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# Chapter

# Etiology, Epidemiology and Management of Asian Soybean Rust (ASR) in Brazil and Vulnerability of Chemical Control of Specific without Multisite Fungicides

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# Abstract

Brazil is the first soybean producer in the world, and the largest exporter. In the 2019/20 harvest, the country produced about 124.85 million tons, representing 30% of world production. Global soy production for 2019/20 reached 337.9 million tons. Asian soybean rust (ASR) is the most pathogen on soybean in Brazil in nowadays. Target spot and Septoria leaf spot plus white mold complete these scenarios. ASR emerged in Brazil in 1979. The use of fungicides in the soybean crop in Brazil intensified after the master of 2002 with the resurgence of soybean rust, where the use of triazoles intensified. The massive sprays to pathogen control reached 3.5 sprays per season. In 2006, the first reports of loss of sensitivity of the fungus to the group appeared, notably for the fungicide flutriafol and tebuconazole used in many situations in a curative way or to eradicate the fungus. From that moment on, the productive system sought to use triazoles and strobilurins. In 2011 came the first reports of loss of sensitivity of the fungus in the group of strobilurins. This fact was due to the use of pyraclostrobin in the vegetative phase of soybeans without protection by multisite. That same year, the introduction of the active ingredients in copper oxychloride, mancozeb and chlorothalonil took place in Brazil. In 2015, the first carboxamides ((benzovindiflupyr) (solatenol and fluxpyroxade) associated in triple mode with triazoles and strobilurins were launched on the Brazilian market. Due to the specific mode of action in the metabolism of the fungus (biosynthesis of ergosterol (triazoles), mitochondrial respiration in the cytochrome oxidase enzyme complex - QOIs (strobilurins) and succin dehydrogenase - SDHIs (carboxamides), the need for their association in the sprayings was seen. To multisite (cuprics, dithiocarbamates and nitriles). For the sustainable management of the disease in Brazil, control strategies are recommended, such as the use of systemic fungicides, with a specific biochemical mechanism of action with the adoption of tank mix with multisite, adoption of cultural practices (sanitary emptiness) and sowing schedule and the use of varieties with quantitative resistance (partial or horizontal resistance). These measures will guarantee the sustainability of the culture and the useful life of systemic fungicides or specific sites.

**Keywords:** soybean rust, specific fungicides, multisite, management, spraying, host resistance.

## 1. Introduction

Soybean [Glycine max (L.) Merrill] is one of the ten most economically important crops worldwide, as it is one of the main sources of protein concentrates and vegetable oil [1]. Brazil is the first soybean producer in the world, and the largest exporter [2]. In the 2019/20 harvest, the country produced about 124.85 million tons, representing 30% of world production [3]. Global soy production for 2019/20 reached 337.9 million tons [4]. The potential of the soybean crop yield can be affected by several factors, among which stand out the soil fertility, water availability during the harvest, the plant population, the sowing time, the productive potential of the cultivar, the occurrence invasive plants, pest infestation and diseases [5–8]. With the increasing incidence and severity of leaf spots in soybean crops [9, 10] in the last harvests greater attention has been paid to multiple soybean epidemics involving brown spot (Septoria glycines), target spot (Corynespora *cassiicola*) and rust itself [9–11]. In this review is appointed the symptoms, etiology and epidemiology of the disease. The calendar system to sowing in Mato Grosso state to seeds production. The use of fungicides in the soybean crop in Brazil intensified after the master of 2002 with the resurgence of soybean rust, where the use of triazoles intensified. The massive sprays to pathogen control reached 3.5 sprays per season. In 2006, the first reports of loss of sensitivity of the fungus to the group appeared, notably for the fungicide flutriafol and tebuconazole used in many situations in a curative way or to eradicate the fungus. From that moment on, the productive system sought to use triazoles and strobilurins. In 2011 came the first reports of loss of sensitivity of the fungus in the group of strobilurins [8]. This fact was due to the use of pyraclostrobin in the vegetative phase of soybeans without protection by multisite. That same year, the introduction of the active ingredients in copper oxychloride, mancozeb and chlorothalonil took place in Brazil. In 2015, the first carboxamides (solatenol and fluxpyroxade) associated in triple mode with triazoles and strobilurins were launched on the Brazilian market. Due to the specific mode of action in the metabolism of the fungus (biosynthesis of ergosterol (triazoles), mitochondrial respiration in the cytochrome oxidase enzyme complex QOIs (strobilurins) and succin dehydrogenase - SDHIs (carboxamides), the needfor their association in the sprayings was seen. to multisite (cuprics, dithiocarbamates and nitriles). For the sustainable management of the disease in Brazil, control strategies are recommended, such as the use of systemic fungicides, with a specific biochemical mechanism of action with the adoption of tank mix with multisite, adoption of cultural practices (sanitary emptiness) and sowing schedule and the use of varieties with quantitative resistance (partial or horizontal resistance). These measures will guarantee the sustainability of the culture and the useful life of systemic fungicides or specific sites [12].

### 1.1 Damage caused by Asian soybean rust (ASR)

The basic cycle of soybean rust is characterized by the phases of pathogen-host relationships in a few days when it does not show partial or horizontal resistance. Genotypes that are among the most active in Brazil, such as BMX Desafio, Foco, Bonus, and others exhibit this behavior, that is, latency between 7 and 10 days. Genotypes with horizontal and/or conventional resistance such as BRS Conquista, IAC 100 that are no longer cultivated have a latent period of more than 20 days [13–15]. For

these genotypes with horizontal or partial resistance, there is greater flexibility in the use of fungicides and or an increase in the range of applications, facilitating programs for the management or monitoring of the pathogen. One of the milestones and systemic action to understand the etiology, epidemiology and control of rust soybean was to hold the 1st Brazilian Workshop on Soybean Rust [16] uniting the entire production chain and companies in an attempt to understand the dynamics of the various interactions in Brazilian biomes and soybean production. Later the first English version was published [17], with the contributions of those involved, in their respective specialties.

### 1.2 Losses

Among the diseases that occur in soybean crops, Asian soybean rust (FAS), caused by the fungus Phakopsora pachyrhizi Sydow, is considered to have the greatest destructive potential, and may cause damage ranging from 10 to 90% in the various geographic regions where it was reported [8, 11, 18–20]. Accounting for the losses related to the decrease in soybean productivity, expenses with chemical control (fungicides and application expenses) and the impact on tax revenue until the 2019/2020 harvest, after its resurgence, rust represented accumulated losses of more than US \$ 30 billion [21], a number that exceeds the foreign currency collected by Brazil in soybean exports from 2019 to 2020 [22]. In Brazil, damage attributed to Asian rust of 70% in the 2001/2002 harvest was reported [23–25]. In the same harvest in the region of Chapadão do Sul, MS, damage of up to 100% was recorded [26, 27]. Losses in the 2002–2003 crop reached almost one billion dollars [11, 28]. According to Juliatti et al. [11, 29–31], rust has reappeared in Brazil, with frequent epidemics, due to the use of benzimidazoles fungicides only to control end-of-cycle diseases, increasing the area cultivated in several biomes and varied sowing time allowing the fungus to survive, as well as the presence of green bridges. The attacked plants undergo defoliation and early maturation, in relation to plants not infected by rust, which causes a reduction in the weight and quality of the grains produced [18]. Currently, due to the limited availability of resistant cultivars, adapted to different cultivation regions, the application of fungicides is the main control tool adopted by farmers. As a result, some populations of the pathogen have already shown an increased reduction in sensitivity to certain fungicides [32–34]. Given this scenario, the search for resistant cultivars is fundamental as part of a long-term sustainable control strategy [35]. However, until now, it has not been possible to find varieties with long-lasting rust. There are promising studies for the use of smaller genes for resistance leading to or allowing the use of them and the reduction of fungicide control in the future [15, 36, 37].

