JÉSSICA SCHMIDT

YIELD LOSS CAUSED BY Phakopsora PACHYRHIZI ON SOYBEAN BASED ON PHYSIOLOGICAL COMPONENTS

Thesis presented to Federal University of Viçosa in partial fulfillment of the requirements to the Plant Pathology Post-graduation program for the degree of *Doctor Scientiae*.

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“WHAT WE KNOW IS A DROP,
WHAT WE IGNORE IS AN OCEAN”

Isaac Newton

To my parents **Cláudio** and **Clara**
for all the opportunities and unconditional support...

To my brother **Adriano Elias**,
for being always my best friend…

To my fiancée **Luciano**,
for being present in all moments….

I dedicate.

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BIOGRAPHY

JÉSSICA SCHMIDT, daughter of Cláudio Schmidt and Clara Bernardete Schmidt, was born in Taquara in the State of Rio Grande do Sul on July 25th of 1977.

In 1995, she started an undergraduate course in Biological Sciences: teaching at the University of Vale do Rio dos Sinos (UNISINOS). From 1996 to 2000, she worked in Botany Department on plant anatomy under the supervision of professor DSc. Nelson Sabino Bittencourt. In 1999, she taught classes in the course of Serviço Social da Indústria (SESI) and at a public high school. Still in 1999, she worked on a final paper on biogeography under the supervision of professor MSc. João la Rocca.

In 2000, Jessica started on undergraduate course in Biological Sciences: bachelor at University of Vale do Rio dos Sinos (UNISINOS). During six months, she was trainee at the Plant Pathology Clinic in Federal University of Rio Grande do Sul (UFRGS) under the supervision of professor PhD Valmir Duarte.

In 2001, she started a Masters of science course at the Federal University of Rio Grande do Sul (UFRGS) in Plant Pathology – epidemiology, advised by professor PhD. Fábio Kessler Dal Soglio. The pathosystem studied was citrus black spot – mandarin. During that time, she had a scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

In 2005, she started doctorate degree at Federal University of Viçosa (UFV) in Plant Pathology – epidemiology, advised by professor DSc. Francisco Xavier Ribeiro do Vale. The pathosystem studied was Asian Soybean Rust – soybean. During that time, she had a scholarship from Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) and during a sandwich in the United States of America, a scholarship from Cordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

In 2008, she spent four months at The Ohio State University under the supervision of professor PhD Pierce Anderson Paul, working on data analysis.

In 2009, Ms. Schmidt started working for Syngenta Seeds, with researching in soybean diseases.

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RESUMO

SCHMIDT, Jéssica, D. Sc., Universidade Federal de Viçosa, fevereiro de 2009. **Quantificação de danos em soja causados por *Phakopsora* pachyrhizi com base em componentes fisiológicos.** Orientador: Francisco Xavier Ribeiro do Vale. Coorientadores: Waldir Cintra de Jesus Junior e Fábio Murilo da Mata.

A ferrugem asiática da soja, agente causal *Phakopsora pachyrhizi*, é a doença mais importante da cultura no mundo nos últimos anos e tem causado danos significativos à produção. A relação entre a ferrugem e a produção da soja foi estudada em experimentos de campo. Quatro experimentos foram conduzidos em Viçosa, MG, Brasil, em área pertencente ao Departamento de Fitopatologia da Universidade Federal de Viçosa, nos anos agrícolas de 2005, 2006, 2007 e 2008. As cultivares utilizadas neste trabalho foram Monarca, Conquista e Vencedora. A soja foi infectada sob condições naturais, e diferentes níveis de severidade foram obtidos no primeiro experimento, utilizando-se 25, 50, 75 e 100% da dose de fungicida recomendada comercialmente; e nos demais experimentos, a partir de nenhuma, uma, duas ou três pulverizações do fungicida tebuconazole, de acordo com o estádio de crescimento das plantas – R1, R3 e R5. Para quantificar o efeito da doença, dois métodos diferentes foram utilizados, um baseado na doença – severidade (x) e área abaixo da curva de progresso da doença (AUDPC) e o outro, baseado em componentes fisiológicos da soja – índice de área foliar sadio (HLAI), duração da área foliar sadia (HAD), absorção da área foliar sadia (HAA), radiação interceptada pelo tecido sadio (HRI) e uso eficiente da radiação (RUE). A relação entre produção e todas as variáveis descritas foi investigada. HAA foi considerada a melhor variável a ser utilizada em um sistema de manejo, devido ao seu comportamento regular entre as estações de cultivo, cultivares e estádios de crescimento da soja. Para determinar o efeito da *P. pachyrhizi* na taxa fotossintética das folhas infectadas, foi empregado o modelo de lesão virtual proposto por Bastiaans (1991). A taxa fotossintética e a severidade foram avaliadas na oitava folha de cada uma das plantas em cada parcela, quinzenalmente, nas estações de cultivo de 2005, 2007 e 2008. A taxa fotossintética foi reduzida, sobretudo, na área lesionada e, dessa forma, houve pouco efeito da ferrugem no tecido verde remanescente das folhas. Os maiores efeitos na redução da fotossíntese aparentemente se deveram à perda de clorofila e à redução da condutância estomática.

ABSTRACT

SCHMIDT, Jéssica, D. Sc., Federal University of Viçosa, February of 2009. **Yield loss caused by *Phakopsora pachyrhizi* on soybean based in physiological components.** Advisor: Francisco Xavier Ribeiro do Vale. Co-advisors: Waldir Cintra de Jesus Junior and Fábio Murilo da Mata.

Asian Soybean Rust (ASR), caused by *Phakopsora pachyrhizi*, has been the most important disease of the soybean in the world, over the last few years, causing significant yield loss. The relationship between ASR and soybean yield was studied in field experiments. Four experiments were conducted in Viçosa, MG in an area belonging to the Plant Pathology department, during the crop seasons 2005, 2006, 2007 and 2008. The cultivars used were Monarca, Conquista, and Vencedora. Soybean was infected under natural conditions and different disease levels were obtained in the first experiment using 25, 50, 75 and 100% of the commercial recommended dose of fungicide and in the other experiments by using none, one, two or three sprays of the fungicide tebuconazole according to plant growth stages – R1, R3 and R5. To quantify the disease effect, two methods were used, one based on disease – severity (x) and Area Under Disease Progress Curve (AUDPC) – and the other based on host physiological components - Healthy Leaf Area Index (HLAI), Healthy leaf Area Duration (HAD), Healthy leaf Area Absorption (HAA), Radiation interception by the healthy tissue (HRI) and Radiation Use Efficiency (RUE). All the above mentioned variables were investigated for their relationship with yield. HAA was considered the best variable for use for the establishment of a management system, due to its consistent behavior among growing seasons, cultivars and growth stages. The virtual model proposed by Bastiaans (1991) was used to determine the *P. pachyrhizi* effect on the photosynthetic rate of infected leaves. Photosynthetic rate and disease severity were assessed on the eighth leaf of each plant in each plot every other week in three experiments during the 2005, 2007 and 2008 growing seasons. Soybean photosynthetic rate was reduced mainly in the lesions and thus there was a small effect of ASR on the remaining green area of the leaf. The greatest effects on photosynthesis apparently were the loss of chlorophyll content and decreased stomatal conductance.

1. GENERAL INTRODUCTION

Soybean (*Glycine max* (L.) MERRIL) is an important commodity responsible for generating approximately US$ 215 billion/year in a production area of 93.9 million hectares (USDA, 2009). In Brazil, the second largest producer, the planted area was 20.69 million hectares in the 2006/2007 growing season, which generated US$ 9.3 billion for the Brazilian economy and represented 6.77% of the total exports (CONAB, 2008).

