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The Asian Soybean Rust in South America

Gustavo B. Fanaro and Anna Lucia C. H. Villavicencio
Instituto de Pesquisas Energéticas e Nucleares (IPEN)
Brazil

1. Introduction

Soybean is infected by two species of fungi that cause the rust: the *Phakopsora meibione* (Arth.) Arth. (American soybean rust), which is native from American continent, existing from Puerto Rico to southern Brazil and not cause concerns for farmers and the *Phakopsora pachyrhizi* Sydow & Sydow (Asian soybean rust), a serious disease which causes a high yield losses. The differentiation of these two species is only possible through DNA testing (Yorinori & Lazzarotto, 2004).

The *P. meibione* is the less aggressive soybean rust species and was reported in the western hemisphere, South and Central America and Caribbean. It was reported in Puerto Rico in 1913, Mexico in 1917 and Cuba in 1926 on hyacinth bean and some other leguminous species, but only in Puerto Rico in 1976 was related on soybean (Pivonia & Yang, 2004). This species occurs under mild temperatures (average below 25 °C) and high relative humidity (Yorinori & Lazzarotto, 2004).

The *P. pachyrhizi* was described as a pathogen on the legume *Pachyrhizus erosus* (L.) Urb. (well-know as jacatupé in Brazil; jícama or pois patate in France; jícama, yam and mexican turnip in English language and jícama, pipilanga, yacón or nabo mexicano in Spanish language) in Taiwan, published by Sydow & Sydow in 1914 and can infect many leguminous species in numerous orders of the family Leguminosae (Deverall et al., 1977).

The *P. pachyrhizi* was first identified in Japan in 1902, and then was detected in India (1906), Australia (1934), China (1940), in Southeast Asia (1950s) and Russia (1957). For many years it remained confined to Asia and Australia, until to be found in Hawaii in 1994 and in Africa continent (from Uganda to South Africa) in 1997 (Begenisic et al., 2004).

P. pachyrhizi was first identified in the America continent in March 2001 in Paraguay, which caused yield reduction of 1,100 kg/hectare. In May, it was also found in Paraná (Brazil). In 2001/02 harvest, the disease recurred throughout Paraguay and was also found in Argentina, Bolivia and in several states of Brazil. In the worst hit places, the reductions in grain yield were estimated between 10% and 80% (Yorinori, 2002).

The fungal inoculum, for the initial outbreak in South America, is thought to originated from southern Africa where soybean rust has been observed since the late 1990s (Scherm et al., 2009). Since 1994, the disease has been identified by several countries, damaging up to 40% of crops in Thailand, 90% in India, 50% in the south of China and 40% in Japan (Hartman et al., 1991; Mendes et al., 2009). In the United States, this disease was first reported at the Louisiana State University AgCenter Research Farm in 2004, but yield loss was not as high as those reported from other countries (Cui et al., 2010).

Soybean plants are susceptible to the fungus at all growth stages. As a general rule, the earlier a crop is attacked, the higher will be the loss (Mendes et al., 2009), however, if the attack occurs at flowering and pod filling stage, which is commonly observed in soybean fields, the yield reducing can be higher than in others stages (Kawuki et al., 2004).

2. Contamination

The Asian soybean rust is one of the most destructive diseases of soybean because it produces a high amount of airborne spores that can infect large areas of soybeans and cause significant yield loss. The fungal spores (uredospores) are deposited on leaves in the lower region of the canopy through the rain or wind transport from nearby plants during the growing season (Huber & Gillespie, 1992). The figure 1 shows a soybean leave contaminated with Asian soybean rust.

The *P. pachyrhizi* is highly moisture dependent, requiring at least 6 hours of free moisture on the lower trifoliolates to starts the contamination. Warm temperature is ideal, but not limiting, since the disease can be established between 15 °C and 30 °C (Embrapa, 2005). These moist conditions can be achieved through any form of wetness as drizzle, mist, fog or dew and the minimum duration of wetness is dependent on the ambient temperature after spore deposition (Schmitz & Grant, 2009).

