

Integrated Management of Asian Soybean Rust

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ABSTRACT

Asian soybean rust is caused by the biotrophic basidiomycete fungus *Phakopsora pachyrhizi*. The disease is considered as highly aggressive. The disease is present in all countries that cultivate this legume. The disease is considered the one with the greatest destructive potential, causing damage ranging from 10 to 90%. In Brazil, the average damage attributed to asian soybean rust was 70% reported in the 2001/2002. *P. pachyrhizi* infect 41 species in 17 genera of the Fabaceae family. Experimentally it can infects 60 plant species belonging to 26 genera. Symptoms (lesions) in general are found on lower leaves, when the plants are in the phenological stage close to or after flowering. The uredia formed on the lesions progressively acquire a light brown to dark brown color which open into a tiny pore, expelling the hyaline colored urediniospores, which turn beige and accumulate around the pores or are removed by the wind. The final stage of the soybean rust epidemic is general yellowing of the foliage, with intense defoliation, reaching the complete fall of the leaves. The fungus causes TAN-type lesion, characterizing resistance and an RB-type lesion, highly susceptible to asian soybean rust. The stages of rust that occur in the field are uredia and telia. The pycnia, aecia and basidium stages have not yet been identified. The disease is favored by light and frequent rains, long dew periods and temperatures between 15 and 28 °C. Temperature higher than 30 °C limits *P. pachyrhizi* infection in soybean. Wind is the main source of dissemination of the urediniospores from the uredia. The greater of the frequency of hours in which the leaves remain wet, the greater the severity of the disease in the field. The latent period of the fungus varies from 7 to 14 days. Integrated control measures must be applied to control the disease. The following management strategies recommended are: use cultivars with horizontal resistance is the most important measure; use of early cycle cultivars; sowing at the beginning

of the recommended season; elimination of voluntary soy plants in cotton, beans and corn plantations; absence of soybean cultivation in the off-season, through the sanitary vacuum (60 to 90 days); monitor of the crop from the end of vegetative stage (V9); monitor the temperature and rainfall to initiate the application; use of fungicides in compliance with anti-resistance strategies when symptoms appear according to the economic damage threshold. Multisite fungicides must be used in mixture with mobile penetrating fungicides with efficiency greater than 70%, to avoid selection of mutants in the population of the fungus in the field.

Key-words: *Phakopsora pachyrhizi*; epidemiology; chemical control; genetic resistance; cultural control.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the ten most economically important crops worldwide because it is one of the main source of protein and vegetable oil (DIAZ et al., 1992). The yield potential of the soybean crop can be affected by several factors, among which the soil fertility, the availability of water during the crop cycle, the plant population, the sowing time, the potential productivity of the cultivar, the occurrence of invasive plants and the infestation of pests and diseases (BROMFIELD, 1984; CASA; REIS, 2004; HENNING; GODOY, 2006). In the 2020/21 harvest, world soybean production was 362.947 million tons, with a planted area of 127,842 million ha (USDA, 2021). In the 2019/20 harvest, Brazil produced around 124.85 million tons, representing 30% of world production. Brazil is the world's largest producer, with 140,499.6 million tons, representing more than 39.0% of world production, with a planted area of 40,399.2 million ha, with a productivity of 3,517 kg/ha.

The main soybean disease in the state of Mato Grosso is asian soybean rust, caused by the biotrophic basidiomycete fungus *Phakopsora pachyrhizi* (SYDOW; SYDOW, 1914).

Asian soybean rust was first described in Japan in 1902 (HENNING, 1903), and by 1914 it had already spread to several countries in Southeast Asia.

In the American continent, the "American" rust (*Phakopsora meibomia* ((Arthur) Arthur) was reported for the first time in 1976, in Puerto Rico (VAKILI; BROMFIELD, 1976; VAKILI, 1978); in Brazil, *P. pachyrhizi* was identified in Lavras, MG, in 1979 (DESLANDES, 1979). Since its first identification in Brazil, in 1979, the only outbreak with economic losses was recorded in 1987/88, in São Gotardo, Minas Gerais (CARVALHO JUNIOR; FIGUEIREDO, 2000). Subsequently, it was confirmed based on the report by Deslandes in 1979 and by evaluating herbarium samples since 1979, both species were confirmed in Brazil (*P. meibomia* and *P. pachyrhizi*), with a predominance of *P. pachyrhizi*. Later, in February 2001, Asian soybean rust was first reported in South America, Paraguay, in February 2001 (MOREL; YORINORI, 2002; YORINORI et al. 2002a; YORINORI et al., 2002b). In 2002, the disease appeared in southern Brazil (REIS et al., 2002; YORINORI et al., 2002b). The disease is now widespread in all states of Brazil where soy is grown. Recently, in July 2021, rust was found in soybean fields in the state of Roraima. In Argentina, the disease was reported by Rossi (2003). In the same year the disease was reported in Bolivia (NAVARRO et al., 2004) and Colombia (REIS et al., 2006), progressing in 2004 to Uruguay (STEWART et al., 2005) and 2005 to Ecuador (SOTOMAYOR HERRERA,

2005), Mexico (CÁRCAMO-RODRÍGUEZ et al., 2006; YÁÑEZ-MORALES et al., 2009) and the United States (SCHNEIDER et al., 2005).

Currently, asian soybean rust is present in all countries where this legume is grown. Its spread was fast around the world, due to the fungus's urediniospores being spread by wind currents (BROMFIELD, 1984; REIS; BRESOLIN, 2004; YORINORI et al., 2004; HARTMAN et al., 2007).

DAMAGE CAUSED BY SOYBEAN RUST

Among the diseases that occur in soybean, the rust caused by the fungus *Phakopsora pachyrhizi* Sydow & Sydow is considered the one with the greatest destructive potential, causing damage ranging from 10 to 90% in the different geographic regions where it was reported (SINCLAIR; HARTMAN, 1999; YORINORI et al., 2005).

The average damage (sensu Nutter et. al., 1993) calculated on the bases of yields of 2006 to 2008 of Embrapa Consortium of Rust was 64 % (Reis, E.M. personnel communication).

The attacked plants undergo defoliation and early maturation, compared to uninfected plants, which causes a reduction in the weight and quality of the grains produced (Figure 1).



Figure 1 – Soybean fields severely defoliated in the maturation phase, due to the intense attack of asian soybean rust (*Phakopsora pachyrhizi*)

Taking into account the losses related to the decrease in soybean productivity, expenses with chemical control (fungicides and expenses with applications) and the impact on tax revenue until the 2006/2007 harvest, rust represented a loss of US\$9.9 billion, a number that coincides with the foreign exchange collected by Brazil, in soy exports in 2006 (AGENCIA BRASIL, 2011).

In Brazil, the damage attributed to asian soybean rust was 70% reported in the 2001/2002 harvest (YORINORI; MOREL, 2002). In the same season, in the region of Chapadão do Sul, MS, damage of up to 100% was recorded (ANDRADE; ANDRADE, 2002). According to Henning and Godoy (2006), losses in the 2002-2003 crop reached almost one billion dollars.

Figure 2 shows the chronology of the damage caused by soybean rust starting on day one when the symptoms of the disease were not visible; day six, soybean leaves have a brownish color due to the fungus attack; on the 13th, intense defoliation of the plants is already observed; and on day 27, total and dry pod defoliation (FRAC 2015). This fact demonstrates how aggressive soybean rust is in the soybean crop.



Figure 2. Evolution of the symptoms caused by asian soybean rust in the field

According to data from the anti-rust consortium (GODOY et al., 2015), soybean rust does not reach a level of control in at least 30% of the country's plantation areas, due to climatic variations. Hence the great importance of adopting integrated management, monitoring the climate, mainly rainfall and the incidence of the disease on the leaves of the lower part of the plants for the decision making of the beginning and interval of atomization of fungicides. The phenotypic phase of the culture must also be taken into account in decision-making.

HOSTS

The causal agent of asian soybean rust is a biotrophic fungus, which survives on green soybeans or other hosts. Hartman et al. (1999) and Slaminko (2008) reported that, unlike other rusts, *P. pachyrhizi* can naturally infect a wide range of plant species, including 41 species in 17 genera of the Fabaceae family. Furthermore, 60 plant species belonging to 26 genera were experimentally infected under controlled conditions (RYTTER et al., 1984), reaching up to 90 species (MISMAN; PURWATI, 1985).

The fungus can also infect and sporulate in species belonging to the sub-family Papilionoideae, family Fabaceae, having as main hosts the species *Glycine max*, *G. soyae*, *Pachyrhizus erosus*, *Pueraria lobata* and *Vigna unguiculata* (CABI, 2001).