Soybean plants are host of a number of pathogens, among them *Phakopsora pachyrhizi*, that causes the disease known as ASR. This disease occurs in almost every region where soybeans are grown, been considered of generalized occurrence (Rossman, 2009). In Brazil, among all producing States, only Roraima still has not register its presence (Embrapa, 2008).

Plant diseases probably have been present among us since the beginning, before humans settled down and started to grown plants. During that time, they most likely already caused yield loss (Agrios, 2005). Even with the development of Plant Pathology as a science 150 years ago, despite all the efforts, today we still try to deal with yield loss without confidence because it is still difficult to make reliable estimates about specific diseases in a particular area and season. Zadocks and Schein (1979) explained that reliable estimates are a prerequisite for establishing any crop protection strategy.

In this sense, this work had as objective to study the effects of ASR on soybean yield to find usefull variables to be used in the future in a model to establish a secure crop management system in this pathosystem.

1.1. Hypothesis

Yield loss caused by ASR is due to early defoliation and consequent reduction of healthy leaf area duration, radiation interception, healthy leaf area absorption and reduction in photosyntheticaly active area, photosynthetic capacity in the healthy area and in radiation use efficiency.

2. LITERATURE REVIEW

2.1. Soybean history

Soybean (*Glycine max* (L.) Merrill) origin is attributed especially in the region of the Yangtze river in China. There is some controversy as to when soybean was first cultivated, but Hymowitz & Shurtleff (2005) investigated facts and myths about it and they explain that there is too much wrong information about soybean history. According to them, soybean was grown for the first time in China 3100 years ago and not 5000 as many authors affirm.

In China, some ancient crops were considered sacred and among them, soybean (CISoja, 2008; Embrapa, 2008). Although it has been planted for so many centuries in Asia, soybean is not the oldest domesticated crop in the world. There are archeological records of at least 30 crops older than it; among them, wheat, corn, chick-pea, beans, pumpkin, fava bean and pepper (Hymowitz & Shurtleff, 2005).

Despite being known and eaten for years in Asia, soybean was introduced in Europe in 1739 and was grown just for curiosity and an ornamental plant. After 1804 it was used in Yugoslavia to mix with cereal grains to fed chickens for increased egg production (CISoja, 2008; Hymovitz, 1990). More than 500 years pass before soybeans started to be used as food for humans in the western world (CISoja, 2008).

Industries started showing interest in soybeans because of its oil and protein value, but the first attempts to introduce plants in commercial fields failed in Russia, England and Germany, probably because of the unfavorable weather conditions (Embrapa, 2008). The first in the western world to be successful at growing soybeans on a large scale were the Americans, who developed new commercial varieties (CISoja, 2008).

2.1.1. Soybean history in Brazil

Soybean introduction in Brazil varies depending on the reference that is used. As Ho (1955) explained, the dissemination of plants has many possible channels, such as traders, travelers, emissaries, and government officials, who have left little or no record. There is little chance that a certain plant is introduced into a new area only once and by a certain route. A new plant may score an immediate success in one region and remain neglected in another for a considerable time, because sometimes only through repeated trial and error, they can strike root. In this way, a new plant may actually be introduced more than once (Ho, 1955).

In Brazil, soybean introduction has two different pathways: the first was from the United States, around the year 1882 in the state of Bahia State by Gustavo Dutra, a researcher at the Bahia Agronomy School (CISoja, 2008). Other path mentioned was from Japan, with the Japanese immigrants to Brazil in 1908, being officially introduced the state of Rio Grande do Sul by the year 1914 (Kamizake et al., 2006; Barreto, 2008, EMBRAPA, 2008).

At the end of the 60's, two internal factors made Brazil pay attention to soybean as a rentable crop: by that time, wheat was the main crop in the South region and soybean was an option to be grown during the summer and, the second factor was that production of pigs and hens was growing, which made the demand necessity for animal food to increase (Embrapa 2008).

In the 70`s, soybean price on the international coupled with the advantage of an advantage of Brazil`s production occurring in between USAs growing season – when the price is highest, made our government invest in technology to obtain cultivars that were adapted to tropical weather conditions. As a result, from being grown only Southern region in the 60`s, after the 80`s soybean started to be grown in the “cerrado”, which is nowadays the biggest area of production in Brazil. Connected to this crop movement from the South to the center of Brazil, is the population movement to areas that were previously uninhabited (CISoja 2008). After the 90`s, the Northwest and Northern region initiated their production and with this, soybean was economically consolidated in all regions of the Country (CISoja 2008). Figure 1 illustrates the increasing area in Brazil where soybean is grown.

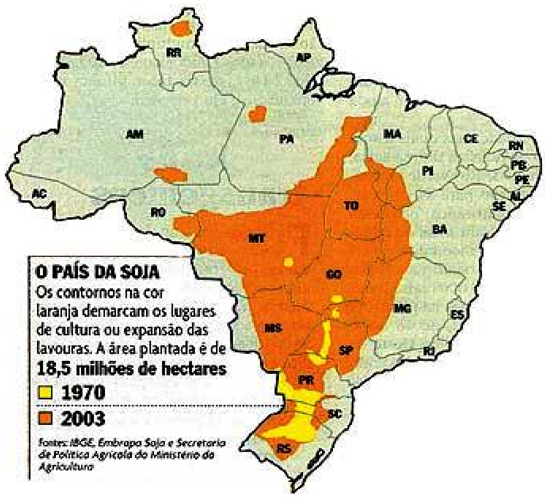

Figure 1 – Comparison of soybean production area between the years of 1970 (yellow) and 2003 (orange) (Delcidio, 2008).

2.2. Soybean significance

Nowadays, the leading countries in order of importance are the United States, Brazil, Argentina, China, India and Paraguay (Embrapa, 2008). Together, USA and Brazil produce 135.69 million tons and are responsible for 60% of the world’s production.

In 2007, United States produced 86.77 million tons and productivity was 2.87 kg/ha (USDA, 2008); in the same year, Brazil produced 58.4 million of tons, at 2.82 kg/ha (CONAB, 2008) and Argentina produced 45 million tons (FAO, 2008).

Soybean production in Brazil has increased 260 fold in the last few decades. This is due mainly to research in developing cultivars that are better adapted to Brazil`s conditions, specially the weather (EMBRAPA, 2009), making possible to increase the area cultivated. The major soybean producing states in Brazil are located in the south, southeast and mid-west regions, states of Mato Grosso, Paraná, Goiás and Rio Grande do Sul, in order of importance (CONAB, 2008).

In the 2007/2008 growing season of, soybeans were grown by 300,000 producers and generated 389,000 direct and 778,000 indirect jobs (CISoja, 2008).

This rapid growth and spread of soybean in Brazil lead to huge changes in the Country`s economy and society. In the beginning, together with wheat, both were responsible for the rising of commercial agriculture and this includes mechanization. Soybean was directly responsible for the expansion of agricultural border, professionalization and increase in international trade, for technological improvement in other cultures (especially maize), as well as stimulated expansion of pigs and poultry production (EMBRAPA, 2008).

Besides the already cited economic improvements, soybean has caused deep changes in population. It has not only caused modifications in the diets of Brazilians, but revolutionized the pattern of population distribution in Brazil. Soybean provoked rapid urbanization of the countryside in the South, Southeast and coasts in the of North and Northeast to regions that were previously unhabited (EMBRAPA, 2009).

2.2.1. Uses

Soybean is a very versatile grain, used by industry to produce many products. Among all uses, there is no doubt that the most known is for fine oil. In the process, lecytin is also produced and is used in the manufacture of sausages, mayonnaises, chocolate, etc. It is consumed in sauce for salads, bakery, products for meat, combined with cereals, beverages, baby food, etc. Soybean is used not only to feed humans, but animals too.

In industry, it is used to prepare seasonings, foam, manufacture fiber, covering and emulsion for inks. Currently, Soybean is being tested to be used as an alternative source of fuel, called biodiesel (CISoja, 2008, EMBRAPA, 2009).