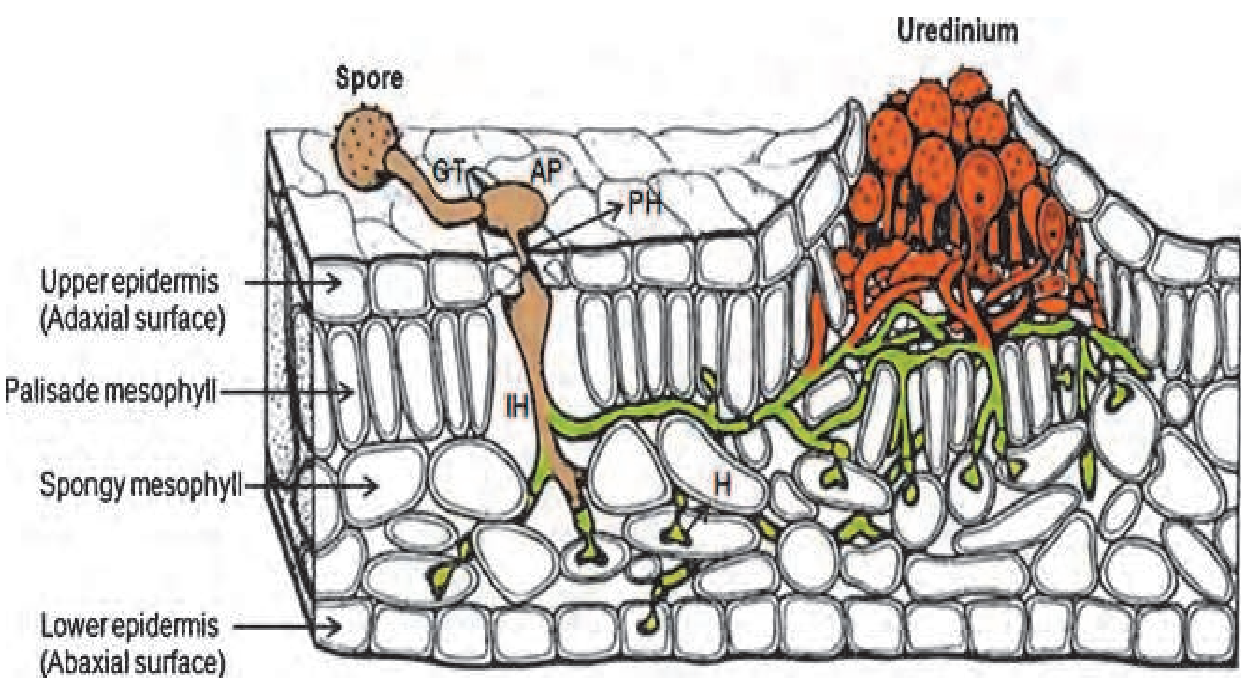
Fig. 1. (Godoy et al. 2009). Soybean leaf contaminated with Asian soybean rust.

Early rust symptoms are characterized by small dots of 1 to 2 mm of diameter, darker than the healthy leaf tissue with a greenish to greenish gray coloration. On the local corresponding at the dark spot, there is initially a tiny lump, like a bubble formed by burning, showing the early formation of the fruiting structure of fungi. As soon as the death of infected tissues, the blemishes increase in size and acquire a reddish brown color. The uredospores, initially has a crystalline color, become beige and accumulate around the pores or are carried by the wind and the number of uredias per point can vary from one to six. The uredias that no longer sporulete shows the pustules with open pores, which allows distinguish them from bacterial pustule, which often causes confusion comparison (Bromfield, 1980; Embrapa, 2004).

The infection causes rapid browning and premature leaf fall, preventing the full grain formation. The earlier the defoliation, smaller is the grain size and lower is the yield and quality. In severe cases, when the disease reaches the stage of the soybean pod formation, it can cause abortion and drop of the pods, resulting in a total loss of income (Constamilan, 2002; Godoy & Canteri, 2004; Soares et al., 2004).

The life cycle is typical of the majority of other rust fungi (Fig. 2) and their uredospores are easily transported by air currents and disseminated hundreds of kilometers in few days (Tremblay et al., 2010).

Once germination occurs, the uredospore produces a single germ tube (GT) that grows across the leaf surface until it reaches an appropriate surface where an appressorium (AP) forms. This penetration occurs between 7-12h after the spore lands on the leaf adaxial surface. Appressoria form over anticlinal walls or over the center of epidermal cells, but rarely over stomata, in contrast to the habit of many other rusts. Thus, penetration is direct rather than through natural openings or through wounds in the leaf tissue. Approximately twenty hours after the spore landing, the *penetration hyphae* (PH), stemming from the appressorium cone, pass through the cuticle to emerge in the intercellular space where a septum is formed to produce the *primary infection hypha* (IH). This IH grows between palisade cells to reach the spongy mesophyll cells where it forms the haustorium (H) (Tremblay et al., 2010).



Where: GT, germ tube; AP, appressorium; PH, penetration hyphae; IH, infection hyphae and H, haustorium.

Fig. 2. (Hahn, 2000 apud Tremblay et al., 2010). Internal structure of a typical dicotyledon leaf showing the different cell layers and infection by a rust fungus.