# 2. Chronology of the disease

Asian rust was first described in Japan, in 1902 [38], and in 1914, it had already spread to several countries in Southeast Asia. On the African continent it was first registered in Togo, in 1980 [39], then in Uganda, in 1996 [40], followed in 1998 in Kenya and Rwanda [41], Zimbabwe and Zambia [42]. In 2001, it was found in South Africa and Nigeria [43], Missiones, Argentina in 2003 [44] reaching an epidemic character [45]. In 2007, rust was also reported in Ghana. In the American continent, it was first reported in 1976 in Puerto Rico [46], followed by Hawaii in 1994 [47]. The first report in South America in Brazil was made by Deslandes [48], in the south of the State of Minas Gerais. At that time the mycologist prof. Deslandes detected both American rust (*P. meibomiae*) and Asian soybean rust (*P. pachyrhizi*) [49] (**Figure 1**) in soybean plantations and in semi-perennial



### Figure 1.

Detection of soybean rust (A) in Brazil (Phakopsora pachyrhizi and P. meibomiae by Deslandes (right) in 1979). Photo from Fernando C. Juliatti's personal file, kindly provided by J.T. Yorinori("in memorian"). (B) Epidemics of soybean rust in late sowing. Personal archives Fernando C. Juliatti. (C).

legumes [49]. The authors, via molecular comparison with specific primers for the two species, confirmed the studies and the been reported in Bolivia [50] and Colombia [51], progressing in 2004 to Uruguay [52] and 2005, in Ecuador [53], Mexico in 2009 [54]) and the United States [55]. Currently, Asian soybean rust (FAS) is present in all countries, where soybean is cultivated. Its spread was rapid throughout the world, due to the fungus's urediniospores being disseminated by wind currents [5, 41, 56–60].

The **Figure 1** shows infection of the pathogen in cotyledons, as happened in Primavera do Leste, MT, in 2004 due to frequent infections in irrigated plantations under central pivot and consecutive sowing times. In nowadays is common near the urban cities around the 163 highway to see "guaxas' plants" (volunteers plants) of soybean with uredines and spores after summer crops in Mato Grosso state (**Figure 2**).

## 3. Host of the pathogen

The causative agent of FAS is a biotrophic fungus, which survives on green soybeans or other hosts. Hartman et al. [61] report that, unlike other rusts,



Figure 2.

Detection of soybean rust (A) in Brazil (Phakopsora pachyrhizi) in guaxas (volunteers' plants) soybeans plants in highway 163 around the cities in the Mato Grosso state. Personal archives from Fernando C. Juliatti.

P. pachyrhizi can aturally infect a ide range of plant species, including 41 species in 17 genera of the baceaefamily. In ddition, 60 plant species belonging to 26 genera were experimentally infected under controlled conditions [62], reaching up to 90 species [63]. The fungus can also infect and sporulate in species belonging to the subfamily Papilionoideae, family Fabaceae having as main hosts, the species Glycine max, G. oyae, Pachyrhizus erosus, Pueraria lobata and Vigna unguiculata More eferences are found in the literature regarding *P. pachyrhizi* hosts, among which the following stand out: Phaseolus vulgaris var. vulgaris, Canavalia gladiate [63, 64], Phaseolus vulgaris [65], Vicia faba, Vigna radiata, V. mungo, Psophocarpus tetragonolobus, Colopogonium muconoides [64], Alysicarpus vaginalis, Securigera varia, Melilotus officinalis, Trifolium repens, T. incarnatum, [62], Phaseolus lunatus var. lunatus, Sesbania exaltata, Trigonella foenum-graicum [5, 6], Phaseolus coccineus [66], Pachyrhizus ahipa, Cajanus cajan [67], Crotalaria anagyroides, C. spectabilis, Macropureum [68]), Pueraria amontana var. lobata [69, 70], Lespedeza cuneata, Kummerowia striata, K. stipulaceae, Pisum sativum, [71], Lipinus albus, L. angustifolius, L. luteus, Lotus spp. [68], among others. In 2015, the pathogen was found in 'jacatupe' (Pachyrhizus erosus), near a soybean field in Federal University of Viçosa, Minas Gerais State (MG), Brazil (Zambolim, 2015, unpublished data).

# 4. Symptoms of the disease

The symptoms caused by FAS, in its initial state, are easily confused with other diseases, such as bacterial pustule (*Xanthomonas axonopodes* pv. *glycines*), bacterial blight (*Pseudomonas savastanoi* pv. *glycinea*) and brown spot (*Septoria glycines*). The

fructifications of the causal agents are not very evident, so that with the naked eye, it is difficult to distinguish ferruginous pustules, which give the common name, to this group of diseases [11, 51]. The same author reports that the symptoms caused by soybean rust are called "lesions", and not pustules, like other rusts, because leaf tissue necrosis occurs and each lesion can present several pustules. Symptoms can appear at any stage of development and in different parts of the plant, such as cotyledons, leaves and stems, with leaf symptoms being the most characteristic [11, 72]. The color of the lesions varies from greenish gray to reddish-brown, with one or more globular uredines, mainly in the abaxial part of the leaf [11, 61]. Sporadically uredines may appear at the top of them [72]. The number of lesions and uredines on a leaf (abaxial part) can reach from 26 to 46 per cm2 under controlled conditions. The initial manifestation of the disease is observed as chlorotic leaf areas in a polygonal shape, because of the delimitation imposed by the veins, which may reach a size of 2–5 mm2 [11, 51]. The first lesions, in general, are found in the low leaves close to the soil, when the plants are in the phenological stage, near or after flowering [11, 51]. Progressively, the uredines acquire a light brown to dark brown color, which open in a tiny pore, expelling the urediniospores (Figures 1 and 2), hyaline in color that become beige and accumulate around the pores or are removed by the wind [11, 72]. As the sporulation proceeds, the leaf tissue around the first uredines acquires a light brown color, called a susceptible lesion or TAN (tanish) and the other reddish brown, known as a resistant lesion or RB (redish-brown). [6, 11, 72–74]. The final stage of the soybean rust epidemic in a crop is characterized by general yellowing of the foliage, with intense defoliation, reaching the complete fall of the leaves [11, 51].

# 5. Causal agent of the disease

Taxonomically the fungus is classified as follows: Kingdom: Fungi; Class: Basid iomycetes; Order: Uredinales; Family: Phakopsoraceae; Current name: *Phakopsora pachyrhizi* Sydow and Sydow; Synonyms: Phakopsoraoyae Fujikuro; *Phakopsora calothea* H. Sydow; Malupaoyae (P. Hennings) Ono, Buritica, and Hennen comb. 06 nov. (Anamorph) Uredooyae P. Hennings [75].

