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How to cope with the vulnerability of site specific fungicides on the control of Asian soybean rust

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Abstract

Asian soybean rust (ASR) caused by *Phakopsora pachyrhizi* is the most important disease of the crop. The pathogen is highly aggressive under field conditions when the environmental conditions favor the disease development. The disease was first described in Japan, in 1902 and in 1914, it had already spread to several countries in Southeast Asia.). The first report in South America was made in Brazil in 1979 in wild soybeans and later in 2001 in the soythern area of the country in comercial plantations. *P. pachyrhizi* can naturally infect a wide range of plant species, including 41 species in 17 genera of the Fabaceae family. The symptoms of the disease can be seen on the abaxial and eventually on the adaxial surface of the lesions. In susceptible varieties the number of uredia per lesion varies from four to eight and the latent period around seven days. *P. pachyrhizi* requires more than 8 hours a day of continuous leaf wetness, and the optimal temperature for maximum germination around 22^{o}C. There is no varieties with complete resistance to the disease available to planting. The main method of control of the disease is the application of fungicides. The main groups of fungicides to control the disease belongs to the demetilation inhibitores (DMI's), quinone outside inhibitor (QoI's) and carboxamides (SDHI). But with the continuous spraying with DMI's and QoI's alone to control ASR resistant mutants of *P. pacHIhyrhizi* multiplied in the population over the country. The use of mixture of DMI'S with QoI's in the beginning proportioned resonable controle of the disease. But few years latter *P. pachyrhizi* acquired resistance to the mixture of triazol with stobolurins. Then it was introduced the carboxamide group (SDHI) to use in mixture with triazol with stobolurins. The triple mixture was then recommended for more a few years giving good results. Finally researchers decided to incorporate multisite fungicides in a mixture with triazol, strobilurin and or carboxamide to minimize the probability to build up resistant mutants in the population of *P. pachyrhizi.*

The addition of multisite fungicides in a mixture with site specific is very important to reinforce the fight against fungal resistance. In conclusion to cope with the vulnerability of *P. pachyrhizi* to site specific fungicides the strategy has to involve integration of measures such as sanitary vacuum, rotation of a mixture of different biochemical mechanisms of action plus multisite, avoid sequential and curative applications, use of early cultivars and sowing at the beginning of the recommended season, eliminate voluntary soybean plants from the field, use of fungicides in the onset of preventively at least once before the planting lines closed, sowing at the beginning of the recommended season, and use of cultivars with resistance gene (s) to reduce the number of spraying. The present overview discuss how difficult is to cope with the resistance of *P. pachyrhizi* to site specific fungicides.

Keywords: *Phakopsora pachyrhizi*, *Glycine max*, site-specific, multissite, fungicides

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 (Díaz *et al*., 1992) [19]. Brazil is the first soybean producer in the world, and the largest exporter (FAO, 2020). In the 2019/20 harvest, the country produced about 124.85 million tons, representing 30% of world production (CONAB, 2020) [14]. Among the diseases that attack soybean, Asian soybean rust (ASR) 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 has been reported (Sinclair; Hartman, 1999; Yorinori *et al*., 2005; Zambolim 2006a) [83, 4, 32, 98] (Figure 1). In Brazil, 70% damage attributed to ASR was reported in the 2001/2002 crop (Yorinori; Morel, 2002) [56].

In the same crop in the Chapadão do Sul region, Mato Grosso do Sul, damage of up to 100% was recorded (Andrade; Andrade, 2002, 2006) [53]. According to Henning and Godoy (2006) [35], losses in the 2002-2003 crop reached almost one billion dollars.

The pathogen is highly aggressive under field conditions when the enviromental conditions favor the disease development. Currently, due to the unavailability of cultivars with complete resistance, the application of fungicides is the main recommended tool for disease control along with cultural practices (Yorinori 2004; Juliatti *et al*., 2004. Silva *et al*., 2007; Silva *et al*., 2011; Mochko *et al*., 2019; Zambolim *et al*., 2019; Reis *et al*., 2021) [96, 81, 80, 53, 92, 67].

The purpose of this review is to discuss and propose measures how to cope with the vulnerability of specific site fungicides in the control of Asian soybean rust.

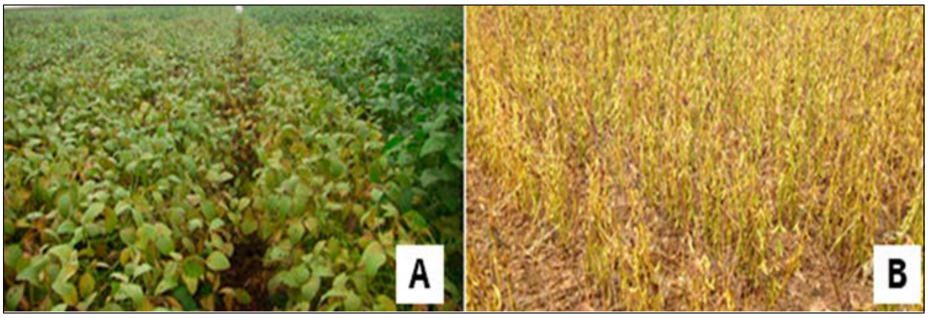


Fig 1: Soybean plants during maturing phase without chemical control of Asian rust (A) and the same plants severely attacked by the disease a week later with severe defoliation (B). Campo Verde city, Mato Grosso state, Brazil, February-2010 (W55°16'30"/S15°35'6"). Source: Rosa *et al*., 2015 [68].