2.3. Botany

Prior to the 20th century, soybean (*Glycine max* (L.) Merrill) seeds were different from the way they are today, they were black, green, brown, yellowish-green or mottled. With breeding, today they are largely yellow. Seeds grow in pods, usually 2-3 in each pod. Leaves are trifoliate and are covered with brown hairs, as are pods and stems (Shurtleff & Aoyagi, 2007).

Soybeans are unique among legumes, because in most plants, flowering and ripening are controlled by air temperature and in soybean, they are governed by photoperiod (Shurtleff & Aoyagi, 2007), which is the number of hours of sun and moonlight. Since day length changes with latitude, there is diversity of cycles, and soybeans will flower and mature later in the North and earlier in the South of the globe. This is very important in choosing the cultivars, because when photoperiod is shorter during plant vegetative growth, flowering occurs precociously and yield is reduced (CISoja, 2008).

Because of the situation described, soybeans are classified in two different maturity groups (MG). In North America, they were classified into 12 maturity groups based on photoperiod sensitivity. Soybeans grown in Canada and the northern parts of the US are classified as MG 00 and 0, respectively. Those grown in the central US belong to MG 1 and 2 through 4, whereas those adapted to the subtropical and tropical zones are classified as MG 8 through 10. Cultivars suited to the northern maturity groups mature quickly, in about 80-90 days after planting, whereas those in southern warm climates take longer, 100-150 days. In general, Brazilian cultivars have cycles between 100 and 160 days and their maturity groups are precocious, semi-precocious, medium, medium-delayed and delayed, depending on the region (Shurtleff & Aoyagi, 2007; CISoja, 2008). International maturity groups for Brazil are illustrated in Figure 2.

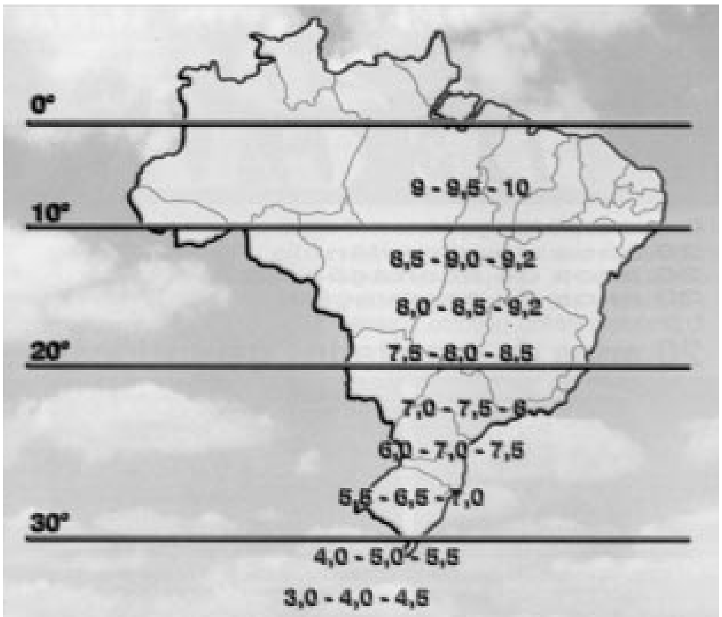

Figure 2 – Soybean Maturity Groups (MG) in Brazil, classified according to the latitude. International convention (Penariol, 2000).

Other important factor in soybeans, that occur with other legumes - for example common bean - is the type of growing: they can have determinate growth, when the vegetative growth is completed prior to flowering and the main stem ends; indeterminate growth, when they continue to increase in height for several weeks after beginning to flower; and intermediate growth, which is semi-determinate (Shurtleff & Aoyagi, 2007; CISoja, 2008). Plant height depends on the interaction between weather conditions in a specific region and the cultivar; for example, those grown in Brazil, vary between 60 and 120 cm (CISoja, 2008).

2.3.1. Soybean growth stages

Soybeans growth stages description can be discussed as (Fehr *et al*.,1971):

<u>Vegetative</u>

V1. Completely unrolled leaf at the unifoliolate node;

V2. Completely unrolled leaf at the first node above the unifoliolate node;

V3. Three nodes on main stem beginning with the unifoliolate node;

V(n). N nodes on the main stem beginning with the unifoliolate node;

<u>Reproductive</u>

R1. One flower at any node;

R2. Flower at node immediately below the uppermost node with a completely unrolled leaf;

R3. Pod 0.5 cm long at one of the four uppermost nodes with a completely unrolled leaf;

R4. Pod, 2 cm long, at one of the four uppermost nodes with a completely unrolled leaf;

R5. Beans beginning to develop (can be felt when the pod is squeezed) at one of the four uppermost nodes with a completely unrolled leaf;

R6. Pod containing full size green beans at one of the four uppermost nodes with a completely unrolled leaf;

R7. Pods yellowing; 50% of leaves yellow. Physiological maturity;

R8. 95% of pods brown. Harvest maturity.

2.3.2. Cultivars used in trials

<u>Monarca</u>

This cultivar named Monarca CS 303, is adapted to Bahia, Goiás, Mato Grosso do Sul, Mato Grosso e Triângulo Mineiro. ‘Monarca’ belongs to maturity group 8.0 with medium cycle in the state of Minas Gerais. The best period for sowing is between October and December (Vasconcelos, 2006, Syngenta Seeds, 2008).

Conquista

In the market since 1995, this cultivar named as MGBR-46, is adapted to Minas Gerais, Rondônia, Tocantins, Roraima, Goiás, Distrito Federal, Mato Grosso, Bahia e São Paulo. Conquista belongs to maturity group 8.1 with a medium to semi-delayed cycle in Minas Gerais. The best period for sowing is between October and December, however, because of its long vegetative period, it can be sown during winter when complementary irrigation is present (EMBRAPA, 2009).

Vencedora

Commercially available since 1998, this cultivar named BRSMG-68, is adapted to São Paulo, Minas Gerais, Goiás, Distrito Federal, Mato Grosso e Bahia. Vencedora is of maturity group 8.0 cycle semi-precocious to medium cycle in Minas Gerais State. The best period for sowing is between October and November (EMBRAPA, 2009).

2.4. Diseases

One of the main factors limiting maximum soybean yields is disease effect. In Brazil, approximately 40 soybean diseases caused by fungi, bacteria, nematodes and viruses have already been identified and this number continues to increase with the expansion of soybean to new areas and increase in monoculture (Embrapa, 2008).

The economic importance of each disease varies from year to year and region to region, depending on the weather conditions of each growing season (Embrapa, 2008). Diseases that occur at the end of the soybean cycle are brown spot (*Septoria glycines*); leaf blight (*Cercospora kikuchii*) and anthracnose (*Colletotrichum dematium* var. *truncata*) (Meyer & Yorinori, 1999). Under favorable ambient conditions, the occurrence of hypocotyl rot and foliar blight (*Rhizoctonia solani / Thanatephorus cucumeris*), downy mildew (*Peronospora manshurica*), target spot (*Corynespora cassicola*), myrothecium leaf spot (*Myrothecium roridum*), sudden death syndrome (*Fusarium solani*), charcoal rot disease (*Macrophomina phaseolina*) and stem rot disease (*Sclerotium rolfsii*) is common.

Other diseases that occur are frogeye leaf spot (*Cercospora sojina*), bacterial leaf blight (*Pseudomonas syringae* pv. *glycinea*), bacterial pustule disease (*Xanthomonas campestris* pv. *glycines*) in areas where it was used susceptible cultivars, presenting considerable yield loss and a particular problem to some areas is soybean cyst nematode (*Heterodera glycines*) (Meyer & Yorinori, 1999; Embrapa, 2008).