### 6. Conditions that favor the disease

The fungus has a short life cycle, under the following conditions: Fine and frequent rains, long periods of dew and temperatures between 15 and 29° C. FAS epidemics can quickly increase from almost undetectable levels to an extremely high incidence, severity and prevalence. Spore production can last at least three weeks [76–78]. The rapid development of the disease has been correlated with canopy closure at the flowering stage (R1+) [76, 79–81]. For this reason, FAS causes a high defoliation rate of the crop, and continues until the environment is no longer conducive to the development of the disease [11, 28, 82]. Then, FAS progresses until there is complete defoliation of the canopy, or until the environment is no longer conducive to the development of the disease [11, 82]. According Bromfield [5, 6], flowering infection can produce high levels of damage, compromising the formation and filling of pods, the final weight of the grains [11, 83], affecting the oil and protein content [59]. After infection, the fungus produces uredinia and urediniospores between seven and 14 days, according to environmental conditions [76, 80]. The symptoms are grouped into staining lesions from 2 to 5 mm in

diameter, with two to five uredines. and abundant sporulation or formation of reddish lesions, with zero to two uredines and sparse sporulation [5, 6].

### 7. Disease control

The control of FAS must be integrated, as an isolated measure will not result in success, as it is an extremely aggressive pathogen. To reduce the risk of rust damage to the crop, the following management strategies in Brazil are recommended: use of early cycle cultivars, sowing at the beginning of the recommended season, the elimination of voluntary soy plants and the absence of soy cultivation in the off season through the sanitary vacuum (60 to 90 days), monitoring the crop since the beginning of crop development, the use of fungicides in the appearance of symptoms or preventively and the use of resistant cultivars, when available [84]. Therefore, the first step in the control of FAS is based on the adoption of cultural measures and genetic resistance [85, 86] including quantitative resistance and tolerant varieties [82]. Therefore, the solution to control Asian soybean rust is in the integration of methods, with emphasis on cultural, genetic and chemical. Monitoring the disease in soybean crops is of great importance. In studies carried out by the rust consortium coordinated by Embrapa Soybean, it was found that, in at least 20 to 30% of the country's planting areas, the disease did not reach a level of control, due to climatic variations [87-89]. The recommendation of chemical control should always be preventive, since the application after the onset of the disease resulted in reduced productivity [90]. The preventive concept, although vague, should be based on spore collection systems (spore-hunters), sown plots ("sentinels"), at least 30 days before sowing in the region [56, 91] and automatic stations that monitor the climate and favorable conditions (temperature and leaf wetness), the occurrence of the pathogen and disease. In addition, it is essential to use an efficient application technology, as well as the choice of correct spray tips, for each situation [11, 92].

# 8. Cultural control

One of the indications for cultural control is to increase the area of crop rotation, because where this measure is practiced for a year, with grasses (corn, sorghum and rice), in place of safrinha soybeans, it has facilitated the control of rust [20, 93–97], as it favors the management of voluntary plants. The presence of "tiguera" or voluntary soybeans inside cotton fields, in the states that sow cotton, after soybeans is a sign of the perpetuation of inoculum from one crop to another since the cotton is harvested in June. Voluntary soy that is on the side of highways in Brazil and in urban areas must also be eliminated, for the reduction of inoculum, for the next harvest. Such fungicides are fundamental in the management of soybean rust fungus resistance. In this case, it reduces the directional pressure on resistant or less sensitive populations to specific sites (triazoles, strobilurins and carboxamides) [11, 33, 98–101]. In this way, stabilizing selection is practiced in the field, preventing the emergence of new pathotypes or breeds of the fungus. Even silicate fertilizers via soil or foliar [102], assist in the management of resistance to systemic or penetrating and mobile fungicides [103]. The use of suitable cultivars at each sowing time is essential for the success and sustainability of control programs [104].

The use of early-cycle cultivars decreases the time of exposure of the plant to the pathogen, as does sowing at preferred times, avoiding late sowing [51]. The load or potential of inoculum is greater due to the multiplication of the fungus, in the first crops [20, 60, 96]. The population arrangement of plants can also contribute to

mitigate rust. Ramos et al. [105] observed that greater spacing between cultivation lines results in less rust severity, and also allows better distribution of the fungicide, during application, facilitating the management of the disease. If the sowing density is high, in smaller spacing and below 0.5 m, there will be difficulty in penetrating the syrup and, consequently, there may be poor coverage of the leaves in the canopy. In this case, the control of the disease will be deficient, even if an efficient fungicide is adopted [28, 88, 97, 106–115].

In addition to these, the most important is the absence of soybean cultivation in the off-season through the sanitary vacuum (60 to 90 days). Considering the high destructive potential of phytopathogen and disease [17], several states have adopted the "sanitary vacuum", a period of 60 to 90 days without soy plants, in the field, as a strategy to reduce the amount of inoculum, in the off-season, delaying the onset of the disease during the summer harvest. Therefore, it becomes an extremely necessary condition, that cultural measures be practiced, to increase the useful scope of the fungicides of the triazole, strobilurin and carboxamide groups. However, any fungicide control program must be adapted to the host's genotype and also includes the appropriate use of fungicides and monitoring of the pathogen [104, 115]. The adoption of multisite fungicides (cupric, mancozeb and chlorotalonil) [33, 88, 98, 99, 106–114] (Figure 3) in Brazil has become if a fundamental tool in the integrated management of the disease, in that case they can be sprayed associated with specific sites or interspersed without limitations of spray tips that suit the condition of the crop and canopy of the crop [92]. At the beginning, a research network was structured with the company UPL (Uniphos Phosphourus Limited), which provided the



#### Figure 3.

Effect of doses of Epoxiconazole with Pyraclostrobin mixtured with multi-site fungicides on the ASR control. Source: Ponce et al. [90]. 1. Control; 2. Epoxiconazole + Pyraclostrobin (33.2 + 10.2 g a.i./ha) associated with Mancozeb (2400 g a.i./ha); 3. Epoxiconazole + Pyraclostrobin (33.2 + 10.2 g a.i./ha) associated with Chlorothalonil (1500 g a.i./ha); 4. Epoxiconazole + Pyraclostrobin (33.2 + 10.2 g a.i./ha) associated with Metiram + Pyraclostrobin (1100 + 100 g a.i./ha); 5. Epoxiconazole + Pyraclostrobin (33.2 + 10.2 g a.i./ha) associated with Metoram + Pyraclostrobin (1100 + 100 g a.i./ha); 5. Epoxiconazole + Pyraclostrobin (33.2 + 10.2 g a.i./ha) associated with Mancozeb (2400 g a.i./ha); 6. Epoxiconazole + Pyraclostrobin (33.2 + 10.2 g a.i. associated)with ha); 7. Epoxiconazole + Pyraclostrobin (33.2 + 10.2 g a.i./ha) associated with Metiram + Pyraclostrobin (1100 + 100 g. a.i./ha); 8. Epoxiconazole + Pyraclostrobin (66.5 + 20.5 g a.i./ha) associated with Mancozeb (2400 g. a.i./ha); 9. Epoxiconazole + Pyraclostrobin (133.0 + 41.0 g a.i./ha) + Chlorothalonil (1500 g. a.i./ha); 10. Epoxiconazole + Pyraclostrobin (66.5 + 20.5 g a.i./ha) + Chlorothalonil (1500 g. a.i./ha); 10. Epoxiconazole + Pyraclostrobin (66.5 + 20.5 g a.i./ha) + Chlorothalonil (1500 g. a.i./ha); 10. Epoxiconazole + Pyraclostrobin (66.5 + 20.5 g a.i./ha) + Chlorothalonil (1500 g. a.i./ha); 10. Epoxiconazole + Pyraclostrobin (66.5 + 20.5 g a.i./ha) + Chlorothalonil (1500 g. a.i./ha); 10. Epoxiconazole + Pyraclostrobin (66.5 + 20.5 g a.i./ha) + Chlorothalonil (1500 g. a.i./ha); 10. Epoxiconazole + Pyraclostrobin (66.5 + 20.5 g a.i./ha) + Chlorothalonil (1500 g. a.i./ha); 10. Epoxiconazole + Pyraclostrobin (66.5 + 20.5 g a.i./ha) + Chlorothalonil (1500 g. a.i./ha); 10. Epoxiconazole + Pyraclostrobin (66.5 + 20.5 g a.i./ha) + Chlorothalonil (1500 g. a.i./ha); 10. E

researchers with the first tests, which determined the first use of mancozeb, at an experimental level in the region of Uberaba - MG, still in the form of wettable powder, in doses of 1 at 3.0 Kg per ha, in the 2011–2012 and 2012–2013 harvests. In this first experiment, the efficiency of dithiocarbamate in substitution to strobilurins was characterized [116].