Chronology of the appearance of the disease in the world

Asian soybean rust was first described in Japan, in 1902 (Henning, 1903) [34], 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 (Mawuena, 1982) [51], shortly after Uganda, in 1996 (Kawuki *et al*., 2003) [42], followed in 1998 in Kenya and Rwanda (Reis and Bresolin, 2004) [65], Zimbabwe and Zambia (Levy, 2005) [46]. In 2001, it was found in South Africa and Nigeria (Akinsani *et al*., 2001), reaching an epidemic character (Pretorius *et al*., 2001) [63]. In 2007, rust was also reported in Ghana (Bandyopadhyay *et al*., 2007) [9]. On the American continent, it was first reported in 1976 in Puerto Rico (Vakili and Bromfield, 1976) [88], followed by Hawaii in 1994 (Killgore; Hell, 1994) [43]. The first report in South America was made in Brazil by Deslandes (1979) [18], in the south of the State of Minas Gerais. At that time, mycologist Josué Deslandes detected both American rust (*P. meibomiae*) and ASR (*P. pachyrhizi*) in soybean plantations and in wild legumes (Deslandes 1979) [18]. In 2001 ARS resurfaced in Campos Gerais do Paraná (Jaccoud Filho *et al*., 2001, in Western Paraná (Yorinori *et al*., 2005) [36, 4], and then in Paraguay (Morel; Yorinori, 2002) [56]. In 2002, the disease appeared again in the southern region of Brazil (Yorinori, *et al*., 2002; REIS *et al*., 2002) [56] and in Argentina in 2003 (ROSSI, 2003) [69]. In the Brazilian up land area (Mid-west) of the country, rust was reported in 2003 and 2004 (Juliatti *et al*., 2004), with the formation of the phase of telia and teliospores, at harvesting time. The disease has also been reported in Bolivia (Navarro *et al*., 2004) [57] and Colombia (REIS *et al*., 2006a) [66], progressing in 2004 for Uruguay (Stewart *et al*., 2005) [79] and 2005, in Ecuador (Sotomayor Herrera, 2005) [78], Mexico (Cárcamo-Rodríguez *et al*., 2007; Yáñez-Morales *et al*., 2009) [13, 85] and the United States (Schneider *et al*., 2005) [71]. Currently, ARS is present in all countries, where soybean is grown. Its spread was rapid throughout the world, due to the fungus urediniospores being disseminated by wind currents (Bromfield, 1984; Hartman *et al*., 2007; Yorinori *et al*., 2004) [72, 96, 81].

Pathogen hosts

The causative agent of ASR (*P. pachyrhizi*) is a biotrophic fungus, which survives on green soybeans and other wild legume hosts. Hartman *et al*., (1999) [32] report 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. In addition, 60 plant species belonging to 26 genera were experimentally infected under controlled conditions (Rytter *et al*., 1984) [69], reaching up to 90 species (Misman; Purwati, 1985) [52].

Symptoms of the disease

The symptoms are grouped into lesions of 2 to 5 mm in diameter, with up to eight uredias and abundant sporulation (Bromfield, 1984) [72]. The leaf tissues around the first uredias may acquire a light brown color, called a susceptible lesion or TAN (tanish) when the variety is susceptible and the other reddish brown, known as a resistant lesion or RB (redish-brown) if the variety is resistant (Bonde *et al*., 2006) [11]. Lesions with uredias usually appear on the leaf's abaxial face (Hartman *et al*., 1999) [32]; sporadically, they may appear at the top of them (Almeida *et al*., 2005; Garcés, 2010) [4, 25]. Uredospores are expelled from the uredias by a tiny pore of hyaline coloration that becomes beige and accumulates around the pores or is removed by the wind (Almeida *et al*., 2005) [4].

The first lesions, in general, are found in the lower leaves close to the soil, when the plants are in the phenological stage near or after flowering. The final stage of the ASR epidemic in a field is characterized by general yellowing of the foliage, with intense defoliation, reaching the complete fall of the leaves (Reis *et al*., 2006) [64].

Causal agent of the disease

Taxonomically the fungus is classified as follows: Kingdom: Fungi; Class: Basidiomycetes; Order: Uredinales; Family: Phakopsoraceae; Current name: *Phakopsora pachyrhizi* Sydow and Sydow; Synonyms: *Phakopsora sojae* Fujikuro; *Phakopsora calothea* H. Sydow; *Malupa sojae* (P. Hennings) Ono, Buritica, and Hennen comb. nov. (Anamorph) *Uredo sojae* P. Hennings (Alexopoulos *et al*. 1996) [3].

Conditions that favour the disease

The fungus has a short life cycle, under conditions of fine and frequent rains, long periods of dew and temperatures between 15 and 29 °C. Spore production can last at least three weeks (Melching *et al*., 1989; Dorrance *et al*., 2005,) [50, 20]. The rapid development of the disease has been correlated with canopy closure at the flowering stage (R1$^+$) (Dorrance *et al*., 2005) [20]. Then, the ASR progresses until there is complete defoliation of the canopy, or until the environment is no longer conducive to the development of the disease (Rupe; Sconyers, 2008) [68]. Flowering infection can produce high levels of damage, compromising the formation and filling of pods, the final weight of the grains, affecting the oil and protein content (Yang *et al*., 1991) [84]. After infection, the fungus produces uredia and urediniospores between seven and 14 days, according to environmental conditions (Dorrance *et al*., 2005) [20]. The infections process of *P. pachyrhizi* requires > 8 hours a day of continuous leaf wetness, being the temperature not limiting for the process (Melching *et al*., 1989; Blum *et al*., 2015) [50, 10]. The uredospores do not germinate in the absence of dew in the surface of the leaves. The lower thermal threshold was 4ºC, the upper 34 ºC, and the optimal temperature for maximum germination 22.2 ºC (Blum *et al*., 2015) [10]. Twizeyimana & Hartman (2010) found that uredospores were killed in four days at 40 to 50 ºC, in eight days at 30ºC and in 18 days at 25 ºC. Godoy and Flausino (2004) [26] showed that the uredospores of *P. pachyrhizi* remained viable for 17 days in the laboratory bench environment, for 60 days in a refrigerator and for 30 days in detached leaflet. In India, Patil *et al*. (1997) [59] reported the viability of the spores for 55 days in detached leaves protected by shade. Soybean infection by *P. pachyrhizi* does not occur at temperatures ≥ 30ºC (Danelli *et al*., (2015) [17]. Directly solar radiation kill in five hours the *P. pachyrhizi* uredospores (Nicolini *et al*., 2010) [58].

Main groups of sistemic fungicides to control Asian soybean rust

There are three main groups of site specific sistemic fungicides (DMI, QoI and SDHI) to control ASR in the world. The demethylator inhibitor (DMI) fungicides are the most important class of compounds for the control of plant fungal pathogens. DMIs are a structurally diverse class of compounds (Chen *et al*. 2015) [15], and act by inhibiting the activity of the enzyme lanosterol 14α-demethylase cytochrome P450 monooxygenase (CYP51), which is involved in the pathway of ergosterol biosynthesis (Ziogas and Malandrakis, 2015) [93]. Ergosterol is the main sterol of the cell membrane in most fungi and is essential for maintaining cell membrane integrity and permeability (Cheng *et al*., 2015) [15].