Although all these diseases occur and cause yield losses, one of the most important diseases of the soybean in the world is Asian Soybean Rust (*Phakopsora pachyrhizi*). It has been a problem in almost all soybean-growing regions of the world (Purdue, 2008). In Brazil, ASR is the worst disease affecting soybeans, and in almost all 15 producing States, except for Roraima, yield losses due to this disease occurrence. However, due to government efforts in the last growing season disease was less severe than it was in previous years (Embrapa, 2008).

2.4.1. Asian Soybean Rust (ASR)

2.4.1.1. Taxonomy of *Phakopsora pachyrhizi*

(NPAG, 2002).

Phylum: Basidiomycota
Class: Uredioniomycetes
Order: Uredinales
Family: Phakopsoraceae
Genera: *Phakopsora*
Species: *pachyrhizi*
Full name: *Phakopsora pachyrhizi* H. Sydow & Sydow (anamorph *Malupa sojae* (P. Hennings) Ono, Buritica & Hennen comb.nov.)

2.4.1.2. Organism information

Soybean Rust (ASR) is caused by two related species of fungi, the most aggressive is *Phakopsora pachyrhizi* Sydow (anamorph *Malupa sojae*), and the less aggressive species, *Phakopsora meibomiae* (Arthur) Arthur (anamorph *Malupa vignae*), has only been found in limited areas in the Western Hemisphere and is not known to cause severe yield losses in soybean (Hartman *et al*., 1996).

Like other rusts, ASR pathogen requires a living host to grow and reproduce; it can survive away from soybean as urediniospores for few days under natural conditions. *P. pachyrhizi* produce only two types of spores: urediniospores and teliospores; the uredinial stage is the repeating stage. Epidemics develop quickly from pustules – spores are produced from seven to ten days after infection, and each pustule can produce hundreds of new urediniospores. Teliospores are produced in old lesions and aecia or spermogonia are unknown (Rupe & Sonyers, 2008).

P. meibomiae, is referred as the American or New World rust. Asian rust caused by *P. pachyrhizi* is extremely aggressive and is listed as a select biological agent (Title 7, Code of Federal Regulations, Part 331.2), determined to have the potential to pose a threat to plant health or plant products (USDA, 2009).

Phakopsora pachyrhizi is ranked as 22nd of the top 100 most dangerous and exotic pests and diseases (Cooper, 2009). It is an air-borne fungal pathogen that, under favorable environmental conditions, cause serious economic and crop losses (Embrapa, 2008).

There are only four known resistance genes for soybean, but all can be overcome by at least one of the many *P. pachyrhizi* isolates (Cooper, 2009). In the USA, in a search for genetic resistance, 940 soybean cultivars currently grown in the US and 12,000 soybean accessions from the USDA germplasm collection were evaluated in seedling assays against soybean rust at the BSL-3 containment greenhouse at Ft. Detrick, MD. None were found to be resistant and fewer than 100 showed any promise for disease tolerance (Cooper, 2009). In Brazil, the government research agency tested a resistant cultivar, but it was overcomed with an isolate from Mato Grosso (Embrapa, 2008).

Until now, chemical control using fungicides has been successful at obtaining good yields, specially myclobutanil and propiconazole in USA (Clayton, 2005). In Brazil, azoxystrobin, ciproconazole, propiconazole, tebuconazole and nine other – in blend or alone - are registered by MAPA to use against ASR (Embrapa, 2008). The problem is that producers have added production costs because of the need for at least three sprays during soybean cycle.

2.4.1.3. Disease cycle and epidemiology

P. pachyrhizi has many alternative hosts the legumes family, which may serve as sources of inoculum, although they are not needed for the fungus to complete its cycle (Rupe & Sonyers, 2008).

ASR epidemics begin with the arrival of airborne urediniospores, and once viable spores have landed on the leaf surface of the host, if environmental conditions are favorable, infection begins with the breaching of the epidermis (Rupe & Sonyers, 2008; Embrapa, 2008; Consórcio, 2009). Generally, infection occurs when leaves are wet and temperatures are between 8°C and 28°C, with an optimum of 16°C to 28°C. At 25°C, some infections occur in as little as 6 hours of leaf wetness, but 12 hours are optimal. After infection, urediniospores appear in seven to eight days, and the cycle re-starts (Rupe & Sonyers, 2008).

2.4.1.4. Symptoms

Symptoms begin as tiny brown or brick-red spots on leaves - especially on the lower surface - in the lower canopy, at or after flowering. Several uredinia are formed in the lesions and when the urediniospores germinate, a germ tube is produced and ends in an appressorium that penetrate directly or through a stoma. ASR begins in the lower canopy, but quickly progresses upward the plant until all the leaves have some level of disease. Severely diseased plants may become completely defoliated (Figure

3) and loss of leaf tissue results in yield reductions from fewer and smaller seeds (Figure 4). Lesions may be either tan - many pustules that produce numerous urediniospores or red-brown - few pustules that produce only few urediniospores. As pustules age, they may turn black due to the formation of a layer of teliospores. Germination of teliospores has been observed only in the laboratory and does not seem to make a significant contribution to the perpetuation of this disease in the field. Besides leaves, soybean rust can also appear on petioles, stems, and even cotyledons, but most rust lesions occur on leaves (Rupe & Sonyers, 2008; Embrapa, 2008; Consórcio, 2009).

Figure 3 – Early defoliation in soybean caused by Asian Soybean Rust (ASR) – on the left treatment with three tebuconazole sprays and on the right, control with no spray. Viçosa, MG, Brazil. 2008.

Figure 4 – Reduction of yield in soybean caused by Asian Soybean Rust (ASR) – on the left control with no fungicide spray and on the right with three tebuconazole sprays. Viçosa, MG, Brazil. 2006.

Soybean rust epidemics can progress from below detectable levels to defoliation within a month and may seem to progress even faster than that, because early infections occur in the lower canopy and are harder to find. Yield losses as high as 30 to 80% have been reported, but the amount of loss depends on when the disease begins and how rapidly it progresses (Rupe & Sonyers, 2008).

2.5. Yield Loss

2.5.1. Quantification of yield loss

Before talking about yield loss, it is necessary to just define yield and loss, because is common in Plant Pathology, some concepts are used in different ways. In this work, yield is considered the plants commercial part, measured as pod and grain number and weight and loss is the reduction in quality and/or quantity of these commercial parts.

2.5.2. Models

To estimate yield loss, information summarized in the form of quantitative relationships called models; consequently, those that can be defined as a simplified representation of a system, in which variables related to disease and loss are used. Models used to estimate yield loss can be one of three kinds (Bergamin Filho *et al*., 1995; Cooke, 2007):

1. **Critical point**: for many pathosystems it is possible to identify a definite stage of host growth when severity can be related to yield loss. In this kind of model, only one assessment is used to represent an entire epidemic. This model, is often criticized because it is based on a small portion of epidemics.

2. **Multiple points**: this model relates yield loss with variables obtained from repeated evaluations (two or more times) during epidemic. This is based on the concept that the entire epidemic potentially influences crop yield.

3. **Integral models**: These models are based in many assessments to represent the entire epidemic, and the importance of a given intensity of disease depends on the time during the season when that disease intensity occurs.

From now on, only integral models are going to be considered.

2.5.3. Integral models use to quantify yield loss

Despite the importance of plant diseases, the relationship between disease and resulting yield was not studied for most diseases, until recent decades. When they finally commenced being studied, researchers were seeking for answers on correlations between crop yield and disease severity. Teng (1985) showed that the traditional method used – a simple correlation between crop yield and disease severity in some host growth stages – was not sufficient to explain what was happening.

The yield loss quantification started with two simple methods, disease incidence and severity (James & Teng, 1979), but the results obtained were disappointing, considering that sometimes they were appropriated and sometimes they were not. There was a need for different methods of assessments.

