies of weather effects and plant resistance expression, to estimate the potential disease occurrence and formulate strategies to control before the epidemics begins in vulnerable geographic regions and help farmers increase yield (Bromfield, 1976; Parlevliet, 1997; Leite and Amorim, 2002; Uchôa et al., 2012). So, we must raise 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 (Clifford, 1974; Parvlievet, 1979). 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, 1983). 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.

CONCLUSIONS

1 - There is a difference among genotypes to AUDPC in the same temperature and leaf wetness duration.

2 - No signals, lesions or symptoms of rust appeared at the temperatures 18 °C and above 30 °C.

3 - The relationship between temperature and leaf wetness duration (LWD) affect the AUDPC and temperature affects incubation period.

4 - The highest AUDPC values occurred at 24 °C and leaf wetting period of 24 hours, and the lower values were achieved at temperatures greater than 27 ºC.

5 - Genotype 1 or cultivar Desafio RR 8473 RSF, showed susceptibility to the monocycle parameters of ASR.

6 - ASR lesions started to appear in average at least 15 days to 20 DAI in partial resistant genotypes: Genotypes 2 - F8 BRSGO Luziânia X Potenza, 3 - F8 BRSGO Caiapônia X Potenza and Genotype 4 - F8 BRSGO Caiapônia X IAC100 who also had lower AUDPC and severity values, during the monocycle experiment.

REFERENCES

AKAMATSU, H.; YAMANAKA, N.; YAMAOKA, Y.; SOARES, R.M.; MOREL, W.; IVANCOVICH, A.J.G.; BOGADO, A.N.; KATO, M.; YORINORI, J.T.; SUENAGA, K. Pathogenic diversity of soybean rust in Argentina, Brazil, and Paraguay. **Journal of General Plant Pathology,** v. 79, p. 28-40, 2013.

ALVES, M. D.E C.; POZZA, E. A.; FERREIRA, J. B.; ARAÚJO, D. V.; COSTA, J. C. B.; DEUNER, C. C.; MUNIZ, M. F. S.; ZAMBENEDETTI, E. B.; MACHADO, J. C. Intensidade da ferrugem asiática (*Phakopsora pachyrizi* H. Sydow & P. Sydow) da soja (*Glycine max* L.) Merr.) nas cultivares Conquista, Savana e Suprema sob diferentes temperaturas e períodos de molhamento foliar. **Summa Phytopathologica**. v. 33, p. 239-244, 2007.

BONDE, M.R.; NESTER, S.E.; AUSTIN, C.N.; STONE, C.L.; FREDERICK, R.D.; HARTMAN, G.L.; MILES, M.R. Evaluation of virulence of *Phakopsora pachyrhizi* and *P. meibomiae* isolates. **Plant Disease.** v.90, p.708-716, 2006.

BONDE, M.R.; BERNER, D.K.; NESTER, S.E.; FREDERICK, R.D. Effects of temperature on urediniospore germination, germ tube growth, and initiation of infection in soybean by *Phakopsora* isolates. **Phytopathology**, v.97, p.997-1003, 2007.

BONDE, M. R., NESTER, S. E., MOORE, W. F.; ALLEN, T. W. Comparative susceptibility of kudzu accessions from the southeastern United States to infection by *Phakopsora pachyrhizi*. **Plant Disease** 93, 593–598, 2009.

BONDE, M. R.; NESTER, S. E.; BERNER, D. K. Effects of soybean leaf and plant age on susceptibility to initiation of infection by Phakopsora pachyrhizi. **Plant Health Progress. Online publication. doi**: 10.1094/PHP-2012-0227-01-RS. v. 10, 2012.

BRADLEY, C., HINES, R., HAUDENSHIELD, J.S., HARTMAN, G.L. First report of soybean rust, caused by *Phakopsora pachyrhizi*, on kudzu (*Pueraria montana* var. lobata) in Illinois. **Plant Disease**. v. 94, p. 477, 2010.

BROMFIELD, K.R. Soybean Rust - **Monograph No. 11**. Saint Paul MN. APS Press, 1984.

BROMFIELD, K.R. World soybean rust situation. In: HILL, L. D. **World Soybean Research: proceedings of the world soybean research conference**. Danville: The Interstate Printers and Publichers, p.491-500, 1976.

BROMFIELD, K.R.; HARTWIG, E.E. Resistance to soybean rust and mode of inheritance. **Crop Science.** v. 20, p. 254-255, 1980.

BROMFIELD, K.R.; MELCHING, J.S. Sources of specific resistance to soybean rust. **Phytopatology.** v. 72, p. 706, 1982.

BROMFIELD, K.R.; MELCHING, J.S.; KINGSOLVER, C.H. Virulence and aggressiveness of *Phakopsora pachyrhizi* isolates causing soybean rust. **Phythopathology**, Lancaster. v. 70, p. 17- 21, 1980.

CALDWELL, P.M.; NUNKUMAR, A.; PRETORIUS, Z.A.; FERNANDES, J.M.C. Development of *Phakopsora pachyrhizi* at different temperatures, relative humidities and leaf wetness duration. In: **International workshop on plant disease epidemiology**, 9., Landerneau, Proceedings. Bordeaux: Societé Francaise de Phytopathologie, 2005.

CASEY, P.S. The epidemiology of soybean rust *Phakopsora pachyrhizi* Syd. **Soybean Rust Newsletter**, Shanhua, v. 4, n. 1, p.3-5, 1980.
CLIFFORD, B.C. The choice of barley genotypes to differentiate races of *Puccinia hordei* Otth. **Cereal Rusts Bulletin**, v. 2, n. 1, p.:5-6, 1974.

DANELLI, A. L. D.; REIS, E. M. Quantification of incubation, latent and infection periods of *Phakopsora pachyrhizi* in soybean, according to chronological time and degree-days. **Summa Phytopathologica**, v. 42, n. 1, p. 11-17, 2016.

DIAS, M. B.; POZZA, E. A.; ABREU, M. S.; MIRANDA, E. O. Efeito da temperatura no crescimento micelial, produção e germinação de conídios de *Colletotrichum* spp. isolados de *Coffea arabica* L. **Ciência e Agrotecnologia**. v. 29, p. 545-552, 2005.

FERREIRA, E. B.; CAVALCANTI, P. P.; NOGUEIRA, D. A. ExpDes.pt: **Experimental Designs package** (Portuguese). 2013.

GALLOTTI, G. J. M.; CASA, R. T. Sobrevivência de *Phakopsora pachyrhizi* em plantas voluntárias de soja nas regiões do Planalto Norte e Planalto Serrano de Santa Catarina. **Agropecuária Catarinense**, Florianópolis, SC, v. 25, n. 2, p. 64-67, 2012.

GODOY, C.V.; KOGA, L.; CANTERI, M.G. Diagrammatic scale for assessment of soybean rust severity. **Fitopatologia Brasileira**, Brasília, v.31, p. 63-68, 2006.

HARTMAN, G.L.; WANG, T.C.; TSCHANZ, A.T. Soybean rust development and the quantitative relationship between rust severity and soybean yield. **Plant Disease**, Saint Paul. v.75, p. 596-600, 1991.

HARTMAN, G.L.; WANG, T.C.; SHANMUGASUNDARAM, S. Soybean rust research: Progress and future prospects. In: **World soybean research conference**, 5., 1997, Bangkok. Proceedings. Bangkok: Kasetsart University Press. p. 180-186, 1997.

HARTMAN, G. L., MILES, M. R., FREDERICK, R. D. Breeding for resistance to soybean rust. **Plant Disease**. v.89, p. 664–666, 2005.

HUBER, L.; IRIER, B. Leaf wetness duration in a field bean canopy. **Agricultural and Forest Meteorology**, v.51, p.281-292, 1990.

JULIATTI, F.C.; POLIZEL, A.C.; BALARDIN, R.S.; VALE, F.X.R. Ferrugem da soja – Epidemiologia e manejo para uma doença reemergente. In: LUZ, W.C.; FERNANDES, J.M.C.; PRESTES, A.M.; PICININI, E.C. **Revisão anual de patologia de plantas** Passo Fundo. v. 13, p. 351-395, 2005.

JULIATTI, F.C.; POLLONI, L.C.; MORAIS, T.P.; ZACARIAS, N.R.S. ; SILVA, E.A.; JULIATTI, B.C.M . Sensitivity of two isolates of *Phakopsora pachyrhizi* to dithiocarmamate, chloronitril, triazoles, strobilurins, and carboxamides fungicides. **Bioscience Journal**, p. 933-943, 2017.

JULIATTI, B.C.M.; BELOTI, I.F.; PENA, L.C.; JULIATTI, F.C. Soybean genotype analysis for cyst nematode resistance. **Genetics and molecular research**, v. 16, p. 1-10, 2017.

JUNIOR, H.B.M.; FERNANDES, J.J. Período de molhamento foliar para ocorrência de ferrugem asiática (*Phakopsora pachyrhizi*) em dois cultivares de soja (*Glycine max*). **Enciclop biosfera**, Centro Científico Conhecer - Goiânia, vol.6, N.11, p. 11, 2010.

KATO, M.; YORINORI, J.T. A study on a race composition of *Phakopsora pachyrhizi* in Brazil: a difficulty of race identification. **JIRCAS Working Report**, v. 58, p. 94-98, 2008.

KAWASHIMA, C.G., GUIMARAES, G.A., NOGUEIRA, S.R., MACLEAN, D., COOK, D.R., STEUERNAGEL, B. A pigeonpea gene confers resistance to Asian soybean rust in soybean. **Nat. Biotechnol**. v.34, p. 661-665, 2016.

KAWUKI, R.S.; TUKAMUHABWA, P.; ADIPALA, E. Soybean rust severity, rate of rust development, and tolerance as influenced by maturity period and season. **Crop Protection**, Guildford. v. 23, p. 447-455, 2004.

KITANI, K.; INOUE, Y.; NATSUME, T. Studies on soybean rust and its control. II. Studies on the control of soybean rust. Shikoku Nogyo Shikenjo Hokoku, **Bulletin of the Shikoku Agricultural** Experiment Station, 5, 343-58, 1960.

KOCHMAN, J. K. The effect of temperature on development of soybean rust (*Phakopsora pachyrhizi*). **Australian Journal of Agricultural Research**, v. 30, n. 2, p. 273-277, 1979.

KOGA, L.J.; CANTERI, M.G.; CALCO, É.S.; UNFRIED, J.R.; GARCIA, A.; HARADA, A.; KIIHL, R.A. de S. Análise multivariada dos componentes da resistência à ferrugem-asiática em genótipos de soja. **Pesquisa Agropecuária Brasileira**, v.43, p.1277-1286, 2008.

LEITE, R. M. V. B. C; AMORIM, L. Influência da temperatura e do molhamento foliar no monociclo da mancha de Alternária em girassol. **Fitopatologia Brasileira**, Brasília, v. 27, p. 193-200, 2002.

LI, S.; YOUNG, L. D. "Evaluation of selected genotypes of soybean for resistance to *Phakopsora pachyrhizi*." *Plant Health Progress* doi: 10.1094/PHP-2009-0615-01-RS, 2009.

LUCK, J.; SPACKMAN, M.; FREEMAN, A.; TREBICKI, P.; GRIFFITHS, W.; FINLAY, K.; CHAKRABORTY, S. Climate change and diseases of food crops. **Plant Pathology**. v. 60, p.113-121, 2011. doi: 10.1111/j.1365-3059.2010.02414.x.

MARCHETTI, M.A.; MELCHING, J. S.; BROMFIELD, K. R. The effects of temperature and dew period on germination and infection by urediniospores of *Phakopsora pachyrhizi*. **Phytopathology**, St. Paul. v.66, p.461-463, 1976.

MARCHETTI, M.A.; UECKER, F.A.; BROMFIELD, K.R. Uredinial development of *Phakopsora pachyrhizi* in soybeans. **Journal of Phytopathology**, Lancaster, v.65, p.822-823, 1975.

MARTINS, J.A.S.; JULIATTI, F.C. Genetic control of partial resistance to Asian soybean rust. **Acta Sci Agron**. Maringá. v. 36(1), p. 11-17, 2014. doi:10.4025/actasciagron. v36i1.16919.

MARTINS, J.A.S.; JULIATTI, F.C.; SANTOS, V.A.; POLIZEL, A.C.; JULIATTI, F.C. Período latente e uso da análise de componentes principais para caracterizar a resistência parcial à ferrugem-da-soja. **Summa Phytopathologica**, v.33, p.364-371, 2007.

MENDIBURU, F.D. agricolae: Statistical Procedures for Agricultural Research. R Package Version 1.2-3, 2015.

MELCHING, J.S.; DOWLER, W.M.; KOOGLE D.L.; ROYER, M. Effect of duration, frequency, and temperature of leaf wetness period on soybean rust. **Plant Disease**. v. 73, p.117-122, 1989.

NIKS, R.E. Comparative histology of partial resistance and the nonhost reaction to leaf rust pathogens in barley and wheat seedlings. **Phytopathology**, v.73, p. 60-64, 1983.

NUNKUMAR, A.; CALDWELL. P.M and PRETORIUS, Z.A. Development of *Phakopsora pachyrhizi* on soybean at controlled temperature, relative humidity and moisture periods. **South African Journal of Plant and Soil**, v.26, p. 225-230, 2013.

PARLEVLIET, J.E.; KUIPER, H. J. Partial resistance of barley to leaf rust, Puccinia hordei. IV. Effect of cultivar and development stage on infection frequency. **Euphytica,** v.26, n.2, p.249-255, 1977.

PARLEVLIET, J.E. Further evidence of polygenic inheritance of partial resistance in barley to leaf rust, *Puccinia hordei*. **Euphytica.** v. 27, p. 369-379, 1978.

PARLEVLIET, J.E. Components of resistance that reduce the rate of epidemic development. **Annu Rev Phytopathol** v. 17, p. 203-222, 1979.

PARLEVLIET, J.E. Models explaining the specificity and durability of host resistance derived from the observations on the barley-*Puccinia hordei* system. In: **Lamberti F, Waller JM, Van der Graaff NA (eds) Durable resistance in crops**. Plenum Press, New York London, p. 57-80, 1983.

PARLEVLIET, J.E. Present concepts in breeding for disease resistance. In: **Brazilian Phytopathology Congress.** Fitopatologia Brasileira, Brasília. v. 22, p. 7-15, 1997.

PAUL, C.; HARTMAN, G.L.; MAROIS, J.J.; WRIGHT, D.L.; WALKER, D.R. First report of *Phakopsora pachyrhizi* overcoming soybean genotypes with Rpp1 or Rpp6 rust resistance genes in field plots in the United States. Plant Dis. v. 97, p. 1379, 2013.

PIEROZZI, P.H.B.; RIBEIRO, A.S.; MOREIRA, J.U.V.; LAPERUTA, L. di C.; RACHID, B. F.; LIMA, W.F.; ARIAS, C.A.A.; OLIVEIRA, M.F. de; TOLEDO, J.F.F. de. New soybean (Glycine max Fabales, Fabaceae) sources of qualitative genetic resistance to Asian soybean rust caused by *Phakopsora pachyrhizi* (Uredinales, Phakopsoraceae). **Genetics and Molecular Biology**, v.31, p.505-511, 2008.

PIVONIA, S., YANG, X.B. Assessment of potential year-round establishment of soybean rust throughout the world. **Plant Disease**, v.88, p.523-529, 2004.

PIVONIA, S.; YANG, X.B. Relating epidemic progress from a general disease model to seasonal appearance time of rusts in the United States: Implications for soybean rust. **Journal of Phytopathology**, Lancaster, v.96, p. 400-407, 2006.

R DEVELOPMENT CORE TEAM. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org. 2017.

REIS, E.M.; SARTORI, A.F.; CÂMARA, R.K. Modelo climático para a previsão da ferrugem da soja. **Summa Phytopathologica**, Jaboticabal. v.30, n.2, p.290-292, 2004.

SANTOS, J.A.; JULIATTI, F.C.; SANTOS, V.A.; POLIZEL, A.C.; JULIATTI, F.C.; HAMAWAKI, O.T. Caracteres epidemiológicos e uso da análise de agrupamento para resistência parcial à ferrugem da soja. **Pesquisa Agropecuária Brasileira**, v.42, p.443-447, 2007.

SHANER, G. and FINNEY, R.E. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. **Phytopathology**, v.70, p.1183-1186. 1977.

SILVA, V. A. S.; JULIATTI, F. C.; SILVA, L. A. S. Interação entre resistência genética parcial e fungicidas no controle da ferrugem asiática da soja. **Pesquisa Agropecuária Brasileira**, Brasília, v.42, n.9, p.1261-1268, 2007.

SINCLAIR, J.B.; BACKMAN, P.A. **Compendium of soybean diseases**. 3rd St. Paul: American Phytopathological Society, p. 24-27, 1989.

SOUZA, A. R.; FERNANDES, J. J. Efeito do período de molhamento foliar na ocorrência da ferrugem asiática (*Phakopsora pachyrhizi*) na soja (*Glycine max*). In: SEMANA ACADÊMICA, 5. **Anais…** Uberlândia. Universidade Federal de Uberlândia, 2008.

TSCHANZ, A.T. and WANG, T.C. Interrelationship between soybean development, resistance, and *Phakopsora pachyrhizi*. Anais, **Fifth International Congress of the Society for the Advanced of Breeding Research in Asia and Oceania**, Bangkok, Thailand. P. 14-20, 1985.

TWIZEYIMANA, M.; HARTMAN, G. L. Pathogenic variation of *Phakopsora pachyrhizi* isolates on soybean in the United States from 2006 to 2009. **Plant disease**, v. 96, n. 1, p. 75-81, 2012.

UCHÔA, C. N.; POZZA, E. A.; ALBUQUERQUE, K. S.; MORAES, W. S. Relação entre a temperatura e o molhamento foliar no monociclo da Sigatoka-negra. **Summa Phytopathologica**, Botucatu, v. 38, n. 2, p. 144- 147, 2012.

VALE, F. X. R.; ZAMBOLIM, L.; CHAVES, G. M. Efeito do binômio temperatura duração do molhamento foliar sobre a infecção por *Phakopsora pachyrhizi* em soja. **Fitopatologia Brasileira**, Brasília DF, v. 15, n. 3, p. 200-202, 1990.

VALE, F.X.R. **Aspectos epidemiológicos da ferrugem (*Phakopsora pachyrhizi* Sydow) da soja (*Glycine max* L. Merrill)**. 104p. Tese (Doutorado em Fitopatologia) Universidade Federal de Viçosa, Viçosa, MG, 1985.

VALLAVIELLE-POPE, C., GIOSUE, S., MUNK, L., NEWTON, A.C., NIKS, R.E., OSTERGARD, H., PONS-KUHNEMANN, J., ROSSI, V. & SACHE, I. Assessment of epidemiological parameters and their use in epidemiological and forescasting models of cereal airborne diseases. **Agronomie**, v. 20, p. 715-727, 2000.

WALKER, D. R., HARRIS, D. K., KING, Z. R., LI, Z., BOERMA, H. R., BUCKLEY, J. B., WEAVER, D. B., SIKORA, E. J., SHIPE, E. R., MUELLER, J. D., BUCK, J. W., SCHNEIDER, R. W., MAROIS, J. J., WRIGHT, D. L.; NELSON, R. L. Evaluation of soybean germplasm accessions for resistance to *Phakopsora pachyrhizi* populations in the southeastern United States, 2009-2012. **Crop Sci.** v. 54, p.1673-1689, 2014.

WRATHER, J. A.; ANDERSON, T. R.; ARSYAD, D. M.; TAN, Y.; PLOPER, L. D.; PORTA-PUGLIA, A.; YORINORI, J. T. Soybean disease loss estimates for the top ten soybean producing countries in 1998. **Canadian Journal of Plant Pathology**, Ontario. v. 23, n. 2, p. 115-121, 2001.

YANG, X.B.; TSCHANZ, A.T.; DOWLER, W. M.; WANG, T.C. Development of yield loss models in relation to reductions of components of soybean infected with *Phakopsora pachyrhizi.* **Journal of Phytopathology**, Berlin, v.81, p.1420-1426, 1991.

YORINORI, J. T. A ferrugem “asiática” da soja no continente americano: evolução, importância econômica e estratégias de controle. In: JULIATTI, F.C., POLIZEL, A.C.,

HAMAWAKI, O.T. (Org.) In: **Workshop brasileiro sobre a ferrugem asiática**, 1., Uberlândia. Coletânea. Uberlândia: EDUFU, 2005. p. 21-37, 2005.

YORINORI, J.T., WILFRIDO, M.P. **Ferrugem da soja: *Phakopsora pachyrhizi* Sydow. Londrina**: Embrapa, 2002. (Folder).

ZAMBENEDETTI, E. B., E.; ALVES, E. A.; POZZA, E D. V.; ARAÚJO, E C.; GODOY, V. Avaliação de parâmetros monocíclicos e da intensidade da ferrugem asiática (*Phakopsora pachyrhizi*) em diferentes genótipos de soja e posições de copa. **Summa Phytopathologica**. v. 33(2), p. 178-181, 2007.

ARTICLE 2

MORPHOLOGICAL, PHYSIOLOGICAL AND CHEMICAL RESPONSE IN SOYBEAN GENOTYPES WITH PARTIAL RESISTANCE AGAINST ASIAN RUST

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ABSTRACT

The objective of this work was to evaluate the activity of enzymes (peroxidases - POX and phenylalanine ammonia liase - PAL), formation of horizontal barriers (epicuticular wax and cell wall thickness) and photosynthetic parameters in plants infected with *P. pachyrhizi* under greenhouse conditions. Six genotypes with partial resistance to soybean Asian rust and one commercial cultivar (Desafio RR 8473 RSF - Check) were tested. The layers of adaxial and abaxial epicuticular wax of the leaflets were analyzed structurally by a scanning electron microscope (SEM). There is a difference among genotypes during the progress of ASR severity in two assays. Genotypes 2, 3 and 6 showed signals of partial resistance and obtained the lowest levels of severity and the cultivar Desafio 8473SFR demonstrated higher susceptibility with higher levels of severity. Photosynthetic parameters were impaired by *P. pachyhizi* infection in the susceptible genotype, but not in its resistant counterpart, as a result of structural and biochemical constraints derived of partial resistance. Impairments to photosynthesis were proportional to the development of the ASR in the susceptible genotypes. The leaf cell wall was ticker in the resistant genotypes. Lignin content was significantly higher in the resistant genotypes before inoculation and the content was equalized four days after inoculation. During 24 to 72 hours after inoculation the activity of PAL enzyme was greatly increase in the resistant genotypes. 8The genotypes that stood out with lower severity values, maintained overall a higher enzyme activity (PAL and POX).

Key words: PAL, photosynthesis, horizontal barriers, epicuticular wax.

INTRODUCTION

Soybean production is an important agricultural activity in Brazil. Future commercial projections show the country as the largest global oilseed player, in terms of production and exportation in a near future (USDA, 2017 and CONAB, 2017). The Asian soybean rust (ASR) caused by the fungus *Phakopsora pachyrhizi* Syd. & P. Syd, was related to a 2001 epidemic outbreak inside Brazilian soybean fields, generating losses close to US$ 10 billion (Yorinori

et al., 2005). Three major strategies are employed to disease management, the spray of chemical fungicides, the adoption of resistant soybean cultivars and specific cultivation practices, such as early sowing, use of cultivars with early maturation cycles, air spore monitoring, reduction in the population of secondary hosts and the adoption of soybean free growth periods called sanitary "void" (60–90 days) (Hartman, Miles and Frederick, 2005, Juliatti et al., 2005, Yorinori and Wilfrido, 2002, Yorinori, 2009). Mutations in different sites confirmed by gene sequencing of *P. pachyrhiz* field populations in Brazil. Revealed a new reality faced by soybean producers, populations of ASR lost sensitivity when exposed to important fungicides groups (Qol, SDHI, SBI and DTC) (Klosowski et al., 2015; FRAC, 2017, EMBRAPA, 2017). So, the alternative of plant resistance is gaining importance in this scenario of uncertainties.

Seven major genes for resistance (Rpp1, Rpp2, Rpp3, Rpp4, Rpp5, Rpp6 and Rpp7) against the ASR agent (*P. pachyrhizi*), already have been identified in soybean plants (Bromfield and Hartwig, 1980; Hartwig, 1986; Calvo et al., 2008; Li et al.,2012; Childs et al., 2017). However, these genes confer resistance to a limited number of ASR isolates and the presence of a pathogen with higher genetic variability, these specific resistance genes can be quickly overcome. Although the occurrence of pathotypes denounces this characteristic, little is known about this variability for ASR (Bromfield, Melching and Kingsolver, 1980; Bonde et al., 2006; Miles et al 2011; Yamanaka et al., 2010). ASR mutations could provoke erosion of Rpp genes in cultivars with complete resistance and can lead to a "vertifolia effect". Also, breeders don't focus in horizontal resistance during field selection with plants under the protection of pesticides So, characateristics expressed by minor genes, like differences in the thickness of cell wall and deposition of epicuticular wax ended being not expressed and reported. (Van der Plank 1963; Parlevliet, 1983). In fact, cultivars or lines without any partial resistance are rare (Parlevliet and Kuiper, 1977; Parlevliet, 1978; Parlevliet et al., 1979; Niks, 1983). The partial resistance is characterized by reducing the epidemic rates, by reduction of number and size of lesions, decrease in spore production and increase on latent period (Parlevliet, 1997). This causes the population of the pathogen to be reduced, and consequently a decline in the amount of inoculum and disease (Wang and Hartman, 1992). In soybean Martins and Juliatti (2014) estimated that rust partial resistance is a characteristic controlled by 2 to 23 genes that are predominantly dominant. These genes when expressed, are related to biochemical pathways and mechanisms developed to combat situations of stress. One of the major systems related to plant defense, is the composition of structural and biochemical "barriers" (Jones and Dangl, 2006; Montesano, Brader and Palva, 2003). The cuticle is one of

these “barriers”, and essentially its composition is complex and consists in cutin, wax (epicuticular and intracuticular waxes) and carbohydrates. This structure coats the outer surface of leaf epidermal cells, to protect the leaves from abiotic and biotic stress. (Martin, 1964; Schreiber, 2010; Nawrath et al., 2013). An increasing number of genes involved in the biosynthesis of the cuticle have been identified mainly in several plants, but many details remain unanswered like the assembly process of extracellular elements (Kunst and Samuels, 2009; Beisson et al., 2012; Lee and Suh, 2013).