Strobilurins, or quinone outside inhibitors (QoI), are an outstanding class of fungicides, whose discovery was inspired by a group of natural derivatives of *β*-methoxy acrylic acid, isolated mainly from basidiomycetes (Cheng *et al*., 2015) [15]. These compounds inhibit mitochondrial respiration by binding to a specific site in the mitochondria, the quinol oxidation (Qo) site (or ubiquinol site) of cytochrome *b* (Cyt *b*; subunit of the Cyt *bc*1 complex) and thereby hamper electron transfer between Cyt *b* and cytochrome *c* (Cyt *c*). This prevents oxidation of reduced nicotinamide adenine dinucleotide (NADH) and synthesis of adenosine triphosphate (ATP), thus leading to the inhibition of the energy production essential for survival (Bartlett *et al*., (2002) [7].

At present, there are numerous synthetic analogues derived from natural strobilurins registered as fungicides in the world market and more are still being developed (Balba 2007) [8]. Since strobilurins have a single-site mode of action, they are prone to the development of resistance.

Succinate dehydrogenase inhibitors (SDHI) are the fastest growing class of fungicides in terms of new compounds launched into the Market (Sierotzki, & Scalliet, 2013) [72]. The SDH enzyme (also termed succinate ubiquinone oxidoreductase) is a mitochondrial heterotetramer composed of four nuclear-encoded subunits. In contrast to other dehydrogenases of the tricarboxylic acid (TCA) cycle, the SDH enzyme transfers succinate-derived electrons directly to the ubiquinone pool of the respiratory chain and not to soluble nicotinamide adenine dinucleotide (NAD$^+$) intermediates. For this reason, SDH, named also complex II, is considered to be an essential component of the respiratory chain. All crop protection SDHI target the ubiquinone-binding pocket. Upon binding, they physically block the access to the substrate, which consequently prevents further cycling of succinate oxidation. Currently, the "overall" spectrum of SDHI fungicides is extremely broad, being comparable with the QoI spectrum. The most recent SDHI fungicides possess high level of activity against the most important pathogens causing diseases in crops (Xiong *et al*., 2015) [83].

Chronogram of events in the control of Asian soybean rust in Brazil.

The history of the ASR control schedule in Brazil is shown in Table 1.

Table 1: History of the ASR control schedule in Brazil.

Year	Event
1976	Identification of American and Asian soybean rust in the south of Minas by Mycologist Josué Deslandes. There was no epidemic of the disease in Brazil at this time.
2001/02	Second finding of Asian soybean rust in Brazil in the southern area was followed by an epidemic of the disease.
After 2002	Beginning of the use of triazole fungicides to control Asian soybean rust.
2005/06	First evidence of a decrease in the performance of the triazoles fungicides.
2006/07	Failure of ciproconazole, flutriafol and tebuconazole to control Asian soybean rust in the state of Goiás, Brazil.
2006/07	Introduction of the sanitary vacuum.
After 2006	Introduction of mixture of active ingredients (DMIs + QoIs)
2007/08	Confirmation of the decrease in the performance of triazoles in the field in the control of Asian soybean rust.
2009/10	Introduction of tolerant soybean germplasm or with partial resistance to Asian soybean rust.
2010/12	Official recommendation of the mixture of triazoles + strobilurins for Asian soybean 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 DMIs + QoIs + SDHI.
2013/15	Recommendation and final registration of the proticonazole from the DMI's group to control Asian soybean rust.
2015/16	Introduction of the group dithiocarbamate (mancozeb), chlorothalonil and copper oxychloride in order to form double or triple mixtures with DMI's, QoI's and SDHIs.

When ASR was discovered in Brazil the disease caused great yield loss. But then the disease was controlled with site specific systemic fungicides using triazole and strobilurin groups (Juliatti *et al*., 2017a) [39]. Despite the high risk of emergence of less sensitive or resistant mutants in the population of the *P. pachyrhizi* fungus, in the field, with the use of fungicides with site specific mode of action, the chemical control of ASR, at present is the only solution available, to reduce the damage caused by the disease.

Risk of using site specific mode of action fungicides to control Asian soybean rust

Site specific fungicides are subject to the risk of developing resistance in the *P. pachyrhizi* populations. The risk factors for the appearance of resistant mutants in the population of *P. pachyrhizi* are:

1. Use of systemic fungicides as the only measure of Asian soybean rust control.
2. Use of only systemic fungicides in extensive soybean cultivation areas.
3. Use of more than two sprays of systemic fungicides to control Asian soybean rust of the same biochemical mechanism of action.
4. Change in the recommended dose. The dose to be used in the spray programs must always be the one recommended by chemical companies.
5. Repetitive application of the active ingredient, with the same biochemical mechanism of action.
6. Application of systemic fungicide during the epidemic of the disease.
7. Characteristics of the pathogen, such as number of generations per crop cycle, sporulation capacity and dissemination by the wind. The *P. pachyrhizi* fungus is a very aggressive pathogen in soybean.
8. Short latent period (5 - 7 days) in susceptible soybean varieties (Martins *et al*., (2007) [49]. Considering seven days the latent period of the fungus, in a period of 45 to 95 days (appearance of the first symptoms of the disease in the field until the beginning of the senescence of the leaves), we would have at least eight cycles of the pathogen which is too much.
9. Extensive time for sowing soybeans for grain production. The sowing period in Brazil is from September to December.
10. In late sowing, the interval of application of fungicides is shorter due to the higher pressure of inoculum in the cultivation fields.

All the conditions above may be very risk to raise resistant mutant in the populations of *P. pachyrhizi.*

Reduced sensitivity of *Phakopsora pachyrhizi* to site specific fungicides

The first chemicals used to control ASR belonged to the triazol fungicides or demethylation inhibitors (DMI's) fungicides with specific mode of action: cyproconazol, epoxiconazol, flutriafol and tebuconazol, difenoconazol, myclobutanil and tetraconazol.

After five seasons of soybean cultivation (from 2002/03) using DMI's alone, failure to control ASR in the state of Goiás in 2006/07 was reported for cyproconazol, flutriafol and tebuconazol (Silva *et al*. 2008) [82]. Until then, flutriafol was used as a standard fungicide, becoming the market leader. As of 2005/06, there was a reduction in the effectiveness of flutriafol, in the State of Mato Grosso (Fundação, 2008) [23]. After the decline of flutriafol, tebuconazol became widely used with high efficiency and was adopted as a reference fungicide for the control of ASR. In the 2005/06 season, the average ASR control by DMI's was 90.3%. After only eight seasons, the control with DMI's was 52.0%, corresponding to 2012/13, 42% reduction in control effectiveness (Godoy *et al*., 2013) [28]. In other states of the federation, such as Minas Gerais, in the 2005 and 2006 crop, the same behavior was observed with the reduction in the efficiency of triazoles (Furtado 2007) [24], which in many situations reached 50% or less effectiveness value.