The presence of voluntary plants at the beginning of the harvest, favor the maintenance of the inoculum (spores) in the field, and they must be desiccated. In Paraguay and southern Brazil (Paraná and Santa Catarina), in addition to soy, the kudzu legume (Pueraria lobata) is an important source of inoculum, as it is a perennial plant, widely established and highly susceptible to rust. Attention is also drawn to the soybean sown in Bolivia in the winter (high levels of water in the soil) and summer in the Santa Cruz de La Sierra region, which perpetuate the inoculum that can be sent to Brazil via air currents, in several months of the year and thus reaching crops in Mato Grosso, Brazil (Juliatti, unpublished data). Another management measure that can be adopted is the sowing schedule, in relation to the crop cycle. Early varieties spend less time in the field, are harvested earlier and, thus, can "escape" the disease or have lower final severities. As in most regions, there is no soy in the winter, in the first sowing, the fungus is not yet present in the field; the tendency is for the inoculum to increase with the advance of the harvest. Thus, earlier sowing also has an escape mechanism regarding the concentration of the inoculum of the fungus in the canopy of plants. Other measures that can result in improved management and control efficiency are:

a. use earlier cultivars, sown at the beginning of the recommended season, for each region, and thus avoid prolonging the sowing period, to escape the greater concentration of the inoculum,

b. monitoring of crops, especially in crop sites subject to high soil moisture, c. observes the temperature conditions (15–28°C) and the wetting period above 6 hours, which are favorable to infection. However, the ideal way to control rust is through genetic resistance. However, until now even though there are resistant cultivars available commercially, using the RPPs genes, for example the "Inox Technology" [117] and others RPPs genes [85, 118–120], this resistance is easily overcome in the field, also requiring protection with fungicides and often the genetic materials already launched have less potential. Productive than the susceptible ones, of the same cycle and maturation group. When the disease is already occurring in the field, chemical control with fungicide is, until now, the main measure. The strategy that has been used most often, is the monitoring of the disease, aiming at the initial inoculum (Xo) that reaches the culture, to support the chemical control. The decision on the time of application, should be based on the factors necessary For the appearance of rust (presence of the fungus in the region, age of the plants And favorable climatic condition), the application logistics (availability of equipment and size of the property), the presence of other diseases and the cost of control. The delay in application, after the initial symptoms are observed, can lead to a reduction in productivity, if climatic conditions favor the progress of the disease. The number and the need for reapplications will be determined by the stage in which the disease is identified in the crop, and by the residual period of the products.

### 9. Genetic resistance

The most efficient method of controlling plant disease is the cultivation of resistant varieties. The use of genetic resistance to soybean rust, is one of the most effective strategies in the long term, to control the disease [121]. However, already

in the 1970s, Van der Plank [122] pointed out the difficulty of finding materials with vertical resistance to rust. In addition, the few materials classified as resistant, had vertical or monogenic resistance [121], lacking horizontal or polygenic resistance, due to the genetic variability of the causal agents. *Phakopsora pachyrhizi* is a pathogen that has high genetic variability. For a long time in Asia, countries have used five sources of genetic resistance in soybean improvement programs, aiming to control rust: Rpp1 [123]; Rpp2 [124]; Rpp3 [6, 125]; Rpp4 [85, 126] and Rpp5 [120]. Genetic and molecular analyzes suggest several alleles or linked genes that govern soybean rust resistance [120]. Zambenedetti et al. [127] studying the genotypes PI 230970 and PI 459025, found that they had a low percentage of germinated urediniospores and appressoria formation. In these genotypes, the formation of appressorium began six hours after inoculation, whereas in the other cultivars occurred four hours after inoculation. Such genotypes may have resistance genes Rpp2 and Rpp4, respectively [85, 123, 126]. Another study by Pham et al. [128] in the evaluation of the known sources of resistance (Rpp1 to Rpp4), to three isolates of Phakopsora pachyrhizi, found that the cultivars PI 200492 (Rpp1) and PI462312 (Rpp3), produced differential response. While PI230970 (Rpp2) and PI459025B (Rpp4) produced susceptible lesions (TAN). Koga et al. [129] evaluating the components of resistance to FAS in 48 cultivars, selected some groups of genotypes, which can be considered promising, as sources of genes of resistance to the disease. Recently, a study was carried out on the reaction of several soybean genotypes to three populations of Phakopsora pachyrhizi, one from Japan and two from Brazil, finding only two resistance genes, being Rpp4, present in the PI 459025 variety, and whether such cultivars are maintained, with adequate levels for quantitative resistance. As mentioned earlier, these genotypes are dependent on fungicides, such as 03 susceptible ones. They require the same care, with gains in application or relaxation 04 intervals and lesser concerns in prolonged rainy periods, where spraying is not possible.

Now, there is not a worldwide variety that has absolute resistance to the disease, that is, a variety with complete resistance. What is understood as an almost impossible task in breeding programs. In the absence of this type of resistance, researchers from abroad and Brazil are looking for cultivars with partial resistance or that have some level of quantitative resistance [13-15, 35, 37, 130]. Strains with partial resistance to rust can be selected from segregating populations, developing under conditions of greater rust severity [131]. The selection by productivity can also be used, in different breeding programs in non-segregating strains, under high severity of the pathogen. Such cultivars with partial resistance and/or tolerance, may require less application of fungicides and, thus, reduce the cost of disease control. According to Silva et al. [121, 132], partial resistance allows the establishment of the commercial use of fungicides allowing stability in the production system. Furthermore, it is possible to use alternative control methods such as foliar silicates based on calcium or magnesium, in fungicide programs [102]. Cultivars with partial, quantitative, or horizontal resistance, will allow adequate rust control, but only with atomization of the plants. Therefore, if there are no cultivars with complete resistance, to be used commercially, chemical control becomes the alternative, more effective in the control of Asian soybean rust combined with cultural practices [58].

Rpp5 in the Shiranui variety which conferred resistance to three populations of the fungus [133]. The Rpps genes for resistance to the fungus *P. pachyrhizi* (Rpp) have been found and described in countless soybean accessions and at least 10 Rpps, genes have been found, or alleles have been mapped in seven gene loci [134]. Obtaining a soybean cultivar resistant to Asian rust has been a challenge for research.