The host adaptation trough development of “barriers” to resist biotic and abiotic stress, is the ongoing evolutionary process resulted after millions of years. This is the basis of the plant innate immunity creation and is characterized by a complex scheme to form the primary defense against pathogens survival (Scheel, 1998; Keen, 1999 and Lygin et al., 2009). This complex defense system is made up of antioxidants and a diverse range of enzymes, such as phenylalanine ammonia liase (PAL) and peroxidases (POX). In the initial phase of an infection, the plant generates numerous cellular changes and activates signaling cascades in the plasma membrane. This leads to interconnection between networks of signaling molecules, such as salicylic acid (AS), jasmonic acid, ethylene and nitric oxide, for the rapid formation of defense responses (PAL, POX and lignin) thus limiting or inhibiting the dispersion of pathogen in the plant (Harrison and Baldwin, 2004).

The peroxidases (POX), catalyze the oxidation and eventual polymerization of alcohol hydroxycinnamic acid in the presence of hydrogen peroxide, resulting in lignin production and depoisiton (Van Loon and Strien, 1999; Monteiro et al., 2016). These enzymes are also involved in the cleaning of compounds related to the oxidative explosion (ROS – reactive oxygen species) and protect the cells from oxidative damage (Kawaoka et al., 2003; Hossain, Uddin, 2011). In addition, this enzyme has been associated with lignification, since it forms cross-links between phenolic groups and wall, pectin’s and other polymers (Mendonça and Guerra, 2003). Lignin biosynthesis also involves the enzyme phenylalanine ammonia liase. PAL catalyzes the conversion of phenylalanine into trans-Cinnamic acid, resulting in phytoalexins, phenolic compounds and lignin, which confer greater resistance to the plant cell wall during fungal infection (Nakazawa, Nozue and Yasuda, 2001). This product of the phenylpropanoid metabolism, includes a complex series of pathways that provide plants with various chemical combinations (Boatright et al., 2004).

Partial resistant plants differ in the gene expression profile before even from the insect attack, indicating that the defense is already activated as a priming mechanism (Cardoso et al., 2014). Van de Mortel et al. (2007) determined through qRT-PCR and microarray analysis, an

overrepresentation of genes associated to the phenylpropanoid pathway at 1 to 2 days earlier in the ASR inoculated genotype PI230970 (Rpp2) in comparison with the genotype Embrapa-48 (susceptible). Fungi like ASR overcome plant resistance through the acquisition of virulence factors or specific races (Resende et al., 2003). These avirulent pathogen infection induces defense responses that frequently results in localized collapse of plant cells known as hypersensitivity reaction (HR), ROS and involves the synthesis of phytoalexins that act as a direct antimicrobial defense (Stakawicz et al., 1995; Salmeron and Vernooij, 1998). The antimicrobial phytoalexins and cell wall reinforcement are encoded by the defense enzyme PAL, liberate elicitor-active oligosaccharides, and peroxidases (POX) by cross-linking macromolecules (lignin) in the cell wall (Rodrigues et al., 2004). This mechanism is part of a plant's innate or basal resistance that helps partial resistant plants to prevent the initial infection and colonization by most fungi, including ASR (Asian Soybean Rust). Phytoalexins like glyceollin formed by the PAL pathway, are described to being correlated with cell wall lignification. PAL also is related to be higher in resistant soybean cultivars in comparison with the susceptible, indicating a possible protective role of lignin in ASR infection development (Dakora and Phillips, 1996; Dixon, 2001; Lozovaya et al., 2007; Lygin et al., 2009). Lozovaya et al., 2004 studying infection of the fungi *Fusarium solani* f. sp. *Glycines* on soybean genotypes reported that, after inoculation, glyceollin accumulated to much lower concentrations in roots of the susceptible cv. Spencer than in the partially resistant genotype PI567374. The lignin also is an important structural component which can affect the susceptibility of cell wall in the presence of degrading enzymes produced by fungi's, preventing the diffusion of mycotoxins and negatively influencing the pathogen infection (Sattler and Funnel 2013, Stintzi et al., 1993 and Mali et al., 2017). Lygin et al., 2009 studying soybean plant–pathogen interactions demonstrated that soybean resistance to the sudden death syndrome (SDS) was correlated with the levels of the antibiotic-like phytoalexin glyceollin. ASR is a biotrophic pathogens, and probably is sensitive by defenses mediated by PAL and POX. So resistant soybean, would present similar responses with the pathways activated in drought periods, like superior formation and distribution of epicuticular wax, increase of lignin content, thicker cell wall and higher defense activity (enzymes).

Foliar diseases also, generally alter physiological functions by decreasing photosynthetic capacity (Tas and Tas, 2007). Tschanz and Wang in 1985 obtained ASR progress curves under different environmental conditions and concluded that ASR resistance was influenced by environmental factors or physiological effects (photosynthetic parameters). Physiological performance and disease resistance can be introduced simultaneously using

phenotypic tools in plant breeding (Jamil et al., 2016 and Lopes et al., 2010). Alterations in leaf gas exchange, water use efficiency (*WUE*), energy dissipation via chlorophyll a and b (*SPAD*), foliar temperature as well as carbon (*Ci*) and stomatal conductance can alter plant metabolism and have been reported to occur in plants during the infection process of pathogens (Baker et al., 2008; Zhao et al., 2011; Resende et al., 2012). It is known that in the presence injury and drought stresses, plants have their antioxidative system enhanced and has their capacity to produce hormones and pigments altered (Jaleel et al., 2008; Wu et al., 2001; Diaz-Espejo et al., 2012). Suitable assessment of photosynthesis in resistant plants infected by ASR can bring new insights into the mechanisms underlying host-pathogen interactions. To date, little is known about the metabolic changes and photosynthesis responses of partial resistant soybean plants during *P. pachyrhizi* infection. The main goal in this research, was to evaluate changes inphotosynthesis, enzymatic activity (PAL and POX), lignin, cell wall thickness and epicuticular wax distribution in soybean genotypes with partial resistance to ASR.

MATHERIAL AND METHODS

Experimental information

The study was conducted during December to April of 2017, in the greenhouse localized at Lavras city on the Minas Gerais state (Brazil), in the geographic coordinates 21°13'35.3"S and 44°58'31.4"W. The greenhouse is equipped with an automated mist sprinkler system and rising temperature control system by air injection. The experimental design adopted was in randomized blocks design (RBD) containing four blocks. The experiment also needed plant destruction to obtain the enzymatic and morphologic analysis. In function of this and to prevent the bias that could be provoked by the stress generated by leaf removal, each block contained a higher number of plants (16 vases in total). Of the eight destructed plants, five plants were used for enzymatic and the other three for morphological (epicuticular wax and cell wall) analysis. The other half were used to conduct the physiological (IRGA and SPAD) and disease evaluations. Each plant (genotype/variety) were sowed separated in their respective 5-liter vase (tagged with plastic platelets), with substrate containing soil, sand (2:1 proportion) and fertilizer (NPK: 0-20-20 - 1.56 g of formulate in each vase). Two pesticides sprays were made throughout the period of the experiment to control caterpillars and sucking insects (141 g. ha^{-1} of thiamethoxam + 50 g. ha^{-1} of lambda-

cyhalothrin). The inoculum consisted in *P. pachyrhizi* urediniospores present in fresh leafy uredium serum collected in plants inside an ASR field nursery plot. Then a suspension was prepared with distilled water containing 0.01% Tween 20 (v: v) and calibrated in a Neubauer chamber for a final concentration of 8.0 x 10^4 urediniospores. ml^{-1}. The suspension was uniformly sprayed on both sides of the leaves until the solution started to run off, when the plants started flowering (R1) at 16/02/2017. Immediately after inoculation, the plants were incubated in a dark environment, inside the greenhouse covered with humid fog for a period of 24 hours. The plants were then kept at 24 ± 3 with 15 min wetting shifts, every 6 hours. The genetic material tested, consisted of 6 promising soybean genotypes with partial resistance from germplasm source program at Federal University of Uberlândia (Lager/UFU) (Table 1), which showed desirable traits in field and greenhouse tests against several pathogens, like Asian rust (*Phakopsora pachyrhizi*) and *Heterodera glycines* (Juliatti et al., 2017) based in Gloria Farm localized in Uberlândia - Minas Gerais state, Brazil, and one more variety considered susceptibility DESAFIO RR - 8473RSF. Seeds of the generation F8, were taken from pods of their respective genotypes, during the season 2015/2016, in an experimental field at the farm Victoria/UFLA based in Lavras – MG.

Table 1 Soybean genotypes of the breeding program LAGER / UFU, used in the assays.

Genotype Code	Lager UFU Code	Variety and Field Crossings	Cycle (days)
1	L200	Desafio 8473SRF - Susceptible Check	120*
2	L224	F8 BRSGO Luziânia X Potenza	120*
3	L266	F8 BRSGO Caiapônia X Potenza	120*
4	L144	F8 BRSGO Caiapônia X IAC100	120*
5	L279	F8 BRSGO Caiapônia X Potenza	120*
6	L254	F8 BRSGO Luziânia X Potenza	120*
7	L218	F8 BRSGO Luziânia X UFU S Impacta	120*

* Approximated values

1- Commercial cultivar screened and evaluated in previous experiment for ASR and described with susceptibility.

2-7 Genotypes developed by the LAGER / UFU improvement program, that were screened and evaluated in previous experiment for ASR with different levels of partial resistance.

Disease assessments

Disease severity was made in a parallel assay and repeated once. The data was assessed on a weekly basis in a period of six weeks starting one week after the inoculation (DAI - days after inoculation). The severity or injured percentage area of the leaf was

determined in the central leaflet of the third and fourth trefoil at the central part of the soybean plant. To estimate the soybean rust severity, the diagrammatic scale of Godoy et al. (2006) was adopted.

Evaluation of chlorophyll and photosynthesis

Two SPAD and IRGA readings were performed in the 1st trifoliate leaves during the conduction of the second assay 15 days before inoculation (V6 stage) and 15 days after inoculation (R4 stage). Triplicate readings were done per plant generating 16 readings per genotype or variety (4 plants used in each block). The plants evaluated, were not submitted to stress or forced leaf removal like the plants used for enzymatic or morphological analysis. The equipment used for the readings, were a portable chlorophyll meter SPAD-502® and an Infra-Red Gas Analyzer (IRGA, Li-6400, Licor Ltda). The IRGA chamber was irradiated with a photosynthetic photon flux density (PPFD) of 1000 μmol m− 2 s− 1 the measurements were performed between 9:00 and 11:00 p.m and the photosynthetic activity (A), transpiration rate (*E*), carbon intake (*Ci*), stomatal conductance (g_s), transpiration (*E*) and water use efficiency (WUE=A/E) rates were obtained.

PAL, POX and Lignin contents.

The genotypes trifoliate leaves inoculated with *P. pachyrhizi* at the second assay R1 stage, were detached and collected to determine the activities of phenylalanine ammonia-lyases (PAL, EC 4.3.1.5), peroxidases (POX, EC 1.11.1.7) and soluble lignin content. The collections consisted in prior to inoculation (0),24, 48, 72 and 96 hours after inoculation (hai). Each period of collection was represented by a single plant, after the destruction, the plant was eliminated of the experiment and other plant non-stressed by leaf collection was used for other assessments. The collected leaves were placed in aluminum foil, frozen in liquid nitrogen and stored at -80 °C until analysis. To obtain the enzymatic extract for the determination of PAL and POX activity, 1 g samples fresh leaf tissue was powdered in liquid nitrogen in a mortar with the addition of 1% polyvinylpyrrolidone (PVP) w/v. The obtained powder was homogenized in 100 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA and 10 mM ascorbic acid. The homogenates were centrifuged at 13,000 g for 25 minutes at 4 °C and the supernatants were used for the enzymatic assays (Biemelt; Keetman, Albrecht, 1998).

PAL activity was initiated after the addition of 5 µl of this extract to a mixture containing 145 µl of Tris-HCl buffer (100 mM - pH 8.8) and 50 µl of Lphenylalanine (50 mM) The reaction mixture was incubated in a microplate spectrophotometer at 30 °C for 1 hour. After incubation, the absorbance of the trans-cinnamic acid derivatives was measured at 290 nm and the molar extinction coefficient of 104 mM-1 cm-1 (Zucker, 1965) was used to calculate the activity of PAL, which was expressed in µM min-1 mg-1 protein. The POX activity was determined by the oxidation of guaiacol, according to the methodology of Urbanek, Kuzniakgebarowska and Herka (1991). 40 µL of the enzyme extract, adjusted to 200 µL of solution containing 100 mM potassium phosphate pH 7.0, guaiacol 7.5 mM and 18.75 mM hydrogen peroxide. After incubation at 30 °C, for 2 minutes, the absorbance was measured at 480 nm. The coefficient of extinction molar ratio of 1.235 mM-1 cm-1 was used to calculate POX activity (Chance, Maehley, 1955).

To determinate the total soluble lignin content, the leaves collected at 0 and 96 hai were powdered in liquid nitrogen and then lyophilized for 24 hours. A 30 mg aliquot of lyophilized material was transferred to 2.0 mL microtube and homogenized with 1.5 mL 80% methanol for 16 hours for tissue depigmentation. After centrifugation at 12,000 g for 5 minutes the solid residue was homogenized with 1.5 mL of water and the mix was centrifuged at 12,000 g for 5 minutes at 4 °C. The supernatant was discarded, and the residue was oven dried at 65 °C for 15 hours. Subsequently, 1.5 mL of thioglycolic acid solution in 2M HCl (1:10) was added. The microtubes were then gently shaken to hydrate the residue and placed in a boiling water bath for four hours. Subsequently, the microtubes were centrifuged at 12,000 g for 10 minutes at 4 °C. The supernatant was discarded, and the precipitate washed with 1.5 mL of ultrapure water and again centrifuged at 12,000 g for 10 minutes at 4 ° C. Thereafter, the supernatant was discarded, and the precipitate was resuspended in 1.5 mL of 0.5 M NaOH and held on a rotary shaker for 15 hours at room temperature. The mixture was centrifuged at 12,000 g for 10 minutes at 4 °C and the supernatant transferred to a new microtube, to which 200 ul of concentrated HCl were added. The suspension obtained was kept in a cold room (4 °C) for four hours to allow precipitation of lignin bound to thioglycolic acid. Thereafter, the mixture was centrifuged at 12,000 g for 10 minutes at 4° C. The supernatant was discarded, and the precipitate was resuspended in 2.0 ml of 0.5 M NaOH. The absorbance of this solution was determined in a spectrophotometer at 280 nm and the values calculated based on the lignin curve and expressed in µg of soluble lignin per milligram of dry mass (Doster and Bostok, 1988).

Cell wall and epicuticular wax distribution

For observation of the leaf preformed barriers and structures such as epicuticular wax and cell wall, plant tissue at the second assay (V6 stage) samples were collected and preserved in Karnovsky fixative solution (2.5% glutaraldehyde, 2.0% paraformaldehyde in 0.05M sodium cacodylate buffer, 0.001M $CaCl_2$, pH 7.2) and stored at 4 °C for 24 hours. After fixation the samples were washed in 0.05 M cacodylate buffer for 10 minutes, this process was repeated twice and then transferred to a solution of 1% osmium tetroxide with water for 1 hour. Subsequently they were washed in distilled water three times and dehydrated in acetone gradient (25%, 50%, 75%, 90% and 100%) remaining for ten minutes in each concentration except for the 100% that was washed three times instead of one according to Bozzola & Russel (1999). Afterwards, the samples were treated in Balzers CPD 030 critical point apparatus, where acetone was replaced with CO_2. The specimens were stick with a double face tape in a stub sputtered with gold in a device SCD 050 Balzers for posterior observation in a scanning electron microscope Zeiss LEO EVO 40. The generated images were edited using Corel Draw software, and the cell wall was calculated using ImageJ software where the thickness measurement of the cell wall was performed in 50 cells of the epidermis of the adaxial face, avoiding cell corners and calculated after the average of the treatments. The formation of epicuticular wax was compared by the formation and observation of the deposition of the wax layer on the adaxial and abaxial faces (Alves and Perina, 2012).

Statistical analysis

The data obtained at each assay were submitted to the Shapiro-Wilk test to evaluate the assumptions of the analysis of variance. The data that obtained a normal distribution, were submitted to analysis of variance. The data was also subjected for cluster comparison by means group test by Scott-Knott (5%) using the R software (R Core Team, 2013) also with the add-on package ExpDes (Ferreira et al., 2003).

RESULTS

Disease assessments

Results of the first assay and the repetition were presented separately. The first signs of disease incidence were observed 7 days after inoculation (7 DAI). The typical symptom (TAN lesion) was not yet visible, and the detection of the disease in its initial phase, was done by visualizing uredinia under the magnifying glass on the abaxial surface of the sampled leaves. The genotypes were at the R2 stage when the lesions started to appear. In this time the first pustules were observed in the trifoliate leaves of the lower third of the plants. Then the disease progressed to the upper canopy of the plant. The epidemics was established from the 35 DAI at the first and from 28 DAI at the second assay, with 30% and 55% of maximum severity reached in the Desafio 8473SFR in the first and second assay respectively (Figure 1).

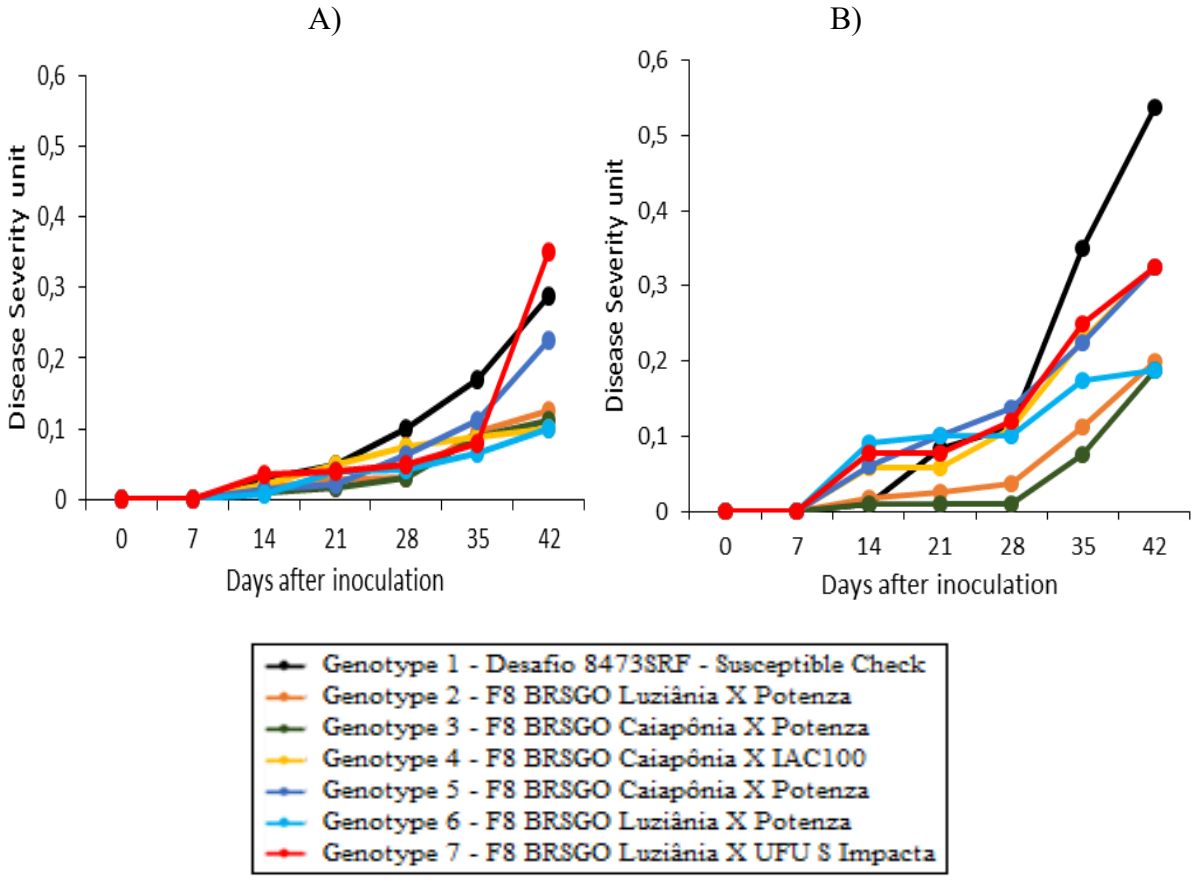


Figure 1 ASR severity observed data for 7 genotypes tested at different assays. A/B - First and second assays respectively.

Evaluation of chlorophyll and photosynthesis

There was significant difference between genotypes for all physiological variables except for the SPAD index (Table 2 and Table S3) before inoculation in the V6 stage. In

relation to the photosynthetic activity (*A*) genotypes 1, 2, 3 and 6 obtained higher values (ranging from 20.49 to 21.80 µmol m^{-2} s^{-1}) and genotypes 4, 5 and 7 (ranging from 13.94 to 16.59 µmol m^{-2} s^{-1}) obtained lower photosynthetic activity. The transpiration rate (*E*) of genotype 4 (3.96 mmol m^{-2} s^{-1}) was lower than the other genotypes, which ranged from 5.06 to 5.58 mmol m^{-2} s^{-1}. The stomatal conductance (g_s) was observed in 3 distinct groups, a first group formed by genotype 2 (1.07 mmol m^{-2} s^{-1}), a second group of lower stomatal conductance composed by genotypes 1, 3, 5 and 6 (0.73 to 0.79 mmol m^{-2} s^{-1}) followed by the lowest stomatal conductance group formed by genotypes 4 and 7 (0.48 and 0.51 mmol m^{-2} s^{-1}). Two groups were formed by the amount of carbon intake (*Ci*), a group with superior values formed by genotypes 2, 5 and 7 (365.65 to 380.46 µmol mol^{-1}) and a group formed by genotypes 1, 3, 4 and 6 (323.60 to 334.41 µmol mol^{-1}) with lower values. Genotypes 5 and 7 (2.80 to 2.85) had lower leaf water use efficiency (*WUE*) in comparison with the rest genetic material (3.74 to 4.44). The leaf *WUE* was 31% less in the genotypes 5 and 7, indicating a moderate water deficit in comparison with other genotypes. There was no significant interaction between genotypes and *SPAD* index related to chlorophyll leaf contents and the values varied from 33.00 to 38.67.

Table 2 Mean values of physiological characteristics evaluated of 6 genotypes of the breeding program LAGER / UFU plus one variety evaluated in soybeans before ASR epidemics (V6 stage) in Lavras - MG, 2017.

Genotype	*A* µmol m^{-2} s^{-1}	*E* mmol m^{-2} s^{-1}	g_s mmol m^{-2} s^{-1}	*Ci* µmol mol^{-1}	*WUE*	*SPAD*
1	21.80 a	5.40 a	0.73 b	334.41 b	4.05 a	38.17 ^{ns}
2	20.64 a	5.36 a	1.07 a	380.46 a	3.85 a	35.00 ^{ns}
3	21.00 a	5.31 a	0.73 b	329.04 b	3.95 a	38.67 ^{ns}
4	16.59 b	3.96 b	0.48 c	323.60 b	4.19 a	36.77 ^{ns}
5	16.27 b	5.58 a	0.79 b	365.65 a	2.90 b	33.00 ^{ns}
6	20.49 a	5.47 a	0.79 b	323.60 b	3.74 a	36.80 ^{ns}
7	13.94 b	5.06 a	0.51 c	368.77 a	2.80 b	32.65 ^{ns}
S-W	**0.91**	**0.63**	**0.18**	**0.80**	**0.62**	**0.49**
F	**0.18**	**0.54**	**0.85**	**0.05**	**0.35**	**0.11**
d	**1.78**	**2.02**	**1.78**	**2.55**	**2.70**	**2.20**

*ns = non-significant

** Original Means.

*** Means followed by the same lowercase letter in the column do not differ by the Scott-Knott test at 5% probability.

**** Values of S-W, F and d in bold mean normal distribution by Shapiro-Wilk test, homogeneity of variance by Levene's test and residue independence by Durbin-Watson at the .05 significance level respectively.

All photosynthetic parameters were significant different comparing genotypes in the middle of ASR epidemics at the R4 stage (Table 3 and Table S4). In relation to the photosynthetic activity (A) genotypes 2, 3, 5 and 6 obtained higher values (ranging from 17.74 to 23.30 µmol m^{-2} s^{-1}) and genotypes 1, 4 and 7 (ranging from 11.19 to 13.39 µmol m^{-2} s^{-1}) obtained lower photosynthetic activity. The susceptible cultivar Desafio 8473SRF had a reduction of 10 µmol m^{-2} s^{-1}in *A* after the begin of the ASR epidemics. Genotypes 2, 3 and 6 maintained similar levels of photosynthetic activity between evaluations. The genotypes 1, 4, 5 and 6 had a higher transpiration rate (varying of 7.48 to 8.48 mmol m^{-2} s^{-1}) in comparison with the genotypes 2, 3 and 7 which ranged from 3.82 to 6.00 mmol m^{-2} s^{-1}. Genotypes 1, 4, 5 and 6 enhanced considerably their *E* after inoculation. A lower stomatal conductance (g_s) was observed in genotypes 1 and 7 (0.30 mmol m^{-2} s^{-1} and 0.47 mmol m^{-2} s^{-1} respectively) in comparison with the rest (0.74 to 1.00 mmol m^{-2} s^{-1}). Genotypes 1 to 5 (313.66 to 326.10 µmol mol^{-1}) had superior values of *Ci* than genotypes 6 and 7 (289.93 to 303.70 µmol mol^{-1}). Genotype 2 had the highest *WUE* ratio (3.56) and genotypes 1, 4 and 7 the lowest (1.49 to 2.59), a difference of 44 %, indicating a severity water deficit in comparison with the first. Genotypes 4 and 5 (39.32 and 42.25 respectively) showed the highest *SPAD* index in comparison with genotypes 2, 3, 6 and 7 (34.17 to 35.63) that showed the lowest values.