Once this first stage has been reached, about 4 days after spore landing, additional hyphae emerge and spread through the entire spongy mesophyll layer of cells where many other haustoria are formed. At approximately 6 days after infection, some necrosis of epidermal cells occurs which is visible at the adaxial surface of the leaves (Fig. 3a). Hyphae aggregate and a uredinium arise in the spongy mesophyll cell layer. Uredinia can develop 6-8 days after spore landing and development might extend up to 4 weeks (Tremblay et al., 2010).

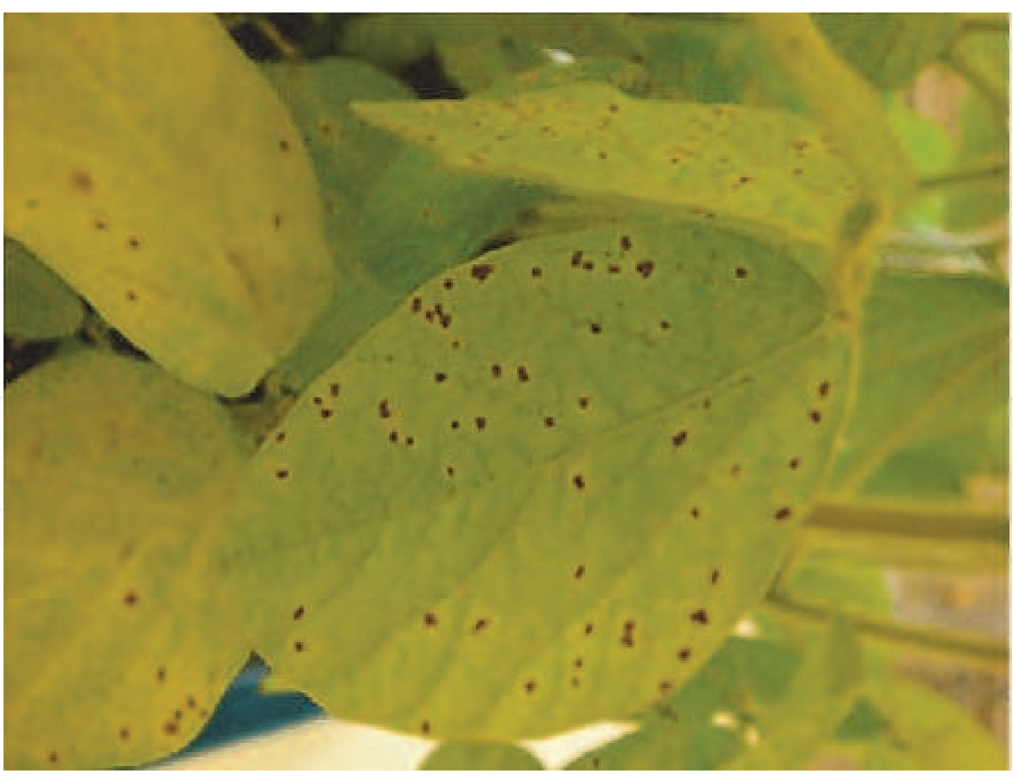
The first uredospores produced by the uredinium emerge at the abaxial leaf surface in 9-10 days after spore landing and spore production can be observed for up to 3 weeks. High rate of sporulation is typical of a susceptible reaction where lesions on the upper surface of the leaf are tan (Fig. 3b). Plants classified as resistant develop a dark, reddish-brown lesion with few or no spores (Fig. 3c) (Tremblay et al., 2010).

Soybean rust diagnosis is usually performed by experienced plant pathologists or plant disease diagnosticians, but nowadays, several technologies are being performed as crop health sensor, either optical or electronic or bio-electronic based to improve the perform crop disease diagnosis (Cui et al., 2010).

A useful tool that can be used as many by experienced professionals as amateurs is the diagrammatic scale to assess the severity of rust. It is very important once provides data on the severity of contamination in the plantation and the result should be informed when the competent organs are contacted, as well as help to define the goals for fungicides treatment. There are several types of scales such as developed by Godoy et al. (2006) (Fig. 4a) and Martins et al. (2004) (Fig. 4b) witch is highly recommended to be used together.