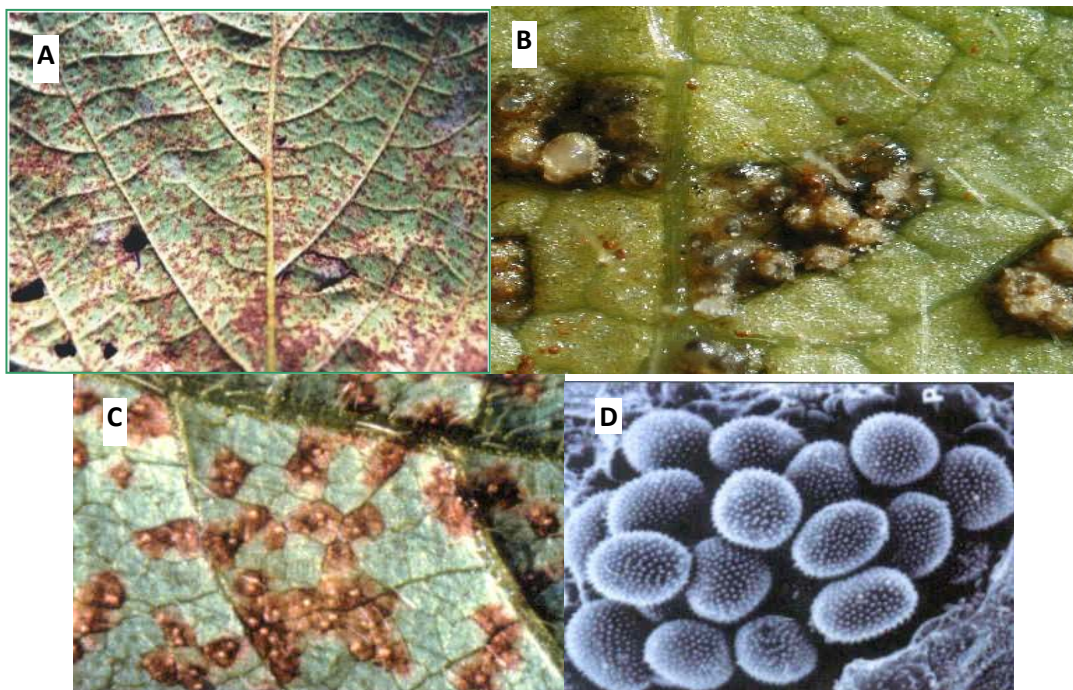
More references are found in the literature regarding the hosts of *P. pachyrhizi*, among which the following stand out: *Phaseolus vulgaris* var. *vulgaris*, *Canavalia gladiata* (STAVELY et al., 1985); *Phaseolus vulgaris* (DU PREEZ et al., 2005); *Vicia faba*, *Vigna radiata*, *V. mungo*, *Psophocarpus tetragonolobus* and *Colopogonium muconoides* (POOLPOL; PUPIPAT, 1985); *Alysicarpus vaginalis*, *Securigera varia*, *Melilotus officinalis*, *Trifolium repens* and *T. incarnatum* (RYTTER et al., 1984); *Phaseolus lunatus* var. *lunatus*, *Sesbania exaltata* and *Trigonella foenum-graicum* (BROMFIELD, 1984); *Phaseolus coccineus* (LYNCH et al., 2006); *Pachyrhizus ahipa* and *Cajanus cajan* (YEH et al., 1981); *Crotalaria anagyroides*, *C. spectabilis* and *Macroptilum*

atropurpureum (KEOGH, 1974); *Pueraria amontana* var. *lobata* (ONO et al., 1992; IVANCOVICH, 2005), *Lespedeza cuneata*, *Kummerowia striata*, *K. stipulaceae* and *Pisum sativum* (SATO; SATO, 1982); *Lipinus albus*, *L. angustifolius*, *L. luteus* and *Lotus* spp. (KEOGH, 1974), among others. In 2015, the pathogen was found in 'jacatupe' (*Pachyrhizus erosus*), near a soybean field, in Viçosa, Minas Gerais state, with asian rust.

However, in Brazil the most common hosts of asian soybean rust are kudzu and perennial soybean. Bean (*Phaseolus vulgaris* L.), the second most important crop after soybean, does not represent a risk of rust maintenance, in fact, there have been no reports of epidemics in this crop.

SYMPTOMS AND SIGNALS

In its initial stage, the symptoms caused by soybean rust are easily confused with other diseases, such as bacterial pustule (*Xanthomonas axonopodis* 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 (REIS et al., 2006). The same authors report that the symptoms caused by soybean rust are called “lesions” and not pustules like other rusts, because leaf tissue necrosis occurs and each lesion may present several pustules (Figure 3). Symptoms can appear at any stage of development and in different parts of the plant, such as cotyledons, leaves and stems, vegetables, with foliar symptoms being the most characteristic (ALMEIDA et al., 2005).



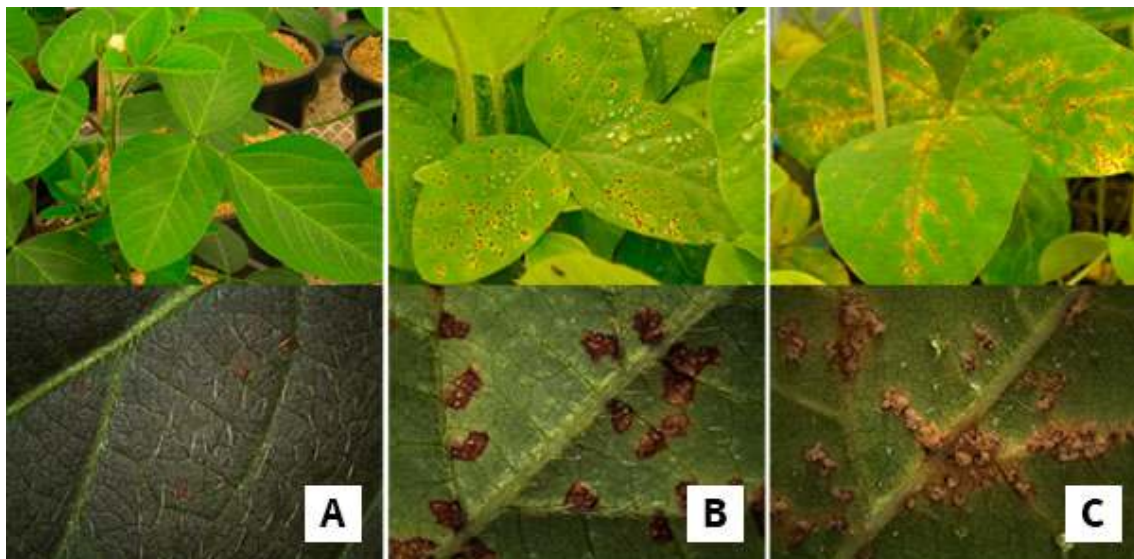
Source: Seixas e Godoy (2004/05)

Figure 3 – Symptoms and signs of *Phakopsora pachyrhizi*. A = Gray lesions on the underside of soybean leaves containing fungus spores; B = *P. pachyrhizi* lesions containing pustules with

spores; C = Lesions with whitish punctuations of *P. pachyrhizi* corresponding to fungal pustules; and D = *P. pachyrhizi* uredospores formed in a pustule.

The color of the lesions varies from greenish-gray to reddish-brown, with one or several globular uredia, mainly in the abaxial part of the leaf (HARTMAN et al., 1999). Sporadically, uredia may appear on top of it. (ALMEIDA et al., 2005; GARCÉS, 2010). The number of lesions and uredia in a leaf (abaxial part) can reach from 26 to 46 per cm² under controlled conditions. In general, the first lesions are found on lower leaves close to the ground, when the plants are in the phenological stage close to or after flowering (REIS et al., 2006). Progressively, the uredia acquire a light brown to dark brown color, which open into a tiny pore, expelling the hyaline colored urediniospores, which turn beige and accumulate around the pores or are removed by the wind. al., 2005).

As sporulation proceeds, the leaf tissue around the first uredia 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) (Figure 4). (ALMEIDA et al., 2005; BONDE et al., 2006). The final stage of the soybean rust epidemic in a crop is characterized by the general yellowing of the foliage, with intense defoliation, reaching the complete fall of the leaves (REIS et al., 2006). Figure 4 shows a TAN-type lesion (B), characterizing resistance and an RB-type lesion (C), typical of materials highly susceptible to asian soybean rust.



Fonte: adaptada de Rosa et al. (2015).

Figure 4 – Photographs of soybean leaves with and without symptoms and signs of asian soybean rust. A = characterizes complete immunity or resistance; B = leaves with RB type lesions (incomplete resistance); and C = TAN-type lesion, susceptible to asian soybean rust.

ETIOLOGY

Soybean rust has two species of the fungus as its etiological agent: *Phakopsora pachyrhizi* (asian soybean rust), the most aggressive, present in soybean fields in the country and *Phakopsora meibomiaie* ("American" rust), which predominated in the country until the end of the year

2000. Distinguishing between the two species is not easy and depends on polymerase chain reaction (PCR) analysis for detection and discrimination of the two species. Frederick et al. (2002) and Harakava (2005) showed how to separate the two species.