Table 3 Mean values of physiological characteristics evaluated of 6 genotypes of the breeding program LAGER / UFU plus one variety evaluated in soybeans at R4 stage during ASR epidemics in Lavras - MG, 2017.

Genotype	*A* µmol m^{-2} s^{-1}	*E* mmol m^{-2} s^{-1}	g_s mmol m^{-2} s^{-1}	*Ci* µmol mol^{-1}	*WUE*	*SPAD*
1	11.19 b	7.48 a	0.30 b	313.66 a	1.49 c	38.85 b
2	17.74 a	4.98 b	0.78 a	316.40 a	3.56 a	35.63 c
3	19.53 a	3.82 b	0.84 a	323.19 a	2.61 b	35.55 c
4	13.39 b	8.48 a	0.74 a	326.10 a	1.58 c	39.32 a
5	23.30 a	7.66 a	1.00 a	324.88 a	3.10 b	42.25 a
6	21.59 a	7.83 a	0.76 a	303.70 b	2.75 b	34.17 c
7	13.17 b	6.00 b	0.47 b	289.93 b	2.19 c	35.16 c
S-W	**0.49**	**0.80**	**0.59**	**0.36**	**0.40**	**0.86**
F	**0.10**	**0.13**	0.01	**0.05**	0.02	0.01
d	**2.04**	**1.87**	**1.74**	**1.34**	**2.20**	**1.98**

*ns = non-significant

** Original Means.

*** Means followed by the same lowercase letter in the column do not differ by the Scott-Knott test at 5% probability.

**** Values of S-W, F and d in bold mean normal distribution by Shapiro-Wilk test, homogeneity of variance by Levene's test and residue independence by Durbin-Watson at the .05 significance level respectively.

Overall, there was reductions in the order of 9, 9.5 and 22 % for the variables *A*, *Ci* and *WUE* respectively after the inoculation of ASR. Also, there was increase of 28 and 10 % in *E* and g_s respectively. The *SPAD* index did not showed percentage differences between evaluations.

PAL and POX activity

In the evaluation of the POX activity in soybean plants, there was significant difference ($p < 0.05$ Table S5) between genotypes. Prior to inoculation (0 hai - hours after inoculation) the genotype 2 (F8 BRSGO Luziânia X Potenza) showed higher activity of this enzyme in relation to the other genotypes. At 48 hai, there was an increase in enzymatic activity for all genotypes except for genotypes 1 (Desafio 8473SRF) and 5 (F8 BRSGO Caiapônia X Potenza) In 72 hai the genotypes showed no significant difference, but there was a general increase in the activity of the POX. In the last collection period (96 hai) the activity dropped again and there was significant difference between genotypes, the genotype 2 reached the highest activity followed by genotype 3 (F8 BRSGO Caiapônia X Potenza) and the genotypes 1, 6 and 7 showed the lowest activity for this enzyme (Figure 1A).

In the analysis of PAL activity, there was a significant difference ($p < 0.05$ Table S6) for all materials. Prior to inoculation in the 0 hai the genotypes 2, 3, 5 and 6 showed higher enzymatic activity in relation to genotypes 1, 4 and 7, in relation to the general activity of this enzymes it was observed that the activity remained below 5 $\mu M\ min^{-1}\ mgP^{-1}$. The activity of the PAL enzyme at 24 hai was more than double of the previous collection, showing an significant frowth in the 24 hours of initial infection period by the rust. In this period, the genotypes 2, 3 and 4 presented higher activity of the enzyme in relation to the other genotypes. In the 48 hai period the enzymatic activity little fluctuated and the genotypes 3, 4, 5 and 6 showed the higher activity of the enzyme. In the period of 72 hai the enzyme activity continued at the same levels as the previous collections, and all genotypes stood out in relation to the check (Desafio 8473SRF) who obtained the worst performance in this variable. In the 96 hai period, there was a great decrease in the activity of this enzyme, which eventually returned to the levels presented before inoculation (Figure 2B).

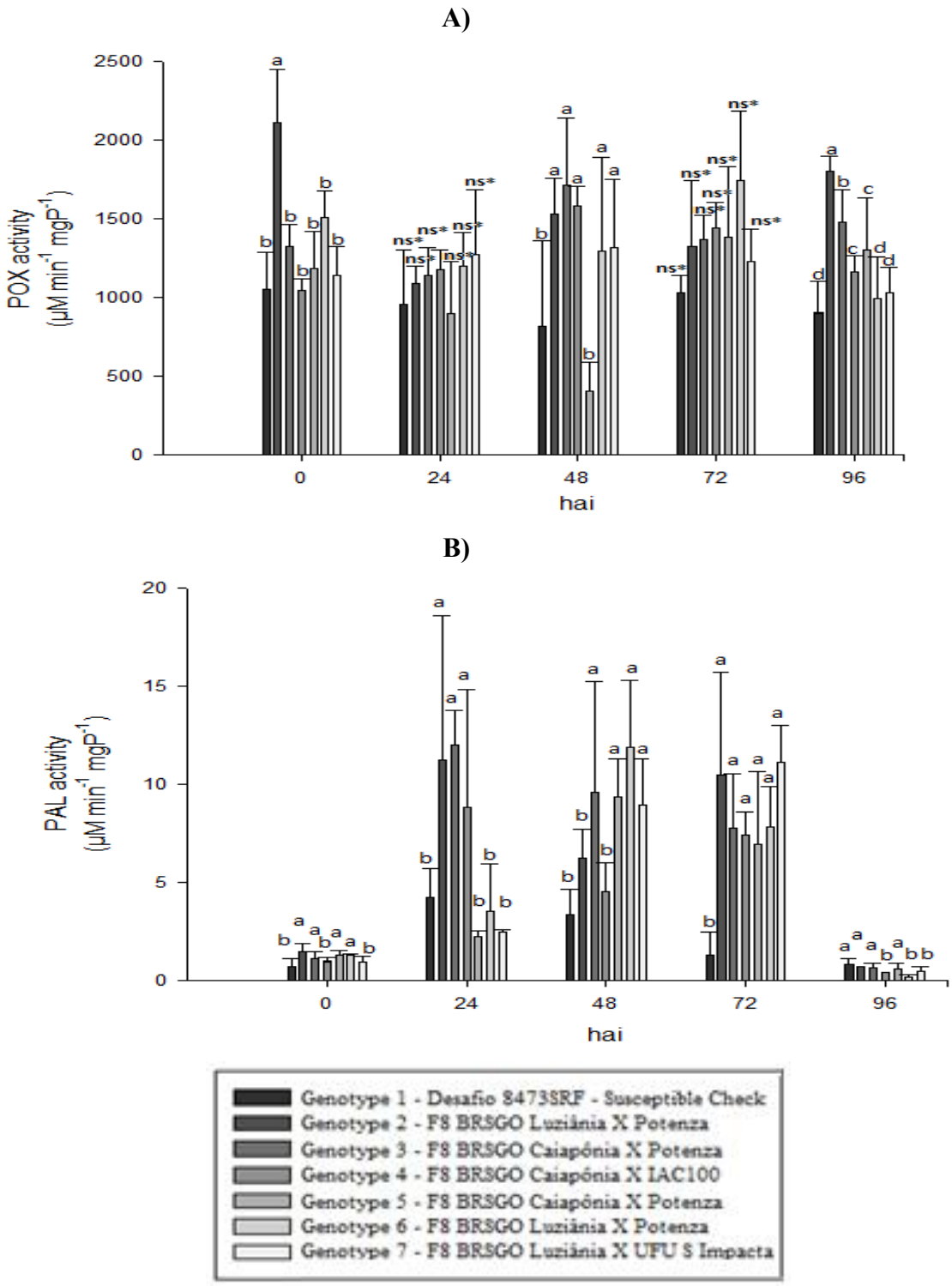


Figure 2 Activity of the enzymes peroxidase (POX) (A) and phenylalanine ammonia-lyases (PAL) (B) in *Glycine max* plants. Inoculation with *Phakopsora pachyrhizi*, occurred immediately after 0 hours after inoculation treatments (hai). Standard deviation bars in relation to the average of four blocks. Bars followed with different letters are statistically different by the Scott Knott test ($P \leq 0.05$).

Cell wall, lignin content and epicuticular wax distribution

There was significant differences between genotypescell wall thickness (Table 4 and Table S7). Three different groups were formed, one composed by genotypes 2 and 3 with the thickest cell wall values (0.89 and 0.95 µm), followed by an intermediate group formed by genotypes 4, 5 and 6 (0.50 to 0.65 µm) and a third group with the lowest values composed by the check and genotype 7 (0.36 and 0.35 µm). All genotypes had cell walls with varying thickness compared to the check. The lignin contents of the genotypes differed significantly (Table S7) only in the collect of 0 (hai). The results obtained showed that genotype 2 (F8 BRSGO Caiapônia X Potenza) has the highest lignin content among the other genotypes, reaching 17.26 µg of lignin per mg of dry matter. Genotypes 3, 4 and 6 presented intermediary content (12.80 to 13.75) and was followed in the ranking sequence by the genotype 7 (F8 BRSGO Luziânia X UFU S Impacta) with 11.32 of µg of lignin per mg of dry matter and lastly the control and genotype 5 presented the lowest values in lignin content in relation to the others (7. 97 and 9.40 µg of lignin per mg of dry matter respectively).

Table 4 Mean values of cell wall thickness evaluated at the V6 stage and lignin content evaluated at R1 stage (0 hai and 96 hai) of 6 genotypes of the breeding program LAGER / UFU plus one variety during rust epidemics in Lavras - MG, 2017.

Genotype	Cell Wall Thickness (µm)	Lignin – 0 hai	Lignin – 96 hai
		µg lignin/mg dry matter	
1	0.36 c	7.97 d	10.40 **ns**
2	0.89 a	17.26 a	12.50 **ns**
3	0.95 a	13.75 b	13.02 **ns**
4	0.65 b	12.80 b	13.31 **ns**
5	0.50 b	9.40 d	11.03 **ns**
6	0.58 b	12.90 b	15.17 **ns**
7	0.35 c	11.32 c	11.63 **ns**
S-W	**0.19**	**0.53**	**0.96**
F	0.02	**0.61**	**0.32**
d	**2.30**	**2.06**	**1.64**

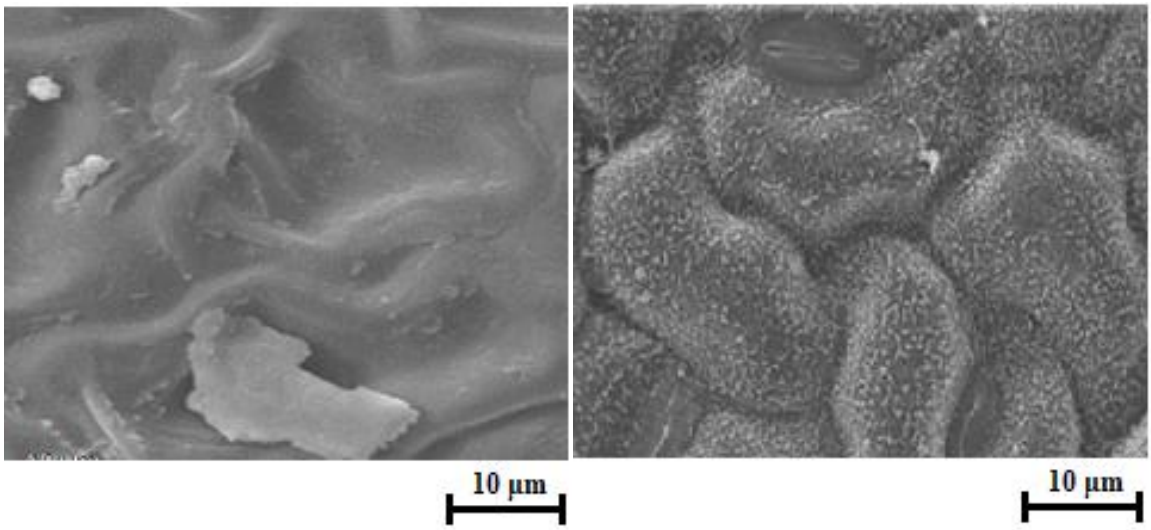
*ns = non-significant

** Original Means.

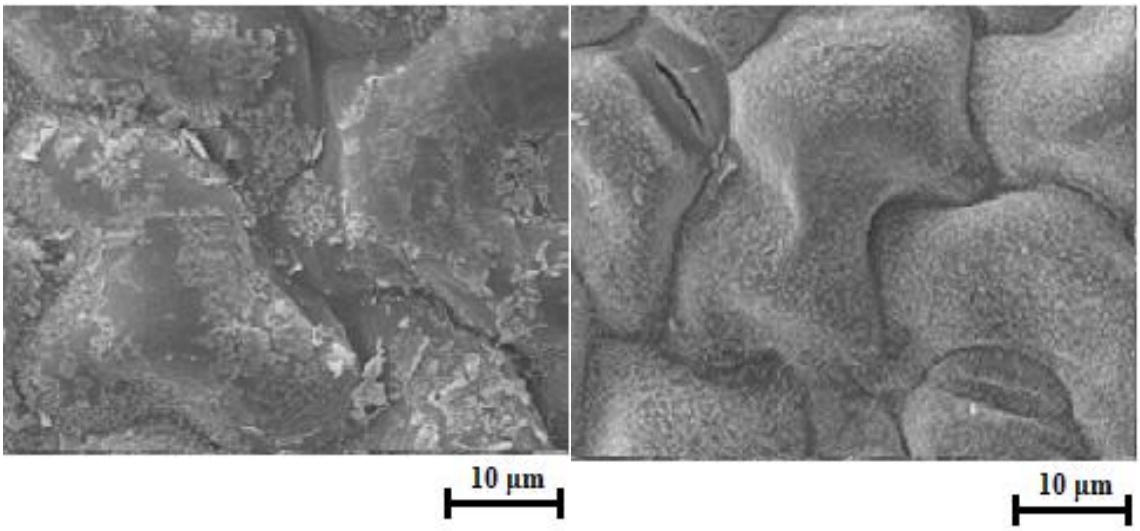
*** Means followed by the same lowercase letter in the column do not differ by the Scott-Knott test at 5% probability.

**** Values of S-W, F and d in bold mean normal distribution by Shapiro-Wilk test, homogeneity of variance by Levene's test and residue independence by Durbin-Watson at the .05 significance level respectively.

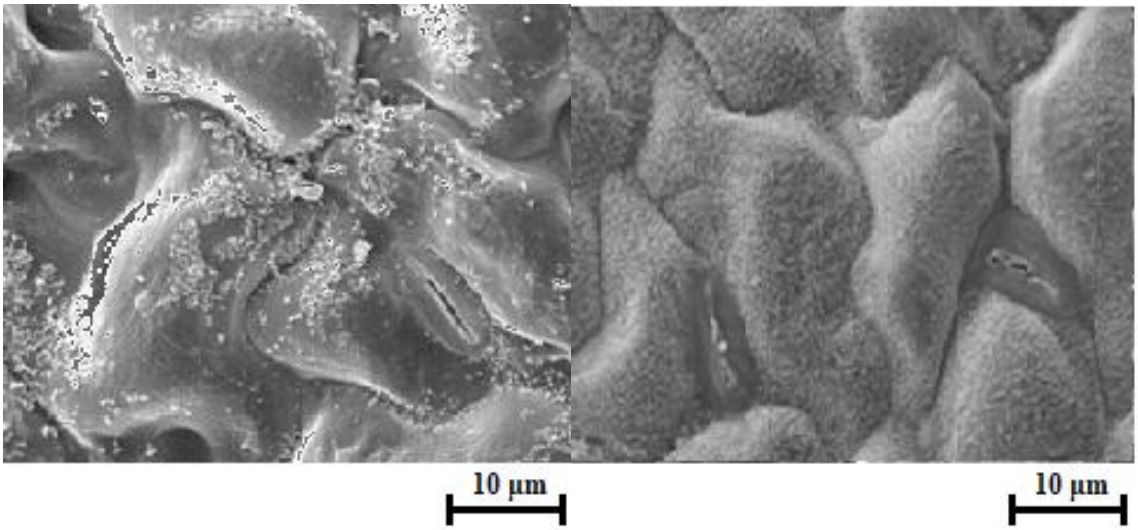
The scanning microscopy image did not show significative difference in epicuticular wax deposition among genotypes. But between surfaces is visible the difference. The upper surface of the different genotypes was covered by amorphous and crystalline epicuticular wax plates (Figure 3). In contrast, the lower surface of leaves was covered by crystalline sheets of wax . Genotypes 1, 4, 5, 6 and 7 do not show significant wax layer deposition being almost absent on the adaxial surface, meanwhile genotypes 2 and 3 seemingly to have wax distribution in few places showing irregularly edged plates structures like a film. In the lower surface areas, all genotypes showed similar thickening wax layer with raised crystals and sheets of wax that tended to appear randomly or either parallel to the leaf axis.



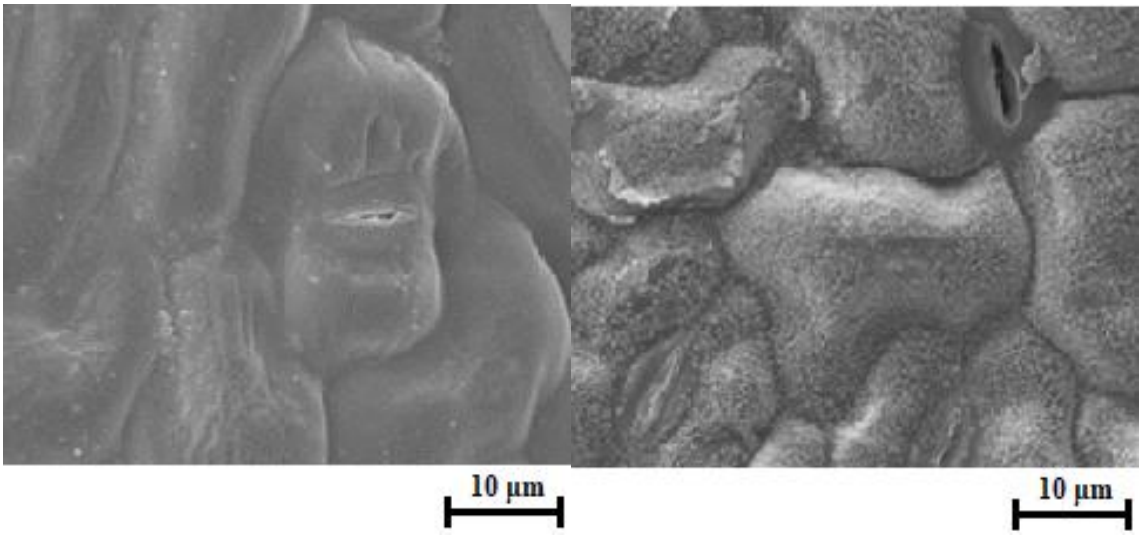
Genotype 1: Desafio 8473SRF Susceptible Check



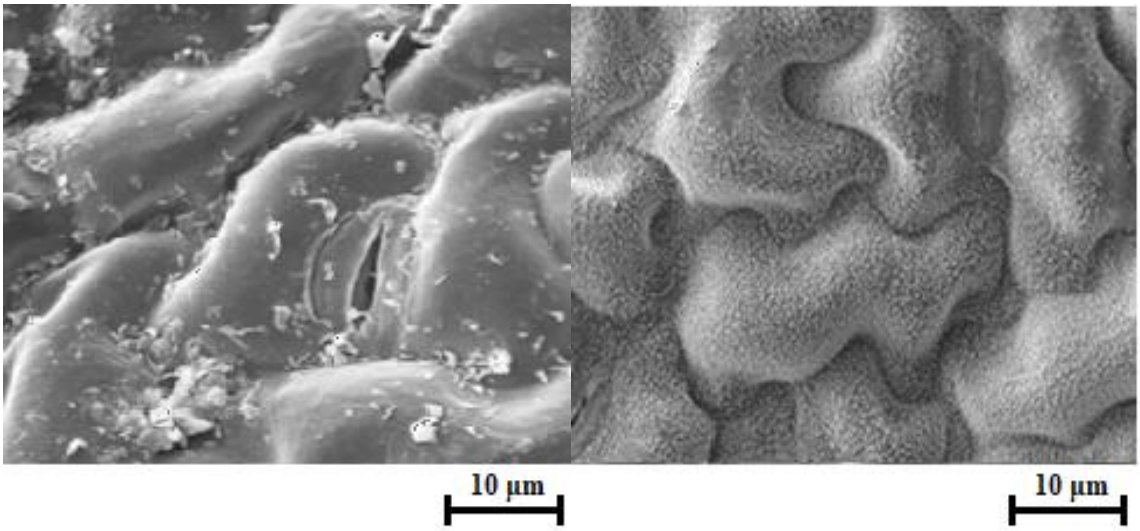
Genotype 2: F8 BRSGO Luziânia X Potenza



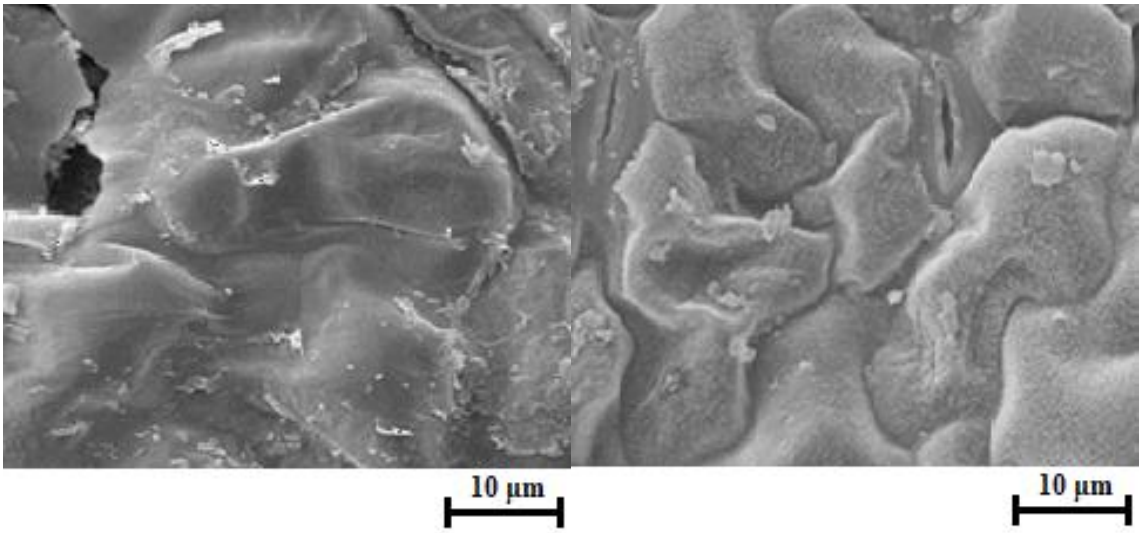
Genotype 3: F8 BRSGO Caiapônia X Potenza



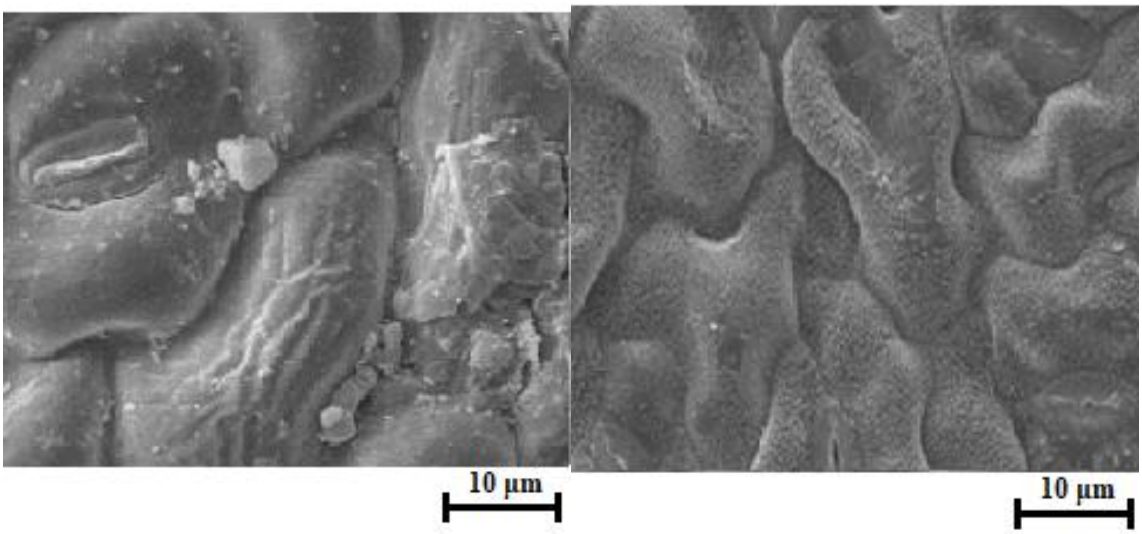
Genotype 4: F8 BRSGO Caiapônia X IAC100



Genotype 5: F8 BRSGO Caiapônia X Potenza



Genotype 6: F8 BRSGO Luziânia X Potenza



Genotype 7: F8 BRSGO Luziânia X UFU S Impacta

Figure 3 Scanning electromyography, showing leaf amorphous and crystalline wax plates on the upper surface (adaxial – left row images) and rosettes in the lower surface (abaxial- right row images) of *Glycine max* genotypes (Bar= 10; µm).

The genotypes that stood out with lower severity values, maintenance of higher enzyme activity (PAL, POX), lignin content and epidermis cell wall thickness were the 2 (F8 BRSGO Luziânia X Potenza) and 3 (F8 BRSGO Caiapônia X Potenza), these genotypes showed chemical traits for horizontal resistance composition. The same genetic material maintained overall, higher values of *A*, g_s, *Ci* and *WUE* and lower values of *SPAD* index and *E* after the beginning of ASR epidemics.