The reduction in the sensitivity of *Phakopsora pachyrhizi* to the fungicides tebuconazol and cyproconazol, with only 42 and 38% of control, respectively, was also confirmed by Godoy & Palaver (2011) [27].

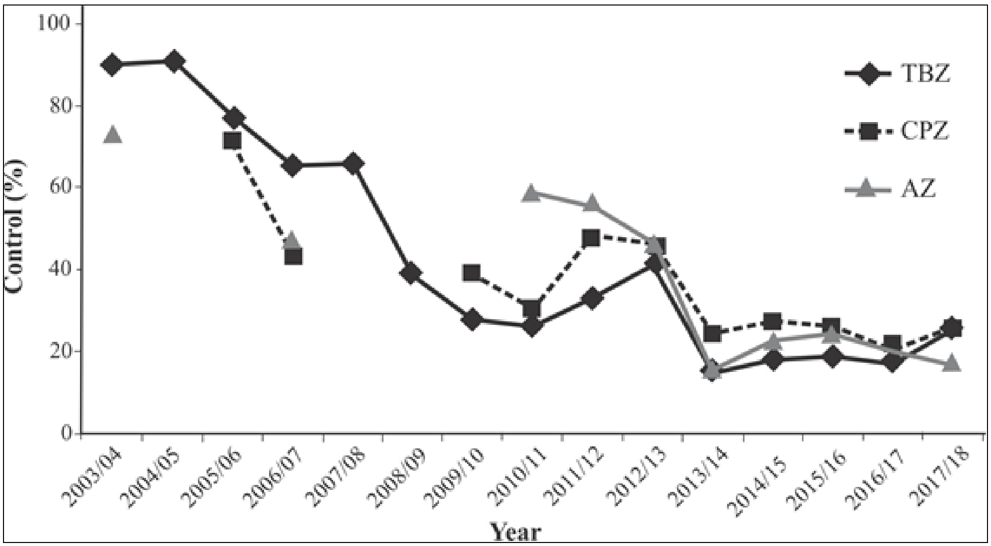
Observations from the 2007/2008 crop showed that the samples collected in the main soybean producing regions of Brazil - in March - predominated populations of *Phakopsora pachyrhizi*, less sensitive to first generation of DMI's, mainly tebuconazol, in some states of the up land area region. In the 2008/2009 crop, the samples collected in the same month and locations of the 2007/2008 crop showed that the predominance of populations less sensitive to first generation DMI's, extended to southeast area of the country (São Paulo and Minas Gerais).

Between the 2009/2010 and 2013/2014 populations of *P. pachyrhizi* less sensitive to first generation DMI's were detected in all Brazilian states that cultivate soybeans. Therefore, over the years, there has been a gradual reduction in the efficacy of tebuconazol in controlling ASR in the production fields in the country. The effectiveness of ASR control with tebuconazol 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 (Godoy and Palaver, 2011, Godoy, *et al*., 2013) [28, 27]. The difficulty in controlling ASR with isolated DMI's fungicides was becoming increasingly evident, proving the high adaptability of *Phakopsora pachyrhizi* to DMI's (Godoy and Palaver, 2011, Godoy *et al*., 2013, Schmitz, 2013) [27, 28,].

What probably happened with the DMI's, in the control of ASR was the fact that, once the resistance of *P. pachyrhizi* to a fungicide of this group emerged, which shows a mode of action in the ergosterol biosynthesis, the resistance was transmitted to the fungus population, for other fungicides with the same biochemical mechanism of action. This phenomenon is called cross resistance (ZAMBOLIM *et al*., 2006b) [99].

In 2010/12 it was approved an official recommendation of the mixture of DMI's + QoI's to control ASR. Fungicides in the group of strobilurins (QoIs) applied alone, have been effective for some time in the control of ASR. Thus due to the lower performance of DMI's fungicides, the strobilurin group have also started to be used in Brazil to control ASR.

The high adaptability of *P. pachyrhizi* in soybean fields makes it difficult to control ASR with specific fungicides alone (Schmitz *et al*., 2013) [70]. The resistance or less sensitivity of the *P. pachyrhizi* fungus to demethylation inhibitors (DMI's) and quinone oxidase inhibitors (QoI's) had already been confirmed in Brazil (Schmitz *et al*., 2014; KLOSOWSKI *et al*., 2016; Godoy *et al*.,2019) [29]. Figure 2 shows the gradual reduction in the sensitivity of *P. pachyrhizi* to the tebuconazole, ciproconazole (DMI) and azoxystrobin (QoI) fungicides from 2003/04 to 2017/18.



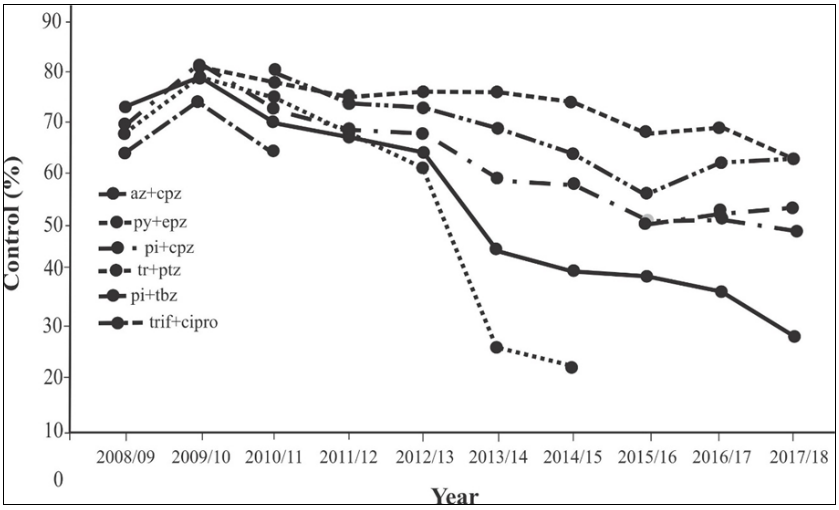
Source: Godoy *et al*., (2019) [29] adapted.

Fig 2: Gradual reduction in the sensitivity of *P. pachyrhizi* to the tebuconazole (TBZ), ciproconazole (CPZ) and azoxystrobin (AZ) fungicides from 2003/04 to 2017/18.