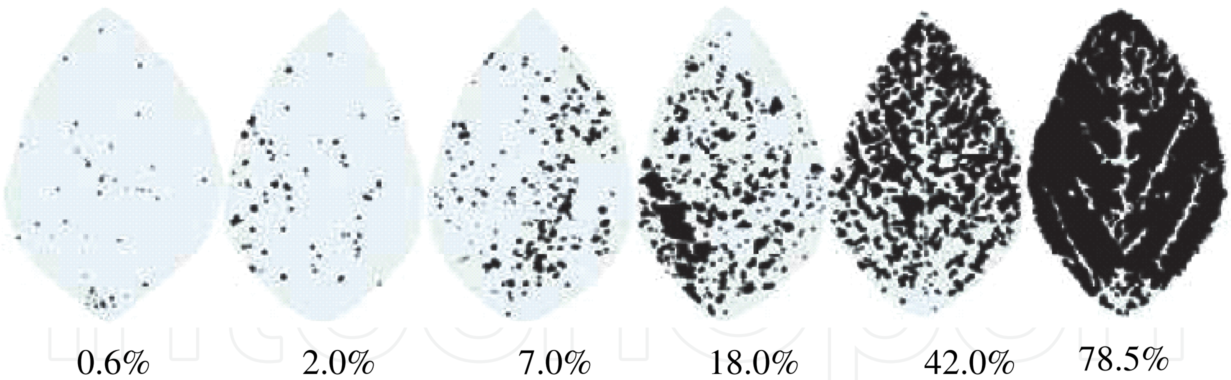
(3a)

(3b)

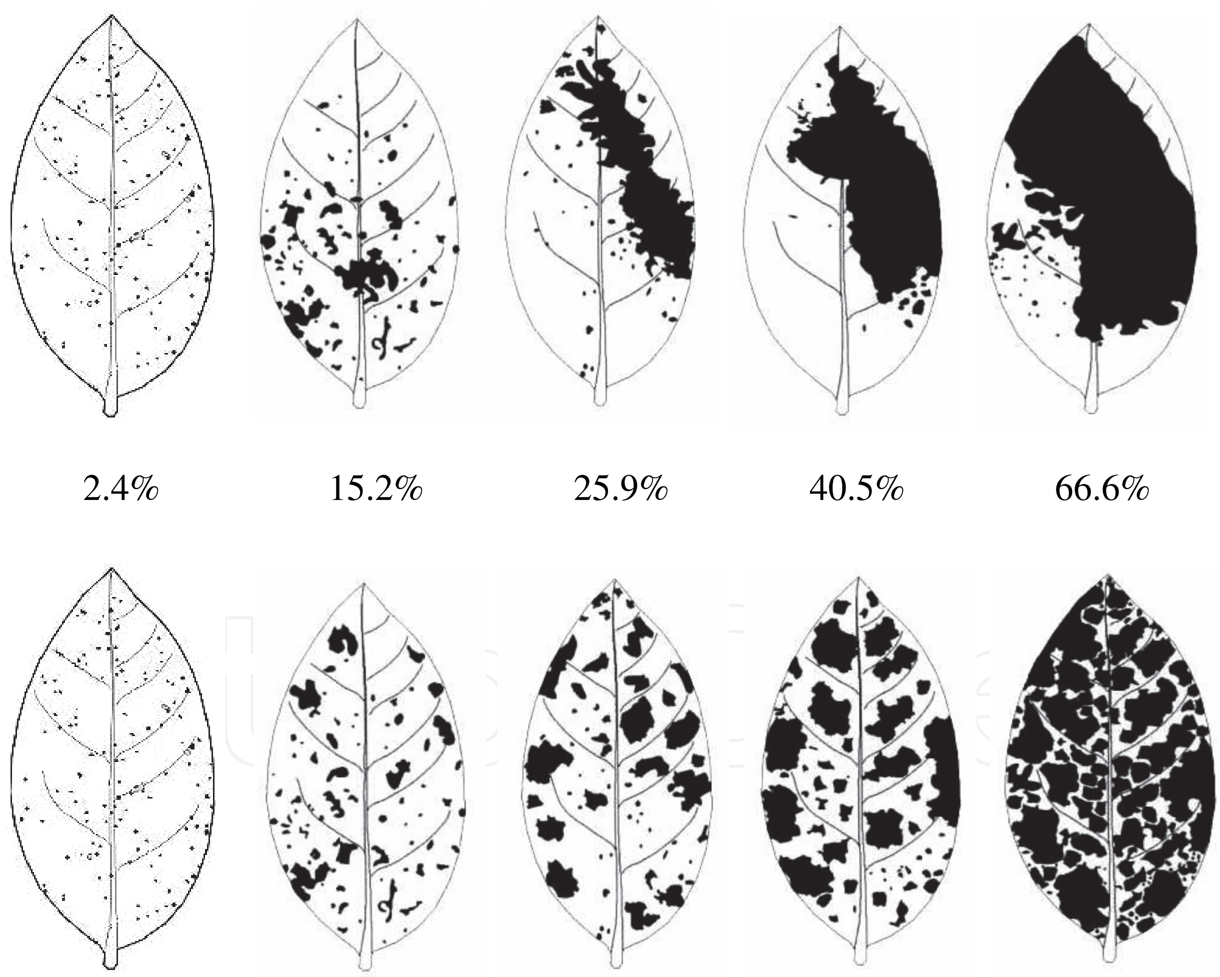


(3c)

Fig. 3. (Tremblay et al., 2010). Symptoms observed on soybean leaves. (a) Yellow mosaic discoloration (b) Tan lesions and (c) reddish-brown lesions.