The causal agent of asian soybean rust is *Phakopsora pachyrhizi* (Sydow & P. Sydow, 1914). Taxonomically, it belongs to the Kingdom Fungi, Division Basidiomycota, Class Urediniomycetes, Subclass Incertae sedis, Order Uredinales, Family Phakopsoraceae, Genus *Phakopsora* and Species *Phakopsora pachyrhizi* Sydow and Sydow, 1914 (KIRK et al., 2001; INDEX FUNGORUM, 2016)

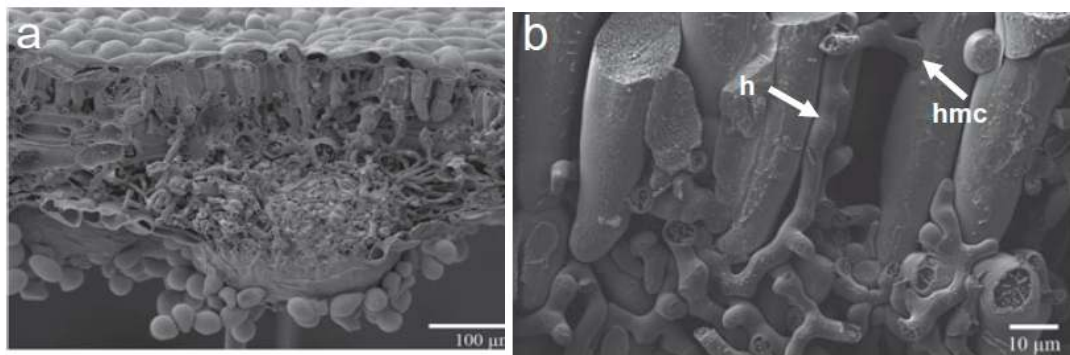
Synonyms: *Phakopsora soyae* Fujikuro; *Phakopsora calothea* H. Sydow; *Malupa soybean* (P. Hennings) Ono Buritica, and Hennen comb. Nov. (Anamorph) *Uredo soybean* and P. Hennings (ALEXOPOULOS et al., 1996; KIRK et al., 2001; INDEX FUNGORUM, 2016).

The stages of rust that occur in the field are uredia and telia. The pycnia, aecia and basidium stages have not yet been identified (Table 1).

Table 1. Fungal life cycle stages

Stadium	Ploidy	Presence/absence
Pycnium (0)	(n, n')	Absence
Écio (I)	(n + n')	Absence
Urédia (II)	(n + n')	Presença
Télia (III)	(2n)	Presence
Basídium (IV)	(n'', n''')	Absence

In Figure 5, the photomycography of a section of uredia can be observed, exposing the urediniospores on the ventral surface (a) and showing a remified hypha (h) and haustorium mother cell (hmc).

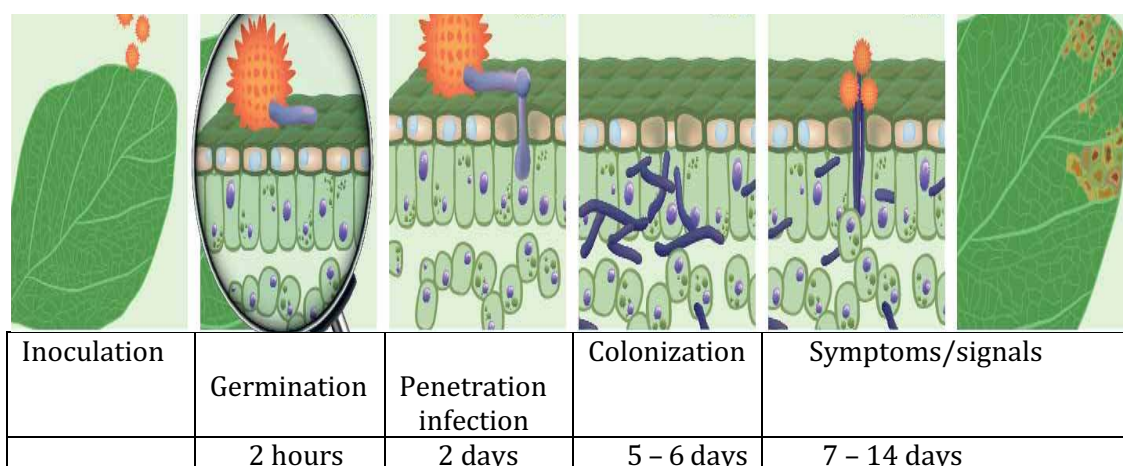


Fonte: Goellmer et al. (2010).

Figure 5. Electronic photomycography showing soybean leaves colonized by *P. pachyrhizi*. a) Section through leaf blade of soybean showing uredia and uredospores. Intercellular space colonized by *P. pachyrhizi* mycelium and urediniospores scattered on the underside of the leaf; b) remified hypha (h), formed in the intercellular space of soybean leaf. Hautorium mother cell (hmc) in contact with mesophyll cells which are invaded by mature haustorium.

EPIDEMIOLOGY

A complete review of the epidemiology of soybean rust caused by *Phakopsora pachyrhizi* in the state of Mato Grosso was published by Reis et al., (2021c). The fungus has a short life cycle under the following conditions: light and frequent rains, long dew periods and temperatures between 15 and 28 °C. Temperature higher than 30 °C limits *P. pachyrhizi* infection in soybean. Light and constant rains favor the dissemination of the urediniospores and infection of plants in the field. However, there are regions of the country, such as ‘Lagoa da Confusão’, in the state of Tocantins, where the disease occurs with scarce rainfall. In this case, the moisture coming from the soil reaches the leaves due to the irrigation system being sub-surface in the planting fields. Therefore, rainfall to trigger the disease is very important, but high relative humidity coming from the soil can also initiate the disease and reach high levels of infection. The high humidity coming from the soil can cause the formation of dew (liquid water on the leaf surface) guaranteeing infection of soybean leaves. As for leaf wetness, the greater of the frequency of hours in which the leaves remain wet, the greater the severity of the disease in the field. The latent period of the fungus varies from 7 to 14 days. If the temperature is around 22 °C, the latent period can reach six days; therefore, in this case, asian soybean rust can be considered as highly aggressive. Figure 6 shows the different stages of development of asian soybean rust until the formation of uredia.



Source: Empresa Brasileira de Pesquisa Agropecuária.

Figure 6. Stages of soybean rust development from the deposition of the inoculum on the leaves to the appearance of symptoms and signs.

Note that germination of urediniospores occurs in at least two hours; penetration and infection occurs within 24 to 48 hours; colonization takes 5 to 6 days and sporulation takes 7 to 14 days, depending on weather conditions. Asian soybean rust epidemics can rapidly increase from almost undetectable levels to very high prevalence. Spore production can last at least three weeks (MELCHING et al., 1989; DORRANCE et al., 2005).

When mature, uredia produce a large amount of urediniospores. Table 2 shows the quantity of spores that are produced on uredia and soybean rust lesions. The data on Table 2 was calculated based on REIS et al., (2006). That is why the soybean rust disease has a great potential for infection, which is considered highly aggressive.

Table 2. Estimated production of spores of *Phakopsora pachyrhizi*

Spores per.....	Número of spores
Spores por uredia	1.000
Spores per cm ² (300 uredias/cm ²)	300.000
Spores per leaflet of soybean (leaflets por 50 cm ²)	15.000.000
Esporos por planta (60 leaflets por planta)	900.000.000
Spores per hectare (250.000 plants/ha)	225 trillions

Fonte: Forcelini (2017) (Personal communication¹).

¹Foot note - Correspondence from the plant pathology professor at the University of Passo Fundo, Rio Grande do Sul, sent to Laércio Zambolim, Federal University of Viçosa, Minas Gerais, on 12/16/2021.

Rapid disease development has been correlated with canopy closure at the flowering stage (R1+) (KOCH et al., 1983; DORRANCE et al., 2005). The disease starts in the older leaves due to the fact they are longer exposed, with lower light intensity and longer leaf wetness and progresses to the middle third and then to the upper third with the closing of the rows. For this reason, asian soybean rust causes high crop defoliation and continues until the environment is no longer conducive to disease development (RUPE; SCONYERS, 2008). Then, asian soybean rust progresses until there is complete defoliation of the canopy or until the environment is no longer conducive to disease development (RUPE; SCONYERS, 2008).

According to Bromfield (1984), infection at flowering can produce high levels of damage, compromising the formation and filling of pods, the final weight of the grains (YANG et al., 1991) and also affecting the protein content (OGLE et al., 1991). al., 1979).

After infection, the fungus produces uredia and uredospores between 7 and 14 days, depending on environmental conditions (KOCH et al., 1983). Symptoms are grouped into lesions of 2 to 5 mm in diameter, with two to six uredia and abundant sporulation or formation of reddish lesions, with zero to two uredia and sparse sporulation (BROMFIELD, 1984).

The pathogen survives in the field on tigueras soybean plants and as a weed in cotton fields and other crops. In addition, it can survive in host plants, including 41 species in 17 genera of the Fabaceae family (HARTMAN et al., 1999; ZAMBOLIM et al., 2021; JULIATTI; ZAMBOLIM, 2021). As the function of the telia and teliospores of the fungus is not known until now, the urediniospores are the structure for the propagation of the fungus. Uredospores survive only on host plants and on live soybeans until the plants remain green; after the leaves dry, the spores die. The lifespan of urediniospores outside the host plants is a maximum of 60 days on the leaves (ZAMBOLIM et al., 2021; JULIATTI; ZAMBOLIM, 2021).

Ascendents air currents can also carry uredospores into the stratosphere, where they can maintain viability for many months. Through descendants currents, spores are deposited on host plants. Spore dispersion occurs mainly by the wind on days with low relative humidity. Light, constant rain with low-intensity wind can also spread the spores.

One wonders how the disease arrived in Paraguay and soon after through all the countries of South America and Brazil? There is still no scientific answer to this question, but it is admitted

that it is due to air currents in the troposphere and stratosphere, as occurred for other rusts, such as coffee rust from the African continent to Brazil and soon after throughout the country. The fungus is greatly influenced by climatic conditions. This fact occurred in the year 2021 in the state of Mato Grosso. By the end of December, the disease had not been detected in the state. Drought is also predominant in Mato Grosso do Sul until the end of December 2021. Hence the question arises: why the disease was not found in the state of Mato Grosso, as it happens every year, at the end of December if the regime rain is normal? Therefore, humidity and rain were not limiting factors for the establishment of rust in Mato Grosso. It is concluded that probably the absence of *P. pachyrhizi* inoculum in Mato Grosso can be explained by the unfavorable climatic conditions in Paraguay and Boliva for the development of rust.