Due to the decline in the effectiveness of both groups of fungicides DMI's and QoI's, applied alone, from the 2007/08 crop, in the up land area (Midwest) of Brazil and in the other regions from the 2008/09 crop, the Commission of Phytopathology of the Research Group of the Central Region of Brazil, started to indicate only the use of commercial mixtures DMI + QoI, for the control of ASR (FRAC, 2015) [21]. The combinations of two or more fungicides with different biochemical mechanisms of action, must be complementary, that is, acting on completely different sites of action in the development of the fungus. The fungicides inhibiting the ergosterol biosynthesis - an important substance for maintaining the integrity of the fungal cell membrane, in addition to QoI's fungicides, which inhibit mitochondrial respiration (complex III) blocks the transfer of electrons between the cytochrome b and the cytochrome c_1, at the QoI site and interferes with ATP production. Thus, the mixture of fungicides from the DMI's + QoI's groups has therefore started to be used in soybean production fields in most producing regions to controle ASR since 2008/09.

In the 2010 to 2012 growing seasons, the mixtures of DMI's + QoI's that was already used to control ASR, showed good efficiency in controlling the disease. Ciproconazol + azoxystrobin and epoxiconazol + pyraclostrobin, showed 72% and 88% control, respectively. The average control for mixtures was 80%. It is likely that the efficacy was ensured by fungicides from the QoI's group, since the control average for DMI was only 40% (Godoy and Palaver, 2011) [27].

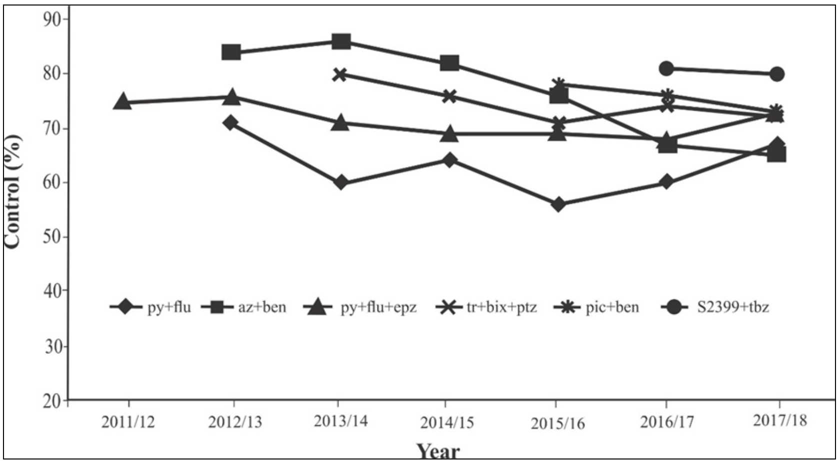
From 2009/10 the percentage of ASR control of the mixture of DMI's + QoI was from 70 - 80%; in 2017/18 the mixture trifloxistrobin + protioconazol and piraclostrobin + tebuconazol decreased the eficacy 13,3 %; two decreased 33% (picoxystrobin + ciproconazol; ciproconazol + trifloxistrobin); one mixture of fungicide decreased 60% (ciproconazol + azoxistrobin) and epoxiconazol + pyraclostrobin 70,6% (Figure 3).



Source: Adapted from Cooperative Testing Network - Anti-rust Consortium (Embrapa).

Fig 3: Gradual reduction in the sensitivity of *P. pachyrhizi* to the mixture of DMI's + QoI's fungicides from 2008/09 to 2017/18. Abreviations: az + cpz = azoxystrobin + ciproconazole; py + epz = pyraclostrobin + epoxiconazole; pi + cpz = picoxystrobin + ciproconazole; tr + ptz = trifloxystrobin + protioconazole; pi + tbz = picoxystrobin + tebuconazole; trif + cipro = trifloxystrobin + ciproconazole.

From 2011/12 to 2017/18 several trials were conducted involving mixture of fungicides of the groups DMI's, QoI and SDHI (Figure 4)



Source: Adapted from Cooperative Testing Network - Anti-rust Consortium (Embrapa).

Fig 4: Gradual reduction in the sensitivity of *P. pachyrhizi* to the mixture of DMI's + QoI's, DMI's + SDHI and DMI's + QoI's + SDHI fungicides from 2011/012 to 2017/18. Abreviations: py + flu = pyraclostrobin + flutriafol; az + ben = azoxistrobin + benzovindiflupir; py + flu + epz = pyraclostrobin + flutriafol + epoxiconazol; tr + bix + ptz = trifloxystrobin + bixafen + protioconazole; pic + ben = picoxystrobin + benzovindiflupir; S2399 + tbz = candidate fungicide + tebuconazole.

From 2012/13 the percentage of ASR control of the mixture of DMI's + QoI was from 72 - 85%; in 2017/18 one fungicide mixture mantained similar eficacy (pyraclostrobin + flutriafol); azoxystrobin + benzovindiflupir decreased 18, 7 %; pyraclostrobin + flutriafol + epoxiconazol decreased 9,3%; trifloxystrobin + bixafen + protioconazol and picoxystrobin + benzovindiflupir 6,2%, respectively (Figure 4).

In the 2013/14 crop, strobilurins reduced efficiency. In the same crop, the first mixtures of strobilurin and carboxamide fungicides were registered for soybean cultivation. In the 2016/17 crop, some fungicides with carboxamides showed reduced efficiency in cooperative trials, in relation to the results of the previous crop, in specific regions. Therefore, due to the reduced sensitivity of *Phakopsora pachyrhizi* to Demethylation Inhibitors (DMI's) + Strobilurins (QoI's) fungicides, several fungicides from the Carboxamide group were launched on the market.

Introduction of the carboxamide group

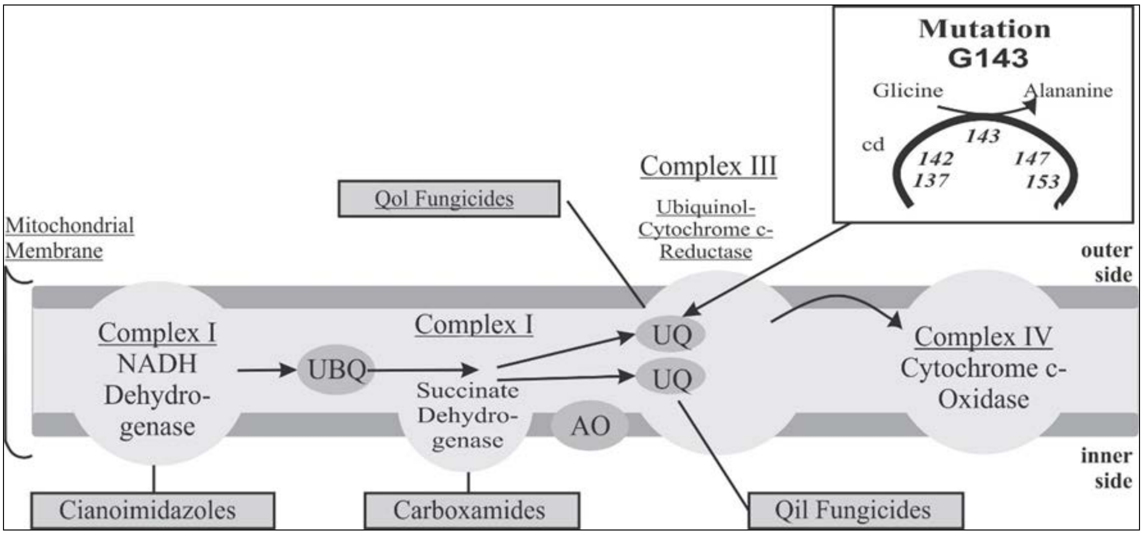
In 2012-13, new fungicides belonging to the group of Carboxamides were introduced to control ASR, which have a specific mode of action, inhibiting fungal respiration, of complex II - succinate dehydrogenase (SDHI) (FRAC, 2015) [21].