(4a)



(4b)

Fig. 4. Two diagrammatic scales of Asian soybean rust severity with percentage that represents the area of disease contamination.

3. The Asian soybean rust in South America

3.1 Argentina

The soybean crop has been converted from the middle of the nineties in the main seasonal crop of Argentina, both in area planted and in its total production (CAS, 2008). During 2004/05 season, the area devoted to soybeans was 14.4 million hectares (51% of the total planted with cereals and oilseeds), producing 38 million tones. In the season 2005/06, the soybean planting grew up to 15 and 15.3 million hectares (PNR, 2011a). In the 2006/07 season, reached a volume of 47.5 million tonnes, representing 50% of total the country grain production estimated at 94.4 million tones (CAS, 2008).

The Asian soybean rust first appeared in Argentina at the end of the 2001/02 season, in a test group in the town of Alem, province of Misiones. The infected plants samples were sent to the United States for identification by molecular analysis techniques and confirmed that the pathogen *P. pachyrhizi* was present. This finding coincided with the species identified in Brazil and Paraguay (Begenisic et al., 2004).

In the following season (2002/03), the soybean rust was detected at the end of the cycle, by a group of technicians from "Instituto Nacional de Tecnología Agropecuaria – INTA" (National Institute of Agricultural Technology) in test samples from the town of Cerro Azul, province of Misiones and two commercial lots located in the town of Gobernador Virasoro, in the province of Corrientes. Although this pathogen has penetrated the national territory, it was far from the main producing areas of the country. Because of the history of the disease, there were high producers and technicians concern about the losses that could result in the coming years (Begenisic et al., 2004).

As a result, at the beginning of the 2003/04 season, bearing in mind that rust had caused heavy losses in neighboring countries and displaying that it could become a serious concerns for Argentina, despite until that moment the rust had a little history in the country, the Ministry of Agriculture launched the "Programa Nacional de Roya de la Soja – PNRS" (National Program for Soybean Rust), coordinating activities with various agencies and public and private institutions in order to minimize the possible impact of the disease in the country (PNRS, 2011a).

The implementation of the PNRS was the first opportunity in which all public institutions have joined forces, in a cooperative manner, carrying out activities in a coordinated way and contributing in those components of the program according to their specific duties incumbent on each institution (PNRS, 2011a).

During crop season of 2004/05, the disease was detected in 13 provinces, including provinces witch the disease was detected for the first time, representing the advance of the disease significantly when compared with the last season. In most of the contaminated provinces, the rust did not cause economic losses due to its late appearance in the crop, with the exception of the province of Entre Rios where the disease was much more severe, reporting significant yield losses up to 30% (PNRS, 2011b).

These low levels of contamination can be explained for (PNRS, 2011b):

- Drought conditions and high temperatures occurring in some regions of Brazil, Paraguay and Bolivia, neighborhood to Argentina, that were not conducive to serious infections and consequently to the high seed production regional;
- The drought in Argentina during the first months of 2005 that prevented the infection conditions for generating a second "weather barrier";
- The limited survival of the fungus because the Argentina has not double cropping of soybeans in the year as in Brazil or Bolivia;

- The high level of adoption of fungicide use in Brazil, Paraguay and Bolivia.

The fungicides recommended by Argentina are the strobilurin, triazoles and their mixtures. The decision to apply is at the first signs and/or when was possible to anticipated the diagnosis in the field or when they are found in areas close to their lots and recorded favorable environmental conditions to ensure at least 7-10 hours of wet leaf and average temperatures of 22°C.

The Argentina has an official monitoring system that allow to analyze a large number of samples for the detection and disease monitoring through the website "www.sinavimo.gov.ar" (in Espanish).

3.2 Bolivia

Bolivia is the eighth country in soybeans production and is the fourth in the South America, after Brazil, Argentina and Paraguay and is one of the most important and is the successful of the national economy, due to growth in primary production, processing and export during the last fifteen years (CAS, 2008).