Fungi are highly variable in nature. Phytopathogenic fungi such as *Phakopsora pachyrhizi* can adapt through mutations, leading to resistance and reduced effectiveness of site-specific fungicides. Mutation therefore is a process that produces variability in microorganisms in the field. The mutation occurs by altering one or more organic bases (A, T, G, C, U) of the fungus's genetic code. This process is verified in the urediniospore of the fungus which is heterokaryotic. The development of resistance is a form of evolution and occurs under two mechanisms: (i) genetic variability – spores of fungi carry genes necessary to resist the toxic substance; (ii) selection – site-specific fungicides are not mutagenic agents, only selectors, due to their repeated use in crop fields. The frequent use of site-specific fungicides creates an adverse environment for a fungus that was previously sensitive to a particular molecule, leading to its adaptation and resistance to the new situation.

As there are no known varieties with vertical or oligogenic resistance to the pathogen, there are no soybean plants that differentiate the isolates, that is, there is no differential interaction. Therefore, the fungus isolates is classified into pathotypes. However, in crop fields, horizontal resistance predominates, which has been used in Brazil associated with chemical management with triazoles, strobilurins, carboxamides and multisites such as mancozeb, cuprics and chlorothalonil (ZAMBOLIM et al., 2021; JULIATTI; ZAMBOLIM, 2021).

The soybean rust cycle begins with the release of *P. pachyrhizi* urediniospores from the uredia formed on the underside and to a lesser extent on the upper side of the leaves. Although telia can be found in crop fields, the role of teliospores in the disease cycle is not yet known. Uredospores are carried by moist air currents in the atmosphere over short and long distances, up to about 500 km, and by convection currents they fall to the surface of host plants to start the new cycle.

There are several factors that make this *P. pachyrhizi* so important in the soybean production system. These factors are (i) ability to cause a reduction in productivity and, (ii) high spore production and ease of dispersal, which result in greater disease severity in the field and (iii) emergence of fungal mutants resistant to site-specific fungicides. (SINCLAIR; HARTMAN, 1999; FANARO et al., 2004; REIS et al., 2018).

Within the epidemiology of *P. pachyrhizi*, the aerobiological phase shows how spores are dispersed in the environment and how climatic factors influence the process. The process of dissemination of spores of *P. pachyrhizi* encompasses the phases of release of spores by the wind that are formed in uredia, dilution of spores in the atmosphere, survival of spores in the

atmosphere (troposphere and stratosphere) and deposition of spores on plant surfaces (DEL PONTE; ESKER, 2008).

The release of spores produced by uredia in the environment can occur by the action of the wind, moving the canopy or raindrops that fall on the surface of the plants, since the pathogen does not have a specific release mechanism where these spores can be dispersed alone or in spore masses (MELCHING et al., 1979; DEL PONTE et al., 2006).

The dilution of spores in the atmosphere occurs with only a low percentage of the spores produced, where they are carried mainly by turbulent diffusion and wind shear, transforming into aerosols, being dispersed in dry or liquid matter in the air. The distance traveled by the spores varies from a few kilometers to continental distances, which depends on a series of climatic factors prevailing at the time of dissemination (ISARD et al., 2006; LI et al., 2009). Heavy rains serve as a vehicle for the transport of spores that are in the atmosphere to the surface of the host plant, serving not only as a way of spreading the pathogen, but also as a source of moisture for the beginning of the infection (NAGARAJAN; SINGH, 1990; PAN et al., 2006; LI et al., 2009).

The survival of spores in the atmosphere is dependent on pathogen and environmental factors. In general, *P. pachyrhizi* spores have few strategies to remain viable in the environment, and the formation of spore masses is perhaps the most important. The agglutination of spores provides physical protection to those spores that are located inside the cluster, and the spores located on the outer surface of the mass will possibly be killed by the effects of solar radiation, low humidity and high temperature (MELCHING et al., 1979).; KIM et al., 2005). The occurrence of storm clouds, such as those that form in hurricanes, have the ability to protect *P. pachyrhizi* spores against UV radiation (PAN et al., 2006).

The environmental factors limiting the viability of spores are mainly temperature, solar radiation and relative humidity, where solar radiation equal to or greater than 27.3 MJ/m², air temperature above 30 °C and relative humidity below 40% drastically reduce or inhibit spore germination (HEGDE et al., 2002; ISARD et al., 2006). Finally, deposition of spores after dissemination occurs on any surface, whether in soil or on plant surfaces. After spore deposition, germination may occur even on surfaces other than plant tissues, if this spore is viable and the conditions of humidity and temperature are favorable (AYLOR, 1986; DEL PONTE; ESKER, 2008). However, as it is an obligate biotrophic pathogen, infection in plant tissue will only occur in the pathogen's host plants if all the conditions of the disease occurrence triangle (susceptible host, viable pathogen, and favorable environment) are in place (MELCHING; BROMFIELD, 1975; ALVES et al., 2006).

The movement of *P. pachyrhizi* spores in soybean producing countries in South America is associated with atmospheric phenomena. The South American Low-Level Jet or SALLJ phenomenon is a humidity corridor formed by the trade winds that enter the Brazilian northeast and travel through the Amazon to the Andes, where the mountains serve as barriers, creating moisture corridors that move southward, passing through Bolivia, Paraguay, central-west, southeastern and southern Brazil, reaching Uruguay and Argentina, mainly during the summer (MARENGO et al., 2004). This humidity corridor, in addition to being responsible for

the distribution of rainfall on the continent, is also responsible for the movement of particles in the north-south direction, such as dust and smoke.

Regardless of the formation process, rain is primarily responsible for cleaning the atmosphere, carrying all suspended particles, including spores, back to the surface. Rainwater is also intercepted by plant leaves and provides, together with the water formed by dew, the necessary moisture for germination and spore infection. On the other hand, rain is also responsible for the impact of the drop on the leaf and the release of spores from the pustule. After that, the wind is responsible for the displacement over long distances. In dry periods, after the formation of the pustule, the release and mobility of the pathogen are hampered. That is why, after the rains, the climatic and phytopathological components add up to potentiate the epidemic.

Knowledge of the aerobiology of *P. pachyrhizi* spores allows relating the level of spore collection with the meteorological conditions of a given region. Thus, in the future, this database may serve as a management tool to anticipate the management of this disease, which is one of the diseases that causes most damage to rural producers.

INTEGRATED MANAGEMENT OF ASIAN SOYBEAN RUST

The control of asian soybean rust must be integrated, as an isolated measure will not result in success, as it is a highly aggressive pathogen. The integrated methods highlighting the cultural, genetic and chemical control (Figure 7) (RUPE; SCONYERS, 2008). To reduce the risk of rust damage to the soybean crop, the following management strategies are recommended: use cultivars with vertical resistance (if available), and if not, use cultivars with horizontal or quantitative resistance, as long as they meet the agronomic characteristics of each region of the country. The option for horizontal resistance may require chemical control. These are the most important measures. The following measures are: i) use of early cycle cultivars; ii) sowing at the beginning of the recommended season when possible; iii) elimination of voluntary soy plants in cotton, beans and corn plantations; iv) absence of soybean cultivation in the off-season, through the sanitary vacuum (60 to 90 days); v) monitoring of the crop from the beginning of the crop development; vi) monitoring of temperature and rainfall; and vi) use of fungicides in compliance with anti-resistance strategies when symptoms appear according to the according to the economic damage threshold.

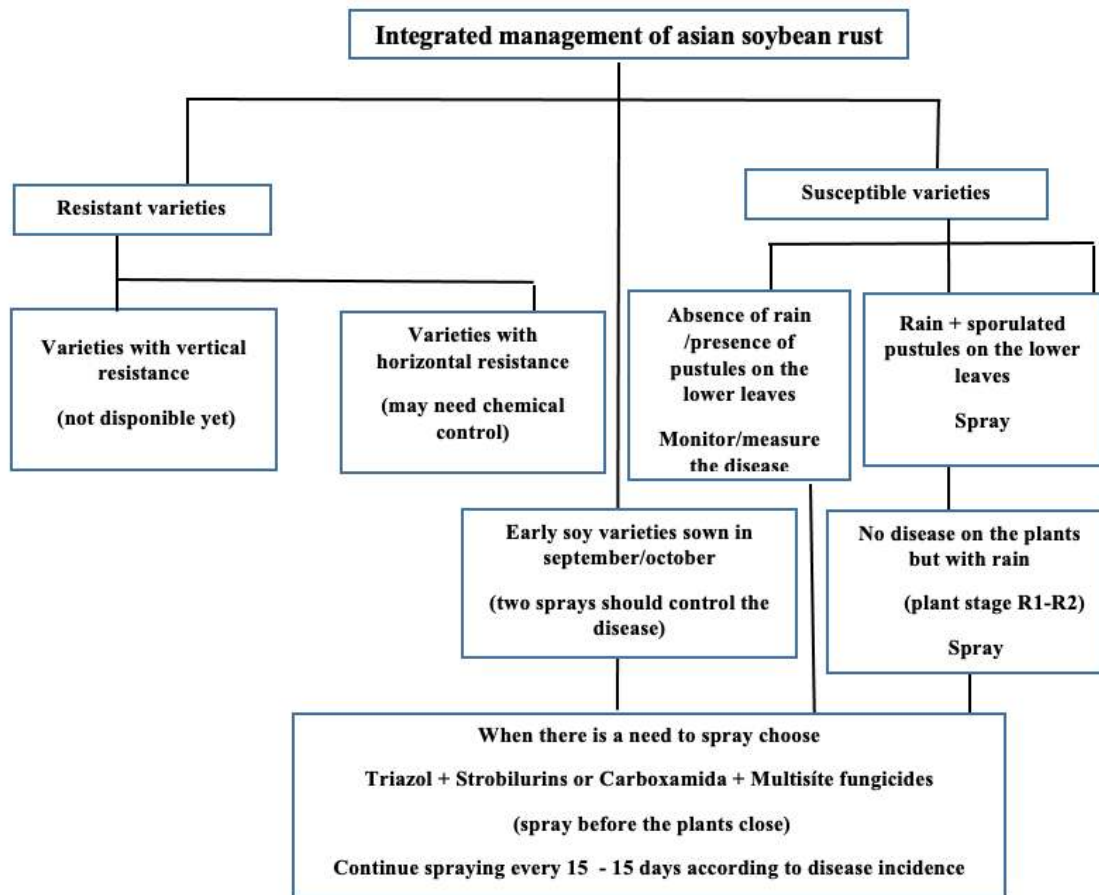


Figure 7. Integrate management of asian soybean rust.