DISCUSSION

Disease assessments

In nature, plants deal with the different pathogens or insects attack. The yield losses caused by Asian soybean rust (ASR), have led producers to use mainly chemical control in the management of this disease. However, the intensive use of chemical fungicides sprays in the soybean crop has some negative effects like microorganism resistance, toxicity and environmental pollution. So, the introduction of cultivars with partial resistance, could be important in a tropical environment under constant change and disease progression. In relation to disease severity, the genotypes performed similar in both assays, but in the second assay were all characterization evaluations were performed, genotypes presented higher levels. This difference in results was due to inoculum viability. The susceptible cultivar (Desafio 8473SFR) presented the highest values of disease and partial resistant genotypes Genotypes 2 (F8 BRSGO Luziânia X Potenza), 3 (F8 BRSGO Caiapônia X Potenza and Genotype) and 6 (F8 BRSGO Luziânia X Potenza) had lower severity values. Martins and Juliatti (2014) related lower values of ASR severity (20 %) in F2 and F3 generations (Caiapônia x IAC-100 and Luziânia x Potenza crosses) in comparison with their respective parental lines (50 %). The parental Potenza also was related to be partially resistant to ASR (Silva, Juliatti and Silva, 2007) but the level of diseases severity for the cultivar Potenza were significantly higher than the cultivar Luziânia (Martins and Juliatti, 2014). The authors attributed these differences in results, to environmental conditions and inoculum variability similar to the ones obtained by Juliatti et al. in 2003. Martins and Juliatti in 2014 suggested that quantitative inheritance controls the partial resistance to ASR, which has been observed in other studies that used if different parents (Garcia et al., 2008; Ribeiro et al., 2007). Another conclusion was about the frequency distribution in all generations, they suggested that partial resistance trait in these lines, is controlled polygenically and this trait is possibly being expressed by genetic dominance. They argued that if the trait were monogenic, without the environmental effect, two or three phenotypic classes would be expected, this absence of a distribution with well-defined phenotypic frequencies was already been noted by other authors (Pierozzi et al., 2008; Ribeiro et al., 2007).

Evaluation of chlorophyll and photosynthesis

Foliar diseases generally alter physiological functions by decreasing photosynthetic capacity (Tas and Tas, 2007). Tschanz and Wang in 1985 obtained ASR progress curves under different environmental conditions and concluded that ASR resistance was influenced by environmental factors or physiological effects (photosynthetic parameters). Theoretically, the physiological cost of host disease resistance can be measured by the changes in the photosynthetic parameters after pathogen infection (Yang et al., 2016). In the present study, we aimed to characterize the effects of *P. pachyrhizi* infection on photosynthetic traits and the physiological cost to host ASR on seven genotypes with contrasting levels of ASR resistance. Our results show that ASR infection substantially changes photosynthetic parameters in the ASR susceptible genotypes than in the partial resistant genotypes, suggesting that host ASR resistance has a greater physiological impact. The photosynthetic parameters affect by ASR infection were *A, Ci, E,* g_s and *WUE*. Unfortunately, studies on the physiological cost of ASR in partial resistance are still scarce. It has been shown that pathogens in several cases like the pathosystem *F. graminearum* in wheat changes the allocation of carbon in leaves (Henkes, 2008 and Yang et al., 2016), *Mycosphaerella sp* in Eucalyptus reduces *A* (Pinkard and Mohammed 2006) and *P. psidii* infection reduces gas exchange and chlorophyll fluorescence parameters in susceptible Eucalyptus clones (Alves et al., 2011). Fifteen days after inoculation, *A* the most important parameter of photosynthesis, decreased considerably for the susceptible cultivar (Desafio 8473SFR), but for partial resistant genotypes 2 (F8 BRSGO Luziânia X Potenza) and 3 (F8 BRSGO Caiapônia X Potenza) the reduction in *A* was trivial. Alves et al in 2011, determined that gas exchange and chlorophyll were virtually unaffected in the resistant clone after inoculation. And reductions in photosynthetic rates were proportional to the diseased leaf area in the susceptible clone. This result were similar to ours, since the partial resistant genotypes, showed little variation in photosynthetic activity. But this result was different with the one obtained by Zou et al. (2005), the authors characterized the soybean response to infection by *Pseudomonas syringae* (bacterial blight) and concluded that infection led to a greater reduction in photosynthesis during the resistance response than during the susceptible response. These differences could be attributed to their period of evaluation being closer to the inoculation in comparison with ours (15 days after inoculation) and the intrinsic necrotic area caused by ASR being more severe. It is known that in the presence injury and drought stresses, plants have their antioxidative system enhanced and has their capacity to produce hormones and pigments altered (Jaleel et al., 2008; Wu et al., 2001; Diaz-Espejo et al., 2012). Plants with higher photosynthetically active green tissues during

ASR infection, can have more pigments and proteins that contribute to retard senescence (Wu et al., 2001; Bartlett et al., 2002; Ruske et al., 2003; Rios et al., 2018).

The transpiration rate (*E*) and stomatal conductance (g_s) was significantly increased during the ASR epidemics for all susceptible genotypes. Koyro and Huchzermeyer, 2018 argued that in any case, stressed plants recover impacts in biomass production keeping higher net photosynthesis and low transpiration simultaneously. Therefore, in attempting to explain the observed differences in transpiration, we must examine the effects of the pathogen on cuticular resistances. Duniway and Durbin argued in 1970, that *Uromyces phaseoli* in common beans, reduced the transpiration rate of leaves during the fleck stage and increase transpiration at the sporulation stage. They explained that when rust sporulated, the epidermis was ruptured and cuticular transpiration became paramount. This was evidenced by the higher transpiration rate of rusted leaves in a situation with dark, and lack of a correlation between stomatal conductance in this situation. So, the probable increase in transpiration on the susceptible cultivar was due to a higher number of uredinias in sporulation process and the increased cell wall permeability. Although the role of guard cell permeability in the inhibition of stomatal conductance and the relation of resistance to ASR reported here is still a hypothesis, other authors in the past already proposed the same idea for other pathosystems (Yarwood, 1947 and Farrel, Preece and Wren, 1969). The stomatal conductance (g_s) in the susceptible cultivar Desafio 8473SFR had a drastic reduction after inoculation in comparison with the partial resistant genotypes, even though the overall analysis indicated an increase. Stomatal conductance for some authors, play a vital role in the regulation of gas exchange and water loss, as well as the ability of plants to cope with pathogen infection (Prats et al., 2007 and Yang et al., 2016). Susceptible Eucalyptus plants inoculated with *P. psidii* showed lower g_s in later stages of infection (Alves et al., 2011). Farrel, Preece and Wren (1969) determined that *Phytophtora infestans* in susceptible potato leaves, usually cause reduces in stomatal aperture and consequently conductance only at the late stages of the disease cycle, when there was an increase in transpiration. In the majority of leaf diseases like ASR, transpiration is increased, and photosynthetic activity is reduced from the beginning of the infection (Berger et al. 2007; Domiciano et al. 2009; Alves et al., 2011). It has been shown that a lower stomatal conductance is one of the major constraints to photosynthesis in diseased plants by limiting CO_2 influx into leaves (Erickson et al. 2003). In our experiment, lower levels of stomatal conductance probably occurred due to pustule development in unhealthy soybean plants. The pustules breaks up the cuticle causing a disruption of the gas exchange and down regulating other physiological process derivate like plant yield. This hypothesis was also

proposed as the most common form of nonhost resistance associated with plant fitness (Withers, Gay and Mur, 2011; Yang et al., 2016). Water deficit occurred after inoculation of ASR, due to decreases in *WUE* for the susceptible cultivar and other five more genotypes. Only the partial resistant genotype 2 kept similar levels of *WUE* showed prior to inoculation. Monitoring the balance between water loss helps to find the weakest link in photosynthesis adjustment against stress sources (Badawi et al., 2004). Water deficit is one major constraint at situations with leaf stress (biotic and abiotic) and can lead to restriction of CO_2 uptake and, as a final consequence, to the development of reactive oxygen species (ROS) (Badawi et al., 2004; Koyro and Huchzermeyer, 2018). It is generally observed that water loss by biotic stress, causes ROS production in parallel to inhibition of photosynthesis. To prevent oxidative damage, plants are equipped with an antioxidative system composed of low-molecular-weight antioxidants and protective enzymes involved in ROS-consuming reaction sequences (Koyro and Huchzermeyer, 2018). The relation between production of ROS and posterior enzymatic activity is important step to understand the reaction of plants under attacks of pathogens or pests. After the stress perception plants like soybean, start signaling via cellular (Ca^{++}; MAP kinases; etc.), tissue, or whole plant level (ABA; jasmonate, etc.) to activate transcription factors and stress-controlled genes to respond via metabolic and physiological responses. So, this cycling process is crucial to understand how partial resistance components acts and reflects in photosynthetic parameter.

PAL and POX activity

PAL, POX enzymes and lignin play important roles and acts as a positive regulator in the composition of the partial resistance against biotic and abiotic stress responses in plants (Liang et al., 1989; Macdonald and D'Cunha 2007). Regarding the composition of partial resistance under influence of enzymatic profile and lignin accumulation, we aimed to characterize genotypes with traits of partial resistance after ASR infection. After investigation of the partial resistant genotypes in relation to the susceptible check, was observed significant differences ($p < 0.0001$) in the activity of the PAL enzyme during the period s from 24 to 72 hai. This period of 2 to 5-day with overexpression of PAL, matches the beginning of ASR infection on leaf tissue. This period is regarded as latent period, the period of initial penetration and colonization of soybean leaf tissues (Sinclair and Backman 1989; Vale et al., 1990; Alves et al., 2007). Phenylalanine ammonia lyase (PAL) is the first and committed step phenylpropanoid biosynthesis pathway and has already been characterized in several

soybean pathosystems (Russel, 1971; Logemann et al., 1995; Marcucci et al., 2010; Zhang et al., 2017). When the L-phenylalanine is converted to trans-cinnamic acid by PAL, it ends synthetizing phenolic compounds (phytoalexins and lignin) that confers greater resistance to the cell wall of plants (Campbell and Sederoff, 1996; Raes et al., 2003). Our study was the first to observe, explain and characterize biochemical responses (PAL and POX) during the composition of partial resistance against ASR.

The susceptible cultivar (Desafio 8473SFR) presented the lower PAL activity in the initial period of evaluation (24 hai) and genotypes 2 (F8 BRSGO Luziânia X Potenza), 3 (F8 BRSGO Caiapônia X Potenza) and 4 (F8 BRSGO Caiapônia X IAC100) presented the higher levels. But during the period of overexpression of PAL during 24 hai to 72 hai, the genotype 3 had and maintained the most consistent levels. The overexpression of the phenylpropanoid pathway reflects in the rise of PAL activity during ASR infection. This process reflects in plant defense characteristics like the composition of primary barriers (lignin and cell wall) and alterations in physiological parameter (photosynthetic activity and stomatal conductance). Increased PAL activity was also reported in induced plants like cowpea against *Fusarium oxysporum* f.sp. tracheiphilum (Rodrigues, Bezerra and Coelho, 2006) and *P. pachyrhizi* in soybean (Cruz et al., 2013).Genotypes 2 and 3 maintained higher levels of PAL activity before inoculation (0 hai). These genotypes parental showed partial resistance traits in field trials for *P. pachyrhizi* (Martins et al., 2007, Silva et al., 2007 and Martins and Juliatti 2014; Santos et al., 2018). Santos et al., (2018) mapped two QTLs in soybean cultivars, the author determined the presence of greater effect (37.0 5%) at the phenotypic variation for resistance to ASR. They determined that crosses derived from seven cultivars in different combinations of the following cultivars IAC 100, Potenza and BRS Caiapônia showed stability with genomic regions contributing to the horizontal resistance at the F4 and F7 generations. The authors concluded that partial resistance is also less subject to environmental variables, a factor that could facilitated field selection of future generations. These genes could be related to the expression of enzymes like PAL. Studies showed PAL genes involved in the response of plants to infection by pathogens, Huang (2010) studying the pal1 through pal4 quadruple knockout genes mutants, obtained susceptibility to the bacterial blight caused by *Pseudomonas syringae* in *Arabidopsis thaliana*. Shadle (2003) working with transgenic tobacco reported that a partial suppression of the PAL gene increased susceptibility to the plant against fungal diseases. Monteiro et al. (2016) suggested in their studies that genes related to the transcription of POX and PAL in plants with induced resistance were related to lower disease levels of coffee rust (*Hemileia vastatrix*). Zhang et al. (2017) determined that

the overexpression of the gene that encodes PAL in transgenic soybean improves resistance to *Phytophtora sojae.* The author suggested that expression levels of *GmPAL2.1* might have an effect on PAL activity and the accumulation of isoflavones and the increase in glyceollin in response to *P. sojae* infection. Previous research suggests that the biosynthesis of glyceollin is via branch of the phenylpropanoid pathway, and more specifically the enzymes involved in flavonoid and isoflavonoid synthesis (Ng et al. 2011). Studies demonstrated that phytoalexins like glyceollin's, reveals a pathway for signaling defense in soybean plants against fungi. This compound with antifungal activity , presents higher activity and production after fungal infection (Paxton, 1991 and Kim et al., 2011). *Aspergillus sojae*, *Sclerotinia sclerotiorum*, *Phytophtora sojae* and *Phytophthora megasperma* var. *sojae* produces lower disease levels in resistant soybean with higher levels of glyceollin's (Bonhoff et al., 1986; Boue, 2009; Kim et al., 2010; Lygin et al. 2010). We can also postulate another hypothesis to explain one of the several factors that contributes to the partial resistant mechanism against ASR. The higher activity of PAL enzyme could lead to higher levels of glyceollin's, thus contributing to the increase latent period and lower severity values obtained in ours work. But this hypothesis should be confirmed in future studies.

Plants of the genotype 2 before inoculation showed the highest POX activity in comparison with the other genotypes, this genotype also showed a great fluctuation in the activity, and otherwise despite this it maintained considerable consistency during the period when the leaves were collected for this assay. The genotypes average enzymatic activity peaked at 72 hai period. We observed fluctuations in the POX activity during the course of all leaf collections (through 0 to 96 hai), meanwhile the check POX activity decrease slow paced until the last day of evaluation (96 hai), reaching the lower activity in comparison with the other genotypes. The genotypes 3, 4 and 7 reached its maximum POX activity at 48 hai and the genotype 6 at 72 hai. Peroxidase (POX) plays a role in the host defense response, this compound catalyzes the oxidation and eventual polymerization of hydroxycinnamic alcohol in the presence of hydrogen peroxide responsible in cell wall lignification in the production of antimicrobial quantities of H_2O_2 and cross-linking with the cell wall proteins (Chittoor et al., 1999; Torres et al., 2006 and Stangarlin et al., 2011). Therefore, the increase in lignin is related to a higher POC activity. Increase in the amount of POX transcripts in a rice cultivar supplied with Si and susceptible to *Pyricularia grisea* was reported by Datnoff et al. (2007). Cruz et al. (2013) characterizing POX activity in inoculated soybean plants with *Phakopsora pachyrhizi,* and previous supplied with resistance inductors formulated with Si, obtained

increase of the activity 24 to 48 hai and a decrease at 72 hai, results very similar to the ones obtained in this work. We can conclude, there was a trend toward increased POX activity in the early onset of ASR. And the contribution of this enzyme to soybean resistance to ASR resulted in the higher production of lignin, that will compose one of the primary cell horizontal barriers against pathogen infection.

We can conclude that of PAL and POX are related to the first defense response in the complex scheme generate by partial resistance plants. Otherwise if enzymatic activity don't rise to counter initial infection, the oxidative burst occurs with higher intensity, and will correspond to generate more ROS inside leaf tissue provoking more cell damage. Plants under stress, generally, use a complex antioxidant defense system and a diverse range of enzymes, such as superoxide dismutase, catalase, peroxidase, glutathione reductase and ascorbate peroxidase. These enzymes are used to clean the ROSs and protect cells from oxidative damage (Hossain and Uddin, 2011). The higher enzymatic activity produces more antioxidants compounds in the plant, which is related to the neutralization of the ROS, reducing the oxidative stress caused by pathogen and, consequently, preventing its colonization (Marcucci et al., 2010 and Monteiro et al., 2016). Also, the genotypes 2 and 3 due to higher enzymatic activity, probably can have a greater expression of elicitors that possibly trigger defense responses in plants also freeing the cells of radical scavengers. Another hypothesis is that these genotypes use with greater efficiency, the calcium in the vegetal tissue, since a higher concentration of this element in leaves, are related to lower severity levels by acting in the recognition of invading pathogens in the plasma membrane, acting as secondary messenger and hindering activity of pectolytic enzymes produced by fungi (Marschner, 2012). Since, enzymatic responses are linked to stress perception and changes in resistance photosynthetic parameters, partial resistant plants probably have more capacity in cleaning ROS with lower leaf damage interference. Cross talk between induced defense-signaling pathways is thought to provide the plant with such a powerful regulatory potential in photosynthesis and enzymes production. It is believed that soybean with partial resistance to ASR would present responses similar to those found in the routes of defense activated by others biotrophic pathogens like downy and powdery mildew.

Cell wall lignin content and epicuticular wax distribution

Resistant plants differ in the gene expression profile even before a pathogen or insect attack, indicating that the defense is already activated as a mechanism of pre-conditioning

(priming) (Cardoso et al., 2014). Based on previous studies, priming is regarded as a part of systemic immunity responses in plants and is improved with the spray of resistance inducers in partial resistance plants depending on the type of pathogens. But the mechanism(s) involved in this broad defense, like the production of reactive oxygen species (hypersensitive response) and physical barrier enhancing with lignin accumulation in the cell walls and epicuticular wax distribution are still not completely understood (Conrath et al., 2011). So, we aimed to quantify these parameters and try to tie a relationship between photosynthesis and enzymatic expression with the enhancement of structural barriers (cell wall) in partial resistant plants. Genotypes 2 and 3 had higher content of preformed lignin before inoculation (0 hai) and thicker cell wall composition, in relation of the other genotypes. The second evaluation for lignin at 96 hai, revealed that lignin concentration was equalized in all genotypes. Probably, his result was due to the samplings (collections) being carried out with leaves exposed to a long period of the initial inoculation, in this period the plant responses to the pathogen had already been produced and the levels of lignin were equalized.

Dordas (2008) observed that wheat with higher resistance to *Geumannomyces graminis*. activate numerous enzymes of the shikimic acid and phenylpropanoid pathways trough the supplement of manganese thus accumulating lignin. Lignin is an aromatic polymer that is mainly deposited in secondary thickened cell walls where it provides strength and imperviousness (Miedes et al., 2014). In dicotyledons plants, lignin is mainly made from the monomers (monolignols). After their biosynthesis, the monolignols are transported to the cell wall and compose the units of the lignin polymer (Morreel et al., 2004; Del Río et al., 2007; Del Río et al., 2012). *Erwinia carotovora* subsp. carotovora and the necrotrophic fungi *Sclerotinia sclerotiorum* transcriptomic analyses revealed that genes putatively encoding enzymes involved in lignin biosynthesis were up-regulated (Zhang et al., 2007; Eynck et al., 2012). In cell suspension cultures of flax plants treated with different fungal PAMPs, had enhanced PAL activity and activation of protein kinase cascades, which regulate downstream immune responses that leaded to cell-wall reinforcement (Engelsdorf and Hamann, 2014; Malinovsky et al., 2014). Menden et al. (2007) studied accumulation of lignin in wheat, acting as a defense response against the infection of rust (*Puccinia graminis*).

Cell walls are important during fungi infection like ASR, because it act as a passive barrier and a reservoir of antimicrobial compounds, which are released during cell wall degradation (Cantu et al., 2008; Hematy et al., 2009). So, a thicker cell wall probably could explain the underlying interactions between pathogens and the plant cell wall. This hypothesis will support the development of plants with optimized lignocellulosic characteristics, without

negatively affecting disease resistance and yield. Micco et al., 2008 working with soybean seedlings under microgravity, related that ultrastructural cell wall disturbance provoked the plant to be more susceptible to stress sources. Our results show that the formation of cell wall is a complex process that usually is accompanied by differences in cell wall thickness distribution. The scanning microscope did not reveal much about the quantity or showed significant differences between epicuticular wax layer between genotypes. In the future biochemical analysis with more accurate tools, should be used to characterize and quantify the amount of epicuticular wax developed by plants. So, we choose to explain only the differences between composition in the different surfaces (adaxial and abaxial). In the comparison of the epicuticular wax morphology of the soybean genotypes leaves, observed in the two collections, shows that the superior face leaf face has a predominance of film-type wax (Figure 3) and the presence of crystalloids isolated which are more frequent in the abaxial leaf face and arranged in rosettes. Lichston and Godoy (2006) analyzed the morphology of the epicuticular foliar wax of cultivars Obatã and 'Catuaí Vermelho' and concluded that differences in the content and morphology of wax, may be related to plant resistance against coffee rust. Probably the differences in the epicuticular wax distribution between leaf surfaces, have resulted in resistance to ASR uredinospores penetration. Further studies regarding the gene expression that is related to the partial resistance composition should be studied for the pathosystem soybean-rust. Therefore, the questions that the adoption of partial resistance cultivars may be conducive to reduce environmental pollution of chemical fungicides and the cost of soybean production need to be further extensive and in-depth study.

CONCLUSIONS

1 - There is a difference among genotypes during the progress of ASR severity.

2- Genotypes 2, 3 and 6 showed signals of partial resistance and obtained the lowest levels of severity and the cultivar Desafio 8473SFR demonstrated higher susceptibility with higher levels of severity.

3 - Photosynthetic parameters were impaired by *P. pachyhizi* infection in the susceptible genotype, but not in its resistant counterpart, as a result of structural and biochemical constraints derived of partial resistance.

4 - Impairments to photosynthesis were proportional to the development of the ASR in the susceptible genotypes.

5 - The cell wall was ticker in the resistant genotypes.

6 – Lignin content was significantly higher in the resistant genotypes before inoculation and the content was equalized four days after inoculation.

9 – During 24 to 72 hours after inoculation the activity of PAL enzyme was greatly increase in the resistant genotypes.

8 - Genotypes that stood out with lower severity values also maintained overall a higher enzyme activity (PAL, POX).

REFERENCES

ALVES, M. D.E C.; POZZA, E. A.; FERREIRA, J. B.; ARAÚJO, D. V.; COSTA, J. C. B.; DEUNER, C. C.; MUNIZ, M. F. S.; ZAMBENEDETTI, E. B.; MACHADO, J. C. Intensidade da ferrugem asiática (*Phakopsora pachyrizi* H. Sydow & P. Sydow) da soja (*Glycine max* L.) Merr.) nas cultivares Conquista, Savana e Suprema sob diferentes temperaturas e períodos de molhamento foliar. **Summa Phytopathologica**. v. 33, p. 239-244, 2007

ALVES, A. A., DA SILVA GUIMARAES, L. M., DE MELO CHAVES, A. R., DAMATTA, F. M., ALFENAS, A. C. Leaf gas exchange and chlorophyll a fluorescence of *Eucalyptus urophylla* in response to *Puccinia psidii* infection. **Acta physiologiae plantarum**. v, 33, n.5, p.1831-1839, 2011.

ALVES, E. and PERINA, F.J. **Apostila do curso introdutório à microscopia eletrônica de varredura e microanálise de raios-X**. UFLA/FAEPE, Lavras, p.63 , 2012.

BADAWI, G.H., YAMAUCHI, Y., SHIMADA, E., SASAKI, R., KAWANO, N., TANAKA, K., TANAKA, K. Enhanced tolerance to salt stress and water deficit by overexpressing superoxide dismutase in tobacco (*Nicotiana tabacum*) chloroplasts. **Plant Sci**. v.166, p. 919-928, 2004.

BAKER N.R Chlorophyll fluorescence: a probe of photosynthesis in vivo. **Annu Rev Plant Biol**. v. 59,p.89–113, 2008.

BARTLETT, D. W., CLOUGH, J. M., GODWIN, J. R., HALL, A. A., HAMER, M., PARRDOBRZANSKI, B. The strobilurin fungicides. **Pest Management Science**. v. 58, p. 649-662, 2002.

BEISSON, F., LI-BEISSON, Y. AND POLLARD, M. Solving the puzzles of cutin and suberin polymer biosynthesis. **Curr. Opin. Plant Biol**. v.15, p. 329-337, 2012.

BERGER, S.; SINHA, A.K.; ROITSCH, T. Plant physiology meets phytopathology: plant primary metabolism and plant–pathogen interactions. **J Exp Bot**. v.58, p. 4019-4026, 2007.

BIEMELT, S., KEETMAN, U. and ALBRECHT, G. Re-aeration following Hypoxia or Anoxia leads to Activation of the Antioxidative Defense System in Roots of Wheat Seedlings. **Plant Physiology.** v.116, p. 651-658, 1998.

BOATRIGHT, J.; NEGRE, F.; CHEN, X.; KISH, C.M.; WOOD, B.; PEEL, G.; ORLOVA, I.; GANG, D., RHODES, D.; DUDSREVA, N. Understanding *in vivo* benzenoid metabolism in petunia petal tissue. **Plant Physiology**, Bethesda. v. 135, n. 4, p. 1993-2011, 2004.

BONDE, M.R.; NESTER, S.E.; AUSTIN, C.N.; STONE, C.L.; FREDERICK, R.D.; HARTMAN, G.L.; MILES, M.R. Evaluation of virulence of *Phakopsora pachyrhizi* and *P. meibomiae* isolates. **Plant Disease.** v.90, p.708-716, 2006.

BONHOFF, A., LOYAL, R., EBEL, J., GRISEBACH, H. Race: cultivar-specific induction of enzymes related to phytoalexin biosynthesis in soybean roots following infection with *Phytophthora megasperma* f. sp. *glycinea*. **Archives of biochemistry and biophysics**. v. 246, n. 1, p. 149-154, 1986.

BOZZOLA, J. J.; RUSSELL, L. D. **Electron microscopy**. Boston: Jones and Bartlett Publishers, p.542 , 1992.

BROMFIELD, K.R.; MELCHING, J.S.; KINGSOLVER, C.H. Virulence and aggressiveness of *Phakopsora pachyrhizi* isolates causing soybean rust. **Phythopathology**, Lancaster. v. 70, p. 17- 21, 1980.

BROMFIELD, K.; HARTWIG, E. 1980. Resistance to soybean rust (*Phakopsora pachyrhizi*) and mode of inheritance. **Crop Science**. v 20, p. 254-255, 1980.

BOUE, S. M., CLEVELAND, T. E., CARTER-WIENTJES, C., SHIH, B. Y., BHATNAGAR, D., MCLACHLAN, J. M., BUROW, M. E. Phytoalexin-enriched functional foods. **Journal of agricultural and food chemistry**. v. 57, n. 7, p. 2614-2622, 2009.