In Bolivia, the Asian soybean rust was first detected in the winter crop season of 2003 at Ichilo, City of Yapacani and is currently distributed throughout in all soybean crops of Santa Cruz and affecting the crop of Tarija (Yacuiba). Before the advent of Asian rust, the number of applications of fungicides for control of diseases ranging from 0 to 1, however, today the value has increased from 3 to 5 applications, increasing the costs of fungicides from 10 to 70 US$ respectively. This disease, year after year is responsible for at least 30 to 50% of loss in total production area, which in economic terms is between US$100 and US$150 million witch concerns to the use of agrochemicals and total yield loss per year (Condori, 2009).

In the 2007 winter cropping season the problems to control the rust emerged from a series of technical and climatic factors as (Condori, 2009):

- Soybean planted between harvests (April-May) generated the Asian rust inoculum that infected soybean fields planted in June until early winter season;
- The prolonged period of drought and the continuous moisture in the months of August and September stressed cultivation, focusing directly into the beginning of flowering that occurred at 65 to 70 days;
- The fungicides applied were exposed to critical climatic conditions as high temperatures (30-35°C) and low relative humidity (50-40%) which affected the residual effect and effectiveness of their control.

Those factors explain that this season (2007/08) was one of the most catastrophic, mainly in the north and east of the Santa Cruz de La Sierra due to continuous rains that prevented raising the winter planting crop, to perform the planting on summer and, the most important fact, the delay of fungicide applications, generating a "explosion" of the rust, forced farmers to make up 7 applications of fungicides per hectare. The economic losses quantified by the "Asociación de Productores de Oleaginosas y Trigo – ANAPO" (Association of Producers of Oilseeds and Wheat), exceed US$ 150 million, for the past two seasons (summer 2007/08 and winter 2008) (Condori, 2009).

The Bolivia are implementing the sanitary break in cities located in the Integrated Zone (Andrés Ibáñez Province, Warnes, Ichilo, Sara, Bishop Santiestevan and Guarayos), Expansion Area (Andres Ibanez, Chiquitos, Ñuflo Chavez, Guarayos) of the Santa Cruz state. This project will benefit more than 14,000 small, medium and large producers, of various nationalities and a planting area between the 700,000 to 1,000,000 hectares. Training, dissemination and sharing of technical and legal measures, through different media

available as workshops, seminars, television and radio messages is also referred in this project (Condori, 2009).
Fungicides recommended for control of Asian rust are bencimidazoles, triazols, triazol + triazol, triazol + benzimidazol and triazol + estrobilurina products. Those products were chose based on research results conducted by different agricultural companies and research institutions (Condori, 2009).

3.3 Brazil

Brazil is the second largest producer of soybeans. In the 2006/07 season, the culture occupied an area of 20.69 million hectares, which totaled a production of 58.4 million tons. The United States, the worldwide producer, accounted for the production of 86.77 million tons of soybean. The yield of soybeans in Brazil is 2,823kg per hectares, reaching about 3,000 kg/ha in Mato Grosso, the largest state producer (EMBRAPA, 2011).
The soybean is the crop which has the higher development in Brazil in the last three decades and accounts for 49% of grains area planted in the country. The grain is an essential component in the manufacture of animal feeds and the growing use food is increasing (MAPA, 2011). Data from the Ministry of Development show that soy has a major share of Brazilian exports. In 2006, were US$ 9.3 billion, representing 6.77% of total exported (EMBRAPA, 2011).
The Asian soybean rust was identified in Brazil in May of 2001 and spread quickly to the main producing regions, becoming a major problem for the national soybean producers. To propose solutions was created in September of 2004, the "Consórsio Antiferrugem - CAF" (Antirust Consortium). The consortium constituents are representative institutions of various soybean segments as foundations, universities, research institutes, representatives of entities of inputs manufacturers and farmer cooperatives. One of the aims of the Consortium is to bring the farmer all available information about the disease and enable him to handle it (Farias, 2009).
The CAF main information and communication vehicle is the consortium website: "www.consorcioantiferrugem.net" (in Portuguese) where the laboratories accredited update information about the disease outbreaks in all producing regions of Brazil during a season. In the system are recorded and presented a map of Brazil, the city of occurrence, date of detection, the fenological phases of culture and type of area (warning unit, commercial field, irrigated area etc). Thus, epidemics of soybean rust have been monitored and the spread of the disease are presented in real time at the consortium website, describing it as the main source of data for the record of events and the spread of the disease in Brazil (Spolti et al., 2009).
When the disease arrives, both farmers and technicians were not prepared to identify soybean rust. Factors such as dry climate, the symptoms likely with other diseases of end of cycle and because it was a new disease in the Americas, their identification was difficult and there was no species resistant to fungus attack (Constamilan, 2002 (2005)). It is estimated that over 60% of soya production in Brazil has been contaminated in the season of 2001/02, causing grains losses estimated at 569.2 thousand of tons or the equivalent of US$ 125.5 million (US$ 220.50/t) (Yorinori, 2004).
In the season 2002/03, the occurrence was different from the last season. In localities where the disease was severe in 2001/02, the high temperatures prevented, despite the high amount of rain, the development of the disease, except in Rio Grande do Sul and Santa Catarina, where the late cultivars were affected. But where the rust had not been reported earlier, favorable