In Brazil, three fungicides of the Carboxamide group available on the market are bixafen, fluxopyiraxade and benzovindiflupyr. However, several several pathogens were reported to be resistant to fungicides of the Carboxamide group, in European countries (FRAC, 2015) [21].

For the Pyrazole-4-carboxamide group (Benzovindiflupyr, Bixafen, Fluxapyroxad, Furametpyr, Isopyrazam, Penflufen, Penthiopyrad and Sedaxane) resistance is known for several species in field populations and laboratory mutants. The site of action of mutations in the SDHI genes, ex. H / Y (or H / L) in 257, 267, 272 or P225L, depends on the species of the fungus. Such fungicides are considered to have a medium to high risk of resistance (FRAC 2015) [21].

It is concluded that the introduction of this fungicide group (Carboxamide), for the control of ASR, probably will not solve the problem, due to the fact that they present a specific mode of action that may cause resistance in the population of *P. pachyrhizi*. Due to these facts, the fungicides of this group, were not recommended for spraying alone, in the control of ASR. Hence the triple mixtures for the control of *P. pachyrhizi* arose.

The biochemical mechanism of action of DMI'S, QoI's e SDHI's on the mitochondrial respiration chain of phytopathogenic fungi is on the Figure 3..



Source: Frac, 2015 [21]

Fig 3: Biochemical mechanism of action of DMI'S, QoI's e SDHI's on the mitochondrial respiration chain of phytopathogenic fungi.

Note that the three different groups of fungicides (DMI'S, QoI's e SDHI's) having a specific site of action act at different locations in the electron transport chain in the mitochondria.

In the years 2013/14, a triple mixtures was registered in Brazil, involving fungicides from the groups DMI's + QoI's + SDHI's to control ASR. The fungicides of the SDHI group, launched on the market, to compose the triple mixtures with triazoles and strobilurins were: benzovindiflupir and fluxpyroxade, bixafen.

Emergence of prothiconazol from the DMI's group

A requirement for multiple mutations to confer resistance, and the diversity of Azoles compounds available to growers, have extended the effective life of this group of fungicides and have ensured that newly developed compounds. From 2013-2015, a new fungicide from the DMI's group, prothioconazole was introduced despite the significant reduction already observed for most fungicides DMI's since 2001/2002. Prothioconazole can still have a profitable share of the market, despite the existence of azole-resistant strains of target pathogens. It was the last DMI registered for control *P. pachyrhizi* in Brazil and is the one that maintains the highest control efficiency (Godoy *et al*., 2020) [30].

Prothioconazole has been very effective to control azole-resistant strains of Asian soybean rust, *Phakopsora pachyrhizi* (Koga *et al*., 2011; Schmitz *et al*., 2013) [45, 70]. From the beginning of monitoring until its launch on the market, protioconazole has shown the lowest effective concentration values 50 (EC_{50}) against *P. pachyrhizi*. The introduction of this fungicide on the market was the result of hundreds of experiments, conducted in demonstration areas, in different soybean producing regions in Brazil. Then the prothioconazole was 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 prothioconazole is a fungicide composed of an innovative active ingredient with differentiated binding at the fungus action site, it constituted the new generation, in the chemical group of DMI's, being chemically classified as triazolintiona (Frac classification on mode of action 2014 - www.frac.info) [21].

Despite the large number of fungicides registered for the control of ASR, only three commercial fungicides (mixtures of active ingredients) showed efficiency above 70% of control in the 2018/2019 crop: (i) tebuconazole + picoxystrobin + mancozeb; (ii) protioconazole + trifloxystrobin + bixafen and (iii) prothioconazole + trifloxystrobin (Juliatti *et al*., 2017b; Godoy *et al*., 2019) [40, 29].

The combination prothioconazole + trifloxystrobin works in two ways: 1. Control of ASR and, 2. Complex of diseases (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 to use foliar fungicides is more than two applications. In this way, it is possible to explore the spectrum of action of this fungicide well, initiating robustly the prevention and control of ASR and, consequently, improving the performance of the subsequent fungicide.

Introduction of the multissite group associated with specific sites to control Asian soybean rust

In the years 2014 to 2015, researchers introduced the use of multissite (MS) fungicides in a spray programs to control ASR (mancozeb, chlorothalonil, metiram and cuprics) (Juliatti *et al*., 2017; Ponce *et al*., 2019; Reis *et al*., 2021) [39, 1, 60, 67]. The introduction of MS fungicides in ASR control programs, could be a very important tool for the management of resistance to *Phakopsora pachyrhizi*. The MS fungicides have the potential to preserve the useful life of specific fungicides of the groups DMI's, QoI's and SDHI's in soybean (Juliatti *et al*., 2017; Ponce *et al*., 2019; Reis *et al*., 2021) [39, 60, 67] The MS fungicides are very cheap compared with the site specific and they act in the fungal cell, interfering with numerous metabolic processes of the fungus, and consequently, resistance to this group of fungicides would be rare or non-existent (ZAMBOLIM *et al*., 2006b) [99].

Trials involving mancozeb in the control of ASR was developed in Minas Gerais, Goiás and Rio Grande do Sul demonstrated that MS has the potential to control the disease, even in isolated applications (Juliatti *et al*., 2014 and 2017; Ponce *et al*., 2019; Reis *et al*., 2021) [60, 67]. Combined with site specific, MS reduced the probability to develop mutants in the populations of *P. pachyrhizi* (Gulino *et al*., 2010).