CANTU, D., VICENTE, A. R., LABAVITCH, J. M., BENNETT, A. B., AND POWELL, A. L. Strangers in the matrix: plant cell walls and pathogen susceptibility. **Trends Plant Sci**. v.13, p. 610–617, 2008.

CAMPBELL, M.M.; SEDEROFF, R.R. Variation in lignin content and composition—mechanism of control and implications for the genetic improvement of plants. **Plant Physiol**, v.110, p. 3-13, 1996.

CARDOSO, D. C.; MARTINATI, J. C.; GIACHETTO, P. F.; VIDAL, R. O.; CARAZZOLLE, M. F.; PADILHA, L.; GUERREIRO-FILHO, O.; MALUF, M. P. Large-scale analysis of differential gene expression in coffee genotypes resistant and susceptible to leaf miner-toward the identification of candidate genes for marker assisted-selection. **BMC Genomics**, London. v. 15, 2014.

CALVO, E.S.; KIIHL R.A.S.; GARCIA, A.; HARADA, A.; HIROMOTO, D.M. Two major recessive soybean genes conferring soybean rust resistance. **Crop Sci**. v. 48, p.1350-1354, 2008.

CHANCE, B.; MAEHLEY, A.C. Assay of catalases and peroxidases. **Methods in Enzymology**. v.2, p.764-775, 1955. http://dx.doi.org/10.1016/S0076-6879(55)02300-8

CHILDS, S.P.; KING, Z.R.; WALKER, D.R.; HARRIS, D.K.; PEDLEY, K.F.; BUCK, J.W.; BOERMA, H.R.; LI, Z. Discovery of a seventh Rpp soybean rust resistance locus in soybean accession PI 605823. **Crop Sciences.** v. 131. p. 27-41, 2018.

CHITTOOR, J. M., LEACH, J. E., WHITE, F. F. Induction of peroxidase during defense against pathogens. **Pathogenesis-related proteins in plants**. p.171-193, 1999.

CONAB. Acompanhamento da safra brasileira: safra 2017/2018 available at [http://www.conab.gov.br/conabweb/download/safra/estudo_safra.pd] Accessed 15 April 2018.

CONRATH, U. Molecular aspects of defence priming. **Trends in plant science**. v. 16, n. 10, p. 524-531, 2011.

CRUZ, M. F. A. D., RODRIGUES, F. Á., POLANCO, L. R., CURVÊLO, C. R. D. S., NASCIMENTO, K. J. T., MOREIRA, M. A., & BARROS, E. G. Inducers of resistance and silicon on the activity of defense enzymes in the soybean-*Phakopsora pachyrhizi* interaction. Bragantia. v. 72, n. 2, p. 162-172, 2013.

DAKORA, F.D and PHILLIPS, D.A. Diverse functions of isoflavonoids in legumes transcend anti- microbial definitions of phytoalexins. **Physiol. Mol. Plant Pathol**.v. 49, p.1-20, 1996. DIXON, R.A. Natural products and disease resistance. **Nature**, v. 411, p. 843- 847, 2001.

DATNOFF, L. E., RODRIGUES, F. A., SEEBOLD, K. W. Silicon and plant disease. **Mineral nutrition and plant disease**. p. 233-246, 2007.

DIAZ-ESPEJO, A., CUEVAS, M.V., RIBAS-CARBO, M., FLEXAS, J., MARTOREL, S., J.E. The effect of strobilurins on leaf gas exchange, water use efficiency and ABA content in grapevine under field conditions, **J. Plant Physiol**. V. 169, p.379-386, 2012.

DEL RÍO, J. C., MARQUES, G., RENCORET, J., MARTÍNEZ, Á. T., & GUTIÉRREZ, A. Occurrence of naturally acetylated lignin units. **Journal of Agricultural and Food Chemistry**. v. 55, n.14, p.5461-5468. 2007.

DEL RÍO, J. C., RENCORET, J., PRINSEN, P., MARTÍNEZ, A. T., RALPH, J., & GUTIÉRREZ, A. Structural characterization of wheat straw lignin as revealed by analytical pyrolysis, 2D-NMR, and reductive cleavage methods. **Journal of agricultural and food chemistry**. v. 60, n. 23, p. 5922-5935, 2012.

DOSTER, M. A. and BOSTOCK, R. M. Quantification of lignin formation in almond bark in response to woundin and infection by *Phytophthora* species. **Phytopathology**, 78: 473-477, 1988.

DOMICIANO, G.P.; RESEND,E R.S.; RODRIGUES, F.A.; DAMATTA, F.M. Alteração na fotossíntese de plantas infectadas por patógenos. **Revisão Anual de Patologia de Plantas** . v.17, p.305-339, 2009.

DORDAS, C. Role of nutrients in controlling plant diseases in sustainable agriculture. **A review. Agronomy for sustainable development**. v. 28, n 1, p. 33-46, 2008.

DUNIWAY, J. and DURBIN, R. The detrimental effect of rust on the water relations of bean. **Phytopathology**, p.60, 1970.

EYNCK, C., SÉGUIN-SWARTZ, G.; CLARKE, W. E., & PARKIN, I. A. Monolignol biosynthesis is associated with resistance *to Sclerotinia sclerotiorum* in *Camelina sativa*. **Molecular plant pathology**. p.13, n 8, p. 887-899, 2012.

EMBRAPA. **Tecnologias de produção de soja Região Central do Brasil 2017**. Empresa Brasileira de Pesquisa Agropecuária available at [http://www.cnpso.embrapa.br] Accessed 15 August 2017.

ENGELSDORF, T. and HAMANN, T. An update on receptor-like kinase involvement in the maintenance of plant cell wall integrity. **Annals of botany**. p. 114, n. 6, p. 1339-1347, 2014.

ERICKSON, J.E.; STANOSZ, G.R.; KRUGER, E.L. Photosynthetic consequences of Marssonina leaf spot differ between two poplar hybrids. **New Phytol** . v.161, p.577-583, 2003.

FRAC. Informação sobre carboxamidas em ferrugem da soja. 2017. 3 p. (Informativo 01/2017)
Available at [http://docs.wixstatic.com/ugd/8 5b1d3_060a6876562140b693f03708057acff2. pdf] Accessed 18 April 2018.

FARRELL, G. M., PREECE, T. F., WREN, M. J. Effects of infection by *Phytophthora injestans* (Mont.) de Bary on the stomata of potato leaves. **Annals of Applied Biology**. v. 63, n. 2, p. 265-275, 1969.

FERREIRA, E. B.; CAVALCANTI, P. P.; NOGUEIRA, D. A. ExpDes.pt: **Experimental Designs package** (Portuguese). 2013.

GARCIA, A.; CALVO, E.S.; KIIHL, R.A.S.; HARADA, A.; HIROMOTO, D.M VIEIRA, L.G.E. Molecular mapping of soybean rust (*Phakopsora pachyrhizi*) resistance genes: discovery of a novel locus and alleles. **Theor Appl Genet**. v. 117, p. 545-553, 2008.

GODOY, C.V.; KOGA, L.; CANTERI, M.G. Diagrammatic scale for assessment of soybean rust severity. **Fitopatologia Brasileira**, Brasília, v.31, p. 63-68, 2006.

HARTWIG, E. Identification of a fourth major gene conferring resistance to soybean rust. **Crop Science**. v.26, p. 1135-1136, 1986.

HARTMAN, G. L., MILES, M. R., FREDERICK, R. D. Breeding for resistance to soybean rust. **Plant Disease**. v.89, p. 664-666, 2005.

HARRISON, M.J.; BALDWIN, I. Biotic interactions: ploy and counter-ploy in the biotic interactions of plants. **Curr Opin Plant Biol**. v.7, p.353-355, 2004.

HEMATY, K., CHERK, C., SOMERVILLE, S. Host–pathogen warfare at the plant cell wall. **Curr. Opin. Plant Biol**. v.12, p. 406-413, 2009.

HENKES, G. **Plant-microbe interactions alter the allocation of carbon in barley** (*Hordeum vulgare*). Master's Thesis, Fachbereich Biologie, Technische Universitat Darmstadt, Germany, 2008.

HOSSAIN, M. A.; UDDIN, S. N. Mechanisms of waterlogging tolerance in wheat: morphological and metabolic adaptations under hypoxia or anoxia. **Australian Journal of Crop Science**, Melbourne, v. 5, n. 9, p. 1094-1101, 2011.

HUANG, J., GU, M., LAI, Z., FAN, B., SHI, K., ZHOU, Y. H., CHEN, Z. Functional analysis of the Arabidopsis PAL gene family in plant growth, development and response to environmental stress. **Plant Physiology**. p. 110, 2010.

JAMIL, M.; ALI, A.; AKBAR, K.F.; GHAFOOR, A.; NAPAR, A.A.; ASAD, S. Relationship among water use efficiency, canopy temperature, chlorophyll content and spot blotch (*Cochliobolus sativus*) resistance in diverse wheat (*Triticum aestivum L*.) germplasm. **Pak J Bot.** v.48, n.3, p. 993-998, 2016

JALEEL, C.A., GOPI, R., PANNEERSELVAM, R. Growth and photosynthetic pigments responses of two varieties of *Catharanthus roseus* to triadimefon treatment. **C. R. Biol**. v.331 ,p.272–277, 2008.

JULIATTI, F.C.; BORGES, E.M.; PASSOS, R.R.; CALDEIRA JÚNIOR, J.C.; BRANDÃO, A.M. Doenças da soja. **Cultivar**, Uberlândia. n.47, p. 13, 2003.

JULIATTI, F.C.; POLIZEL, A.C.; BALARDIN, R.S.; VALE, F.X.R. Ferrugem da soja – Epidemiologia e manejo para uma doença reemergente. In: LUZ, W.C.; FERNANDES, J.M.C.; PRESTES, A.M.; PICININI, E.C. **Revisão anual de patologia de plantas** Passo Fundo. v. 13, p. 351-395, 2005.

JULIATTI, B.C.M.; BELOTI, I.F.; PENA, L.C.; JULIATTI, F.C. Soybean genotype analysis for cyst nematode resistance. **Genetics and molecular research**, v. 16, p. 1-10, 2017.

JONES, J. D. G.; DANGL, J. L. The plant immune system. **Nature**, London, v. 444, n. 7117, p. 323-329, 2006.

KAWAOKA, A. et al. Ectopic expression of a horseradish peroxidase enhances growth rate and increases oxidative stress resistance in hybrid aspen. **Plant Physiology**, Rockville, v. 132, n. 3, p. 1177-1185, 2003.

KEEN, N.T. Plant disease resistance: progress in basic under-standing and practical application. **Adv. Bot. Res**. v.30, p.291-328, 1999.

KIM, H. J., SUH, H. J., KIM, J. H., PARK, S., JOO, Y. C.,KIM, J. S. Antioxidant activity of glyceollins derived from soybean elicited with *Aspergillus sojae*. **Journal of agricultural and food chemistry.** v. 58, p. 22, p. 11633-11638, 2010.

KLOSOWSKI, A.C.; MAY DE MIO, L.L.; MIESSNER, S.; RODRIGUES, R.; STAMMLER, G. Detection of the F129L mutation in the cytochrome b gene in *Phakopsora pachyrhizi*. **Pest Management Science**, v. 72, p. 1211-1215, 2016.

KOYRO, H. W. and HUCHZERMEYER, B. Coordinated Regulation of Photosynthesis in Plants Increases Yield and Resistance to Different Types of Environmental Stress. In **Plant Metabolites and Regulation Under Environmental Stress**. p. 281-309, 2018.

KUNST, L. and SAMUELS, L. Plant cuticles shine: advances in wax biosynthesis and export. Curr. Opin. **Plant Biol**. v. 12, p. 721-727, 2009.

LEE, S.B. AND SUH, M.C. Recent advances in cuticular wax biosynthesis and its regulation in Arabidopsis. **Mol. Plant** v.6, p. 246-249 , 2013.

LI, S.; SMITH, J. R.; RAY, J. D.; FREDERICK, R. D. Identification of a new soybean rust resistance gene in PI 567102B. Theoretical and Applied Genetics, Berlin. v. 129. p. 863- 877, 2016.

LICHSTON, J. E. and DE GODOY, S. A. P. Morfologia e teor de cera de folhas de café após aplicação de fungicida. **Pesquisa Agropecuária Brasileira**, v. 41, n.6, p. 919-926, 2006.

LIANG, X.W.; DRON, .; CRAMER, C.L.; DIXON, R.A.; LAMB, C.J. Differential regulation of phenylalanine ammonia-lyase genes during plant development and by environmental cues. **Journal of Biological Chemistry.** v.264, p.14486-14492, 1989.

LOGEMANN, E., PARNISKE, M., & HAHLBROCK, K. Modes of expression and common structural features of the complete phenylalanine ammonia-lyase gene family in parsley. **Proceedings of the National Academy of Sciences**. v. 92, n. 13, p. 5905-5909, 1995.

LOPES, M., M. REYNOLDS, X. DRAYE, J. FOULKES, M. HAWKESFORD AND E. MURCHIE. Dissecting drought adaptation into its phenotypic and genetic components in wheat. **Asp. Of App. Biol**. v. 105; p. 7-11, 2010.

LOZOVAYA, V.V., LYGIN, A.V., LI, S., HARTMAN, G.L. and WIDHOLM, J.M. Biochemical response of soybean roots to *Fusarium solani* f. sp. glycines infection. **Crop Sci**. v. 44, p.819-826, 2004.

LOZOVAYA, V.V.; LYGIN, A.V.; ZERNOVA, O.V.; ULANOV, A.V.; LI, S.X.; HARTMAN G.L. Modification of phenolic metabolism in soybean hairy roots through down regulation of chalcone synthase or isoflavone synthase. **Planta**. v. 225, p. 665-679, 2007.

LYGIN, A.V., LI, S., VITTAL, R., WIDHOLM, J.M., HARTMAN, G.L., LOZOVAYA, V.V. The importance of phenolic metabolism to limit the growth of *Phakopsora pachyrhizi*. **Genet. Resist**. v. 99, p. 1412-1420, 2009.

LYGIN, A. V., HILL, C. B., ZERNOVA, O. V., CRULL, L., WIDHOLM, J. M., HARTMAN, G. L., LOZOVAYA, V. V. Response of soybean pathogens to glyceollin. **Phytopathology**. v. 100, n. 9, p. 897-903, 2010.

MALI. K., MIRAJKAR, K. K., BIRADAR, S., PATIL. R.S. Role of defence related enzymes and lignin in host resistance of durum wheat cultivars infected by *Bipolaris sorokiniana.* International Journal of Agriculture Sciences. v.9 , p. 3886-3890, 2017.

MALINOVSKY, F. G., FANGEL, J. U., & WILLATS, W. G. The role of the cell wall in plant immunity. **Frontiers in plant science.** v.5, p. 178, 2014.

MARTIN, J.T. Role of cuticle in the defense against plant disease. **Annu. Rev. Phytopathol**. v.2, p. 81-100, 1964 10.1146/annurev.py.02.090164.000501.

MARTINS, J.A.S.; JULIATTI, F.C.; SANTOS, V.A.; POLIZEL, A.C.; JULIATTI, F.C. Período latente e uso da análise de componentes principais para caracterizar a resistência parcial à ferrugem-da-soja. **Summa Phytopathologica**, v.33, p.364-371, 2007.

MARTINS, J.A.S.; JULIATTI, F.C. Genetic control of partial resistance to Asian soybean rust. **Acta Sci Agron**. Maringá. v. 36(1), p. 11-17, 2014. doi:10.4025/actasciagron. v36i1.16919.

MACDONALD, M.J.; D'CUNHA, G.B. A modern view of phenylalanine ammonia lyase. **Biochemistry and Cell Biology**.v.85, p. 273-282, 2007.

MARCUCCI, E., ALEANDRI, M.P., CHILOSI, G., MAGRO, P. Induced resistance by -aminobutyric acid in artichoke against white mould caused by *Sclerotinia sclerotiorum*. **J. Phytopathol**. v.158, p. 659-667, 2010.

MARSCHNER, P. Marschner's Mineral Nutrition of Higher Plants (Third Edition) p. 369-388, 2012.

DE MICCO, V., ARONNE, G., JOSELEAU, J. P., RUEL, K. Xylem development and cell wall changes of soybean seedlings grown in space. **Annals of botany**. v. 101, n 5, p. 661-669, 2008.

MILES, M.R.; BONDE, M.R.; NESTER, S.E.; BERNER, D.K.; FREDERICK, R.D.; HARTMAN, G.L. Characterizing resistance to *Phakopsora pachyrhizi* in soybean. **Plant Disease.** v. 95, p. 577-581, 2011.

MIEDES, E., VANHOLME, R., BOERJAN, W., MOLINA, A. The role of the secondary cell wall in plant resistance to pathogens. **Frontiers in plant science**. v.5, p. 358, 2014.

MENDONÇA, S.C. ; GUERRA, N.B. Métodos físicos e químicos empregados no controle do escurecimento enzimático de vegetais. **Boletim SBCTA**, Campinas, v. 37, n. 2, p. 113-116, 2003.

MENDEN, B., KOHLHOFF, M., & MOERSCHBACHER, B. M. Wheat cells accumulate a syringyl-rich lignin during the hypersensitive resistance response. **Phytochemistry**. v.68, n 4, p. 513-520, 2007.

MONTEIRO, A.C.A, RESENDE, M.L.V.; VALENTE, T.C.T.; JUNIOR, P.M.R.; PEREIRA, V.F.; COSTA, J.R.; SILVA, J.A.G. Manganese Phosphite in Coffee Defence against *Hemileia*

vastatrix, the Coffee Rust Fungus: Biochemical and Molecular Analyses. **Journal of Phytopathology**. v. 164, n.11-12, p. 1043-1053, 2016.

MONTESANO, M.; BRADER, G.; PALVA, E. Pathogen derived elicitors: searching for receptors in plants. **Molecular Plant Pathology**, London. v. 4, n. 1, p. 73-79, 2003.

MORREEL, K., RALPH, J., KIM, H., LU, F., GOEMINNE, G., RALPH, S., BOERJAN, W. Profiling of oligolignols reveals monolignol coupling conditions in lignifying poplar xylem. **Plant Physiology**. v.136, n 3, p. 3537-3549, 2004.

NAKAZAWA, A.; NOZUE, M.; YASUDA, H. Expression pattern and gene structure of phenylalanine ammonia-lyase in *Pharbitis nil.* **Journal of Plant Research**, Tokyo. v. 114, n. 2, p. 323-328, 2001.

NAWRATH, C., SCHREIBER, L., FRANKE, R.B., GELDNER, N., REINA-PINTO, J.J., KUNST L. Apoplastic diffusion barriers in *Arabidopsis*. Arabidopsis Book v.11, p.167, 2013. 10.1199/tab.016.

NG, T. B., YE, X. J., WONG, J. H., FANG, E. F., CHAN, Y. S., PAN, W., WANG, H. X. Glyceollin, a soybean phytoalexin with medicinal properties. **Applied microbiology and biotechnology**. v. 90, n. 1, p.59-68, 2011.

NIKS, R.E. Comparative histology of partial resistance and the nonhost reaction to leaf rust pathogens in barley and wheat seedlings. **Phytopathology**, v.73, p. 60-64, 1983.

PARLEVLIET, J.E.; KUIPER, H. J. Partial resistance of barley to leaf rust, Puccinia hordei. IV. Effect of cultivar and development stage on infection frequency. **Euphytica,** v.26, n.2, p.249-255, 1977.

PARLEVLIET, J.E. Further evidence of polygenic inheritance of partial resistance in barley to leaf rust, *Puccinia hordei*. **Euphytica.** v. 27, p. 369-379, 1978.

PARLEVLIET, J.E. Components of resistance that reduce the rate of epidemic development. **Annu Rev Phytopathol** v. 17, p. 203-222, 1979.

PARLEVLIET, J.E. Models explaining the specificity and durability of host resistance derived from the observations on the barley-*Puccinia hordei* system. In: **Lamberti F, Waller JM, Van der Graaff NA (eds) Durable resistance in crops**. Plenum Press, New York London, p. 57-80, 1983.

PARLEVLIET, J.E. Present concepts in breeding for disease resistance. In: **Brazilian Phytopathology Congress.** Fitopatologia Brasileira, Brasília. v. 22, p. 7-15, 1997.

PRATS, E., CARVER, T., GAY, A., AND MUR, L. Enemy at the gates interaction specific stomatal responses to pathogenic challenge. **Plant Signal. Behav**. v. 2, p. 275-277, 2007.

PINKARD, E.A.; MOHAMMED, C.L. Photosynthesis of *Eucalyptus globulus* with *Mycosphaerella* leaf disease. **New Phytol**. v.170, p.119-127, 2006.

RESENDE, M. L. V.; SALGADO, S. M. L.; CHAVES, Z. M. Espécies ativas de oxigênio na resposta de defesa de plantas a patógenos. **Fitopatologia Brasileira**, Brasília, v. 28, n. 1, p. 123-130, 2003.

RUSSELL, D. W. The metabolism of aromatic compounds in higher plants X. Properties of the cinnamic acid 4-hydroxylase of pea seedlings and some aspects of its metabolic and developmental control. **Journal of Biological Chemistry**. V. 246, n. 12, p. 3870-3878, 1971.

R DEVELOPMENT CORE TEAM. R: **A language and environment for statistical computing. R Foundation for Statistical Computing**, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org, 2017.

RAES, J., ROHDE, A., CHRISTENSEN, J. H., VAN DE PEER, Y., BOERJAN, W. Genome-wide characterization of the lignification toolbox in Arabidopsis. **Plant Physiology**. v. 133, n. 3, p. 1051-1071, 2003.

RESENDE, R.S.; RODRIGUES, F.A.; CAVATTE, P.C.; MARTINS, S.C.; MOREIRA, W.R.; CHAVES, A.R.; DAMATTA FM. Leaf gas exchange and oxidative stress in sorghum plants supplied with silicon and infected by *Colletotrichum sublineolum*. **Phytopathol**. v.102, p.892-898, 2012.

RIBEIRO, A. S.; MOREIRA, J. U. V.; PIEROZZI, P. H. B.; RACHID, B. F.; TOLEDO, J. F. F.; ARIAS, C. A. A.; SOARES, R. M.; GODOY, C. V. Genetic control of Asian rust in soybean. **Euphytica**, v. 157, n. 1-2, p. 15-25, 2007.

RIOS, V. S., RIOS, J. A., AUCIQUE-PÉREZ, C. E., SILVEIRA, P. R., BARROS, A. V., RODRIGUES, F. Á. Leaf gas exchange and chlorophyll a fluorescence in soybean leaves infected by *Phakopsora pachyrhizi*. **Journal of Phytopathology** v. 166, n. 2, p.75-85, 2018.

RODRIGUES, F.A., MCNALLY, D.J., DATNOFF, L.E., JONES, J.B., LABBÉ, C., BENHAMOU, N., MENZIES, J.G., and BÉLANGER, R. R. Silicon enhances the accumulation of diterpenoid phytoalexins in rice: a potential mechanism for blast resistance. **Phytopathology**. v. 94, p. 177-183, 2004. http://dx.doi.org/10.1094/PHYTO.2004.94.2.177

RODRIGUES, A. A., BEZERRA NETO, E., & COELHO, R. S. Indução de resistência a *Fusarium oxysporum* f. sp. *tracheiphilum* em caupi: eficiência de indutores abióticos e atividade enzimática elicitada. **Fitopatologia Brasileira.** v**.** 31, n. 5, p. 492-499, 2006.

RUSKE, R. E., GOODING, M. J., JONES, S. A. The effects of triazole and strobilurin fungicide programmes on nitrogen uptake, partitioning, remobilization and grain N accumulation in winter wheat cultivars. **Journal of Agricultural Science**. v. 140, p. 395-407, 2003.

SALMERON, J.M.; VERNOOIJ, B. Transgenic approaches to microbial disease resistance in crop plants. **Current Opinion in Plant Biology**. v. 1, p.347-352, 1998.

SATTLER, S.E AND FUNNELL-HARRIS D.L. Frontiers in Plant Science, v.4, n.70, p. 1-8, 2013. doi:10.3389/fpls.2013.00070.

SHADLE, G. L., WESLEY, S. V., KORTH, K. L., CHEN, F., LAMB, C., & DIXON, R. A. Phenylpropanoid compounds and disease resistance in transgenic tobacco with altered expression of L-phenylalanine ammonia-lyase. **Phytochemistry**. v. 64, n. 4, p. 153-161, 2003.

SCHREIBER, L. Transport barriers made of cutin, suberin and associated waxes. **Trends Plant Sci**. v. 15, p. 546-553, 2010 10.1016/j.tplants.2010.06.004.

SCHEEL, D. Resistance response physiology and signal trans-duction. **Curr. Opin. Plant Biol**. v.1, p.305-31, 1998.

SILVA, V. A. S.; JULIATTI, F. C.; SILVA, L. A. S. Interação entre resistência genética parcial e fungicidas no controle da ferrugem asiática da soja. **Pesquisa Agropecuária Brasileira**, Brasília, v.42, n.9, p.1261-1268, 2007.

SINCLAIR, J.B.; BACKMAN, P.A. **Compendium of soybean diseases**. 3rd St. Paul: American Phytopathological Society, p. 24-27, 1989.

STANGARLIN, J. R., KUHN, O. J., TOLEDO, M. V., PORTZ, R. L., PASCHOLATI, S. F. A defesa vegetal contra fitopatógenos. **Scientia Agraria Paranaensis**. v. 10, n. 1, p. 18, 2011.

STASKAWICZ, B.J., AUSUBEL, F.M., BAKER, B.J., ELLIS, J.G. and JONES, J.D.G. Molecular genetics of plant disease resistance. **Science**. v.268, p.661-67, 1995.

STINTZI, A.; HEITZ, T.; PRASAD, V.; WIEDEMANN-MERDINOGLU, S.; KAUFFMANN, S.; GEOFFROY, P.; LEGRAND, M.; FRITIG, B. 'pathogenesis-related' proteins and their role in defense against pathogens. **Biochimie**. v.75, p.687-706, 1993.