climatic conditions and a new strain of *P. pachyrhizi* caused major losses. The states of Bahia, Goiás, Minas Gerais and Mato Grosso were severely affected (Yorinori, 2004).
However, despite the intensive campaigns to alert and guidance on methods for identification and control, held in 2002 and in January and February of 2003, through lectures, publications and other means of dissemination, the technical assistance and most producers were not prepared to control the rust. In many crops, the fungicide application was delayed due to lack of product and/or excessive rain which precluded the spraying (Yorinori, 2004). In this season the damage caused by the rust (amounting the grain losses, control expenses and revenues falling) were approximately US$ 1.29 billion (Soares et al. 2004).
The beginning of 2003/04 season was characterized by irregular rainfall and high temperatures, which probably not favored the outbreak of rust as expect. Moreover, the experience of loss in the previous crop left farmers in the areas previously affected readiness and "armed" for the chemical control. However in the southern region, the beginning of the harvest was characterized by mild temperatures and frequent rainfall, which favored the early emergence of *P. pachyrhizi*. The total damage caused by rust, in this year, adding the grain losses, control spending and falling revenue was approximately US$ 2.28 billion (Yorinori, 2004).
Among the crops of 2005/06 to 2008/09 were recorded, respectively, 1,369; 2,766; 2,107 and 2,880 reports of the occurrence of soybean rust. While there is an increase in the number of reports over the years, it is not possible to assert that the severity of epidemics is related to the number of outbreaks, since it is observed only presence of disease in crops, once in 2006/07, when they were registered the greatest losses in productivity caused by rust due to the higher disease severity, the number of reported outbreaks was lower than in 2008/09 when, according to regional information, the attack of the disease was not as severe as that year (Spolti et al., 2009).
Since the disease monitoring, the Asian rust was not observed before the month of October, whether in the commercial field or in units of alert. The progress of the number of reports of disease presents a sigmoid pattern with a logarithmic phase and a stationary phase when approaches the end of growing season. The maximum rate of increase, indifferent to the season, was observed between January and March (90 to 150 days after October 1st), this period can be defined as critical in the epidemics development, being responsible for the differentiation of the final number of focus reported in the cycle (Spolti et al., 2009).
At present, around 70 fungicides are registered in the Brazilian Ministry of Agriculture for managing soybean rust and many of these have been evaluated annually since 2003/2004 in a nationwide network of standardized coordinated by Embrapa Soja, a research unit of the Brazilian Agricultural Research Corporation (Godoy et al., 2010; Scherm et al., 2009). The fungicides registered for control of Asian soybean rust belong into two main groups: Triazoles and strobilirins (Godoy & Flausino, 2008).

3.4 Paraguay

The soybean in Paraguay is the main agricultural export item, with a market of 70% of national output in the form of grain. This is due to high charges imposed by the European Union, the main buyer, for other soy subproducts such as soybean oil. Today, Paraguay is the sixth soybean production in the world (preceded by USA, Brazil, Argentina, China and India) and has a weighted average of 2,600kg/ha, performance similar to Argentina and Brazil (CAS, 2008).