From 2018 - 2021 several experiments demonstrated that mancozeb associated to triazol, carboxamide and strobilurin fungicides increased the efficiency on the control of ASR (Reis *et al*., 2021; Alves & Juliatti, 2018; Ponce *et al*., 2019, Zambolim *et al*., 2019) [1, 53, 92, 60, 67]. Multissite fungicides have the potential to preserve the useful life of site specific fungicides, such as (DMI, QoI and SDHI), in soybean crops (Alves & Juliatti, 2018; Ponce *et al*., 2019; Reis *et al*., 2021) [1, 53, 60, 67].

Ponce *et al*., (2019) [60] evaluated the performance of triazols with strobilurins in several concentrations associated with MS (mancozeb, chlorothalonil and metiram). The hypothesis was that the DMI's and QoI's can be mixed with MS fungicides to improve ASR control and increase productivity. The results showed that the average ASR control with the application of triazol + strobilurin associated with protective fungicides (mancozeb, chlorothalonil and metiram) was 70.2%. The efficiency of ASR control was not higher due to the fact that the fungicides were applied after the beginning of the disease epidemic in the field. Field experiments were sprayed, when the disease severity had already reached 2.0 to 5.0%, on the leaves of the lower part of the plants. Any of the three protective fungicides can be used in the mixture with epoxiconazol with piraclostrobin or cyproconazol with azoxystrobin (Ponce *et al*., 2019) [60]. In general, the fungicides DMI's + QoI's associated with MS had an efficiency greater than 68.0% of control and yielded more than 70.0% over control. These results showed that it is possible to control ASR even after the disease severity has reached 2.0 to 5.0%, at the time of spraying. Protective fungicides mancozeb and chlorothalonil associated with epoxiconazole + pyraclostrobin (0.5 kg/ha) or cyproconazol + azoxystrobin (0.30 kg/ha) increased soybean yield by 89.5% and 109, 0%, respectively.

Recent report showed an increase in the efficiency of ASR control due to the addition of mancozeb in all treatments involving DMI's, QoI's and SDHI's (Reis *et al*., 2021) [67]. Control above 80% was obtained with tebuconazol + picoxystrobin, fluxpyroxade + pyraclostrobin, benzovindiflupir + azoxystrobin and protioconazol + trifloxystrobin plus 2.0 kg/ha of mancozeb. The average ASR control without the addition of MS fungicide was 46% (21 to 71%).

Several authors showed efficient control of ASR with DMI's + QoI's, SDHI's + QoI's added to mancozeb (Table 5).

Table 5: Control (%) of Asian soybean rust due to the addition of mancozeb to the triazol + strobilutin and carboxamide + strobilurin fungicides.

	Control (%)		Reference
Treataments	**Mancozeb (-) ***	**Mancozeb (+) ***	
Tebuconazole + picoxistrobin	46(21-71)	>80	Reis *et al*., (2021) [67]
Fluxapiroxade + pyraclostrobin	46(21-71)	>80	Reis *et al*., (2021) [67]
Benzovindiflupir + azoxistrobin	46(21-71)	>80	Reis *et al*., (2021) [67]
Protioconazole + trifloxistrobin	46(21-71)	>80	Reis *et al*., (2021) [67]
Benzovindiflupir + azoxistrobin	79	87	Alves & Juliatti (2018) [1]
Protioconazole + trifloxistrobin	73	82	Alves & Juliatti (2018) [1]
Epoxiconazole + Piraclostrobin	48	>68	Ponce *et al*., (2019) [60]

*Mancozeb (-) = no; Mancozeb (+) = yes.

In the greenhouse, triazol fungicides mixed with strobilurin associated with FMS 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 (Godoy *et al*., 2013; Juliatti *et al* 2014) [28, 1]. In this situation, the use of multisite fungicides such as mancozeb was providential (Juliatti *et al* 2014; Juliatti *et al*., 2017b) [40]. Probably *P. pachyrhizi* acquired resistance to triazole or strobilurin in the field, where soybeans were grown extensively, in the cerrado region, when such fungicides were applied alone extensively. The anastomosis of germ tubes, and the migration of nuclei from the hypha of germ tubes of *Phakopsora 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) [82]. It is possible that this mechanism could occur in nature, because millions and millions of urediniospores are produced in soybean leaves, in the field and are then dispersed by the wind.

Based on the information above, it is suggested that the application of triazol and strobirulin associated with MS fungicides, starting at the soybean crop stages (V9 or R1, R2), may promote better disease control, especially in the leaves in the lower third of plants, which is the main source of inoculum for the upper third and for the entire field. Therefore, the combination of fungicides from the DMI + QoI or SDHI group, associated with MS fungicides, 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 MS fungicides (mancozeb, chlorothalonil and metiram) on soybean leaves, they can promote greater longevity of the DMI, QoI and SDHI molecules and decrease the number of applications.

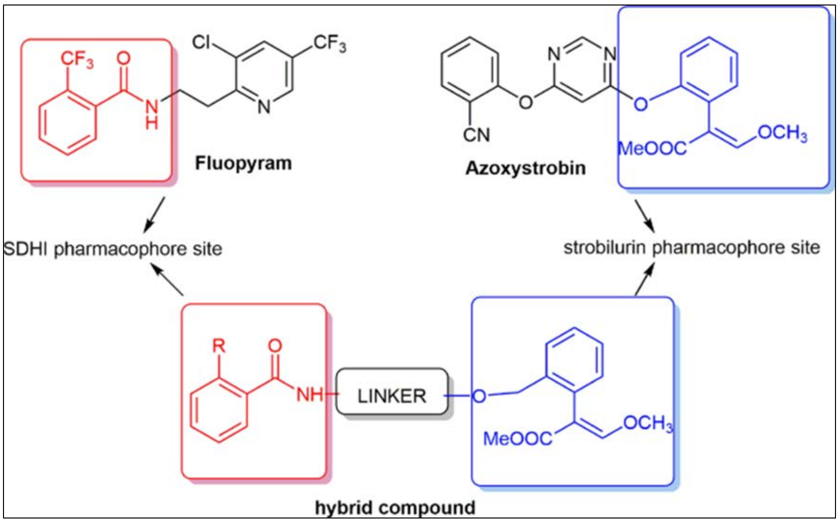
The addition of mancozeb to reinforce the fight against fungal resistance is not a new strategy. Mancozeb has been included in mixtures, to contribute to the management of resistance, and to expand the spectrum of fungicides with a specific mode of action, for numerous plant diseases. To stabilize ASR control, the same strategy could be used for soybeans, to chemically manage the disease. 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. In the same way, this could be followed in the control of ASR. To reinforce the role of mancozeb in the strategy against resistance in the control of fungal diseases, in more than six decades of continuous use, they have led to records in more than 70 cultures and in 400 different diseases (Gulino *et al*., 2010).