The existence of a large number of pathotypes, makes control difficult through vertical resistance. Dominant genes for resistance, called Rpp1 to Rpp4, identified in plant introductions (PI's) reported in the literature [6, 126] have doubtful stability, due to the great variability of the pathogen. Eighteen pathotypes were identified, in samples collected from soybean plants, and wild hosts in Japan [135]. Studies carried out in Taiwan, showed the existence of at least one patotype, containing three virulence genes [136]. In Brazil, studies carried out by Embrapa Soybean, Brazil, have identified 11 cultivars with resistance to rust [95]; however, this resistance was quickly superseded, with isolates of the fungus from the state of Mato Grosso. Of the four sources of resistance already described in the literature, only those with the Rpp2 and Rpp4 genes remained resistant to rust in Brazil, until the year 2004 [118]. Today, these genes no longer provide vertical resistance to the phytopathogen. Martins and Juliatti [35] determined that partial resistance in IAC 100 can be governed by more than a dozen smaller genes depending on the cross or parent lineage involved. Other RPPs genes have been described in the world and added to germplasm banks for future use and manipulation in breeding programs. Only time will tell about their stability.

Another type of resistance to be explored is the horizontal one, which involves a reduction in the rate of development of the disease, being more effective against agreater number of races of the pathogen. However, the quantification of this type of resistance is more difficult, thus limiting its use [137]. After 20 years of working with smaller and allele genes from the IAC 100 cultivar [14, 15, 35, 130, 131], the authors report success in the generation of soybean strains adapted to various cultivation regions in Brazil, in different maturation groups. Due to the instability of vertical strength and the difficulties associated with the identification and quantification of horizontal strength, other methods have been used, aiming to avoid reductions in productivity caused by rust., highlighting the tolerance of cultivars. A review was presented on the control of soybean rust and studies on the management of the disease and the use of Rpps genes [138]. The authors demonstrated the leadership of the USA, followed by Brazil, Germany, Japan and Argentina in the study of the pathosystems.

Tolerance can be defined as the capacity of the plant to support the development of the pathogen, without showing a significant reduction, in productivity or product quality [139]. Losses in productivity are generally used to assess the level of tolerance and therefore, the lower the loss, the greater the level of tolerance. Losses can be quantified by the difference in productivity between severely infected plots and plots without disease [140]. Epidemiologically, tolerance and resistance are different concepts [122]. Normally, the genetic material that is tolerant is under the same intensity or severity of the disease as the susceptible, so the differences are quantified in numbers or productivity values in the tolerant genotype in relation to the susceptible.

Melo et al. [141] studied the tolerance in Brazilian soy germplasm and separated this type of classification from the reaction of the genotypes was for partial resistance [142]. The genotypes BRS7560 and BRS 239 were classified as tolerant because they did not show a reduction in productivity with and without chemical control. In Brazil, for the 2009–2010 harvest, soybean cultivars TMG 801-INOX® and TMG 803-INOX® with some incomplete resistance, Asian rust, were made available to farmers in some Brazilian regions [117, 119]. However, it is not known until now.

# 10. Sowing times, production of quality seeds in Mato Grosso and chemical rust control

There is a great debate in Brazil without scientific evidence that in the sowing schedule from December to February, the frequency of mutants increases, such as

F129L [143] (mutation for loss of sensitivity to triazoles or inhibitors of demethylation) and I86F (mutation for loss sensitivity to carboxamides). The genetic adaptation occurred in the Quinone oxidase enzyme gene at position 129 of the sequence, where the amino acid phenylalanine was replaced by leucine, in the first case. In the second case in the Succinate Dehydrogenase - C enzyme, the amino acid isoleucine (I) was replaced by phenylalanine (F), at position 86 of the protein sequence (sdhC-186F mutation) [144]. In the study of monitoring of raccoon soybeans carried out by FRAC-BR, the presence of resistant Asian rust inoculum with high frequency of the 186F mutation was detected in the years 2017, 2018 and 2019, in practically all soybeans' regions of Brazil. The data reinforce the importance of the sanitary vacuum for the reduction of resistant populations and warn of the importance of another phytosanitary measure, which has become essential for soybean production in Brazil: the sowing schedule. In relation to the sowing schedule, this is particularly important, as long as the producer is not forbidden to obtain his own seed. December sowing, as a limit for seed production, leads to low physiological and health quality of this, in addition to harvesting in Mato Grosso, under rain and with lower yields. In addition to the greater severity of soybean rust. When sowing of February to make seeds in Mato Grosso, the producer will have better health quality, higher yields compared to December sowing. There will also be a mini sanitary vacuum from December to February (60 days). As less leaf wetness occurs, fewer fungicides were applied, compared to December, which is the sowing deadline, for making seeds in Mato Grosso [145]. Therefore, it is questioned the scientific and Mato Grosso authorities, in addition to MAPA, to prevent such practice without scientific validation. The results by Reis et al. [145], point out the opposite of technical and political decisions, in relation to seed production in the state of Mato Grosso. It is well known that sowing in February would not enter the sanitary void and would allow the farmer to make his seeds of quality and at a lower cost compared to the month of December, which would remain within the calendar and without advancing in the sanitary void, as happens in the states of Tocantins (Lagoa da Confusão) and Goiás (Luis Alves and São Miguel do Araguaia), where large seeders harvest their seeds in September–October and advance in the Brazilian sanitary vacuum.

### 11. Chemical control

The main groups of fungicides registered for the control of Asian soybean rust (ASR) are: demethylation inhibitors (DMI's - tebuconazole, cyproconazole, protioconazole (triazolintione), epoxiconazole and others); quinone oxidase inhibitors (QoI's - azoxystrobin, trifloxystrobin, picoxystrobin and pyraclostrobin), succinate dehydrogenase inhibitors (SDHI's - fluxpyroxade, bixafen and benzovin-diflupyr), cupric (oxychloride), nitriles (e 3), until the year 2020. Currently, more than 120 molecules are registered with the Ministry of Agriculture, Livestock and Supply (MAPA), for the control of Asian soybean rust. Most records are composed of fungicides, of the triazole groups formulated with strobilurins. The fungicides of the DMI's, QoI's and SDHI's groups act only at a specific location, out of thousands of biochemical reactions in the fungal cell [143]. Therefore, such groups of fungicides are vulnerable to fungal strains for reduction or loss of sensitivity. They are of high risk for the development of resistance [143] and, therefore, their use in isolation, for the control of any fungal disease, notably ASR is not recommended.

In Brazil, since the discovery of ASR in the south of the country, losses in grain production have only been reduced thanks to chemical control, carried out with triazole and strobilurins fungicides [11, 33, 142]. Despite the high risk of emergence

of less sensitive or resistant mutants, in the population of the fungus *P. pachyrhizi*, in the field, with the use of fungicides with specific mode of action, chemical control of ASR, at present is the only solution available, for reduce the damage caused by the disease, due to the absence of cultivars with an adequate level, basal resistance and low efficiency of prophylactic strategies. However, in atomization programs, multisite and protective fungicides must be employed, formulated or not with systemic and strobilurins.