TAS, S. and B. TAS. Some physiological responses of drought stress in wheat genotype with different ploidity in turkiya. **World J. Agri. Sci**. v. 3, p. 178-183, 2007.

TSCHANZ, A.T. and WANG, T.C. Interrelationship between soybean development, resistance, and *Phakopsora pachyrhizi*. Anais, **Fifth International Congress of the Society for the Advanced of Breeding Research in Asia and Oceania**, Bangkok, Thailand. P. 14-20, 1985.

TORRES, M. A., JONES, J. D., DANGL, J. L. Reactive oxygen species signaling in response to pathogens. **Plant physiology**. v. 141, n. 2, p.373-378, 2006.

VALE, F. X. R.; ZAMBOLIM, L.; CHAVES, G. M. Efeito do binômio temperatura duração do molhamento foliar sobre a infecção por *Phakopsora pachyrhizi* em soja. **Fitopatologia Brasileira**, Brasília DF, v. 15, n. 3, p. 200-202, 1990.

VAN DE MORTEL, M.; RECKNOR, J.C.; GRAHAM, M.A.; NETTLETON, D.; DITTMAN, J.D.; NELSON, R.T.; GODOY, C.V.; ABDELNOOR, R.V.; ALMEIDA, A.M.R.; BAUM, T.J. Distinct biphasic mRNA changes in response to Asian soybean rust infection. **Mol Plant Microbe Interact** . v.20, p. 887-899, 2007.

VAN DER PLANK, J. E. **Plant diseases**. Elsevier Science, 1963.

YAMANAKA, N, YAMAOKA, Y, KATO, M, LEMOS, NG, PASSIANOTTO, ALL, SANTOS, JVM, BENITEZ, ER, ABDELNOOR, RV, SOARES, RM; SUENAGA K. Development of classification criteria for resistance to soybean rust and differences in virulence among Japanese and Brazilian rust populations. **Tropical Plant Pathology**. v. 35, p. 153-162, 2010.

YARWOOD, C. E. Water loss from fungus cultures. **American journal of Botany**. v. 34, n. 9, p. 514-520, 1974.

YANG, S., LI, X., CHEN, W., LIU, T., ZHONG, S., MA, L., LUO, P. Wheat resistance to fusarium head blight is associated with changes in photosynthetic parameters. **Plant Disease**. v. 100, n.4,p. 847-852, 2016.

YORINORI, J. T.; YUYAMA, M. M.; SIQUERI, F.V. **Doenças da soja**. Boletim de Pesquisa de Soja 2009. Rondonópolis. 2009. p. 180-222. (Boletim, 13).

YORINORI, J.T., WILFRIDO, M.P. **Ferrugem da soja: *Phakopsora pachyrhizi* Sydow. Londrina**: Embrapa, 2002. (Folder).

YORINORI, J. T. A ferrugem "asiática" da soja no continente americano: evolução, importância econômica e estratégias de controle. In: JULIATTI, F.C., POLIZEL, A.C., HAMAWAKI, O.T. (Org.) In: **Workshop brasileiro sobre a ferrugem asiática**, 1., Uberlândia. Coletânea. Uberlândia: EDUFU, 2005. p. 21-37, 2005.

URBANEK, H.; KUZNIAKGEBAROWSKA, E.; HERKA, K. Elicitation of Defense Responses in Bean-Leaves by *Botrytis Cinerea* Polygalacturonase. Acta Physiol. Plant. 13:43-50, 1991.

USDA (2017) Overview United States Department of Agriculture available at [http://wwwersusdagov/topics/crops/soybeans-oil-cropshtml] Accessed 20 October 2017.

WANG T.C.; HARTMAN G.L. Epidemiology of soybean rust and breeding for host resistence. **PI. Prot Bull**. v. 34, p. 109-149, 1992.

WITHERS, C. M., GAY, A. P., AND MUR, L. A. J. Are stomatal responses the key to understanding the cost of fungal disease resistance in plants? **J. Sci. Food Agric**. v.91, p.1538-1540, 2011.

WU, Y.X., VON TIEDEMANN, A. Physiological effects of azoxystrobin and EPO on senescence and the oxidative status of wheat. **Pestic. Biochem**. Physiol. v.71, p.1-10, 2011.

ZHANG, S. H., YANG, Q., MA, R. C. *Erwinia carotovora* ssp. *carotovora* infection induced "defense lignin" accumulation and lignin biosynthetic gene expression in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). **Journal of Integrative Plant Biology.** p**.** 49, n. 7, p. 993-1002, 2007.

ZHANG, C., WANG, X., ZHANG, F., DONG, L., WU, J., CHENG, Q., LI, N. Phenylalanine ammonia-lyase2. 1 contributes to the soybean response towards *Phytophthora sojae* infection. **Scientific reports**. V.7, n.1, p.7242, 2017.

ZHAO, D.; GLYNN, N.C.; GLAZ, B.; COMSTOCK, JC.; SOOD, S. Orange rust effects on leaf photosynthesis and related characters of sugarcane. **Plant Dis**. v.95, p.640-647, 2011.

ZOU, J. J., RODRIGUEZ-ZAS, S., ALDEA, M., LI, M., ZHU, J., GONZALEZ, D. O., VODKIN, L. O., DELUCIA, E., AND CLOUGH, S. J. Expression profiling soybean response to *Pseudomonas syringae* reveals new defense-related genes and rapid HR-specific downregulation of photosynthesis. **Mol. Plant Microb. Interact**. v.18, p.1161-1174, 2005.

ZUCKER, M. Induction of phenylalanina deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. **Plant Physiology**, v.40, p.779-784, 1965. DOI: 10.1104/pp.40.5.779

ARTICLE 3

ASSOCIATION OF PARTIAL RESISTANCE AND CHEMICAL CONTROL IN THE MANAGEMENT OF SOYBEAN ASIAN RUST

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ABSTRACT

Plant resistance is characterized by an alteration in pathogen survival patterns and perpetuation process, this mechanism is activated when the parasitism process starts. The development of new resistant cultivars against diseases is a primordial step in the preservation of plant yield. The objective of this study was to identify the reaction and interaction of a single fungicide spray (R1 stage) with 6 soybean genotypes and one susceptible standard (Desafio RR 8473 RSF) of the soybean breeding and improvement program developed by the Germplasm Laboratory on the Federal University of Uberlândia (LAGER / UFU), after inoculation of *Phakopsora pachyrhizi*. The variables evaluated made before and after inoculation, consisted on agronomic traits, defoliation and disease severity progress. Disease severity data were transformed in four different mathematic models to obtain the apparent infection rate (r). The study was conducted in two different seasons inside a greenhouse. The results indicated significant difference between genotypes and interaction between split-plots ($p<0.05$). The Gompit model explained better the observed variability in disease severity data with an overall average of 90.47% in agreement between field-observed and model-predicted disease data. Genotypes 2 (F8 BRSGO Luziânia X Potenza) and 3 (F8 BRSGO Caiapônia X Potenza) showed traits of partial resistance with overall lower AUDPC values than the susceptible check in both assays. The same groups of genotypes also, presented overall lower apparent infection rates than the check in both assays. In both experiments, there was

significant reduction of apparent infection rate, AUDPC and defoliation with the fungicide spray (Solatenol + Azoxistrobin 200 g / ha^{-1}). The cultivar Desafio 8473SFR demonstrated higher susceptibility with higher disease levels and defoliation. Genotype 5 (F8 BRSGO Caiapônia X Potenza) had the best agronomic attributes.

Key words: Genetic resistance, *Phakopsora pachyrhizi*, defoliation, epidemiology.

INTRODUCTION

Soybean is the major crop produced in Brazil also is the second largest producer in the world with 113.923 million tons produced in 2016/2017 harvest season against 117.208 million tons produced by USA. Future projections put Brazil as the largest global oilseed player, in terms of production and exportation in a near future (USDA, 2017 and CONAB, 2017). According to Juliatti et al. (2005), among the main factors limiting the efficiency, profitability and success of soybean Brazil production are diseases during the crop cycle. Worldwide, soybean has over 100 diseases (Sinclair and Backman, 1989), of which about 50 is identified inside Brazilian borders. With the increase and expansion of the productive areas of a single crop, the prevalence of the disease increases annually the risk of considerable economic impacts, along with the fluctuating weather conditions. The average production losses caused by diseases are estimated by 15-20%, however, some diseases may cause losses close to 100% (EMBRAPA, 2017; Juliatti et al., 2005). Among these diseases, the Asian soybean rust (ASR), caused by the fungus *Phakopsora pachyrhizi* Syd., is one of the main phytosanitary problem of this crop (Juliatti et al., 2003). The symptoms are particularly evident in leaves, evolving from isolated uredinia to areas with pronounced coalescence of pustules, yellowing and premature foliar abscission (Bromfield, 1984). Infections at the onset of the R1 stage (blooming), if not managed, can subsequently produce high levels of crop damage, also affecting protein content in the grain (Ogle et al., 1979, Broomfield, 1984). In the first epidemics of this disease in Brazil, despite the intense alert campaign and guidelines for identification and control and the excessive rainfall and lack of fungicides, the rust generated in the 2002/03 harvest season an equivalent of US $ 737,453 million (EMBRAPA, 2005). Then, it's necessary to improve the control of this disease. The management of soybean rust requires a combination of methods to minimize damages and losses. Among these, the most accessible and adopted now, is the use of protective and curative chemical fungicides, mainly the Frac groups QoI, DMI and SDHI. Followed by complementary

methods such as early sowing, use of early cultivars, adoption of a sanitary void and destruction of soybean debris and lastly the use of resistant cultivars. However, rust samples collected in Brazil have been tested for sensitivity to these fungicides since 2007 by FRAC. These fungicides still have good performance, 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 populations collected at these sites, indicated a mutation in the C subunit at position I86F (FRAC, 2017). With the report and confirmation of other cases of resistance, and due to the high cost and time demand in the development of new molecules to control ASR, the alert was raised, and the alternative of resistant cultivars and other methods, is gaining importance in this scenario of uncertainties.

The genetic resistance to diseases can be defined as a host ability to prevent the growth and development of the pathogen (Parlevliet, 1997). One of these types of resistance, is the partial resistance. This resistance is characterized by the delay 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 and Hartman, 1992). This type of resistance became evident and important when a monogenic resistance is overcome by a new breed of pathogen (Parlevliet, 1997). 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 (Tschanz, Wang, Tsai, 1983, Hartman, Wang, Shanmugasundaram, 1997). Marchetti, Uecker and 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. Therefore, the most widely used breeding strategy programs for resistance to Asian soybean rust, has been the incorporation selection of genotypes from populations originated from crosses among resistant genotypes and adapted cultivars. The objective of this study was to evaluate partial resistant soybean genotypes, under a program of ASR management with a chemical fungicide spray.

MATERIAL AND METHODS

Experimental information

The study was conducted at two times, from July to October of 2016 and repeated in January to April of 2017, inside a greenhouse with a controlled environment in the city of Lavras, Minas Gerais State, Brazil, geographic coordinates 21°13'35.3"S and 44°58'31.4"W. The greenhouse is equipped with an automated mist sprinkler system that is programed with 15 min of wetting shifts every 6 hours, also a rising temperature control system by air injection that maintained an average of 24 °C and 75 % of relative Humidity. The experimental design adopted was split-plot in randomized blocks containing four blocks, each repetition contained 4 plants. Each plot was split in two plots (Split Plot Design – SPD), one with a single fungicide spray (Solatenol + Azoxistrobin 200 g / ha^{-1} + Mineral Oil 0,5% v.v) at R1 stage and another without the fungicide spray. Each genotype/variety were tagged with plastic platelets and sowed in a different 5-liter vase with substrate containing soil and sand (2:1 proportion) and the fertilizer (NPK: 0-20-20) used was homogenized with the substrate. Two pesticides sprays were made throughout the period of the experiment to control caterpillars and sucking insects.

Genotypes evaluated

The genetic material consisted of six promising soybean genotypes developed by the LAGER / UFU improvement program, based in Gloria Farm localized in Uberlândia - MG, and one more variety considered susceptible to rust, the Desafio RR - 8473RSF. These genotypes showed partial resistance traits in field trials for *P. pachyrhizi* (Martins et al., 2007, Silva et al., 2007 and Martins and 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 (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 et al., 2007). The seeds of the generation F8, were taken from pods of their respective genotypes, during the season 2015/2016, in an experimental field at the farm Victoria/UFLA based in Lavras – MG. Each lineage was characterized depending on its field rust resistance response and classified according to their cycle (days) and degree of relative maturity (early maturity groups between 6 and 7).

Table 1 Soybean genotypes of the breeding program LAGER / UFU, used in the assays.

Genotype Code	Lager UFU Code	Variety and Field Crossings	Cycle (days)
1	L200	Desafio 8473SRF - Susceptible Check	120*
2	L224	F8 BRSGO Luziânia X Potenza	120*
3	L266	F8 BRSGO Caiapônia X Potenza	120*
4	L144	F8 BRSGO Caiapônia X IAC100	120*
5	L279	F8 BRSGO Caiapônia X Potenza	120*
6	L254	F8 BRSGO Luziânia X Potenza	120*
7	L218	F8 BRSGO Luziânia X UFU S Impacta	120*

* Approximated values

Inoculation

P. pachyrhizi urediniospores present in fresh leafy uredium serum were collected, prepared in a suspension with distilled water containing 0.01% Tween 20 (v: v) and calibrated in a Neubauer chamber for a final concentration of 8.0 x 10^4 urediniospores.ml^{-1}. The suspension was uniformly sprayed on both sides of the leaves until the solution started to be running off, when the plants started flowering (R1) at 21/08/2016 for the first assay and 16/02/2017 at the second assay. Immediately after inoculation, the plants were incubated in dark inside the greenhouse covered with fog for a period of 24 hours. Plant inoculation was performed 48 hours after the single fungicide spray on the split-plots.

Data assessment

Disease severity was assessed on a weekly basis in a period of six weeks starting one week after the inoculation (DAI - days after inoculation). The severity or injured percentage

area of the leaf was determined in the central leaflet of the third and fourth trefoil at the central part of the soybean plant. In order to estimate the soybean rust severity, the diagrammatic scale of Godoy et al. (2006) was adopted. With the values of disease severity, the area under the severity progress curve (AUDPC) was calculated in according to Shanner and Finey (1977), and then subjected to analysis of variance.

Defoliation was calculated, using the total number of leaves collected in the flowering period. These data were crossed later, with the total leaf count present in the last severity assessment (42 DAI) to obtain the average percentage of defoliation (%) in this period. The agronomic attribute average number of leaves (ALN) was counted twice, one in the R1 stage after the expansion of the last triplets, and the second in R4-R5 stage, and also in this stage one plant per plot had all leaves collected and photographed to compute the average leaf area (ALA - cm^2) using the image tool software ImageJ.

Linear models adjust to obtain soybean rust rate

Disease severity data were fitted to the linearized forms of Exponential, Logistic, Gompertz and Monomolecular mechanistic models to obtain the disease rate (r) (Table 2). The model that gave the highest coefficient of determination (R^2), low residual means square (RMS) and best fit residual plot distribution, was selected as the most appropriate model for describing the temporal pattern of the disease (Madden, 2007). Primary infection "β0" or initial inoculum and apparent infection rates (r) of the disease, were estimated from the selected models for each genotype and split-plot treatments. A table of estimated parameters from the selected empirical and mechanistic models were generated using the least squares method.

Table 2 Linearized forms of the non-linear mechanistic growth models used in transforming the disease intensity (y)

Model	Linearized form	Author (year)
Exponential	Exponit $(y) = ln\, y$	Campbell and Madden (1990)
Monomolecular	Monit $(y) = ln\,(\frac{1}{1-y})$	Campbell and Madden (1990)
Logistic	Logit $(y) = ln\,(\frac{y}{1-y})$	Van der Plank (1963)
Gompertz	Gompit $(y) = -\,ln\,[-\ln(y)]$	Berger (1981)

Adapted from Jesus Junior, Pozza, Vale e Aguilera (2004)

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 Life Time" (RLT) cycle (Tschanz and Tsai, 1983). However, in the experiment, the lineages and cultivar presented a very similar cycle, so there was no need to compute this value.

Statistical analysis

The data obtained at each assay were submitted to the Shapiro-Wilk test to evaluate the assumptions of the analysis of variance. The data that obtained a normal distribution, were submitted to analysis of variance. There was a need to transform the leaves number to log [x] only in the second season data. Both assays were compared by conjoint analysis to verify the presence of differences between them for all variables. The data also was subjected in a mean cluster comparison by Scott-Knott ($p<0,05$), all statistical analyses were performed using the R software (R Core Team, 2013) also with the add-on packages ExpDes (Ferreira et al., 2003) and agricolae (Mendiburu, 2005).

RESULTS

There was difference between repeated experiments over time. Then, the results of the assays were presented separately. The first signs of disease incidence were observed 7 days after inoculation (7 DAI). The typical symptom (TAN lesion) was not yet visible, and the detection of the disease in its initial phase, was done by visualizing uredinia under the magnifying glass on the abaxial surface of the sampled leaves. The genotypes were at the R2 stage when the lesions started to appear. In this time the first pustules were observed in the trifoliate leaves of the lower third of the plants and the disease progress to the upper third of the plant. The epidemics was established from the 35 DAI at the first and from 28 DAI at the second assay, with 30% and 55% of maximum severity reached in the Desafio 8473SFR in the first and second assay split-plot without fungicide spray, respectively (Figure 1). The difference between assays probably was due to inoculum viability.

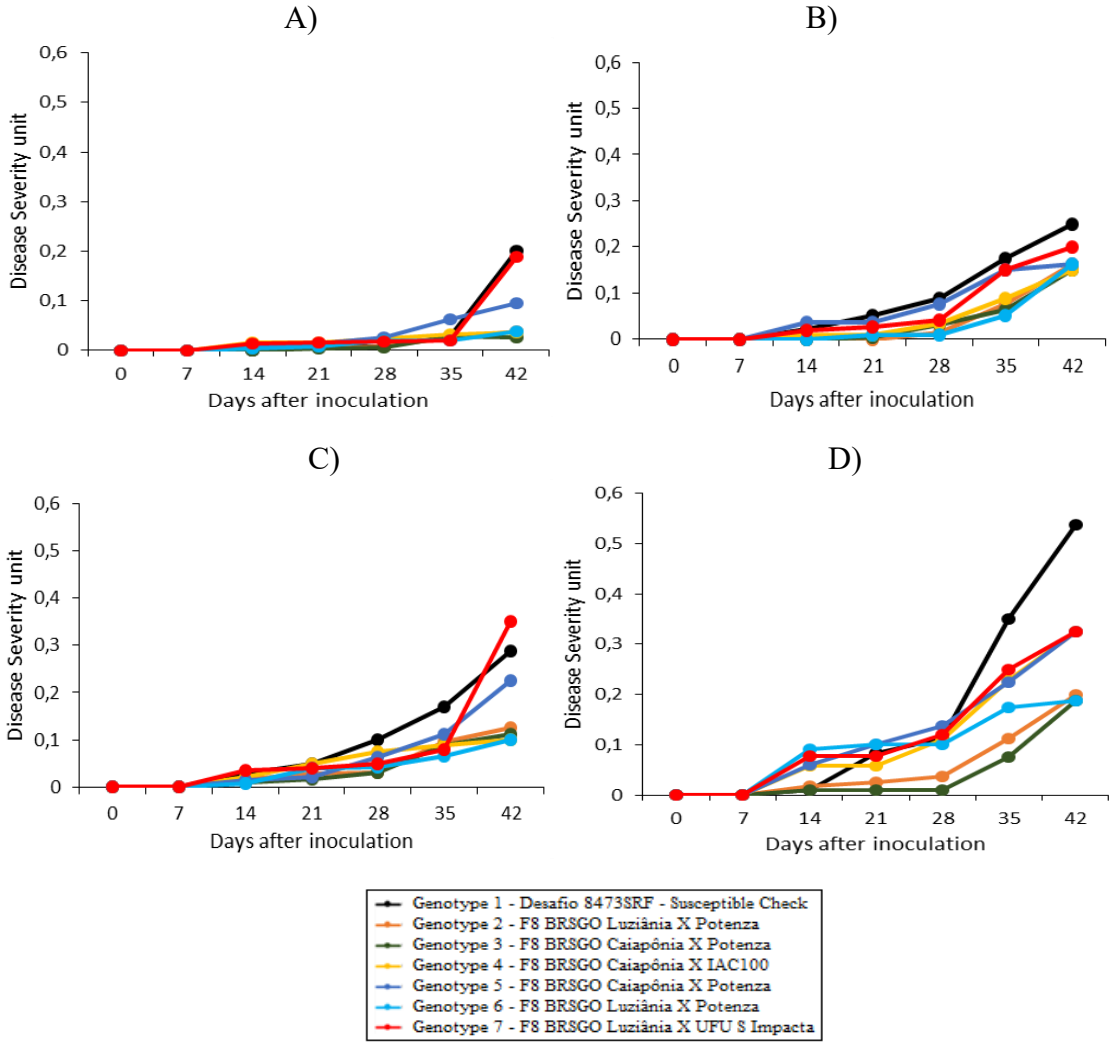

Figure 1 Disease severity observed data for 7 genotypes tested at different assays. A/B - Split-plot with a single fungicide spray in the first and second assays respectively; C/D - Split-plot without a fungicide spray in the first and second assays respectively.

To obtain the apparent disease rate (r), mathematic linear models were adjusted (Table 3). The Logistic (logit) and Exponential (exponit) model transformations had coefficients of determination very similar to the gompertz (gompit), but the parameters low residual mean square (RMS) and β0 were not suitable to explain the disease progress like the previous one. The gompit model overall had a coefficient of determination (R^2), ranging in 76.05 to 97.27 %

among genotypes in the first and 79.24 to 98.91% at the second season at the split-plot S. For the split-plot WS the R^2 varied among genotypes from 85.80 to 99.54 % at the first and 85.49 % to 99.47 % in the second assay. In addition, the best residual distribution was of the gompit model. The disease rate for Gompertz models varied among 0.011 to 0.039 at the first and 0.014 to 0.074 foliar area/day at the second assay. The first assay overall, had low values in apparent infection rate (r) in comparison with the second.

Table 3 Estimated disease parameters from the soybean rust (*Phakopsora pachyrhizi*) epidemics from fitted linear models (interaction genotype x split-plot treatment) in two assays of 2016 and 2017 at Lavras, MG.