Genetic Resistance

The most efficient method of controlling a plant disease is the cultivation of resistant varieties (Figure 7). SHUXIAN (2012) reported that the use of genetic resistance to asian soybean rust is one of the most effective long-term strategies to control the disease. However, already in the 1970s, VAN DER PLANK (1968) pointed out the difficulty of finding materials with vertical resistance to rust. In addition, the few materials cataloged as resistant had vertical or monogenic resistance, lacking horizontal or polygenic resistance, due to the genetic variability of the causal agents. *Phakopsora pachyrhizi* is a pathogen with high genetic variability. JULIATTI; ZAMBOLIM, 2021 reported that horizontal resistance from the IAC 100 genotype has been used for the development of new genotypes with polygenic resistance.

For a long time in Asia, countries used five sources of genetic resistance in soybean breeding programs to control rust: Rpp1 (CHENG; CHAN, 1968, McLEAN; BYTH, 1980, HARTWIG; BROMFIELD, 1983); Rpp2 (HIDAYAT; SOMATMADJA, 1977); Rpp3 (BROMFIELD; HARTWIG, 1980, VAN DE MORTEL et al., 2007); Rpp4 (HARTWIG, 1986) and Rpp5 (GARCIA et al., 2008). Today there are eight RPP genes (JULIATTI; ZAMBOLIM, 2021) available in the world's germplasm; however, after its use in commercial soybean germplasm, vertical resistance was supplanted by races of the pathogen.

Genetic and molecular analyzes suggest several closely linked alleles or genes that govern soybean rust resistance (GARCIA et al., 2008). ZAMBENEDETTI et al. (2007), studying the genotypes PI 230970 and PI 459025, found that *P. pachyrhizi* had a low percentage of germinated urediniospores and appressoria formation. In these genotypes, appressorium formation started six hours after inoculation, while in the other cultivars it occurred four hours after inoculation. These genotypes may present resistance genes Rpp2 and Rpp4, respectively (HARTWIG; BROMFIELD, 1983; HARTWIG, 1986). Another research carried out by PHAM et al. (2009) found that when each of the known sources of resistance (Rpp1 to Rpp4) was evaluated with three *Phakopsora pachyrhizi* isolates; cultivars PI200492 (Rpp1) and PI462312 (Rpp3) produced a differential response, while PI230970 (Rpp2) and PI459025B (Rpp4) produced susceptible lesions. Koga et al. (2008), evaluating the components of asian soybean rust resistance in 48 cultivars, selected some groups of genotypes, which can be considered promising as sources of disease resistance genes. 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, Rpp4, present in the PI459025 variety, and Rpp5 in the Shiranui variety. , which conferred resistance to three populations of the fungus (YAMANAKA et al., 2010).

Obtaining a soybean cultivar resistant to Asian rust has been a challenge for research. The existence of a large number of races (pathotypes) makes control difficult through vertical resistance. Dominant genes for resistance, called Rpp1 to Rpp4, identified in plant introductions (PI) reported in the literature (BROMFIELD; HARTWIG, 1980; McLEAN; BYTH, 1980; HARTWIG, 1986), have dubious stability, due to the great variability of the pathogen. . Eighteen pathotypes of the fungus were identified in samples collected from soybean plants and wild hosts in Japan (YAMAOKA et al., 2002). Studies carried out in Taiwan showed the existence of at least one pathotype, containing three virulence genes (BROMFIELD, 1981). In Brazil, studies carried out by Embrapa Soja identified 11 cultivars with resistance to rust (YORINORI et al., 2002a); however, this resistance was quickly overcome 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 2004 (ARIAS et al., 2004). Today, these genes no longer confer vertical resistance to the disease. Another type of resistance to be explored is the horizontal one, which involves a reduction in the rate of disease development, being more effective against a greater number of races of the pathogen. However, the quantification of this type of resistance is more difficult, thus limiting its use (TSCHANZ; WANG, 1985).

Due to the instability of vertical resistance and the difficulties associated with the identification and quantification of horizontal resistance, other methods have been used, aiming to avoid reductions in productivity caused by rust, highlighting the tolerance of cultivars. Tolerance can be defined as the ability of the plant to support the development of the pathogen, without presenting a significant reduction in productivity or product quality (SCHAFFER, 1970). Yield losses are generally used to assess the tolerance level and, therefore, the smaller the loss, the higher the tolerance level. Losses can be quantified by the difference in productivity between severely infected plots and plots without disease (ZADOCKS, 1985). Epidemiologically, tolerance and resistance are different concepts (VAN DER PLANK, 1963). As at the moment there is no variety at the world level that has absolute, vertical or complete resistance to the disease, researchers from abroad and from Brazil are looking for tolerant cultivars.

Rust-tolerant strains can be selected from segregating populations, growing under conditions of severe rust attack, simply by selection for productivity. These tolerant cultivars may require fewer fungicide applications and, therefore, reduce the cost of disease control.

Therefore, since cultivars with complete resistance are not available to be used commercially, chemical control becomes the most effective alternative for asian soybean rust control (NAVARINI et al., 2007; ZAMBOLIM et al., 2021; JULIATTI; ZAMBOLIM, 2021).

Cultural control

Cultural measures alone do not control the disease. However, they become an extremely necessary condition to increase the useful life of fungicides of the triazole, strobilurin and carboxamide groups and also of the varieties with vertical and horizontal or quantitative resistance (ZAMBOLIM et al., 2021; REIS et al., 2021a).

The use of early cycle varieties sown at the end of September and beginning of October reduces the time of exposure of the plant to the pathogen, as well as sowing at preferred times, avoiding late sowing (REIS et al., 2006). , because the inoculum load is higher due to the multiplication of the fungus in the first cultures (YORINORI, 2004) (Figure 7).

Normally, sowings in December, due to the longer time the leaves remain wet, present a greater risk of infection and development of epidemics, when compared to the cultivation of February for seed production. In soybean cultivation in February, there is a shorter time of leaf wetness and a reduction in the size of the plants, as a consequence, there will be less chance of the emergence of epidemics of the disease. In February sowing, disease control is usually done with systemic and multisites, with a lower number of atomizations (ERLEI et al., 2021a), in addition, the quality of the seeds will have higher quality due to lower rainfall intensity.

The population arrangement of plants can also contribute to attenuate the disease. The greater spacing between crop lines results in less rust severity and also allows for better distribution of the fungicide during application, facilitating disease management (FERREIRA, 2009; RAMOS, 2009). If the sowing density is high in dense spacings, there will be difficulty in penetration of the fungicide spray and, consequently, there may be deficient coverage of the leaves in the canopy. 2006; JULIATTI; ZAMBOLIM, 2021).

The presence of volunteer soybean plants at the beginning of the season favors the maintenance of the inoculum (spores) in the field, and they must be desiccated. In Paraguay and in the Campos Gerais of the state of Paraná, in addition to soybean, the legume kudzu (*Pueraria lobata*) is an important source of inoculum, as it is a perennial plant, widely established and highly susceptible to rust. Throughout the country, except in Mato Grosso fields, perennial soybean is a source of inoculum for the disease.

Considering the severity of the disease, several states have adopted the sanitary vacuum, a period of 60 to 90 days without soybean plants, in the field, as a strategy to reduce the amount of inoculum in the off-season, delaying the appearance of the disease, during the spring crop. /summer. The 'sanitary vacuum' must be adopted as a mandatory requirement by all states.

Another cultural measure that can be adopted is the staggering of sowing in relation to the crop cycle. Early varieties spend less time in the field, are harvested earlier and thus may “escape” the disease or be less affected. As in most regions there is no soybean in winter at the first sowings, the fungus is not yet present in the field; the tendency is for the inoculum to increase with the evolution of the crop. Thus, earlier sowings also constitute an escape mechanism in terms of fungal inoculum concentration.

Another very important cultural measure is the mineral nutrition of soybean plants. Studies carried out at the Federal University of Viçosa, Minas Gerais, showed that adequate nutrition of both potassium and phosphorus promoted a greater 'tolerance' of plants to rust (MOCHKO, et al., 2019; ZAMBOLIM et al., 2019). Therefore, nutrition with potassium and phosphorus does not control rust but promotes greater plant productivity, since phosphorus is part of essential amino acids, proteins and enzymes, making plant metabolism more efficient.