Over the years, as seen above, the efficiency of DMI's, QoI's and SDHI's have been losing effectiveness in controlling ASR. Godoy *et al*. (2019) [29] reported that only three commercial fungicides showed efficiency above 70% of disease control in the 2018/2019 crop. To complicate, the chemical industry has not launched a new group of fungicides to control ASR since the introduction of the Strobilurin group on the marketing about 20 years ago. For these reasons it is very important to associate soybeans cultivars with partial resistance with good strategy of chemical control (Juliatti *et al*., 2019) [38].

New approach: hybrid compounds

The number of fungal strains resistant to multiple antifungal compounds is dramatically increasing (Sparks & Lorsbach, 2017) [77]. To overcome this drawback, a well-established approach is the use of tank-mix combination of molecules with diferent sites of action. The design of hybrid bifunctional compounds, i.e. conjugates resulting from merging the pharmacophores of active molecules with diferent mechanisms of action, appears to be a promising alternative to the combination approach, since it displays several advantages (Morphy *et al*., 2004; Cincinalli *et al*., 2018) [54]. Synergistic interaction of the two active components able to inhibit simultaneously multiple targets, improved bioactivity and lower risk of resistance are expected (Muller-Schittmar *et al*., 2012). Even though the use of co-formulations or tank-mixes of fungicides with diferent modes of action is a well established strategy, their conjugation into a single molecule is a relatively underexplored approach. Synthetic studies directed to find dual-action pesticides are very (Liet *et al*., 2009; Jiang *et al*., 2014; Li *et al*., 2019) [72, 37]. Cheng *et al*. (2015) [15] described 1,2,4-triazole-1,3-disulfonamides as dual inhibitors of mitochondrial complex II and complex III, whereas other groups reported examples of strobilurins functionalized with a 1,2,3-triazole moiety or with N-phenylpyrimidin-2-amines. Strobilurins, or quinone outside inhibitors (QoI), are an outstanding class of fungicides, whose discovery was inspired by a group of natural derivatives of β-methoxy acrylic acid, isolated mainly from (Cheng *et al*., 2015) [15].

The Figure 1 shows the design of hybrid compounds proposed by Zuccolo *et al*., (2019) [94]. The addition of multissite fungicides to the hybrid formulation can be a good strategy to manage ASR to avoid epidemy of the disase.



Source: Zuccolo *et al*., (2019) [94].

Fig 4: Design of hybrid compounds. The SDHI (red) and the strobilurine (blue) pharmacophoric groups are joined by a linker.

Final considerations to cope with the vulnerability to site specific fungicides

To cope with the vulnerability of *P. pachyrhizi* to site specific fungicides, is a great chalenge. The strategy has to involve integration of measures and strategies to achieve good results on ASR control. The measures that could cope with the vulnerability of ASR to site specific fungicides are:

1. Make the sanitary vacuum, with the absence of soybean plants in the off-season.
2. Rotation of a mixture of different biochemical mechanisms of action has to be done to unfavorable formation of mutants of *P. pachyrhizi* in the field.
3. It is mandatory to include in tank mixture, multissite fungicides such as mancozeb or chlorothalonil with site specific DMI's, QoI's and SDHI's to reduce the population of resistant mutants of *P pachyrhizi*.
4. Addition of multissite fungicides to hybrid formulations can be a good strategy to manage ASR to avoid epidemy of the disase.
5. Avoid spray soybean with the first generation of DMI's such as ciproconazol, tebuconazol, expoxiconazol, tratraconazol and flutriafol alone to avoid resistant mutants arise in the population of *P. pachyrhizi* in the field. New generation of DMI's such protioconazol is giving better results on the control of ASR. But if strategy anti resistance is not applied resistant mutants to protioconazol will arise soon.
6. Avoid spray soybean with strobilurin fungicides alone such as axoxistrobin, piraclostrobin and trifloxystrobin due to the buildup *P. pachyrhizi* resistant mutants. New generation strobilurin fungicides could be used in a mixture with site specific fungicides and or multissite fungicides to avoid *P. pachyrhizi* mutants in the field populations.
7. Sequential and curative applications should be avoided to reduce the selection pressure on the population of *P. pachyrhizi*. ASR control must always be preventive, due to the agressiveness of the pathogen.
8. Use of early cultivars and sowing at the beginning of the recommended season.
9. Eliminate voluntary soybean plants from the field. After soybean harvesting thousands and thousands of seeds fall down into the soil and germinate mantaining the *P. pachyrhizi* uredospores in the field for many planting seasons.
10. Do not cultivate cotton after soybean. At harvesting soybeans seeds go to the soil. If cotton is seeded, by the time of flowering, soybean will germinate and the leaves become infected with ASR. The rust fungus is maintained inside cotton plantation on the leaves till august/september when cotton is harvested. September is the season when soybean is seeded again for the first planting. Furthermore triazol and strobilurins fungicides are recommended to control cotton diseases such as target soybean spot (*Corynespora cassiicola*). Soybean and cotton are susceptble to target spot.
11. Due to this fact there is a possibility that *P. pachyrhizi* populations incorporate more resistant mutants genes to the DMI and QoI fungicides.
12. The off-season must be free of soybean cultivation (pay attention to the sanitary measures)
13. Use of fungicides in the onset of symptoms or preventively (R1/R2) at least once before the planting lines closed.13. Sowing at the beginning of the recommended season. Avoid late sowing in relation to the recommended season. Early sowing season avoid high pressure of the rust fungus.
14. 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 of each location (Juliatti *et al*., (2017a) [39].
15. 15-The use of fungicides must be planned, according to the risk factors. It is mandatory to use multissite in all the spraying programs of control.
16. The timing of application and reapplication, at the right time is of fundamental importance in controlling the disease. There are several factors to be observed before the decision to spray: phenological phase, time of planting, environmental conditions, soybean cycle, previous crop and disease incidence in the field,
17. Use of cultivars with resistance gene (s) to reduce the number of spraying (Silva *et al*., 2007; 2011) [80, 81].

In conclusion the management of ASR using site specific fungicides, with specific biochemical mechanism of action without the adoption of tank mix with the MS, cultural practices and without the use of varieties with quantitative resistance as it has been done so far, it does not guarantee the sustainability of the crop or the useful life of systemic fungicides.

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