Waggoner & Berger (1987) worried about this lack of good results, probably were the first plant pathologists at the end of the last century to look back to another branch of science: plant physiology. Crop physiologists started to look for the principles of yield physiological processes a long time before plant pathologists. The biggest advance was obtained by West *et al*. (1920) and Briggs *et al*. (1920) when they developed growth functions that combined physiology with field work. One of them was net assimilation rate (NAR), defined as weight increased by leaf surface in time. “NAR” can be considered photosynthesis minus loss due to plant breathing (Gregory, 1926):

$$NAR = ((w_2 - w_1) (\ln (L_2) - \ln (L_1)) / (t_2 - t_1) (L_2 - L_1)) ,$$

in which w_1 and w_2 are the dry weight, L_1 and L_2 leaf area, in time t_1 e t_2, respectively.

Twenty seven years after Gregory's work, it was found high correlation between yield and leaf area index (LAI), showing the remarkable role of LAI in the growth on many crops, among them wheat, barley, sugar beet and potato (Watson, 1947). Furthermore, Watson realized that more important than just the leaf area, was the integral of LAI during all growth stages of the plants that should be taken into account, because yield was even better correlated to leaf area duration (LAD). This was because LAD considers the size of the leaves and how long they persist. This is the concept of Leaf Area Duration = LAD, calculated as

$$LAD = \Sigma ((LAI_i (t_i - t_{i-1}) / 2)),$$

in which LAD is the duration of leaf area (in days), LAI is leaf area index (without dimension), and t, is time.

These concepts of LAI and LAD represented a remarkable progress toward the comprehension of the bases of production and opened the doors to other concepts that would be based on them more than twenty years later.

With this in mind, the knowledge of a healthy host phenology is also necessary because the action of senescent parts can clearly be differentiated from diseased parts lost due to pathogens.

Last (1955) was almost certainly, the first Plant Pathologist to defend an approach based on the host instead of only considering the area covered by lesions. He suggested that the healthy leaf area was better correlated to yield than disease parameters. This totally new approach based on Barley did not have the repercussions that it deserved. Bergamin Filho *et al.* (1995) mentioned that maybe this was due to the huge amount of work that was needed to reproduce it. Some authors (Rea & Scott, 1973), in subsequent years, with the same pathosystem confirmed the pioneering results of Last. They noticed that protecting barley chemically against powdery mildew, this crop presented bigger Leaf Area Duration (LAD), which was well correlated with yield. These results were later confirmed by Jenkyn (1976).

This completely new way to look at yield loss trough host-pathogens relationship was left aside until Lim & Gaunt`s (1981) work. With their research on leaf area, they highlighted the role of leaf area in yield quantification. In the same year (1981), Carver and Griffiths had similar conclusions as Lim and Gaunt (1981) about the importance of leaves. These authors were the first to use concept of Green Leaf Area, which would be the same concept as HAD (Healthy Area Duration) defined two years later by Waggoner and Berger (1987).

The traditional approach did not consider the size and quantity of leaves, neither the radiation intercepted by them, nor their capacity to carry out photosynthesis. Most of the physiological processes related to host growth to the later storage occur in the leaves. When a pathogen interrupts this natural process, all or at least part of it is interrupted, generating disease and this affects yield.

Now it is possible to understand why the relationship between disease and yield was weak and it explains the disappointing results obtained when plant pathologists considered only the pathogen and disease instead of the host when qualifying yield loss.

Waggoner & Berger (1987) called attention to the fact that yield, or dry matter of a plant, is function to a large extent, of the photosynthesis that occurs in the leaves. They gathered data from many previous articles from different places and pathosystems and showed that photosynthesis is more directly related to the absorption of solar radiation by leaves than LAI.

Generally, the Beer`s law is used to express the transmission of solar radiation I (MJ m^{-2}) through the foliage. Thus, the absorbed fraction '*f*' is given by:

$$f = (1 - \exp(-k\mathrm{LAI})),$$

in which k is the extintion coefficient (value close to one for plants with horizontal leaves and around to 0.3 for plants with erect leaves) and depends on LAI. The production of dry matter w (g m^{-2}) becomes related to LAI and solar radiation through the equation:

$$w = e \int If dt.$$

Previous studies on yield loss quantification had focused on the increase in lesions number or in disease severity progress in time. Disease progress curves are summarized by means of rate changes, r (day^{-1}), or area under disease progress curve (AUDPC) (days) (Vanderplank, 1963). None of these variables used (r and AUPDC) supply information regarding foliage size, how long it remains functional or how much solar radiation it absorbs.

To relate disease progress curve to host growth, it should be deducted the lesioned area from LAD value and by the integration of the healthy and operational leaf area with host growth over the time, results in what Waggoner & Berger (1987) called Healthy Area Duration (HAD) (days):

$$HAD = \Sigma \left(1 - y_1 \right) LAI_i \; (t_i - t_{i-1}) / 2)$$

A similar thing can be done with LAA, which includes diseased area and gives rise to the Healthy Area Absorption (HAA) (MJ m^{-2}) Waggoner & Berger (1987):

$$HAA = \Sigma \left(I \left(1 - y_1\right) \left(1 - \exp\left(-kLAI_i\right) (t_i - t_{i-1}) / 2 \right)\right.$$

Waggoner & Berger`s (1987) ideas provoked interest in the scientific community, leading to a peak in the number of studies conducted in this area (Johnson, 1987; Campbell & Madden 1990). This interest generated research in diverse pathosystems relating HAD or HAA to yield: potato-*Phytophthora infestans* (Van Oijen, 1990); potato-BMYV-MYV (Rossing *et al*., 1992); Potato-*Alternaria solani* (Jonhson & Teng, 1990; Shah *et al.*, 2004a, 2004b); potato-*Globodera rostochiensis* (Shah *et al.*, 2004a); wheat-*Puccinia recondita* f.sp. *tritici* (Subba Rao *et al.*, 1989); wheat -*Mycosphaerella graminicola* (Shaw & Royle, 1989); wheat -*Erysiphe graminis* f.sp. *tritici* (Daamem & Jorritsma, 1990); soubean-Phakopsora *pachyrhizi* (Yang *et al*., 1991, 1992; Kumudini *et al.*, 2008); soybean-*Erysiphe diffusa* and *Cercospora kikuchii* (Godoy & Cantery, 2004); peanut-*Cercosporidium personatum* (Aquino *et al*., 1992; Savary & Zadoks, 1992); rice-*Pyricularia oryzae-Thanatephorus cucumeris*-insects-rats (Pinnschmidt & Teng, 1993);

rice-*Pyricularia grisea (= oryzae)* (Bastiaans, 1991; Pinnschmidt *et al*., 1994); corn-*Phaeosphaeria maydis* (Godoy *et al*., 2001); common bean-*Colletotrihum lindemuthianum* (Gianasi, 2002), *Phaeosariopsis griseola* (Jesus Junior *et al*., 2003; Cantery & Godoy, 2005), *Uromyces appendiculatus* (Bassanezi *et al*., 2001; Jesus Junior *et al*., 2001a, 2001b), *Xanthomonas axonopodis* pv. *phaseoli* (Díaz *et al*., 2001); sunflower-*Alternaria helianthi* (Leite *et al.*, 2006); alamus-*Melampsora medusae* (May-de Mio *et al*., 2006).

Johnson (1987) replied to Waggoner & Berger`s (1987) letter, stating that the effect of the pathogens could be even worse, because they don`t only affect radiation interception and absorption by causing the hosts to drop their leaves through defoliation, but they could also affect the use of the radiation intercepted, showing the importance of the pathogen`s action on radiation use efficiency (RUE).

Griffiths (1984) perceived that not all pathosystems present the same high correlation between the integral of the green leaf area and yield mentioned by Waggoner & Berger (1987). As possible explanations, are photosynthesis reduction in areas distant from the lesions, variations in harvest index, increase of plant breathing due to infection, the peculiar contribution to yield by specific leaves and changes in growth regulators. Bastiaans (1991) found the same results with rice-leaf blast and more than this, the area that pathogens affect can be greater than one can see. This effect can be due to toxins, for example and called this "virtual lesion", because it can be measured, but it cannot be seen.