Model	Fungicide Treatment				Without Fungicide Treatment				Fungicide Treatment				Without Fungicide Treatment			
	RMS	R^2	β0	r	RMS	R^2	β0	R	RMS	R^2	β0	r	RMS	R^2	β0	r
	Genotype 1 - Desafio 8473SRF – 2016 Assay								Genotype 1 - Desafio 8473SRF – 2017 Assay							
Linear	0.00	**62.9**	**-0.06**	0.006*	0.00	92.7*	**-0.06**	**0.009**	0.00	90.9**	**-0.17**	**0.019**	0.00	82.0*	**-0.07**	**0.006**
Exponit	0.12	83.3*	-5.81*	0.102*	0.19	98.1**	-4.33**	0.090**	0.02	91.9**	-5.04**	0.135**	4.07	91.9**	-15.4**	0.403**
Logit	0.13	82.2*	-5.89*	0.109*	0.24	99.2**	-4.42**	0.101**	0.04	95.3**	-5.31**	0.162**	4.09	92.1**	-15.5**	0.410**
Monit	0.00	**61.4**	**-0.07**	0.007*	0.00	89.8*	**-0.09**	**0.011**	0.00	87.2**	**-0.27**	**0.027**	0.00	81.5*	**-0.07**	**0.006**
Gompit	0.01	76.0*	-1.95*	0.034*	0.03	99.5**	-1.63**	0.039**	0.00	97.1**	-2.02**	0.070**	0.08	92.9**	-3.20**	0.074**
	Genotype 2 - F8 BRSGO Luziânia X Potenza - 2016 Assay								Genotype 2 - F8 BRSGO Luziânia X Potenza - 2017 Assay							
Linear	0.00	80.0*	-0.00*	0.001*	0.00	85.4**	-0.02**	0.004*	0.00	89.0**	-0.03**	0.010**	0.00	90.9**	-0.01**	0.005**
Exponit	0.04	88.3*	-6.00**	0.074**	0.75	93.5**	-5.38**	0.094*	0.06	92.1**	-3.20**	0.059**	3.19	89.6**	-5.78**	0.128**
Logit	0.04	88.2*	-6.00**	0.075**	0.80	93.3**	-5.40**	0.098*	0.08	91.9**	-3.25**	0.070**	3.23	90.7**	-5.80**	0.134**
Monit	0.00	80.0*	-0.00*	0.001*	0.00	84.9**	-0.02**	0.004*	0.00	88.1**	-0.05**	0.012**	0.00	91.3**	-0.02**	0.006**
Gompit	0.00	87.8*	-1.84**	0.017**	0.05	91.6**	-1.76**	0.029*	0.01	90.9**	-1.30**	0.032**	0.08	96.2**	-1.72**	0.035**
	Genotype 3 - F8 BRSGO Caiapônia X Potenza - 2016 Assay								Genotype 3 - F8 BRSGO Caiapônia X Potenza - 2017 Assay							
Linear	0.00	79.6*	0.00*	0.001*	0.00	90.4*	-0.03*	**0.004**	0.00	83.5*	0.05*	0.004*	0.00	83.5*	-0.04*	0.005*
Exponit	0.02	81.9*	-6.06*	0.067*	0.37	94.8*	-5.44*	0.093*	0.03	86.5*	-2.69*	0.028*	0.09	86.5*	-5.69*	0.108*
Logit	0.02	81.9*	-6.05*	0.068*	0.41	94.7*	-5.47*	0.098*	0.04	85.8*	-2.64*	0.033*	0.10	85.8*	-5.75*	0.114*
Monit	0.00	79.6*	0.00*	0.001*	0.00	89.8*	-0.03*	**0.004**	0.00	84.9*	0.05*	0.004*	0.00	84.9*	-0.05*	0.006*
Gompit	0.00	81.5*	-1.84*	0.015*	0.03	93.8*	-1.80*	0.029*	0.00	85.4*	-1.02*	0.014*	0.00	85.4*	-1.89*	0.034*
	Genotype 4 - F8 BRSGO Caiapônia X IAC100 - 2016 Assay								Genotype 4 - F8 BRSGO Caiapônia X IAC100 - 2017 Assay							
Linear	0.00	79.6*	**0.01**	0.001*	0.00	99.8**	**-0.01**	0.004*	0.00	**73.5**	**-0.06**	**0.006**	0.00	86.9*	-0.06*	0.007*
Exponit	0.24	77.6*	-4.50*	0.042*	0.17	82.7*	-4.24**	0.063**	0.04	80.0*	5.97*	0.112*	7.92	91.8*	-9.80**	0.244*
Logit	0.24	78.0*	-4.49*	0.043*	0.20	84.2*	-4.25**	0.067**	0.04	79.9*	-6.05*	0.118*	8.01	91.8*	-9.87**	0.252*
Monit	0.00	79.6*	**0.01**	0.001*	0.00	99.9**	**-0.01**	0.004*	0.00	**73.2**	**-0.07**	**0.007**	0.00	86.4*	-0.07*	0.008*
Gompit	0.01	79.8*	-1.50*	0.011*	0.02	91.7**	-1.48*	0.022**	0.00	79.2*	-1.99*	0.037*	0.19	91.6*	-2.38*	0.055*
	Genotype 5 - F8 BRSGO Caiapônia X Potenza - 2016 Assay								Genotype 5 - F8 BRSGO Caiapônia X Potenza - 2017 Assay							
Linear	0.00	89.5*	**-0.02**	**0.003**	0.01	72.0*	-0.05*	0.008*	0.00	81.8*	-0.05*	0.006**	0.00	84.7*	-0.05*	0.005*
Exponit	0.22	93.7**	-5.39**	0.082**	0.50	89.6*	-4.09**	0.072*	7.15	96.9**	-8.64*	0.215**	3.68	90.3*	-12.91*	0.348*
Logit	0.24	93.7**	-5.42**	0.086**	0.70	88.8*	-4.17**	0.083*	7.28	97.4**	-8.70*	0.221**	3.73	90.8*	-12.97*	0.353*
Monit	0.00	88.9*	**-0.02**	**0.003**	0.00	74.2*	-0.05*	0.008*	0.00	81.5*	-0.06*	0.007**	0.00	82.9*	-0.06*	0.006*
Gompit	0.02	93.4**	-1.77**	0.024*	0.08	87.3*	-1.50**	0.029*	0.19	97.0**	-2.22*	0.049**	0.08	97.6*	-2.83*	0.065*
	Genotype 6 - F8 BRSGO Luziânia X Potenza - 2016 v								Genotype 6 - F8 BRSGO Luziânia X Potenza - 2017 Assay							
Linear	0.00	90.9*	-0.00*	0.001*	0.00	95.3**	-0.01*	0.003*	0.00	95.1**	-0.02**	0.009**	0.00	95.5**	-0.05**	0.008**
Exponit	0.30	94.8*	-5.34*	0.065*	0.53	84.1*	-5.13*	0.081*	0.27	97.3**	-3.43**	0.068**	0.13	95.8**	-4.62**	0.098**
Logit	0.31	94.8*	-5.34*	0.067*	0.58	84.7*	-5.14*	0.084*	0.32	98.5**	-3.48**	0.079**	0.14	96.9**	-4.69**	0.107**
Monit	0.00	90.9*	-0.00*	0.001*	0.00	93.1**	-0.01*	0.003*	0.00	92.7**	-0.05**	0.012**	0.00	94.5**	-0.07**	0.010**
Gompit	0.02	94.9*	-1.71*	0.017*	0.04	85.8*	-1.67*	0.023*	0.03	98.9**	-1.34**	0.034**	0.01	99.4**	-1.67**	0.039**
	Genotype 7 - F8 BRSGO Luziânia X UFU S Impacta - 2016 Assay								Genotype 7 - F8 BRSGO Luziânia X UFU S Impacta - 2017 Assay							
Linear	0.00	86.2*	-0.04*	0.006**	0.00	87.6**	-0.06*	0.007*	0.00	90.0*	-0.05*	0.010*	0.00	**72.8**	**-0.06**	**0.005**
Exponit	0.48	99.8**	-5.10*	0.092*	0.28	95.2**	-5.16*	0.101*	5.57	88.6*	-6.12*	0.153*	0.00	77.6	-11.4**	0.300*
Logit	0.54	99.6**	-5.15*	0.099*	0.36	95.5**	-5.24*	0.110*	5.60	90.3*	-6.20*	0.165*	0.00	78.6	-11.4**	0.306*
Monit	0.00	84.2*	-0.05*	0.007*	0.00	84.8**	-0.08*	0.009*	0.00	88.6*	-0.08*	0.013*	0.00	**71.3**	**-0.06**	**0.006**
Gompit	0.05	97.2**	-1.76*	0.032*	0.05	94.7**	-1.82*	0.038*	0.13	94.7*	-1.79*	0.048*	0.00	91.9*	-2.67*	0.059*

*,** Significant by t-test at 5% and 1% probability respectively. Numbers in bold Not-significant.

There was interaction between genotypes and fungicide spray to AUDPCs and infection rates to both assays (Table S8 and S9 - p<0,05). For the first assay, there were significant differences among the AUDPCs averages only in the WS split-plot. From the data obtained, it was possible to separate the germplasm materials into two distinct groups. A first group composed of genotypes 2, 3, 4, 5 and 6, whose AUDPC ranged from 141.00 to 225.75 and a second group composed of the check and genotype 7, in which the AUDPC ranged from 266.00 to 344.00. Also, there was significant difference for the variable AUDPC at the second assay (Figure 2 and Table 4) the means were grouped into two distinct groups at the split-plot. One group formed by the genotypes 2, 3, 4 and 6 (105.87 to 150.50), followed by a second group containing the check and genotypes check, 7 and 5 too (232.75 to 320.25 of variation). On the split-plot WS, occurred the formation of three groups with different means, where the check (1) achieved the highest AUDPC value (576.62), followed by a group with genotypes 4, 5, 6 and 7 (391.12 to 481.25) and a third group characterized by genotypes 2 (204.75) and 3 (139.12). There was significant reduction of the disease, between the overall average (OA) of a single fungicide spray split-plot and the split-plot without fungicide for both assays.

Table 4 Area under severity progress curve (AUDPC), apparent infection rates (r) caused by *Phakopsora pachyrhizi* in 7 genotypes at 2 split-plots scenarios during the epidemics in both assays, under greenhouse conditions.

Genotype	**First Assay**				**Second Assay**			
	AUDPC (%-days)		r (gompit. day^{-1})		AUDPC (%-days)		r (gompit. day^{-1})	
	S	**WS**	**S**	**WS**	**S**	**WS**	**S**	**WS**
1	110.25 ^{ns} B	344.00 a A	0.036 a ^{NS}		320.25 a B	576.62 a A	0.070 a ^{NS}	0.074 a ^{NS}
2	59.50 ^{ns} B	162.75 b A	0.021 c ^{NS}		120.75 b ^{NS}	204.75 c ^{NS}	0.032 c ^{NS}	0.035 d ^{NS}
3	35.00 ^{ns} B	141.00 b A	0.016 e ^{NS}		105.87 b ^{NS}	139.12 c ^{NS}	0.014 d B	0.034 d A
4	94.50 ^{ns} B	198.00 b A	0.023 c ^{NS}		150.50 b B	430.50 b A	0.037 c B	0.055 c A
5	110.50 ^{ns} B	225.75 b A	0.026 b ^{NS}		263.37 a B	479.50 b A	0.049 b B	0.065 b A
6	50.00 ^{ns} B	143.75 b A	0.019 d ^{NS}		119.87 b B	391.12 b A	0.034 c ^{NS}	0.039 d ^{NS}
7	111.25 ^{ns} B	266.00 a A	0.035 a ^{NS}		232.75 a B	481.25 b A	0.048 b B	0.059 c A
Average	81.57 B	211.28 A	0.021 B	0.030 A	187.62 B	386.12 A	0.040 B	0.051 A
S-W=	**0.84**		**0.30**		0.0005		**0.08**	
VC-Plot	56.16 %		10.33%		20.02 %		8.12 %	
VC-SplitPlot	29.23 %		14.07%		21.39 %		8.15 %	

* S means Split-plot with a single fungicide spray and WS split-plot without fungicide spray.

**ns = non-significant interaction among genotypes; NS = non-significant interaction between split-plot treatments.

*** Original Means.

**** Means followed by the same lowercase letter in the column do not differ by the Scott-Knott test at 5% probability.

***** Means followed by the same uppercase letter in the line do not differ by the Scott-Knott test at 5% probability.

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

*******VC = Variance coefficient; OA= Overall average of the seven genotypes in each split-plot treatment.

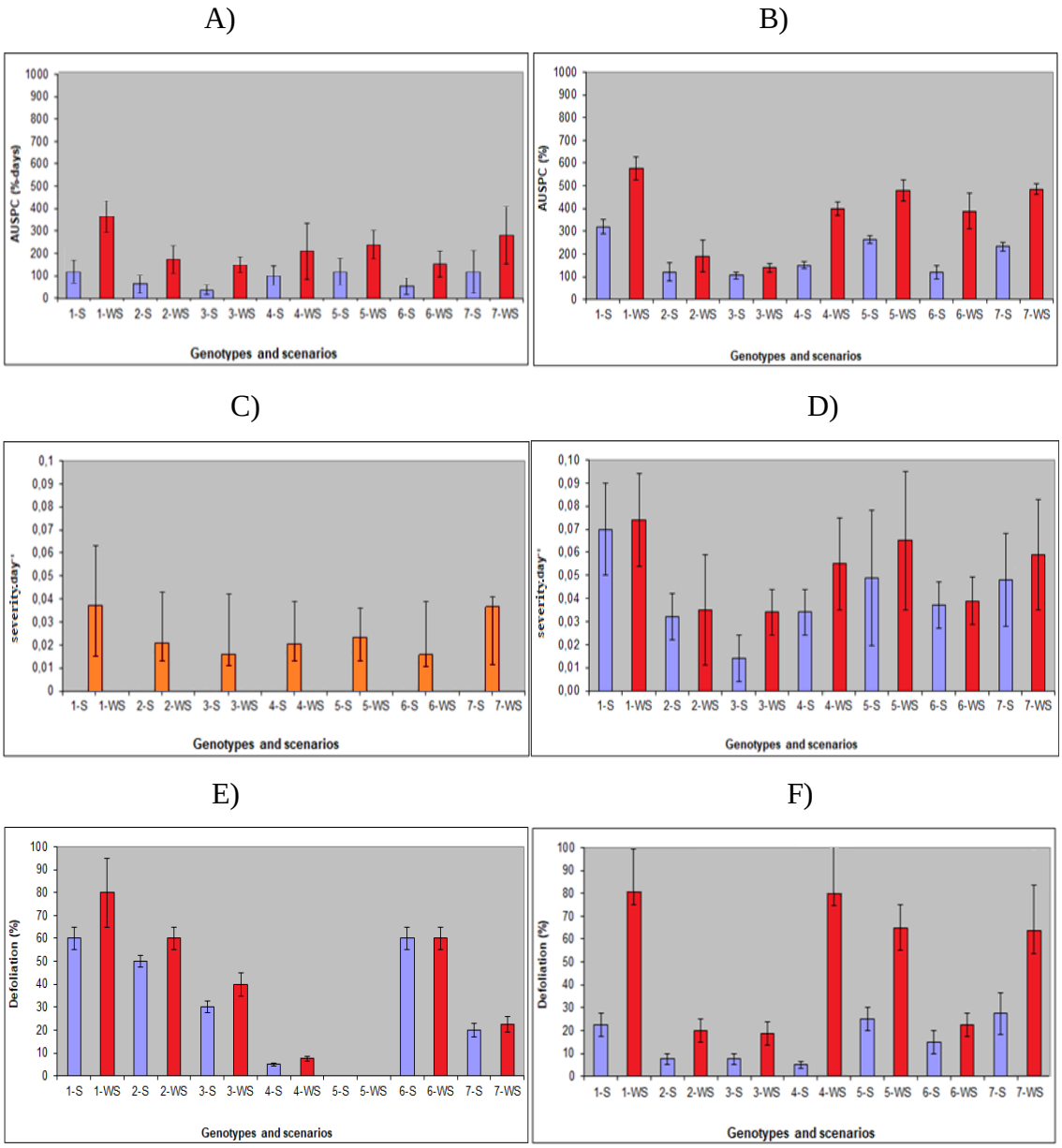

Figure 2 A/B) AUDPCs in the first and second assay respectively; C/D) Infection rates (r) of the first and second assay respectively; E/F) Defoliation (%) in the first and second assay respectively. Split-plots scenarios during the epidemics.

There was a significant difference among the rates in the two assays (Table 4, Figure 2 and Table S8 and S9). In the first and second assay the overall average of the seven genotypes in the split-plot S were 0.021 and 0.030 and, in the Split-plot, WS 0.040 and 0.051 foliar area/day, respectively. Although, no interaction occurred in the first experiment, r followed the AUDPC results, with the Desafio check (1) and genotype 7 with the highest r (> 0.035) (Table 4 and Figure 2) To the second assay interaction occurred between genotypes and spraying ($p<0,001$), with apparent infection rates at least 50% greater than the first (>0,040).

So, even the sprayed genotypes were different. The highest rates were of the genotypes check (0.070), 7 (0.048) and now the 5 (0.048). There was also a significant reduction of the disease rate, with one fungicide spray, where the OA in the split-plot S reached a lower value (0.021) compared to the value reached by the of split-plot WS (0.030).

There was significant interaction between genotypes and the fungicide spray in plant defoliation ($p < 0.05$). For both tests the defoliation was lower in sprayed genotypes (Table 5 and Figure 2). There was no significant difference between the genotypes sprayed in the second assay, only for the second. In the first assay, the OA defoliation had no significant difference, only at the second. For this assay, the genotypes 4 (<7.5%) and 5 (0) had the lower defoliation, while check reached 60% even sprayed.

Table 5 Defoliation (%) caused by *Phakopsora pachyrhizi* in 7 genotypes at 2 split-plots scenarios, with and without fungicide spray.

Genotype	First Assay(%)		Second Assay(%)	
	S	WS	S	WS
1	60.00 a B	80.00 a A	22.50 ^{ns} B	80.00 a A
2	50.00 b B	60.00 b A	7.50 $^{ns\ NS}$	20.00 c ^{NS}
3	30.00 c B	40.00 c A	7.50 $^{ns\ NS}$	18.75 c ^{NS}
4	5.00 e B	7.50 e A	5.00 ^{ns} B	80.00 a A
5	0.00 e ^{NS}	0.00 f ^{NS}	25.00 ^{ns} B	65.00 b A
6	60.00 a ^{NS}	60.00 b ^{NS}	15.00 $^{ns\ NS}$	22.50 c ^{NS}
7	21.25 d B	23.75 d A	27.50 ^{ns} B	65.00 b A
OA	32.32 ^{NS}	38.75 ^{NS}	15.71 B	50.17 A
S-W=	**0.29**		**0.28**	
VC-Plot	19.99 %		41.29 %	
VC-Split-plot	3.07 %		27.49 %	

* S means Split-plot with a single fungicide spray and WS split-plot without fungicide spray.

**ns = non-significant interaction among genotypes; NS = non-significant interaction between split-plot treatments.

*** Original Means.

**** Means followed by the same lowercase letter in the column do not differ by the Scott-Knott test at 5% probability.

***** Means followed by the same uppercase letter in the line do not differ by the Scott-Knott test at 5% probability.

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

*******VC = Variance coefficient; OA= Overall average of the seven genotypes in each split-plot treatment.

No significant difference interaction was obtained between genotypes and the fungicide spray for average leaf number (ALN) and average leaf area (ALA) in the first assay (Table S10 - $p > 0.05$). In the second assay the interaction was significant only for the average leaf number (Table 6, Figure 2 and Table S11). The genotypes 4 (F8 BRSGO Caiapônia X IAC100), 5 (F8 BRSGO Caiapônia X Potenza),6 (F8 BRSGO Luziânia X Potenza) and 7 (F8 BRSGO Luziânia X UFU S Impacta) obtained the higher average leaf area in both assays

reaching 11.77 to 15.78 in the first and 23.55 to 31.56 on the second assay. For the first assay the overall leaf area was higher in the sprayed genotypes (13.40) in comparison of the non-sprayed (9.90). In the second assay the genotypes had difference interaction in the average leaf number (ALN). The overall leaf number for spray and non-spray treatments was more than the double in comparison of the first assay. The genotypes with higher leaf number when sprayed and not sprayed was the genotype 5 (F8 BRSGO Caiapônia X Potenza).

Table 6 Mean values of soybean agronomic attributes of 6 genotypes of the breeding program LAGER / UFU plus one variety evaluated at the first and second assay during the rust epidemics in Lavras, MG.

Genotype	**First Assay**				**Second Assay**			
	ALA (cm²)		ALN		ALA (cm²)		ALN	
	S	**WS**	**S**	**WS**	**S**	**WS**	**S**	**WS**
1	7.97 d ^{NS}		14.62 $^{ns\ NS}$		15.95 d ^{NS}		35.33 e B	36.66 b A
2	10.40 c ^{NS}		17.37 $^{ns\ NS}$		20.81 c ^{NS}		59.33 b A	31.00 c B
3	9.10 d ^{NS}		13.12 $^{ns\ NS}$		18.20 d ^{NS}		47.66 c A	36.66 b B
4	13.61 b ^{NS}		13.87 $^{ns\ NS}$		27.23 b ^{NS}		41.00 d A	30.00 c B
5	15.78 a ^{NS}		22.50 $^{ns\ NS}$		31.56 a ^{NS}		65.00 a A	60.00 a B
6	11.77 b ^{NS}		19.50 $^{ns\ NS}$		23.55 b ^{NS}		34.50 e A	30.00 c B
7	12.95 b ^{NS}		20.12 $^{ns\ NS}$		25.92 b ^{NS}		30.50 f ^{NS}	30.00 c ^{NS}
OA	13.40 a	9.90 b	17.98 ^{NS}	16.62 ^{NS}	26.81 a	19.81 b	44.76 a	36.33 b
S-W=	**0.07**		0.0001		**0.07**		0.0001	
Plot - VC	14.36 %		39.28 %		14.37 %		9.36 %	
Split-plot VC	16.48 %		61.57 %		16.49 %		2.04 %	

*ALN- Average Leaf Number; ALA- Average Leaf Area.

** S means Split-plot with a single fungicide spray and WS split-plot without fungicide spray.

***ns = non-significant interaction among genotypes; NS = non-significant interaction between split-plot treatments.

**** Original Means. Averages, but the values were changed, for average leaves number to log [x]

***** Means followed by the same lowercase letter in the column do not differ by the Scott-Knott test at 5% probability.

****** Means followed by the same uppercase letter in the line do not differ by the Scott-Knott test at 5% probability.

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

********VC = Variance coefficient; OA= Overall average of the seven genotypes in each split-plot treatment.

In both experiments, there was significant difference between the genotypes and positive interaction in the reduction of the disease apparent infection rate and AUDPC with the fungicide spray. In the first assay, the genotypes that stood out and showed desirable traits in the reduction of the disease progress was the genotypes 2 (F8 BRSGO Luziânia X Potenza), 3 (F8 BRSGO Caiapônia X Potenza), 4 (F8 BRSGO Caiapônia X IAC100) and 6 (F8 BRSGO Luziânia X Potenza) with lower AUDPC values than the check (59.00, 35.00, 94.50 and 50.00 respectively) and lower apparent infection rates (0.021, 0.016, 0.023 and 0.019 respectively). For the second assay with higher disease pressure, the genotypes 2 (F8

BRSGO Luziânia X Potenza) and 3 (F8 BRSGO Caiapônia X Potenza) had the lower AUDPC (204.75 and 139.12) and lower rates of disease progression (0.034 and 0.035) compared with the susceptible check (Desafio 8473SRF) in the Split-plot without fungicide spray. The genotype 5 (F8 BRSGO Caiapônia X Potenza) had the best agronomic attributes, with higher leaf area and lower defoliation but obtained a low score in disease progression with higher AUDPC values and apparent infection rates.

DISCUSSION

The variation in genotype performance with partial resistance shows the importance of selection for many isolates or for pathogen variation, besides the specific adoption to the given regions, since environments and genotype interaction with other strategies like a fungicidal spraying. This interaction is resolved with either by selection of broad adaptation cultivars or by stratification of the production area and release of cultivar specifically adapted to each stratum (Ramalho et al., 1993; Toledo et al., 2006). Some plants have evolved mechanisms that allow them to perceive external stresses and rapidly regulate their physiology, metabolism and gene expression to cope with them; this could lead differences in plant development, cycle and yield (Kyei-Boahen and Zhang, 2006). This behavior could be observed in the different progress curves of the disease among genotypes or cultivars over time. The soybean genotypes developed by the LAGER / UFU improvement program, showed partial resistance traits in field trials for *P. pachyrhizi* (Santos, 2007; Martins et al., 2007; Silva et al., 2007; Martins and Juliatti 2014). The parental variety's IAC100, Luziânia, Caiapônia and Potenza, are the base for several crossings to obtain some of the genotypes. The parental IAC100 also was related to have partial resistance against soybean rust infection sharing this trait with the cultivar Potenza (Silva et al., 2007).

In our experiment, the disease progress curve begins at 7 DAI for both seasons, but the epidemics starts at 35 and 28 DAI for the first and second assay respectively, the major explanation to this result, is the difference in the inoculum, viability and virulence. There was significant interaction between the genotypes tested and the fungicide spray in plant AUDPC, apparent infection rates (r), defoliation and leaf number. The genotypes 2 (F8 BRSGO Luziânia X Potenza) and 3 (F8 BRSGO Caiapônia X Potenza) had consistency lower apparent infection rate (r), AUDPC and higher average leaf number. This interaction with partial resistant genotypes and fungicides has great importance for rust management in field conditions. Soybean fields under intensive use of fungicides of the SDHIs group, during the

Brazilian 2016/2017 crop season and with conditions of high levels of rust pressure, showed a loss of fungicide performance. So, leaves infected with rust populations collected at these sites, after molecular analysis, indicated a mutation in the C subunit at position I86F (FRAC, 2017). Klosowski et al. (2016) after molecular analysis of leaves infected with *P. pachyrhizi* collected from Brazilian soybean fields in the 2012/2013 and 2013/2014 seasons, showed the presence of the F129L mutation in monouredinial isolates and related this the reduction of the fungi sensitivity to QoI fungicides. So, in this new scenario, where fungicides are losing efficacy and Asian soybean rust are under fast genetic alterations, disease management with partial resistant genotypes interacting with fungicide sprays is important to protect plant yield potential and manage rust resistance to fungicides sprays.

The genotypes tested in our experiment, are derived from crossings between BRSGO Luziânia, UFUS Impacta, BRS Caiapônia, Potenza, BRSGO Caiapônia, IAC100 and Cristalina. These progenies had the highest partial resistance to soybean rust, in field and greenhouse conditions (Martins et al. 2007; Oliveira and Juliatti, 2017). Santos in 2018 phenotyped crosses between IAC 100, BRS Caiapônia, BRS Caiapônia and UFUS Impacta with rust severity in field, and concluded that these parental varieties have high selection accuracy and high heritability for resistance to Asian rust. The same author mapped the QTL 6 in IAC 100, that later was identified in a region separated by approximately 20 cM from the region where the resistance gene Rpp5 was mapped by other authors. Also, she mapped other QTLs in other regions than the Rpps genes. QTLs are chromosomal regions where several genes of small effect are found and need not necessarily be in the same regions of the genes that confer vertical resistance to the disease (Garcia et al., 2008). Santos in 2010, also reported the existence of QTLs in regions other than those mapped to rust resistance genes and concluded that these QTLs are important in the composition of horizontal resistance against the pathogen and proposed that these genotypes requires new analyzes in different environmental conditions.

Disease rate progression is related to the expression of partial resistance, since this variable is inserted on the contribution in the reduction or tolerance for disease damage, being part of the horizontal resistance instead the immunity generated by the vertical resistance. Among five models employed to adjust the disease rate (r), the linearized Gompertz model provided the best fit to the disease severity rate, with the higher average coefficient of determination. In previous works (Berger, 1981; Vuori, 2006; Mohapatra, 2008; Gyamera et al., 2015) have indicated the Gompertz model are noted for its consistent and stable parameter to estimate and describe progress curves of foliar diseases. The genotypes 2 (F8 BRSGO

Luziânia X Potenza) and 3 (F8 BRSGO Caiapônia X Potenza) had the lowest apparent infection rates (r) of 0.032 and 0.014 respectively in the conditions of a single fungicide spray (QoI + SDHI mixture), in comparison with 0.070 showed in the check (Desafio 8473SRF). In the situation without the spray, the same genotypes stood out with the lower rates (0.035 and 0.034 respectively) and the check showed the highest (0.074). For AUDPC, the genotypes 2 and 3 also showed in the experiment and its repetition, constant lower disease levels, with 120.75 and 105.87 respectively with the fungicide spray interaction and 204.75 and 139.12 without fungicide spray. The check showed the highest AUDPC levels, with 320.25 and 576.62 in the interaction with and without fungicide spray, respectively.

Kawuki, Tukamuhabwa and Adipala (2004) studying the rate of progress of Asian soybean rust in early, middle and late cycle genotypes at three growing seasons in Uganda, Africa, estimated by logit regression between severity and a Relative Life Time (RLT). The RLT indicates the percentage of soybean cycle that has been completed on a certain date, this measurement was different form used in our work, that took in count growth of lesions per day. On average, early maturing lines registered the lowest rates (0.07), followed by medium maturing lines (0.15), and then the late maturing lines (0.17). On average, early maturing genotypes registered the lowest severity (54%), followed by medium maturing lines (61%), and then the late maturing lines (65%). They concluded that most of the genotypes had higher rates and severity than that of the local check Nam 2. In the joint analysis of the data, the authors concluded that the greatest variation among taxa occurred as a function of the influence of the growing season. The results also indicated that high rates of disease progression were not associated with high susceptibility of the genotypes, as low rates were not associated with resistance. We can argue that maturing cultivars, which have a short cycle and suffer less from rust epidemic and can have lower yield losses. Bromfield (1984) pointed out that early maturing cultivars can therefore be utilized to minimize yield losses attributable to soybean rust.