Since the appearance of Asian soybean rust in 2001 in Paraguay, there have been major changes in the soybean production system and it also contributed to better care for the crop, getting even better yields by protecting against various diseases of economic importance appellant in soybean. The productivity losses were very important in years when climatic conditions were favorable for the disease, especially during the breeding season and when constant rainfall recorded during the months of January and February, considered the most critical for the development of an epidemic Paraguay (Morel & Bogado, 2009).
In the first year where the disease was recorded yield losses were estimated, in the cultivars most affected, at more than 60%. On the next seasons, 2001/02 and 2002/03, the severity of the disease was not very important because of the drought but the late sown soybean crop showed severe losses of more than 50% of performance. This epidemic is especially observed when the rains season from the month of March to May (Morel & Bogado, 2009).
In all the years that the rust has been detected early, it was observed in plants of 30-35 days from sentinel plots in the region of Pirapó, considered an endemic area once is possible to detect the strong presence of the volunteer plant, being a fungus host, named Kudzu (*Pueraria lobata*), but the severity level ever has thrived in the vegetative phase. This demonstrates the importance of the survival of the disease during the winter, which has made a strong campaign of awareness among farmers aiming the elimination of inoculum source in areas with no winter crop, in order to avoid the primary infection in an early period of soybean cultivation (Morel & Bogado, 2009).
The crop of 2005/06, was the largest epidemic in the normal planting season, resulting in a loss of more than US$ 400 million. This strong impact due to multiple factors, neglect of producers, the time control and problems in application of the technology. The number of fungicide applications was a maximum of 5 and a minimum of 2. In the season of 2008/09, the rust incidence was reported again in a very early (second fortnight of October) in crops planted in September, but the severe drought that affected the whole area of soybean production allowed the progress of the disease (Morel & Bogado, 2009).
In rare cases and in regions where rains started to become evident, controls measures have been made, but around the country more than 1 application of fungicides should be done for each producer. This drought was so important that did not allow the progress of the disease throughout the production area, except in some regions of late-sown soybean (Morel & Bogado, 2009).

4. Disease control

Once commercial soybean cultivars used in the major soybean producing countries are susceptible to soybean rust, management of the disease is done using fungicides, although some cultural and crop management practices also may decrease disease risk at field and regional scales. Early research in Asia indicated that mancozeb and, to a more limited extent, the benzimidazole fungicides suppressed soybean rust but required three to five applications to be effective. The disease control was significantly improved after the introduction of the triazole fungicides (Scherm et al., 2009).
Scherm et al. (2009) studying the efficacy of several fungicides on a soybean crop in Brazil showed that triazole fungicides had significantly efficiency than strobilurins classes. The combination of triazoles with strobilurins improved disease control and yield gain compared with triazoles or strobilurins alone. However the combination of triazoles with a benzimidazole fungicide did not improve the desease control when compared with triazoles

alone. They either conclude that the two fungicides with the best disease control efficacy were combinations of two active ingredients as flusilazole + carbendazim and azoxystrobin + cyproconazole.

The triazoles group acts to inhibit the ergosterol biosynthesis and have the primary site of action the C-14 demethylation and the strobilurins interfere with mitochondrial respiration by blocking electron transfer by the cytochrome bc1 complex, formulated alone or in mixtures (Godoy & Flausino, 2008).

Besides the fungicides application, other measures could be taken as the use of earliest varieties, seed at the beginning of recommended time for each region, avoid prolonging the period of sowing, inspect crops and verify if there are temperature and high humidity favorable to the pathogen (Reunião de Pesquisa da Soja da Região Sul, 2002).

One way to anticipate the presence of this fungus before it reaches the crop would be the establishment of sentinel plots in one or more locations, depending on the area of the property. These traps, seeded with 15 to 20 days in advance of the first crops are intensively monitored to identify the first symptoms. Once detected the presence of disease, the traps must be destroyed or heavily treated with an effective fungicide. From this initial detection, the commercial areas should be treated or monitored more carefully for making treatment decisions to be made (Yorinori & Lazzarotto, 2004).

Another method for disease control is the adoption of absence of living plants in the field of this culture denominated sanitary break. This technician aiming to reduce the amount of uredospores in the environment on off-season and then, inhibit the early attack to soybean plants, trough the smaller inoculum presence (Seixas & Godoi, 2007) and is adopted in many countries. The general rule is that all regions are forbidden to cultivate soybean in the period established and the remaining plants from the last crop should be eradicated with chemicals or other means. The producer who does not obey the sanitary break will be required to pay large fines. Another caution that the producer should be is to remove the soy plants that may grow due to grain that fell in the soil and germinate during the harvest.

Also, the kudzu (*Pueraria lobata*), a leguminous plant witch is highly susceptible to the Asian soybean rust, founded in Paraguay and Brazil, shown to be an efficient source of inoculum, presenting the first symptoms and fungal growth before the first crops of soybean (Yorinori & Lazzarotto, 2004). In those countries where kudzu is found, control policies of the rust also include the control of this plant.

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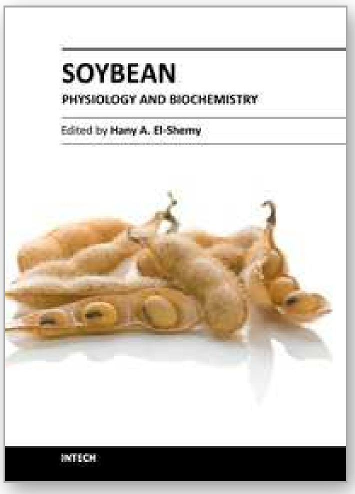

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