# 12. Risk factors for the use of fungicides with specific mode of action

Due to the fact that systemic fungicides, acting specifically in a single action site, are subject to risk factors for the development of resistance in the population of fungi. The risk factors are: 1. Use of fungicides specific, systemic or mobile and penetrating sites, as the only form of control, 2. Use of specific or systemic fungicides in extensive cultivation areas, 3. Use in more than two sprayings of mobile and penetrating fungicides, called specific sites, 4. Change in the recommended dose. The dose to be used in the atomization must always be the one that is in the product leaflet, 5. Repetitive application of the active ingredient, with the same mode of action, 6. Application of systemic fungicide after the onset of the epidemic as a dressing, 7. Characteristics of the pathogen, such as number of generations per crop cycle, sporulation capacity and ease of dissemination, 8. Extensive window for sowing soybeans for grain production. The sowing period runs from September to December. In some regions the sowing window may even double, 9. In late sowing, the interval of application of fungicides should be shorter due to the greater pressure of inoculum in the fields of cultivation. Growers must also use the specific site fungicide in more than one application, associated with multisites [115]. Give preference to the use of alternating or associated multisites in the initial applications and not at the end of the spraying schedule. Considering the latent period of the fungus from seven to nine days, in a period of 45 to 55 days (appearance of the first symptoms of the disease in the field until the senescence of the leaves), we would have eight to ten cycles of the pathogen.

### 13. Use of triazoles to control Asian soybean rust

The first chemicals used to control ASR belonged to the triazole fungicides or demethylation inhibitors (DMI), especially cyproconazole, epoxiconazole, flutriafol and tebuconazole. Diphenoconazole, myclobutanil and tetraconazole, fungicides with specific mode of action, were also used. There is also triazolintione (protioconazole [103]. The fungicide is a combination of cyproconazole and sulfur at the molecular level). Until 2020, the most commercialized triazole in Brazil is cyproconazole and soon this leadership should be changed to protioconazole (It was used in isolation in Brazil for the first time in the 2006–2007 harvest [103]), under experimental conditions compared to assets present in the Brazilian market and at the time it proved to be the most effective in controlling rust in isolation. Alves and Juliatti [98] published the beneficial effects of mancozeb in isolation and in association with other fungicides used in soy. The increase in chlorophyll A and B, carbon fixation in the plant and the green effect and increase in the mass of the grains stand out, allowing in addition to increasing productivity, the management of fungal resistance [34, 103], as *Phakopsora pachyrhizi*.. Certainly, the green effect on the plant is due to zinc (3% in the formulation) and manganese (12%) which are added to the effect of the fungicide. The mancozeb fungicide is an active carrier into the

plant, in addition to its reducing effect on the phytotoxicity of some more aggressive triazoles. After five seasons of growth (from 2002/03), using DMI alone, Silva et al. [121] reported failure to control ASR, in the state of Goiás, in 2006/07, for cyproconazole, flutriafol and tebuconazole. Until then, flutriafol had been highly effective and used by researchers as a standard, becoming the market leader. As of 2005/06, there was a reduction in the effectiveness of flutriafol, in the State of Mato Grosso [119]. After the decline of flutriafol, tebuconazole became widely used with high efficiency and was adopted as a reference fungicide to control ASR In the 2005/06 season, the average ASR control by DMI's was 90.3%. After just eight seasons, corresponding to 2012/13, the control with DMI's was 52.0, therefore there was a 42% reduction in control effectiveness [111, 112]. In other states of the federation, such as Minas Gerais, in the 2005 and 2006 harvest, the same behavior was found with the reduction in the efficiency of triazoles [11, 16, 29–31, 33, 103, 146], which in many situations reached 50% or less of effectiveness.

# 14. Reduction of the sensitivity of *Phakopsora pachyrhizi* to triazole Fungicides

Observations from the 2007/2008 harvest showed that the samples collected in the main soy producing regions of Brazil - in March - predominated populations of 03 Phakopsora pachyrhizi, less sensitive to DMI's, first generation, mainly tebuconazole, in some states of the Midwest. In the 2008/2009 harvest, the samples collected in the same month and the locations of the 2007/2008 harvest, showed that the predominance of populations less sensitive to first generation DMI's, extended to other locations in the country, in addition to states in the regions. Midwest, Southwest (São Paulo and Minas Gerais) also the southern states (Paraná and Rio Grande do Sul). Between the 2009/2010 and 2013/2014 harvests, it was observed that populations less sensitive to the first generation DMI's were detected in all Brazilian states, which produce soybeans [147–150]. Therefore, over the years, there has been a gradual reduction in the effectiveness of DMI (tebuconazole) in controlling ASR in soybean production fields in the country [147-150]. The effectiveness of ASR control with tebuconazole was 90 and 91% in 2003/05 soybean crops, 77% in 2005/06, 58% in 2006/08, 39% in 2008/09 and only 24% in 2009/10 [110].

Fungicides	Control efficiency of Asian soybean rust (%)							
	Year							
	2009	2010	2011	2012	2013	2014	2015	
Cyp. + Azo.*	72	80	70	67	63	47	40	
Epo. + Pyr.	70	79	76	70	62	28	_	
Cyp. + Pico.	71	80	72	70	69	60	59	
Teb. + Pico	72	80	80	77	75	70	67	

<sup>\*</sup>*Respectively: Cyp. + azo. (cyproconazole + azoxystrobin); Epo. + pyr. (Epoxiconazole + Pyraclostrobina); Cyp. + peak. (Cyproconazole + picoxystrobin); Teb. + peak. (Tebuconazole + picoxystrobin). Source: Godoy et al. [87–89, 151, 152].* 

### Table 1.

Reduction in the sensitivity of Phakopsora pachyrhizi (control%) to groups of demethylation inhibitor (DMI's) and DMI's + Strobilurin (QoI) from 2003/2004 to 2014/2015.

The difficulty in controlling ASR with isolated DMI's fungicides was becoming increasingly evident, proving the high adaptability of *Phakopsora pachyrhizi* [110] (**Table 1**).

The reduction in the sensitivity of *Phakopsora pachyrhizi* (Pp) to the fungicides tebuconazole and cyproconazole, with only 42 and 38% of control, respectively, was also confirmed by Godoy et al [109, 110].

# 15. Introduction of the group of carboxamides and triple formulations for the control of soybean rust

In 2012–2013, new fungicides belonging to the Carboxamide group were introduced, which have a specific mode of action, inhibiting fungal respiration, of complex II - succinate dehydrogenase (SDHI), to control ASR [153]. In Brazil, the three fungicides of the carboxamide group available on the market are bixafen, fluxopyiraxade and benzovindiflupyr. Fungicides benzovindiflupyr, bixafen, fluxapyroxad, furametpyr, isopyrazam, penflufen, penthiopyrad and silkxane, resent a medium to high risk for resistance. Numerous cases of resistance to other pathogens have already been documented for the carboxamide group in other countries [147–150, 153]. It is concluded that the introduction of this fungicide group, for the control of ASR, probably will not solve the problem, since they present a specific mode of action, subject to resistance in the population of Phakopsora pachyrhizi. Due to these facts, the fungicides of this group, were not recommended for use alone, in the control of rust. Hence the associations or triple mixtures, for use in soybean crops and for the target *Phakopsora pachyrhizi*, arose. In the years 2013/14, there was the registration of triple mixtures, involving fungicides from the groups DMI's + QoI's + SDHI's. The fungicides of the SDHI group, launched on the market, to compose the triple mixtures with triazoles and strobilurins were: benzovindiflupir and fluxpyroxade, bixafen [33, 36, 147–150].