Yang et al. (1990) studying the rust epidemics on Taiwan, reported that the cultivar TK 5 and G 8587 (PI 230971), had an average apparent infection rates of 0.12/day and 0.08/day respectively in a trial made during the year of 1980, and 0.12/day and 0.11/day during 1981. In the first year, the authors confirmed that the cultivar TK had higher rates in comparison with the cultivar G8587, but during the second year the rates didn't have significant difference. And in relation to the relative AUDPC (RAUDPC), the cultivar TK5 in an average, higher values during both years and all seasons evaluated. Tchanz, Wang and Tsai (1983) to obtain AUDPC curves under different environmental conditions observed that some

lineages were considered partially resistant because they presented a displaced curve to the right. Also, they determined the daily apparent rate of infection and severity on resistant cultivars RE-Z-11A 0.1083, SRE-Z-11B and SRE-Z-15A have 0.1083 / 34.9%, 0.1014 / 18.6% and 0.099 / 22.8% respectively, consistently they presented low rates of rust development and low predicted rust severities during the assays. The authors stated that they were being used as parents in soybean improvement program. But they also found that the cultivar TK 5 (susceptible cultivar) had 0.1113 of daily rate and average severity of 40.3 and the cultivar KS 535 showed a higher rate 0.2376 and lower average severity 30.6, they attributed this occurrence to the difference of maturation between the cultivars and not due to differences in the resistance reaction among them. In relation to yield, there was positive interaction of cultivars and fungicide spray, since the susceptible cultivar TK 5 had superior values (2.27 t. ha^{-1}) when a fungicide treatment was applied in comparison an untreated plot (0.23 t. ha^{-1}), revealing a protection of 89.9% in yield loss. For all cultivars and genotypes, including the lines that showed resistance, the treatment with fungicide increase yield, but the authors didn't disclaim about the amount of fungicide sprays and the type of product used in the treatments. Levy (2005) studying AUDPC, of the soybean rust severity (log-transformed) on unsprayed and sprayed (azoxystrobin) soybean cultivars LS666 Soprano, and Sonata, related the benefit of a single earlier spray of fungicide. All cultivars sprayed at 50 days after sowing had significantly less disease than those sprayed at 70 days after sowing.

Kim, Wang and Yang (2005) calculates r values for the cultivars TK 5 (0.004 to 0.454 logit. day-1), whereas those for G 8587 (0.008 and 0.774 logit. day-1). Nevertheless, the r values for TK 5 generally exceeded those for G 8587. The authors made 7-day simulation, applying daily apparent infection rate estimates for FLAIR models to the logistic growth, and this explained 86.5% of variation of disease severity for validation sets, they also concluded that different rates were due to temperature variation. Tchanz and Wang (1980) reported that apparent infection rate of soybean rust ranged from 0.11 to 0.22 in four cultivars on Taiwan. McLaren (2008) calculating the rate by RLT in several cultivars, determined the lowest mean apparent infection rate for the cultivars LS444 at 0.150 per unit per day (pupd) and highest for the susceptible cultivar SNK500 at 0.270 pupd. But even with this difference the AUDPC didn't show significant difference 2007.35 and 2029.15 respectively. The commercial soybean cultivars were evaluated for rust and were interacted with 26 entries in fungicide sprayed and unsprayed plot, rust AUDPC in all unsprayed plots had higher values (2488.5) in comparison with average threated plots (1760.1). Bromfield, Melching and Kingsolver (1979) related apparent rates of lesion area per day in several cultivars under *P. pachyrhizi*

infection. The American cultivar Pr-Comp obtained the lowest value and highest resistance (0.0054), followed by Australia-71-1 (0.0219), India 73-1 (0.314) and Taiwan-72-1 (0.369) had the highest rate and consequently was the most susceptible cultivar is his trial. Silva et al. (2007) testing the fungicide mixture of azoxystrobin and cyproconazole with rust resistant genotypes, obtained with the Potenza cultivar, low values of AUDPC for incidence. For AUDPC of severity the cultivar Potenza also maintained the lowest values of AUDPCS, in several treatments conjugated with different fungicides, with this information the author concludes that there is indicative of partial genetic resistance in this material. Silva et al. (2011) working with the reactions of early, medium and late cycle soybean cultivars against ASR interacting with fungicide sprays, reported significantly reductions in yield of soybean cultivars inoculated with ASR, with values varying from 65.1% to 76.5% in the untreated plots, in comparison to the best chemical control program (monitoring).

In relation to defoliation the genotype 5 (F8 BRSGO Caiapônia X Potenza) had the best agronomic attributes with the higher leaf area and lower defoliation but obtained a low score in disease progression with higher AUDPC values and apparent infection rates. We can infer that the higher number of leaves, and the higher area for lesions growth could be attributed to the reason the disease had scattered faster generating higher levels of severity. Also, the leaves not every genotype with lower values for disease, had lower values for defoliation. This reason is attributed to another effects that also could is related to defoliation, like differences of physiology related to maturation periods, wind turbulence mixed with mist generated by irrigation, could accelerated this process. The values for rate with and without fungicide spray, reveal that the interaction of resistance and fungicide spray, could contribute to lower levels and apparent infection rates during the Asian rust epidemics. These data corroborate to our hypothesis of the importance of partial resistance in the management of this disease. According to the results obtained in this work, it is assumed that the partial resistance to soybean Asian rust, should be evaluated under conditions of average epidemic, which happened in this work at 21 days after inoculation for all characters evaluated. It is important to highlight that the use of soybean rust resistance partial genotypes may be useful in reducing the number of fungicide applications, for the integrated management to control the soybean Asian rust. Silva et al (2007) recommended that genetic breeding programs for genetic resistance to ASR should be based on genotypes with the earliest possible cycle, as the smaller cycle and the existence of genes that confer partial resistance will act in favor of higher productivity. As the materials used in this work have an early / medium cycle, these

materials are of fundamental importance in future disease management strategies, aiming higher yield under conditions of high pressure of rust.

CONCLUSIONS

1 - There is significant difference among genotypes during the progress of ASR severity in two assays.

2 - The Gompertz model explained better the observed variability in ASR severity data with an overall average of 90.47% in agreement between field-observed and model-predicted disease data.

3 - Genotypes 2 (F8 BRSGO Luziânia X Potenza) and 3 (F8 BRSGO Caiapônia X Potenza) showed traits of partial resistance with overall lower AUDPC values than the susceptible check in both assays.

4 - The same groups of genotypes also, presented overall lower apparent infection rates than the check in both assays.

5 - In both experiments, there was significant reduction of apparent infection rate, AUDPC and defoliation with the fungicide spray (solatenol + azoxistrobin 200 g / ha^{-1}).

6 - The cultivar Desafio 8473SFR, demonstrated higher susceptibility with higher disease levels and defoliation.

7 - Genotype 5 (F8 BRSGO Caiapônia X Potenza) had the best agronomic attributes, with higher leaf area and lower defoliation but obtained a low score in disease progression.

REFERENCES

BONDE, M. R.; NESTER, S. E.; AUSTIN, C. N.; STONE, C. L.; FREDERICK, R. D.; HARTMAN, G. L.; MILES, M. R. Evaluation of virulence of *Phakopsora pachyrhizi* and *P. meibomiae* isolates. **Plant Disease**. v.90, p.708-716, 2006.

BROMFIELD, K. R. **Soybean Rust** - Monograph No. 11. Saint Paul MN. APS Press, 1984.

BROMFIELD, K. R.; HARTWIG, E. E. Resistance to soybean rust and mode of inheritance. **Crop Science**. v. 20, p. 254-255, 1980.

BROMFIELD, K. R.; MELCHING, J. S.; KINGSOLVER, C. H. Virulence and aggressiveness of *Phakopsora pachyrhizi* isolates causing soybean rust. **Phythopathology**, Lancaster. v. 70, p. 17- 21, 1980

BERGER, R. D. Comparison of the Gompertz and Logistic Equations to Describe Plant Disease Progress. **Phytopathology**, v. 71, n. 7, p. 716-719, 1981.

CAMPBELL, CL; MADDEN,LV. **Introduction to Plant Disease Epidemiology**. New York:JohnWiley, 1990.

CONAB. Acompanhamento da safra brasileira: safra 2017/2018 available at [http://www.conab.gov.br/conabweb/download/safra/estudo_safra.pd] Accessed 15 April 2018.

FRAC. Monitoring results and use recommendations available at [http://www.frac.info/working-group/sdhi-fungicides/general-use-recommendations] Accessed 04 September 2017.

EMBRAPA. Centro Nacional de Pesquisa de Soja. Tecnologia de produção de soja na região central do Brasil – **Sistema de Produção**, Londrina, n. 11, 2005.

EMBRAPA. Tecnologias de produção de soja Região Central do Brasil 2017. Empresa Brasileira de Pesquisa Agropecuária available at [http://www.cnpso.embrapa.br] Accessed 15 August 2017.

GARCIA, A.; CALVO, E. S.; KIIHL, R. A. S.; HARADA, A.; HIROMOTO, D. M VIEIRA, L.G.E. Molecular mapping of soybean rust (*Phakopsora pachyrhizi*) resistance genes: discovery of a novel locus and alleles. **Theor Appl Genet**. v. 117, p. 545-553, 2008.

GODOY, C. V.; KOGA, L.; CANTERI, M. G. Diagrammatic scale for assessment of soybean rust severity. **Fitopatologia Brasileira**, Brasília, v.31, p. 63-68, 2006.

GYAMERA, E. A., AMOATEY, H. M., & OWUSU, G. K. Spatial and Temporal Spread Patterns of Viral Diseases on a Zucchini Squash Field in the Coastal Savannah Zone of Ghana. **Plant Pathology Journal**, v. 14*,* n. 2, p. 58, 2015.

HARTMAN, G. L.; WANG, T. C.; SHANMUGASUNDARAM, S. Soybean rust research: Progress and future prospects. In: **World soybean research conference**, 5., 1997, Bangkok. Proceedings. Bangkok: Kasetsart University Press. p. 180-186, 1997.

HARTWIG, E. E. Identification of a 4th major gene conferring resistance to soybean rust. **Crop Science**. v. 26, p. 1135-113, 1986.

JESUS JUNIOR WC, POZZA EA, VALE FXR AND AGUILERA GM. Análise temporal de epidemias. In: VALE FXR, JESUS JUNIOR WC AND ZAMBOLIM L (Eds), **Epidemiologia aplicada ao manejo de doenças de plantas**. Belo Horizonte: Perfil, p.127-191, 2004.

JULIATTI, F.C.; BORGES, E.M.; PASSOS, R.R.; CALDEIRA JÚNIOR, J.C.; BRANDÃO, A.M. Doenças da soja. **Cultivar**, Uberlândia. n.47, p. 13, 2003.

JULIATTI, F.C.; POLIZEL, A.C.; BALARDIN, R.S.; VALE, F. X. R. Ferrugem da soja: epidemiologia e manejo para uma doença reemergente. **Rev. Anual Patol**. Planta v.13 p.351-395, 2005.

JULIATTI, B.C.M.; BELOTI, I.F.; PENA, L.C.; JULIATTI, F.C. Soybean genotype analysis for cyst nematode resistance. **Genetics and molecular research**, v. 16, p. 1-10, 2017.

KAWUKI, R. S.; TUKAMUHABWA, P.; ADIPALA, E. Soybean rust severity, rate of rust development, and tolerance as influenced by maturity period and season**. Crop Protection**, Guildford. v. 23, p. 447-455, 2004.

KIM, K. S.; WANG, T. C.; YANG, X. B. Simulation of apparent infection rate to predict severity of soybean rust using a fuzzy logic system. **Phytopathology**, v. 95, n. 10, p. 1122-1131, 2005.

KYEI-BOAHEN, S.; ZHANG, L. Early-maturing soybean in a wheat–soybean double-crop system: yield and net returns. **Agronomy Journal**, v. 98, n. 2, p. 295-301, 2006.

KLOSOWSKI, A. C.; BRAHM, L.; STAMMLER, G.; DE MIO, L. L. M. Competitive Fitness of *Phakopsora pachyrhizi* Isolates with Mutations in the CYP51 and CYTB Genes. **Phytopathology**, v. 106, n. 11, p. 1278-1284, 2016.

Levy, C. (2005). Epidemiology and chemical control of soybean rust in southern Africa. **Plant Disease**. p. 89, n.6, p. 669-674, 2005.

MARCHETTI, M. A.; UECKER, F. A.; BROMFIELD, K. R. Uredinial development of *Phakopsora pachyrhizi* in soybeans. **Journal of Phytopathology**, Lancaster, v.65, p.822-823, 1975.

MARTINS, J. A. S.; JULIATTI, F. C.; SANTOS, V. A.; POLIZEL, A. C.; JULIATTI, F. C. Período latente e uso da análise de componentes principais para caracterizar a resistência parcial à ferrugem da soja. **Summa Phytopathologica**, v. 33, n. 4, 2007.

MARTINS, J. A. S.; JULIATTI, F. C. Genetic control of partial resistance to Asian soybean rust. **Acta Scientiarum**. Agronomy, v.36, n. 1, p.11-17, 2014.

MCPHERSON, R. M.; BUSS, G.R.; ROBERTS P. M. Assessing stink bug resistance in soybean lines containing genes from germplasm IAC100. **J. Econ. Entomol**. V.100, p.1456-1463, 2007.

MENDIBURU, F.D. agricolae: Statistical Procedures for Agricultural Research. R Package Version 1.2-3, 2015.

MOHAPATRA, N. K., MUKHERJEE, A. K., SURIYA RAO, A. V., NAYAK, P. Disease progress curves in the rice blast pathosystem compared with the logistic and Gompertz models. **Journal of Agricultural and Biological Science**, v. 3, n. 1, p. 28-37, 2008.

OGLE, H. J.; BYTH, D. E.; MCLEAN, R. Effect of rust (*Phakopsora pachyrhizi*) on soybean yield and quality in south-eastern Queensland. **Aust. J. Agric**. Res. v. 30, p. 883-893, 1979.

PARLEVLIET, J. E. Present concepts in breeding for disease resistance. In: Brazilian Phytopathology Congress. **Fitopatologia Brasileira**, Brasília. v. 22, p. 7-15, 1997.

RAMALHO, M. A. P.; SANTOS, J. B.; ZIMMERMANN, M. J. O. **Genética quantitativa em plantas autógamas: aplicações ao melhoramento do feijoeiro**. Goiânia: UFG, 1993.

SANTOS, J.A.; JULIATTI, F.C.; SANTOS, V.A.; POLIZEL, A.C.; JULIATTI, F.C.; HAMAWAKI, O.T. Caracteres epidemiológicos e uso da análise de agrupamento para resistência parcial à ferrugem da soja. **Pesquisa Agropecuária Brasileira**, v.42, p.443-447, 2007.

SANTOS, J. V. M. Mapeamento de QTLs de características sob influência da ferrugem asiática da soja. Dissertação (Mestrado em Genética e Biologia Molecular) – Universidade Estadual de Londrina, Londrina. 107f, 2010.

SILVA, V. A. S.; JULIATTI, F. C.; SILVA, L. A. S. Interação entre resistência genética parcial e fungicidas no controle da ferrugem asiática da soja. **Pesquisa Agropecuária Brasileira**, Brasília, v.42, n.9, p.1261-1268, 2007.

SILVA, J. V. C., JULIATTI, F. C., DA SILVA, J. R. V., & BARROS, F. C. Soybean cultivar performance in the presence of soybean Asian rust, in relation to chemical control programs. **European journal of plant pathology**v. 131, n. 3, p. 409-418, 2011.

SINCLAIR, J.B.; BACKMAN, P.A. **Compendium of soybean diseases**. APS Press, Saint Paul, 1989.

TOLEDO, J. F. F. D.; CARVALHO, C. G. P. D.; ARIAS, C. A. A.; ALMEIDA, L. A. D.; BROGIN, R. L.; OLIVEIRA, M. F. D.; HIROMOTO, D. M. Genotype and environment interaction on soybean yield in Mato Grosso State, Brazil. Pesquisa **Agropecuária Brasileira**, v. 41, n. 5, p. 785-791, 2006.

Tschanz, A. T., Wang, T. C., & Tsai, B. Y. Recent advances in soybean rust research. In **International Symposium on Soybean in Tropical and Sub-tropical Cropping Systems***, 1983*.

TSCHANZ, A. T.; WANG, T. C. Soybean rust development and apparent infection rates at five locations in Taiwan. **Protection Ecology**, 2(3), 247-250, 1980.

TSCHANZ, A.T., TSAI, M.C.Evidence of tolerance to soybean rust in soybeans. Soybean Rust Newslett. V.6 n. 1, p. 28-31, 1983.

USDA (2017) Overview United States Department of Agriculture available at [http://wwwersusdagov/topics/crops/soybeans-oil-cropshtml] Accessed 20 October 2017.

VAN DER PLANK, J. E. **Plant diseases**. Elsevier Science, 1963.

VUORI, K.; STRANDÉN, I.; SEVÓN-AIMONEN, M. L.; MÄNTYSAARI, E. A. Estimation of non-linear growth models by linearization: a simulation study using a Gompertz function. **Genetics Selection Evolution**, v.38, n.4, p.343, 2006.

YANG, X. B., ROYER, M. H., TSCHANZ, A. T., & TSAI, B. Y. Analysis and quantification of soybean rust epidemics from seventy-three sequential planting experiments. **Phytopathology**, v.80, n.12, p.1421-1427,1990.

WANG T. C.; HARTMAN G. L. Epidemiology of soybean rust and breeding for host resistence. **PI. Prot** Bull. v. 34, p. 109-149, 1992.

SUPPLEMENTARY MATERIALS

Table S1 ANOVA of AUDPC in function of the three-way factorial: 7 genotypes x 5 temperatures (18 °C, 21 °C, 24 °C, 27 °C and 30 °C) x 4 wetting periods (0, 6, 12, 24) in Lavras - MG, 2017.

Source of Variation	DF	Sum of Square	Mean Square	F value	P value
Genotype	6	55096.07	9182.68	227.22	0.001
Temperature	4	744448.26	186112.06	4605.26	0.001
Wetting Period	3	118478.07	39492.69	977.23	0.001
Genotype*Temperature	24	63050.02	2627.08	65.01	0.001
Genotype*WP	18	19810.45	1100.58	27.23	0.001
Temperature*WP	12	106832.81	8902.73	220.29	0.001
Genotype*Temperature*WP	72	32862.66	456.42	11.29	0.001
Residue	420	16973.44	40.41		
Total	559	1157551.77			
		VC: 5.89%			

WP: Wetting Period; DF: Degrees of freedom

Table S2. ANOVA of Severity in function of the two-way factorial: 7 genotypes x 5 temperatures (18 °C, 21 °C, 24 °C, 27 °C and 30 °C) in Lavras - MG, 2017.

Source of Variation	DF	Sum of Square	Mean Square	F value	P value
Genotype	6	186.8	31.14	21.25	0.001
Temperature	4	2319.6	579.90	395.86	0.001
Genotype*Temperature	24	208.2	8.67	5.92	0.001
Residue	525	769.1	1.46		
Total	559	3483.7			
		VC: 51.56%			

DF: Degrees of freedom

Table S3 ANOVA of mean square and variance coefficient of : Photosynthetic activity (*A* - µ mol m^{-2} s^{-1}), transpiration (*E* - mmol m^{-2} s^{-1}), carbon intake (*Ci* - µmol mol^{-1}), stomatal conduc tance (g_s - mmol m^{-2} s^{-1}), water use efficiency (*WUE*) and *SPAD* evaluated at the V6 stage in Lavras - MG, 2017.

		Mean Square					
SV	**DF**	***A***	***E***	***Gs***	***Ci***	***WUE***	***SPAD***
Genotypes	6	40.12 *	1.23*	0.16 *	1738.34 *	1.48 *	22.81 ^{ns}
Block	3	4.12 ^{ns}	0.17 ^{ns}	0.02 ^{ns}	118.13 ^{ns}	0.20 ^{ns}	23.35 ^{ns}
Residue	18	11.34	0.07	0.01	241.37	0.25	11.62

VC: %	-	17.9	5.34	13.69	4.44	13.44	9.50

ns Not significant; * Significant at 5% of probability; DF: Degrees of freedom

Table S4 ANOVA of mean square and variance coefficient of : Photosynthetic activity (*A* - µ mol m^{-2} s^{-1}), transpiration (*E* - mmol m^{-2} s^{-1}), carbon intake (*Ci* - µmol mol^{-1}), stomatal conduc tance (Gs - mmol m^{-2} s^{-1}), water use efficiency (*WUE*) and *SPAD* evaluated at the R4 stage in Lavras - MG, 2017.

		Mean Square					
SV	**DF**	***A***	***E***	***Gs***	***Ci***	***WUE***	***SPAD***
Genotypes	6	86.02 *	11.73 *	0.24 *	693.18 *	1.98 *	34.11 *
Block	3	11.55 ^{ns}	3.34 ^{ns}	0.07 ^{ns}	313.26 ^{ns}	0.33 ^{ns}	5.87 ^{ns}
Residue	18	19.51	2.67	0.06	183.71	0.26	0.79
VC: %	-	25.78	24.75	34.68	4.32	18.89	2.39

ns Not significant; * Significant at 5% of probability; DF: Degrees of freedom

Table S5 ANOVA of POX activity in function of the two-way factorial: 7 genotypes x 5 hours after inoculation (0, 24, 48, 72, 96 hours) in Lavras - MG, 2017.

Source of Variation	DF	Sum of Square	Mean Square	F value	P value
Genotype	6	5527411	921235	11.84	0.0001
Block	3	1057393	352464	4.53	0.005
Hai	4	1159737	289934	3.72	0.007
Genotype*hai	24	7107534	296147	3.80	0.0001
Residue	102	7935669	77801		
Total	139	22787744			
VC: 22.2 %					

Hai: hours after inoculation

Table S6 ANOVA of PAL activity in function of the two-way factorial: 7 genotypes x 5 hours after inoculation (0, 24, 48, 72, 96 hours) in Lavras - MG, 2017.

Source of Variation	DF	Sum of Square	Mean Square	F value	P value
Genotype	6	235.50	39.25	6.77	0.0001
Block	3	67.82	22.61	3.90	0.01
Hai	4	1405.40	351.35	60.68	0.0001
Genotype*hai	24	684.88	28.54	4.93	0.0001
Residue	102	590.60	5.79		
Total	139	2984.19			
VC: 51.4 %					

Hai: hours after inoculation

Table S7 ANOVA of mean square and variance coefficient of : Cell Wall Thickness (µm) and Lignin (0 and 96 hai) at the V6 and R1 stages in Lavras - MG, 2017.

		Mean Square		
SV	**DF**	Cell Wall	Lignin – 0 hai	Lignin – 96 hai
Genotypes	6	0.56 *	36.85 *	10.25 ^{ns}
Block	9	0.02 ^{ns}	2.34 ^{ns}	7.91 ^{ns}
Residue	54	0.02	1.67	7.20
VC: %	-	23.83	10.6	21.57

ns Not significant; * Significant at 5% of probability; DF: Degrees of freedom

Table S8 ANOVA of mean square and variance coefficient of : Area under disease progress curve (AUDPC), apparent infection rates (r) and Defoliation (%) in the assays with Split-plot design made in Lavras - MG, 2016.

		Mean Square		
SV	**DF**	AUDPC	r	Defoliation
Genotypes	6	21172 *	0.000470 ***	3127.8 ***
Block	9	8284 ^{ns}	0.000007 ^{ns}	98.1 ^{ns}
Residue a	18	6779	0.000007	212
Split-plot (S/WS)	1	236730 ***	0.001037 ***	21804 ***
Genotypes*Split-plot	6	4967 *	0.000031	1900 ***
Residue b	21	1837	0.000013	116
VC: Plot %	-	56.16	10.33	41.29
VC: Split-plot %	-	29.23	14.07	27.49

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

DF: Degrees of freedom

Table S9 ANOVA of mean square and variance coefficient of : Area under disease progress c urve (AUDPC), apparent infection rates (r) and Defoliation (%) in the assays made with Split-plot design in Lavras - MG, 2017.

		Mean Square		
SV	**DF**	AUDPC	r	Defoliation
Genotypes	6	108793 ***	0.002142 ***	5941.1 ***
Block	9	273 ^{ns}	0.000013 ^{ns}	36.3 ^{ns}
Residue a	18	3300	0.000014	46.0
Split-plot (S/WS)	1	551632 ***	0.001705 ***	578.6 ***
Genotypes*Split-plot	6	19504 **	0.000103 ***	107.7 ***
Residue b	21	3766	0.000014	1.2
VC: Plot %	-	20.02	8.12	19.09
VC: Split-plot %	-	21.39	8.15	3.07

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

DF: Degrees of freedom

Table S10 ANOVA of mean square and variance coefficient of : Average leaf area (ALA - c m²) and average leaf number (ALN) with Split-plot Design in Lavras - MG, 2016.

		Mean Square	
SV	**DF**	ALA	ALN
Genotypes	6	58.93 ***	101.58 ^{ns}
Block	9	16.73 **	318.48 **
Residue a	18	2.80	46.20
Split-plot (S/WS)	1	171.25 ***	3.50 ^{ns}
Genotypes*Split-plot	6	4.60 ^{ns}	44 ^{ns}
Residue b	21	3.69	113.54
VC: Plot %	-	14.36	39.28
VC: Split-plot %	-	16.48	61.57

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

DF: Degrees of freedom

Table S11 ANOVA of mean square and variance coefficient of : Average leaf area (ALA - c m^2) and average leaf number (ALN) with Split-plot Design in Lavras - MG, 2017.

		Mean Square	
SV	**DF**	ALA	ALN
Genotypes	6	58.93 ***	969.22 ***
Block	9	16.73 **	0.76 ^{ns}
Residue a	18	2.80	14.42
Split-plot (S/WS)	1	171.25 ***	994.49 ***
Genotypes*Split-plot	6	4.60 ^{ns}	198.26 ***
Residue b	21	3.69	0.69
VC: Plot %	-	14.36	9.36
VC: Split-plot %	-	16.48	2.04

ns Not significant; *** Significant at 0.1% of probability; ** Significant at 1% of probability; * Significant at 5% of probability
DF: Degrees of freedom