To obtain beta value, the interaction on the photosynthetic rates of healthy and diseased leaves are determined by gas-exchange analysis and used in the equation (Bastiaans,1991):

$$P_x/P_0 = (1 - x)^{\beta}$$

in which P_x is the photosynthetic rate on a diseased leaf and P_0 on the healthy ones, X is disease severity and β is the ratio between virtual and visual lesion.

In virtual lesion concept, when beta values are equal to 1, the area that is affected by pathogen is just the same that can be seen as diseased: visual and virtual areas are the same. The longer the beta value is above 1, the bigger is the area affected in relation to visually lesioned area on the leaves.

Plant pathogens can be divided into groups, based on their nutritional behavior, such as biotrophic and necrotrophic. Those that do not kill their hosts immediately and depend on viable tissue to complete their development are called biotrophic; rusts are examples (Agrios, 2007). Lopes (1999) explains that the pattern of response and the reduction in photosynthesis are related to the type of trophic relationship, but it cannot be assumed that all biotrophic agents act in a similar manner concerning the mechanisms of infection.

Generally, when necrotrophic microorganisms are involved, they use toxins that diffuse into the tissue and result in bigger values of β, being similar to hemibiotrophic pathogens (Bassanezi *et al.*, 2001). In the case of biotrophic microorganisms, as they are less destructive to the tissue because of their mode of living, they usually present values of β equal or close to one. Some research that corroborate this idea are Bastiaans (1991) – wheat rust; Bastiaans & Roumen (1993) – rice blast; Bassanezi *et al*. (2001) – rust, angular leaf spot and common bean anthracnose; Lopes & Berger (2001) – bean rust; Diaz *et al.* (2001) – common bacterial blight on bean.

It is important to highlight that every pathosystem is unique and pathogen`s effect on green leaf tissue vary and may present different results, for example McGrath & Pennypacker (1990) work with wheat rust, Elings *et al.* (1999) with bacterial blight on rice and Robert *et al.* (2005) with septoria on wheat.

2.5.4. Yield losses in soybean caused by Asian Soybean Rust

Solar radiation is one of the factors that is most limiting for plant growth and development, because all the energy necessary to make photosynthesis comes from it (Taiz & Zieger, 2004). In soybean, solar radiation is related to photosynthesis among other important processes

(Câmara, 2000) which depend on radiation intercepted and the right use of this intercepted radiation (Shibles & Weber, 1965). Thus, any factor that causes reduction in leaf area, can affect yield (Diogo, 1997).

One of the most important factors is ASR, because it affects the photosynthetic potential of plants due to yellowing and early defoliation (Yorinori, 2003). Under favorable weather conditions and depending on the time of infection, ASR cause yield loss of up to 80% (Hartman *et al.*, 1991; Dorrance *et al*., 2005).

Such yield loss occurs due to leaf reduction by accelerated defoliation (Kumudini *et al*., 2008). However, considering that ASR causes lesions in advance of defoliation, it is unclear to what degree the disease induces yield loss from premature leaf loss or, from direct or indirect effects of lesions on light absorption and the rate of dry matter accumulation.

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ASIAN SOYBEAN RUST EFFECTS ON PHYSIOLOGICAL COMPONENTS AND YIELD

1. ABSTRACT

The effects of Asian Soybean Rust (ASR) on host yield were quantified at different phenological stages of the crop using three different cultivars. Four field experiments were carried out during the 2005/2006, 2006, 2006/2007 and 2007/2008 growing seasons, where different disease levels were obtained using fungicide sprays. Rust severity was highest and yield was the lowest during the 2007/2008 growing season. Based on the analysis of 250 plants in the first and 320 in each of the second, third and fourth experiments, yield was related to Area Under Disease Progress Curve (AUDPC, R^2=25.2; 51.0; 22.3 for ‘Monarca’, ‘Conquista’ and ‘Vencedora’, respectively), Healthy Leaf Area Duration (HAD, R^2= 49.2; 47.6; 26.3) and Healthy Leaf Area Absorption (HAA, R^2= 50.3; 43.8; 51.8). HAA was considered the best variable because it was the only one that kept the same trend among cultivars, seasons and growth stages. According to all experiments combined, ASR lead to early defoliation which decreased leaf area and radiation interception, plus reduced absorption of healthy area surrounding lesions and Radiation Use Efficiency (RUE).

2. INTRODUCTION

The United States is the world's leading soybean producer and exporter (70.70 million metric tons), followed by Brazil (58.19 million metric tons) and Argentina (45.5 million metric tons) (FAO, 2008).

These figures show the soybean importance for these country economics, and among the causes that reduce productivity, are the losses caused by diseases.

One of the most important diseases of the soybean is the Asian Soybean Rust (ASR). It has been a problem to almost all soybean-growing regions of the world (Purdue, 2008). The ASR is a severe disease caused by the fungus *Phakopsora pachyrhizi* that leads to significant crop losses, mainly because the infected leaves fall prematurely, which is preceded by yellowing and necrotic areas, limiting grain formation. When ASR occurs early in reproductive growth, the intense defoliation cannot be overcome from the plants and this can lead to pod abortion and fallen (Yorinori & Paiva, 2002). These kinds of losses have been attributed to reduction in the Leaf Area Index, because leaf necrosis and defoliation reduces light interception and photosynthesis (Aquino *et al.*, 1992).

One of the largest problems with this disease is that for soybean have a good development, the optimal temperature is between 15 and 22ºC (Liu *et al*., 2008) and to *P. pachyrhizi*, it is between 16 to 28 ºC (Rupe & Sonyers, 2008); very close and therefore, good conditions for the host, imply good conditions to pathogen, thus ultimately making disease management a difficult task. The effect on yield depends on the disease severity earliness in the host growth stage and can reduce yield as much as 80% (Ogle *et al.*, 1979).

The crop loss assessment had been made in the past by correlating yield with disease severity, but most of the times, they cannot be used in other seasons and locations due to the indirect relationship between yield and disease, especially when a crop is grown under different production environments (Shah *et al.* 2004). Principally because of this problem, plant pathologists started to search for other variables that could conduct to a better understanding of yield loss.

The method proposed by Waggoner & Berger (1987) changed the focus from the pathogen to the host. Instead of disease severity, the parameters started to be based on host attributes, such as the leaves total area projected on a square meter of soil - Leaf Area Index (LAI) and healthy leaves (HLAI); how much time they persist in the plant - Leaf Area Duration (LAD) and the persistence of the healthy tissue - Healthy Leaf Area Duration (HAD); how much radiation can their leaves intercept – Radiation Interception (RI) and that intercepted by the leaves that still remain green in the plant - Healthy Leaf Area Absorption (HAA). Finally, Johnson (1987) replied that the pathogens not only affect the host capacity of radiation interception, but in their capacity to use it too – Radiation Use Efficiency (RUE).

The Crop Field researchers already used the RUE concept for a long time, since Watson (1947) could find good relation between LAI and Yield and Monteith (1977) between biomass accumulation and radiation interception (RUE), introducing the importance of the radiation use. The RUE is a good variable, because it is a simple and efficient method to estimate the productivity of a crop, being influenced by the radiation available and how much it is intercepted. Because of this, RUE value changes according to the stresses that the host suffer (Costa *et al.*, 2000), including diseases that interfere in the foliage. Previous studies are in accordance and analysis have consistently indicated a dependence of RUE on leaf photosynthetic activity (Sinclair & Muchow, 1999).