Chemical control

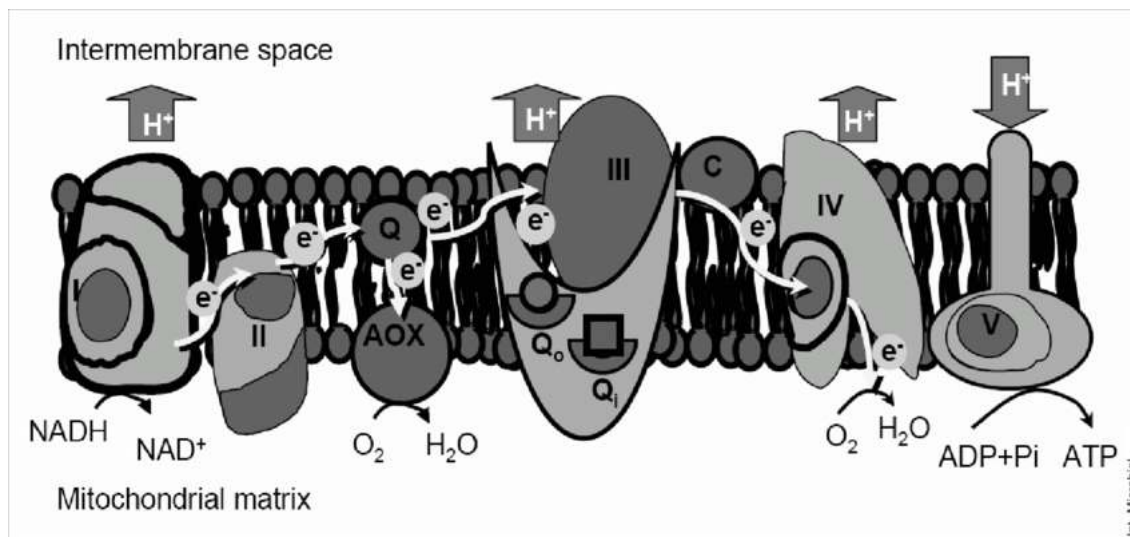
Nowadays the only viable measure is chemical control. The strategy that has been used most frequently is disease monitoring, to support chemical control. However, most growers spray at pre-closing, flowering and often preventively. The decision on the moment of application must be based on the factors necessary for the appearance of rust (presence of the fungus in the region, short spacing, flowering age of the plants and favorable climatic conditions), the logistics of application (availability of equipment and size of the property), the presence of other diseases and the cost of control by different methods.

The delay in the application, after the initial symptoms are observed, can lead to a reduction in productivity, if the weather conditions favor the progress of the disease, mainly rains. The number and need for reapplications will be determined by the stage at which the disease is identified in the crop and by the residual period of the products, which is usually around 15 days (ZAMBOLIM et al., 2021; JULIATTI; ZAMBOLIM, 2021).

When applying fungicides to control the disease, the adoption of anti-resistance strategies becomes essential. Figure 7 shows the main disease control methods using disease control tactics in susceptible varieties. The chemical control of the disease must comply with certain epidemiological criteria in order to be rational, economical with respect to the environment. First, the criterion of absence of rain/presence of symptoms in the lower leaves must be observed. In this case, the disease must be monitored and quantified for decision-making on chemical control. Second, it should be observed if the rains are frequent, with the presence of symptoms and sporulated lesions on the lower leaves of the plants. In this case, the recommendation is to atomize. Third, even in the absence of the disease but with frequent rains, the recommendation is to atomize the plants in a preventive way.

The main groups of fungicides registered for the control of Asian soybean rust are: demethylation inhibitors (DMI – tebuconazole, cyproconazole, prothioconazole, epoxiconazole and others), which are specific inhibitors of ergosterol biosynthesis; succinate dehydrogenase inhibitors (SDHI – fluxapyroxad, bixaphene and benzovindiflupyr), which are inhibitors of mitochondrial respiration, of complex II – succinate dehydrogenase (SDHI); equinone oxidase inhibitors (eQI – azoxystrobin, trifloxystrobin, picoxystrobin, pyraclostrobin and metominostrobin), which are specific inhibitors of quinone in complex III mitochondrial

respiration (Figure 8); and dithiocarbamate (mancozeb) which are multisite inhibitors of enzymes and proteins with SH group.



Source: Fernández-Ortuño et al., 2008.

Figure 8. Schematic representation of the mitochondrial electron transport system. I, II, III, IV are the different complexes of the transfer chain. V is the ATP synthase complex; Q, the ubiquinone pool; and C, the peripheral protein cytochrome c. The arrows inside the membrane indicate the direction of electron flow. Q₀ and Q_i binding sites of the cytochrome bc₁ enzyme complex (complex III) are delineated by a circle and a square representing Q₀- and Q_i-inhibitor molecules, respectively. In some fungi, inhibitors of the respiratory pathway induce the synthesis of alternative oxidase (AOX), which diverts electrons at the ubiquinone pool (Q), although generating much less energy.

The fungicides of the demethylation inhibitors, quinone inhibitors and succinate dehydrogenase inhibitors groups act only at a specific site of thousands of biochemical reactions in the fungal cell. Therefore, these groups of fungicides are vulnerable to the fungus races, for reduction or loss of sensitivity. They are considered high risk for the development of resistance and, therefore, their use alone is not recommended for the control of any fungal diseases, notably soybean rust. These groups of fungicides act directly on the selective pressure on the emergence of resistant mutants in the *P. pachyrhizi* fungus population (REIS et al., 2021b).

The history of the fungicide molecules used to control Asian soybean rust in Brazil is shown in Table 3.

Table 3. History of asian soybean rust control in Brazil

Year	Event
1979	Identification of the disease in Brazil
2001/02	Resurgence of the disease in Brazil
After 2002	Beginning of the use of triazole fungicides in the control of asian soybean rust.
2005/06	First evidence of a decrease in the performance of triazoles of isolated active ingredients.
2006/07	Rust control failure in Goiás state, for cyproconazole, flutriafol and tebuconazole.
2006/07	Introduction of the sanitary measure
After 2006	Introduction of dual formulations (DMI + QoI).
2007/08	Confirmation of reduced performance of triazoles in the field
2009/10	Introduction of soy tolerant germplasm to rust
2010/12	Official recommendation of the mixture of triazoles + strobilurins for rust control
2012/13	Introduction of the carboxamide group to control Asian soybean rust.
2013/14	Introduction of triple formulations for the control of Asian soybean rust formed by DMI + QoI + SDHI.
2013/15	Emergence of proticonazole from the DMI group.
2015/16	Record of azoxystrobin plus mancozeb mixture.
2015/16	Introduction of the dithiocarbamate group, cuprics and chlorothalonil aiming to form triple mixtures with DMI + QoI + mancozeb.

In Brazil, from the discovery of the disease until the year 2010-12, the damages in the production of soybeans were only reduced thanks to the chemical control carried out with fungicides of the triazoles and strobilurins (GODOY, 2012). However, today triazoles formulated with strobilurins without multisites are not efficient in controlling rust. Despite the high risk of emergence of resistant mutants in the *P. pachyrhizi* fungus population in the field, with the use of site-specific mode of action fungicides, the chemical control of Asian soybean rust at present is the only available solution to reduce the damage caused by the disease, due to the absence of cultivars with an adequate level of horizontal resistance and the low efficiency of prophylactic strategies.

However, because site-specific fungicides act specifically at a single site of action, they are subject to risk factors for the development of resistance in the fungal population. The risk factors are:

1. Use of site-specific fungicides as the only form of control;
2. Use of site-specific fungicides in extensive areas of cultivation;
3. Use of site-specific fungicides alone;
4. Change in recommended dose up or down;
5. Repetitive application of the site-specific active ingredient with the same mode of action;
6. Application of site-specific fungicide after the beginning of the epidemic as a curative;
7. Characteristics of the pathogen, such as number of generations per crop cycle, sporulation capacity and ease of dissemination. This is the case of *P. pachyrhizi* the causal agent of soybean rust.

The first chemicals used to control asian soybean rust belonged to triazole fungicides or demethylation inhibitors (DMI), especially flutriafol and tebuconazole. Difenconazole, myclobutanil and tetraconazole, fungicides with a specific mode of action were also used.

After five harvests, starting in 2002/03, of using DMI alone, Silva et al. (2008) reported failure to control Asian soybean rust in the state of Goiás in 2006/07 for cyproconazole, flutriafol and tebuconazole. Until then, flutriafol had been very 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 (FUNDAÇÃO MT, 2008). After the decline of flutriafol, tebuconazole became widely used with high efficiency and was adopted as a reference fungicide to control Asian soybean rust. In the 2005/06 crop, the average control of Asian soybean rust by DMI was 90.3%. After only eight harvests, corresponding to 2012/13, the control with DMI was 52.0, thus 42% reduction in control effectiveness (GODOY et al., 2013). The reduction in the sensitivity of *Phakopsora pachyrhizi* to the fungicides tebuconazole and cyproconazole, with only 42 and 38% of control, respectively, was also confirmed by GODOY; WORD (2011).