# 16. Emergence of proticonazole from the DMI's group

In the period comprising 2013–2015, a new fungicide from the DMI's group, protioconazole, appeared. From the beginning of monitoring until its launch on the market, protioconazole has shown the lowest effective concentration values 50 (EC50) in the rust monitoring program. The introduction of this fungicide on the market was the result of hundreds of experiments, conducted in demonstration's areas, from different soy producing regions in Brazil. The protioconazole was then evaluated in a mixture with the QoI fungicide, trifloxystrobin. The comparison was made with fungicides launched on the market, such as the combinations of strobilurins (QoI) and carboxamides (SDHI). Because it is a fungicide, composed of an innovative active ingredient with differentiated binding at the fungus action site, protioconazole constituted the new generation, in the chemical group of DMI's, being chemically classified as triazolintione (Frac classification on mode of action 2014 - www.frac.info). The first studies in Brazil with protioconazole to control soybean rust were carried out by Furtado et al. [103]. The combination protioconazole + trifloxystrobin acts in two ways: 1. in the control of Asian soybean rust, and 2. in the disease complex (such as target spot, powdery mildew, molasses, anthracnose, and end-of-cycle diseases). Therefore, its use is recommended preventively, in the first application or in the first two, when the plan of use of foliar fungicides, is of more than two applications. In this way, it is possible to explore the spectrum of action of this fungicide well, starting in a robust way the prevention

and control of soybean rust and, consequently, an improvement in the performance of the subsequent fungicide.

# 17. Introduction of the dithiocarbamate group to control Asian soybean rust

In the years 2014 to 2015, the idea of introducing multi-site fungicides into the ASR control programs began to be considered, such as mancozeb, chlorothalonil and others. The introduction of multisite fungicides in ASR control programs could be an especially important tool for the management of resistance to *Phakopsora pachyrhizi*, with the potential to preserve the useful life of specific fungicides DMI's, Qo'sI and SDHI ', s in soybean culture [144, 153]. Multisite fungicides (mancozeb, chlorothalonil, metiram and others) have the great advantage, because in addition to being of low price, they act in multiple sites of action in the fungal cell, interfering with numerous metabolic processes of the fungus, and consequently, resistance to fungicide group would be rare or non-existent [97]. Recent work involving mancozeb, in the control of ASR was developed in Uberlândia - MG and in Rio Verde - Goiás, demonstrated that the product has the potential to control the disease, even in isolated applications [101, 116] (**Figure 4**). Multisite fungicides, such as mancozeb, therefore have the chance to be strong allies in the defense against Asian soybean rust.

Studies carried out at the Federal University of Viçosa on the control of ASR, with fungicides from the triazole group (DMI) + strobilurins (QoI) associated with multisite fungicides (MSF) mancozeb, chlorothalonil or met, in the agricultural years 2013 to 2015, performed by Ponce et al. [90], showed that the association of DMI and QoI with MSF (Alves and [99]), in different environmental conditions,



### Figure 4.

Residual effects of DMI and QoI, alone or in mixture and multi-site fungicides on ASR in the greenhouse. Source: Ponce et al. [90]. 1. Control; 2. Epoxiconazole + Pyraclostrobin (33.0 + 10.2 g a.i./ha); 3. Cyproconazole + Trifloxystrobin (16.8.0 + 7.2 g a.i./ha); 4. Protioconazole + Trifloxystrobin (28.0 + 16.0 g a. i./ha); 5. Cyproconazole + Azoxystrobin (18.0 + 7.2.0 g a.i./ha) + Nimbus (0.5% v/v); 6. Pyraclostrobin (75.0 g a.i./ha); 7. Mancozeb (240 g a.i./ha); 8. Chlorothalonil (150 g a.i./ha); 9. Metiram + Piraclostrobin (400 g a.i./ha), 10. Tebuconazol + Trifloxistrobina (100.0 + 50.0 g a.i./ha). The bars represent the standard errors of the means. Two experiments were conducted with consistent results; results from one representative experiment are shown. AUDPC – Area under disease progress curve; \*leaf fall – (%). Source: Ponce et al. [90].



#### Figure 5.

Effect of doses Epoxiconazole with Pyraclostrobin mixtured with multi-site fungicides, on soybean grain yield. Source: Ponce et al. [90]. 1. Control; 2. Epoxiconazole + Pyraclostrobin (33.2 + 10.2 g a.i./ha) associated with Mancozeb (1400 g a.i./ha); 3. Epoxiconazole + Pyraclostrobin (33.2 + 10.2 g a.i./ha) associated with Chlorothalonil (1500 g a.i./ha); 4. Epoxiconazole + Pyraclostrobin (33.2 + 10.2 g a.i./ha) associated with Metiram + Pyraclostrobin (1100 + 100 g a.i./ha); 5. Epoxiconazole + Pyraclostrobin (49.8 + 15.3 g a.i./ha) associated with Mancozeb (1400 g a.i./ha, 3.0 kg c.p./ha); 6. Epoxiconazole + Pyraclostrobin (opera SC; 33.2 + 10.2 g a.i./ha, 0.5 L c.p./ha) associated with Chlorothalonil (1500 g a.i./ha); 7. Epoxiconazole + Pyraclostrobin (33.2 + 10.2 g a.i./ha) associated with Metiram + Pyraclostrobin (1100 + 100 g. a.i./ha); 8. Epoxiconazole + Pyraclostrobin (66.5 + 20.5 g a.i./ha) associated with Mancozeb (1400 g. a.i./ha); 9. Epoxiconazole + Pyraclostrobin (133.0 + 41.0 g a.i./ha) associated with Chlorothalonil (1500 g. a.i./ha); 9. Epoxiconazole + Pyraclostrobin (66.5 + 20.5 g a.i./ha) associated with Metiram + Pyraclostrobin (1500 g. a.i./ha); 10. Epoxiconazole + Pyraclostrobin (66.5 + 20.5 g a.i./ha) associated with Metiram + Pyraclostrobin (1100 + 100 g a.i./ha). CV = 18.3%. The bars represent the standard errors of the means. Two experiments were conducted with consistent results; results from one representative experiment are shown. \*yield gain – Was obtained in relation to the control treatment. Source: Ponce et al. [90].

improved the efficacy in the control of the disease and the productivity of the soybean (Figure 5). In the greenhouse, triazole fungicides mixed with strobilurin associated with MSF effectively controlled FAS, applied before inoculation (protective effect). On the other hand, triazoles or strobilurins were not effective in controlling ASR in some cultivation areas in Brazil [116]). In this situation, the use of multisites such as mancozeb was providential [33, 116]. There are two possible explanations for this hiring: 1st. failures in the application of fungicides in the field. The application technology is based on three premises: time of application, environmental conditions, and the target to be reached. These are the points that are generally overlooked by producers when applying fungicides, 2nd. the fungus may have acquired resistance to triazole or strobilurin in the field, where soybeans were grown extensively, in the savana's region, when such fungicides were applied alone. The anastomosis of germ tubes, and the migration of nuclei from the hyphae of germ tubes of the soybean rust pathogen, *Phakopsora pachyrhizi*, may explain, how the fungus recombines its genetic material, and develops resistance to fungicides with specific mode of action [154]. It is possible that this mechanism could occur in nature, due to the fact that millions and millions of urediniospores are produced in soybean leaves, in the field and are then dispersed by the wind.

Based on the results obtained, three field experiments were carried out (doses of triazoles with strobilurins associated with MSF (mancozeb, chlorothalonil and metiram) [90]. The hypothesis is that the fungicides of the DMI's and QoI's groups may mixed with MSF's to improve ASR control and increase soybean productivity.