Since this approach was proposed, many pathosystems have been studied and better relations between disease and production have been found. Advances have been made especially with beans (Bergamin Filho *et al.*, 1997; Silva *et al.* 1998; Díaz *et al.*, 2000; Jesus Junior *et al.*, 2001; Bassanezi *et al.*, 2001; Gianasi, 2002; Jesus Junior *et al.*, 2003), soybean (Hartman & Sinclair, 1995; Koga *et al.*, 2007), sunflower (Leite *et al.*, 2005); potato (Shah *et al.* 2004a, 2004b, 2004c), peanut (Aquino *et al.*, 1992); wheat and oats (McKirdy *et al.*, 2002) and *Populus* spp. (May-De Mio *et al.*, 2006).

Most of these studies did not take measurements of defoliation when it occurred, because it is so hard to take. Even so, Rotem *et al.* (1983) and Aquino *et al.* (1992) made some considerations about the importance to consider that plants can have the same disease severity, but different amounts of healthy foliage and the replacement or not of the fallen leaves. This is very important on the *P. pachyrhizi* - soybean pathosystem, because the disease comes from the bottom to the upper part of the plant and the most diseased leaves fell off in the soil and then, disease can seem to decrease or to be stable, when it is getting higher.

This study focuses on the quantification of the effects of ASR on soybean physiology and yield to achieve the objective to determinate the best variable related to yield - independent of location and season - to quantify yield loss caused by this disease. In the future, it can be used in models to better predict yield.

3. MATERIALS AND METHODS

3.1. Field experiments

Four field experiments were conducted at the Universidade Federal de Viçosa, Viçosa county, Minas Gerais State, Brazil. The experiments, independent from each other, were conducted in the rain and dry seasons: from November 2005 to March 2006 (rain), August to November 2006 (dry), October 2006 to February 2007 (rain) and October 2007 to January 2008 (rain) with soybean cv. ‘‘Monarca’’ in the first one, ‘‘Conquista’’ in the second and ‘‘Vencedora’’ for third and fourth trials. All cultivars are susceptible to rust and have determinate habit of growth. ‘‘Conquista’’ was used during winter season, because it is a medium-delayed cultivar and there is no need for too many sunlight hours, while ‘‘Vencedora’’ had needs longer days.

The soil was prepared in traditional way. The fertilization was based on chemical soil analysis. Before sowing, seeds were treated with fungicide (Thiram) and inoculated with *Bradyrhizobium japonicum*.

Each plot (14 m^2) consisted of 4-m-long rows, spaced 0.5 m apart. There was 1.5 m between adjacent plots. To minimize interplot interference, only the five central rows of each plot were used for assessment (0.5 m at the end of each row was omitted).

Eighteen seeds were sown and fifteen plants were kept per linear meter of row. The plots were maintained with conventional cultural practices used in commercial fields, which included planting, foliage fertilization by spray, insecticide sprays, weeding and irrigation.

Plants were naturally infected by *Phakopsora pachyrhizi* and irrigated during winter when the weather condition was dry. Irrigation was necessary to provide infection`s moisture conditions, ensuring enough leaf wetness to have the infection guaranteed. In order to obtain a range of disease levels, plants in different plots were sprayed with a fungicide (Tebuconazole) with a manual applicator until the running point. The treatments were, for the first experiment: (1) n spray = control; (2) 25% of the recommended fungicide dose (rfd) = 0.125 l/ha, (3) 50% rfd = 0.25 l/ha, (4) 75% rfd = 0.375 l/ha and (5) 100 rdf = 0.5 l/ha; for other experiments: 1: control (no sprayed); 2: 30 days after emergence (DAE); 3: 60 DAE; 4: 90 DAE = ; 5: two sprays, 30 and 60 DAE; 6: two sprays, 30 and 90 DAE; 7: two sprays, 60 + 90 DAE; and three sprays: 8: 30 + 60 + 90. The experiments were set in a randomized complete block design and five replications.

Every measurement was taken in three plants in each plot, randomly harvested, total of 75 plants in the first trial and 96 plants in the other trials, assessed weekly.

3.2. Crop growth

Crop growth was quantified by measuring soybean leaves. The increase in growth was measured in cm^2 in all leaves of the harvested plants, using a Leaf Area Meter LI-3100C (LICOR Biosciences, Lincoln, NE).

3.3. Disease severity and defoliation

Disease severity was accessed using a diagrammatic scale (Godoy *et al.*, 2006) on every leaf of three plants sampled weekly (n=96), starting with the appearance of the first symptoms. These total readings were divided by the number of leaves present to give an average rust for each plant (in percentage).

Defoliation was determined in each evaluation. The values were obtained counting the marks on the stem left by the fallen leaves in relation to their total number. Defoliation data were used to correct disease severity values according to Aquino et al (1992):

$$y_t = [(1 - d) y_v] + d$$

in which y_t represents total disease severity, y_v is the proportion of visible disease, and d is the proportion of defoliation.

A correction of the natural defoliation was made through discounting the values obtained in the plots without disease incidence from the diseased ones.

Growth stage of plants harvested in each plot was determined and recorded at every assessment date, according to the descriptive scale of Fehr and Caviness (1977).

3.4. Yield and yield loss

Yield (*Y*) was determined at harvest maturity (growth stage R8). Ten relatively uniform plants were removed randomly from the 5 center rows, in each plot, to determinate the yield components. The yield components per plant were directly counted: filled and unfilled (shriveled) pods per plant, grains per pod, 100-seed weight, and total seed weight (g/plant), and total yield (g/m^2). Weight was always determined after grains were dry to correct moisture to 12%.

Yield loss was determined by the comparison of yield (g/m^2) on the plots with lower disease values against all the others and converted to percentage to allow comparisons, since cultivars have different actual yields.

3.5. Integral variables

The area under disease progress curve (AUDPC) was calculated by trapezoidal integration (Shaner and Finney, 1977) for each plant. The numbers of observations were 9, 8, 5 and 9, respectively, for the first, second, third and fourth trials. Considering that this work intends to provide reproducible data in any other location (soybean development changes according to cultivar, season and latitude) and because of the unequal number of observations among experiments, all the measurements made, were standardized (by average) by plant growth stage.

AUDPC was calculated as:

$$AUDPC = \sum_{i=1}^{n-1} \left[\frac{Y_{i+n1} + Y_i}{2}\right] [X_{i+1} - X_i]$$

In which Y_i= disease severity (% per unit) at the *ith* observation, X_i= time (days) at the *ith* observation, and n= total number of observations.

With 24 plants per m^2, Leaf Area Index (LAI) was calculated, multiplying the average leaf area by 24. From the LAI, the Healthy Leaf Area Index (HLAI) (Waggoner and Berger, 1987) could be obtained as follows:

$$HLAI = LAI\ (1 - X)$$

in which, X = asian soybean rust severity. And through LAI values, its integration over time reflects the Leaf Area Index Duration (LAD), which was defined by Watson (1947) as:

$$LAD = \sum_{i=1}^{n} \left[\frac{LAI_i + LAI_{i+1}}{2}\right] [t_{i+1} - t_i]$$

Healthy Leaf Area Duration (HAD) in days was calculated for each plant accordingly to Waggoner and Berger (1987):

$$HAD = \sum_{i=1}^{n-1} \left(\frac{LAI_i\ (1 - X_i) + LAI_{i+1}\ (1 - X_{i+1})}{2}\right) (t_{i+1} - t_i)$$

Being the Radiation Intercepted (RI) in (MJ m^{-2}) given by:

$$RI = I_i[1 - \exp(-k\ LAI)]$$

in which I= average of the incident solar radiation (MJ m^{-2}) in the period between evaluations; k = extinction coefficient, for soybean k = 0.6 (Pengelly *et al.* 1999).