Observations from the 2007/2008 harvest showed that the samples collected in the main soybean producing regions of Brazil - in March - predominated populations of *Phakopsora pachyrhizi*, less sensitive to DMI, of the 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 the first-generation DMI extended to other locations in the country, in addition to states in the regions Midwest, Southwest (São Paulo and Minas Gerais) as well as 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 first-generation DMI were detected in all Brazilian states, which produce soybeans. Therefore, over the years, there was a gradual reduction in the effectiveness of DMI (tebuconazole) in the control of Asian soybean rust in soybean production fields in the country. The effectiveness of disease control with tebuconazole was 90 and 91% in soybean crops in 2003/05, 77% in 2005/06, 58% in 2006/08, 39% in 2008/09 and only 24% in 2009 /10 (GODOY; PALAVER, 2011, GODOY et al., 2013). The difficulty in controlling the disease with

isolated DMI fungicides was becoming increasingly evident, proving the high adaptability of *Phakopsora pachyrhizi* (GODOY; PALAVER, 2011; GODOY et al., 2013, SCHMITZ et al., 2014; ZAMBOLIM et al., 2021; JULIATTI; ZAMBOLIM, 2021).

With the emergence of fungicides from the strobilurin group (QoI) applied alone, for some time they were efficient in the control of asian soybean rust. However, fungicides of this group used in Europe to control other diseases showed reduced efficiency. After a short time of using fungicides from the strobilurin group in Brazil, they found that the performance of the products of this group was not satisfactory.

What probably happened with the DMI's in the control of asian soybean rust in the country, was the fact that, once the loss of sensitivity or resistance of *P. pachyrhizi* to a fungicide of this group, which has a mode of action in the biosynthesis of the ergosterol, resistance was transmitted from the fungus population to other fungicides with the same mode of action. This phenomenon is called cross-resistance (ZAMBOLIM et al, 2007; ZAMBOLIM et al., 2021).

The high adaptability of *P. pachyrhizi* in soybean fields in the country made it difficult to control Asian soybean rust with site-specific fungicides alone (SCHMITZ et al., 2013). The resistance or lower sensitivity of the fungus *P. pachyrhizi* to demethylation inhibitors (DMI) and quinone oxidase inhibitors (QoI) fungicides had already been confirmed in Brazil (SCHMITZ et al., 2014; KLOSOWSKI et al., 2016 a, b) . It is possible that resistance to triazole or strobilurin alone in fields where soybeans are cultivated extensively may have occurred because of germ tube anastomosis and migration of germ tube hyphae nuclei of the soybean rust pathogen, *P. pachyrhizi*, may explain how the fungus recombines its genetic material and develops resistance to fungicides with a specific mode of action (VITTAL et al., 2011). It is possible that this mechanism may occur in nature, as millions upon millions of urediniospores are produced on soybean leaves in the field, then dispersed by the wind.

Due to the decline in the effectiveness of both groups of DMI's and QoI fungicides, applied separately from the 2007/08 harvest in the Midwest region and in other regions from the 2008/09 harvest, the Phytopathology Commission of the Research Meeting from the Central Region of Brazil started to indicate only the use of commercial mixtures of triazoles with strobilurins for the control of asian soybean rust (FRAC, 2015).

In the 2010 to 2012 harvests, the mixtures of DMI + QoI, which were already used to control the disease, showed good control efficiency. Cyproconazole + azoxystrobin and epoxiconazole + pyraclostrobin showed 72 and 88% control, respectively. The average control for the mixtures was 80%. It is likely that the effectiveness was assured by fungicides of the QoI group, since the average control for DMI was only 40% (GODOY; PALAVER, 2011). About 27 million hectares of soybean, in the 2012/13 season, were treated with fungicide to control rust, reaching 93% in 2012. The highest percentage of the area was treated with DMI + QoI (60%). However, the area treated with DMI alone was 24%, and the area treated with QoI alone increased, reaching 3.6% in 2012 (SOYA RESEARCH MEETING OF THE SOUTH REGION, 2012). The isolated use of fungicides from both groups was not recommended by FRAC (2012). In this sense, it is important to emphasize that when two or more fungicides are combined, they must have different biochemical modes of action but always with multisite in the mixture. Fungicides that inhibit the biosynthesis of ergosterol – an important substance for maintaining

the integrity of the cell membrane of fungi, in addition to QoI fungicides that inhibit mitochondrial respiration (complex III), which blocks the transfer of electrons between cytochrome b and c cytochrome c1, at the site QoI, interfere with ATP production, were important in the control of Asian soybean rust. (ZAMBOLIM et al., 2021; JULIATTI; ZAMBOLIM, 2021).

The mixture of fungicides of the DMI + QoI groups, therefore, started to be used in the fields of soybean production in most producing regions. However, certain triazole and strobilurin fungicides did not show good efficiency in controlling the disease in mixtures. Hence, a fungicide of the carboxamide group was introduced on the market. In the 2012-13 harvest, new fungicides belonging to the chemical group carboxamide were introduced, which have a specific mode of action, inhibiting fungal respiration, of the complex II – succinate dehydrogenase (SDHI), to control Asian soybean rust (FRAC, 2015).

However, numerous cases of resistance to other pathogens had already been documented for this fungicide group in other countries (FRAC, 2015). It is concluded that the introduction of this group of fungicides to control the disease would probably not solve the problem, due to the fact that they present a specific mode of action, subject to resistance in the population of *P. pachyrhizi*. Due to this fact, the fungicides of this group were not recommended for use alone in the control of rust. Then came the triple mixes.

In the 2013/14 crop, there was a record of triple mixtures involving fungicides of the DMI + QoI + SDHI groups. The fungicides of the SDHI group launched in the market to compose the triple mixtures with triazoles and strobilurins were: bixaphene, fluxpiraxade, fluindapyr and benzovindiflupyr. Two to three years later, prothioconazole from the group of demethylation inhibitors appeared on the market.

In the period 2013-2015, a new fungicide from the DMI group emerged, prothioconazole. From the beginning of monitoring until its market launch, prothioconazole has shown the lowest values of effective concentration 50 (EC₅₀) in the rust monitoring program. The introduction of this fungicide on the market was the result of experiments conducted in demonstration areas in different soybean producing regions in Brazil. Prothioconazole was then evaluated in admixture with the QoI fungicide, trifloxystrobin. The comparison was made with fungicides launched on the market, such as combinations of strobilurins (QoI) and carboxamides (SDHI). As it is a fungicide composed of an innovative active ingredient with differentiated binding at the site of action of the fungus, prothioconazole constituted the new generation, in the chemical group of DMI, being chemically classified as triazolinthion (Frac classification on mode of action 2014 – available at: www.frac.info).

The combination prothioconazole + trifloxystrobin acts in two ways: i) in the control of Asian soybean rust; and ii) in the complex of diseases, such as target spot, powdery mildew, mildew, 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 for more than two applications. In this way, it is possible to fully explore the spectrum of action of this fungicide, initiating in a robust way the prevention and control of soybean rust.

However, as the recommendation for rust control involved three site-specific active ingredients (triazole, strobilurin and carboxamide) that could result in high selection pressure in the *P. pachyrhizi* population, the use of multisite fungicides in the mixture was considered. Hence, due to the reduction in the sensitivity of specific sites with control of less than 50%, from 2014 to 2015, fungicides with a multisite mode of action, such as mancozeb, chlorothalonil and oxychloride, were introduced in the control programs of asian soybean rust. copper. The introduction of multisite fungicides in disease control programs is the most important tool for the management of resistance to *P. pachyrhizi*, with the potential to preserve the effective shelf life of specific DMI, QoI, SDHI fungicides in the soybean crop (ZAMBOLIM et al., 2021).

Multisite fungicides (mancozeb, chlorothalonil, copper oxychloride) have a great advantage because, in addition to being inexpensive, they act at multiple sites of action in the fungal cell, interfering with numerous metabolic processes of the fungus. Consequently, resistance to site-specific fungicides would be rare or non-existent (ZAMBOLIM, 2006; ZAMBOLIM et al., 2021; JULIATTI; ZAMBOLIM, 2021). So far, no resistance of fungi causing rust to multisite fungicides has been found in the literature.

Recent work involving mancozeb in the control of asian soybean rust has shown that the product has the potential to control the disease, even in isolated applications (SILVA et al., 2015). Multisite fungicides, such as mancozeb, therefore, are strong allies in the defense against asian soybean rust (GULLINO et al., 2010; ZAMBOLIM et al., 2021). Based on the results obtained, three experiments were carried out in the field: doses of triazoles with strobilurins associated with the multisites mancozeb, chlorothalonil and methiram. (PONCE et al., 2019). The hypothesis is that fungicides from the DMI and QoI groups can be mixed with the multisites to improve rust control and increase soybean production. The results showed that the average rust control with the application of triazole with strobilurin associated with protective fungicides - mancozeb, chlorothalonil and methiram - was 70.2%. The rust control efficiency was not higher only because the products were applied after the beginning of the disease epidemic in the field. The tests carried out with soybeans in the field were sprayed, when the severity of the disease had already reached 2.0 to 5.0% in the leaves of the lower part of the plants. This result shows that any of the three protective fungicides can be used in a mixture with epoxiconazole with pyraclostrobin or cyproconazole with azoxystrobin (PONCE et al., 2019). In general, the DMI + QoI fungicides associated with multisites had an efficiency greater than 68.0% of the control and produced more than 70.0% of the control (control). These results show that it is possible to control the disease even after the disease severity has reached 2.0 to 5.0% before the first spraying of the plants. In this work, the protective fungicides mancozeb and chlorothalonil associated with epoxiconazole + pyraclostrobin (0.5 kg /ha) and cyproconazole + azoxystrobin (0.30 kg/ha) increased the soybean grain yield of 89.5 and 109, 0%, respectively (PONCE et al., 2019).