The results showed that the average ASR control with the application of triazolewith strobilurin associated with protective fungicides (mancozeb, chlorothalonil and metiram) was 70.2%. The efficiency of rust control was not higher, because the 04 products were applied after the beginning of the disease epidemic in the field. The 05 field tests were sprayed, when the disease severity had already reached 2.0 to 5.0%, 06 on the leaves of the lower part of the plants. This result shows that any of the three 07 protective fungicides can be used in the mixture with epoxiconazole with piraclostrobin or cyproconazole with azoxystrobin [90]. In general, the DMI's + QoI's fungicides associated with MSF had an efficiency greater than 68.0% of control and produced more than 70.0% of the control (control). These results show that it is possible to control ASR even after the disease severity has reached 2.0 to 5.0%, at the time of plant spraying. In this work, the protective fungicides mancozeb and chlorothalonil associated with epoxiconazole + pyraclostrobin (0.5 kg bw/ha) or cyproconazole + azoxystrobin (0.30 kg bw/ha) increased the yield of soybeans by 89.5% and 109, 0%, respectively. Based on the information above, it is suggested that the application of triazole and strobirulin associated with FMS, starting at the stages of soybean culture (V9 or R1, R2), may promote better control of the disease, especially in the leaves at the bottom of the plant, which is the main source of inoculum, for the upper part and for the whole field. Therefore, the combination of fungicides from the DMI + QoI or SDHI group, associated with MSF, can be recommended as a new strategy for the control of ASR in the short and long term. In addition, due to the residual effect of multisite fungicides [10, 33, 98] on soybean leaves, (mancozeb, chlorothalonil and metiram) can promote greater longevity of the DMI, QoI and SDHI molecules and decrease the number of applications.

The addition of mancozeb to reinforce the fight against fungal resistance is not a new strategy. Mancozeb has been included in mixtures, to contribute to the management of resistance, and to expand the spectrum of fungicides with specific mode of action, for numerous plant diseases. To stabilize ASR control, the same strategy could be used for soybeans, to chemically manage ASR. Examples of fungicides that are already used in mixture with mancozeb include benalaxyl, cymoxanil, dimetomorph, famoxadone, fenamidone, folpet, fosetil-aluminum, iprovalicarb, mandipropamide, metalaxyl and zoxamide. Likewise, this could be followed in the control of ASR, both in Brazil and elsewhere. To reinforce the role of mancozeb, in anti-resistance strategies, in the control of fungal diseases, in more than six decades of continuous use, they have been recorded in more than 70 cultures and in 400different diseases [144, 153]. The introduction of MSF in the ASR control program can be an important tool for managing resistance. Such fungicides have the potential to preserve the useful life of specific fungicides, such as (DMI, QoI and SDHI), in soybean crops.

It was observed that the fungicides of the DMI's group (tebuconazole, epoxiconazole) and the QoI's (azoxystrobin) alone, lost about 40 to 50% of efficiency, in the control of ASR, during this period. The percentage of control of mixtures of DMI's with QoI's ranged from 75% (highest level of control) to 55% (lowest level of control). For the fungicides tebuconazole, cyproconazole and azoxystrobin applied alone, the highest level of control in 2010/2011 was 56% and the lowest 19%. The introduction of fungicides of the SDHI group (bixafen, benzovindiflupir and fluxapiroxad) associated with DMI (protioconazole) and Qo's (picoxystrobin, trifloxystrobin) in triple mixes and in double mixtures

(trifloxystrobin + fluxapiroxad + benzoxystrin + maxoxystrobin of control ranging from 69 to 82% (**Table 2**). The most efficient combinations in the control of ASR were those that 08 had the fungicides of the SDHI group in double mixture, with fungicides of the QoI 09 group (pyraclostrobin and azoxystrobin). Therefore, these results suggest that the addition of fungicides from the SDHI group were more efficient both in the control and in the productivity of soybeans (**Table 3**). The highest yields corresponded to the groups' fungicides, which provided the best controls for ASR. Therefore, there was a correlation between ASR control and productivity. The highest yields also corresponded to the mixtures, which had the participation of the fungicides of the SDHI group (bixafen, benzovindiflupyr and fluxapiroxad). In disease control programs, they are often applied as 'dressings' (**Table 2**). **Table 2** shows us that rust control must be done preventively.

Location	Fungicides Sprays without symptoms (0) and with symptoms (1)	Yield (Redution - %)
Tomarara – PR	0	39
Londrina – PR	1	72
Paulinia – SP	1	24
Planaltina – DF	1	74
Ipameri – GO	1	37
Alvorada – TO	0	14
Dourados - MS	1	22
Riachão – MA	0	25
Chapadão do Sul – MS	1	68
Senador Canedo – GO	1	69

Source: Godoy et al. [113].

Difference between the production of the check and the best treatments were: Application without symptoms) - 52% and with symptoms - 34%.

#### Table 2.

Reduction of soybean productivity, in different regions with application of fungicides, before the first symptoms and after the Asian soybean rust (ASR) entry in the crop in many states of Brazil.

Duble and triple mixes	(%) of Control	Yield (Kg/ha)	
2015/2016			
Piraclostrobin + benzovindiflupir	78,0	(3479,0)	
Azoxystrobin + benzovindiflupir <sup>*</sup>	82,0	(3428,0)	
Piraclostrobin + epoxiconazole + fluxapiroxade	69,0	(3246,0)	
Trifloxystobin + protioconazole + fluxopiraxade	71,0	(3303,0)	
Picoxystrobin + tebuconazole + mancozeb	64,0	(3126,0)	
Azoxystrobin + tebuconazole + mancozeb	59,0	(3065,0)	

<sup>\*</sup>In 2016–2017 the efficiency reduction was 40–50% after fungus mutation to carboximides (gene I86F).

#### Table 3.

Percent of control (%) and Yield for double mixtures and casings used to control Asian soybean rust, in 2015/2016.

**Table 2** summarizes the results of three agricultural years of studies, with fungicides DMI's, QoL's, SDHI's and dithiocarbamate in various regions of the country carried out in 2010/2011 until 2015/2016 [36, 113, 114, 155].

# 18. Conclusions

To reduce the risk of damage to the crop, the management strategies recommended in Brazil for this disease are:

1. Prioritize the rotation of fungicides with different biochemical mechanisms of action and adapting the programs to the time of sowing. It is mandatory to include multisite and/or protective or residual fungicides in tank mixes.

- 2. Sequential and curative applications should be avoided to decrease the pressure of selection of resistance to the fungus. Rust control must always be preventive, as it is an aggressive pathogen.
- 3. Use early cycle cultivars and sowing at the beginning of the recommended season when possible.
- 4. Eliminate voluntary soybeans plants and raccoons.
- 5. The off-season must be free from soybean cultivation (pay attention to the sanitary vacuum that varies from 60 to 90 days according to the region).
- 6. Monitor the crop, since the beginning of the development of the crop. Control will only be effective if the disease is diagnosed early.
- 7. Atomize the crop to control Asian soybean rust, at least once, before planting lines are closed.
- 8. Avoid late sowing in relation to the recommended season.
- 9. The adoption of a single model for the management of the disease is not justified, and it is important that this be done in a rational manner depending on the situation in each location.
- 10. The use of fungicides must be planned, according to the risk factors, which are monitored during the harvest.
- 11. The timing of application and reapplication, at the right time, is of fundamental importance in controlling the disease.
- 12. The use of fungicides with a prolonged residual effect such as multisite/ protectors is fundamental in preserving the useful life of systemic fungicides in the field.
- 13. Use cultivars with partial resistance already available in Brazil, when these allow levels of productivity and adaptation to the different macro-regions of Brazil. Even in these genotypes, the use of the fungicide is fundamental, given the variability of the pathogen.

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