Based on the information above, it is suggested that the application of triazole and strobilurin associated with multisites, starting at the soybean crop stages (V9 or R1, R2), according to the monitoring of the disease and rainfall, may promote better control of the disease, especially in the leaves of the lower part 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 multisites, can be recommended as a new strategy for the management of soybean rust control in the short and long term. In addition, due to the residual

effect of multisite fungicides on soybean leaves (mancozeb, chlorothalonil and methiram) they can promote a longer effective life of DMI, QoI and SDHI and decrease the number of applications (ZAMBOLIM et al., 2021).

The addition of mancozeb to strengthen the fight against fungal resistance is not a new strategy. Mancozeb has been included in mixtures to contribute to resistance management and to broaden the spectrum of fungicides with specific mode of action for numerous plant diseases (GULLINO et al., 2010). To stabilize control of asian soybean rust, the same strategy could be used on soybeans to chemically manage asian soybean rust. Examples of fungicides that are already used in mixture with mancozeb include benalaxyl, cymoxanil, dimethomorph, famoxadone, fenamidone, folpet, fosetyl-aluminium, iprovalicarb, mandipropamide, metalaxyl and zoxamide (GULLINO et al., 2010). Likewise, this could be followed in the control of soybean rust, 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, it has been registered in more than 70 cultures and in 400 different diseases (GULLINO et al., 2010).

The introduction of multisites in the asian soybean rust control program can be an important tool for resistance management. These fungicides have the potential to preserve the effective lifetime of specific fungicides such as DMI, QoI and SDHI in soybean. (ZAMBOLIM et al., 2021).

The introduction of fungicides of the SDHI group (bixaphene, benzovindiflupyr, fluindapyr and fluxapyroxada) associated with DMI (prothioconazole) and QoI (picoxystrobin, trifloxystrobin) in triple mixtures and in double mixtures (trifloxystrobin + fluxapyroxada, azoxystrobin + benzovindiflupyr or picoxystrobin + benzovindiflupyr obtained the control percentages, varying from 69 to 82%. The most efficient combinations in the control of asian soybean rust were those that had the fungicides of the SDHI group in a double mixture, with the fungicides of the QoI group (pyraclostrobin and azoxystrobin) suggest that the addition of fungicides from the SDHI group were more efficient both in controlling and in soybean yield.

The highest yields corresponded to the fungicides of the groups, which provided the best rust control. Therefore, there was a correlation between disease control and soybean yield. The highest yields also corresponded to the mixtures, which had the participation of fungicides from the SDHI group (bixaphene, benzovindiflupyr and fluxapyroxada) (GODOY et al., 2015). But nowadays all the recommendations to control asian soybean rust must have multisite fungicides in a mixture.

CONSIDERATIONS ABOUT FUNGICIDES MIXTURES IN DISEASE CONTROL

All products registered with the Ministry of Agriculture, Livestock and Supply (Mapa) to control a given disease have passed the agronomic efficiency test. However, when they passed the test, many of them, carried out more than 15 years ago, did not have a high population of plants in the field and high inoculum pressure in the cultural remains and in guaxa plants and in the air, which in this case would be the urediniospores of *P. pachyrhizi*. In addition, there was no fungus in the population, resistant mutants in sufficient quantity capable of causing a reduction in the efficiency of the pathogen. Over time, due to the intense use of mobile penetrating fungicides with a specific mode of action and from the same chemical group, such as triazoles, the process of reducing the efficiency of these groups began. However, the reduction in efficiency occurred with all products, both the most efficient and those with reduced action in controlling the

disease. In the following years, a new group of fungicides was introduced to control rust, but again the same mistake was made, the group also had a specific mechanism of action, in this case strobilurins. Over time, both triazoles and strobilurins, used alone or in mixture, reduced efficiency. Then, later on, another specific systemic group, carboxamides, emerged. Double and triple mixtures began to be used with these three specific groups of fungicides. A short time later, it was also found that the efficiency of this mixture was no longer effective. The mixtures launched have already shown that they are potent inducers of resistance in the population of *P. pachyrhizi*. Allied to these facts, in disease control programs, they are often applied as 'curative'. What will be the future, if in the short and medium term, there will no longer be a new chemical group of fungicides on the market, with a site-specific mode of action, to be recommended for the control of asian soybean rust? Therefore, there will no longer be the possibility of using fungicides, with a specific site-specific mode of action, alone or in a double or triple mixture. Hence, there is a need to use products with a generalized mode of action the multissite fungicides. The dithiocarbamate (mancozeb) and nitriles (chlorothalonil) groups are available on the market; there are also coppers but with less efficiency. The great advantage of using these products is that they reduce the probability of the emergence of resistant mutants of the fungus in the *P. pachyrhizi* population. Formulated blends or tank blending of site specific fungicides with mancozeb or chlorothalonil is of great importance, in increasing the shelf life of mobile penetrating fungicides. Studies have shown that four to five applications of mancozeb have been efficient in controlling the disease (SILVA et al., 2015). Studies conducted at the Universidade Federal de Viçosa also demonstrated that mancozeb and chlorothalonil in a tank mixture with triazoles were very efficient in controlling asian soybean rust, in addition to significantly increasing productivity (PONCE et al., 2019; MOCHKO et al., 2019; ZAMBOLIM et al., 2019). The path, therefore, lies in the introduction of protective or residual fungicides in site-specific fungicide formulations formulated with triazoles, strobilurins and carboxamides. It is worth mentioning that the inefficiency in the control of asian soybean rust in the field is often due to failures in the application of fungicides. The application technology is based on three premises: time of application, environmental conditions and the target to be achieved. These are the points often overlooked by growers when applying fungicides.

CONCLUSIONS

To reduce damage to the soybean crop and minimize the emergence of resistance in the fungus population, the management strategies recommended in Brazil for this disease are:

- i. i) Currently, there are no recommendations for varieties based on a greater or lesser degree of susceptibility.
- ii. ii) Sequential and curative applications should be avoided to reduce selection pressure on the fungus population. Control should be based on disease monitoring in the field. Monitor mainly the humid sites in the field crop where the humidity is more intense, starting with the lower leaves. After the first application, continue monitoring the crop for decision making.
- iii. iii) Use early cycle cultivars and sowing at the beginning of the recommended season, when possible for each region. In this way, the extension of sowing to escape from the highest concentration of inoculum of the pathogen is avoided.
- iv. iv) Eliminate voluntary soy plants and guaxas in maize, cotton and bean crops.
- v. v) The off-season must be free of soybean cultivation (pay attention to the sanitary vacuum that varies from 60 to 90 days, according to each state). In the state of Mato Grosso, due to the sanitary vacuum, there will be no green bridges due to the water

- deficit in the winter period, except in areas for research with soybean breeding programs.
- vi. vi) Monitor the disease in the field, by the end of V9 stage of the crop development. Control will only be efficient if the disease is diagnosticated early. The monitoring of the soybean crop must be done mainly in humid sites, to verify if the disease is present or not in the plants.
 - vii. vii) Observe temperature conditions (15 to 28 °C) and wetness period above 6 hours, which are favorable to infection. It is important to observe the duration of daily leaf wetness in hours, resulting from light and constant rains to verify the possibility of infection by the pathogen. The first symptoms appear on the lower leaves of the plants and then rise in the canopy.
 - viii. viii) The atomization of the soybean crop can be based on three possibilities: (1) Atomize the crop, if the spacing is 50 cm between rows, for the control of asian soybean rust, at least once, before closing the planting lines and the following application every 14 days; if the spacing is greater than 50 cm, there is probability that there is no need to atomize the plants, however, there is always a need to monitor the plants in the field; (2) Atomize the culture at the R1/R2 phenological stage and the other atomizations being every 14 days. There are cases in which the disease begins in the vegetative period, hence the great importance of monitoring the disease on the plants; (3) Atomize only when the disease is detected in the field, the following applications being also based on disease intensity.
 - ix. ix) The recommended number of site-specific fungicide applications would be two in mixture with multissite fungicides; however, in cases where the disease is severe, up to three atomizations and a maximum of four can be achieved. Multissite fungicides should always be part of the anti-disease resistance strategy, either mixed with site-specific or alternating.
 - x. x) Avoid sowing later than the recommended time.
 - xi. xi) The use of multissite fungicides with a genral mode of action is fundamental in preserving the effective life of mobile penetrating fungicides on plants in the field.
 - xii. xii) Take into account the application logistics (availability of equipment and size of the area to be atomized), the presence of other diseases and the cost of control by atomizers, drones and aircraft.

It is concluded that the integrated management of asian soybean rust will only be efficient, guaranteeing the sustainability of the crop, when groups of mobile penetrating fungicides with efficiency greater than 70% (triazoles, strobilurins and carboxamides) with site-specific mode of action are used always mixed in a tank with multissite fungicides (mancozeb, chlorothalonil, copper oxychloride), in all applications and in all areas (ZAMBOLIM et al., 2021). In addition, it is necessary to adopt cultural practices such as good nutrition with potassium and phosphorus, soybean varieties with quantitative or horizontal resistance and appropriating spacing of the plants. Early cycle cultivars and sowing at the beginning of the season, escape to the highest concentration of inoculum of the pathogen.

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