Healthy Leaf Area Absorption (HAA) for each plant was calculated as follows (Waggoner and Berger, 1997):

$$HAA = \left(\frac{RI_i + RI_{i+1}}{2}\right)(t_{i+1} - t_i)$$

3.6. Data analysis

Calculations of the integral variables were made with Excel (Microsoft Office, 2005). All analysis of covariance were performed on the software SAS system (SAS Institute, Cary, NC), including the check of experiments three and four, with the same cultivar, to verify if they could be polled together.

3.7. Climate data

Climate data (temperature and incident solar radiation) were obtained from INMET automatic station in Viçosa, distant 2 km from the experiments. To obtain the average of radiation on each day there were used data from 9 A.M. to 5 P.M, to standardize the number of hours considered, because it is the period of the day when normally sunlight is present even during winter.

4. RESULTS

4.1. Temperature and solar radiation

Usually, in Brazilian southeast region, the summer (December to March) season is wet and it is called “the rainy season”, while it is dry in the winter (June to September) it is dry.

The average incident solar radiation values during the experiment period, from the sowing to R7 in the years 2005/2006, 2006 and 2007/2008 ranged from 12.41 (July, 2006) to 22.01 (January, 2006).

4.2. Disease severity and defoliation

Healthy plants were not observed in any of the experiments at the end of epidemics. Plants sprayed three times were healthier for a longer period, but could not be maintained in this condition until the end of the host cycle (Figure 1).

In the first trial, during 2005/2006 summer, despite the ideal conditions to *P. pachyrhizi*, epidemic was slow and kept lower – maximum of 54% in unprotected plants, comparing with the other trials (Figure 1 and Table 1).

Table 1 – Temperature (°C) during trials for cultivars ‘Monarca’ (summer 2005/2006), ‘Conquista’ (winter 2006) and ‘Vencedora’ (summer 2006/2007 = 1 and 2007/2008 = 2). Viçosa, MG, Brazil

Cultivar	Season	Av Max* ± SD	Av min* ± SD
‘Monarca’	summer	32.46 ± 3.43	14.80 ± 2.07
‘Conquista’	Winter	25.90 ± 2.94	12.90 ± 9.63
‘Vencedora’ 1	summer	31.55 ± 1.17	16.30 ± 2.05
‘Vencedora’ 2	summer	32.25 ± 1.65	15.70 ± 0.40

AV max and AV min = average among maximum and minimum temperatures during the season, respectively. SD= standard deviation.

Epidemics duration varied in all four trials, leading to different assessment results, because evaluations started with the appearance of the first symptoms. To make comparisons, a standardization of the values was performed by averaging the values obtained in the same host growth stage. These averages were used in this work to weight host phenology instead of days after sown, because disease and host growth vary in different seasons and producing regions as weather conditions change.

During the experiments, natural infections occurred in all plots, and lesions caused by *P. pachyrhizi* were first noted right before or during bloom (between V8 and R1). The ASR severity was significantly different between non sprayed and sprayed treatments, irrespective of the experiment and cultivar ($P<0.01$).

Defoliation varied among experiments, but the maximum visual rust severity on attached leaves was higher in all experiments when defoliation was considered (Figure 1). As occurred with severity, the defoliation was significantly different between non sprayed and sprayed treatments, irrespective of the experiment and cultivar ($P<0.01$).

4.3. Crop growth

The crop growth was evaluated by the increase of leaf area. The maximum values for LAI and HLAI decreased as epidemics progressed

(Figure 2) because of the early defoliation provoked by the action of pathogens. Plants located in control plots (unprotected) reached stages R7 and R8 between 10 and 15 days before the protected ones, in all experiments, regardless of the cultivar.

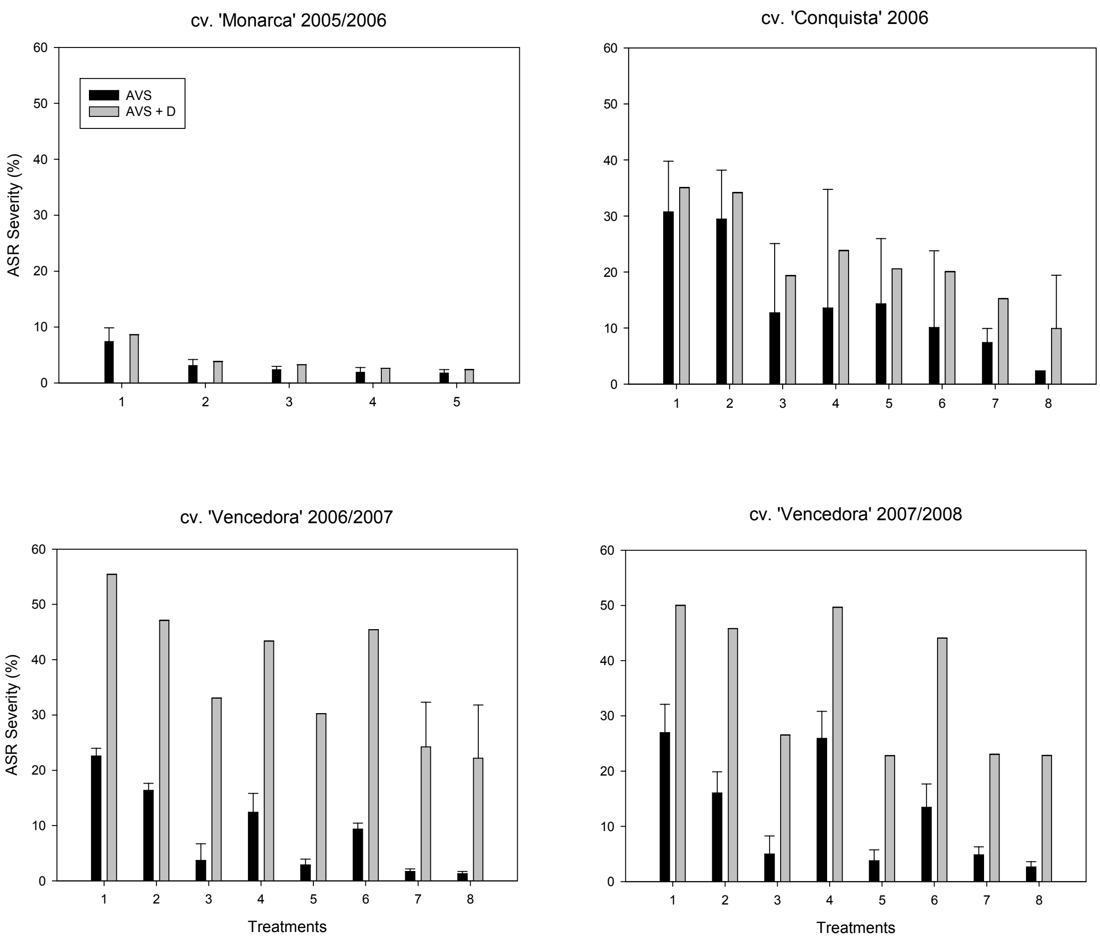

Figure 1 – Average visual disease severity (AVS, in black columns) and disease severity accounting defoliation percentage (AVS+D, in grey columns) of Asian Soybean Rust (ASR) in three different soybean cultivars in three different years. Viçosa, MG, Brazil. Treatments were obtained with fungicide tebuconazole: 'Monarca' = 1: no fungicide spray, 2: 0.125 l/ha, 3: 0.25 l/ha, 4: 0.375 l/ha and 5: 0.5 l/ha; 'Conquista' and 'Vencedora' = 1: no spray, 2: 0.5 l/ha, 30 days after emergence (DAE); 3: 0.5 l/ha, 60 DAE; 4: 0.5 l/ha, 90 DAE; 5: 0.5 l/ha, two sprays, 30 and 60 DAE; 6: 0.5 l/ha, two sprays, 30 and 90 DAE; 7: 0.5 l/ha, two sprays, 60 + 90 DAE; and 0.5 l/ha, three sprays: 8: 30 + 60 + 90. Vertical bars represent ± standard error.

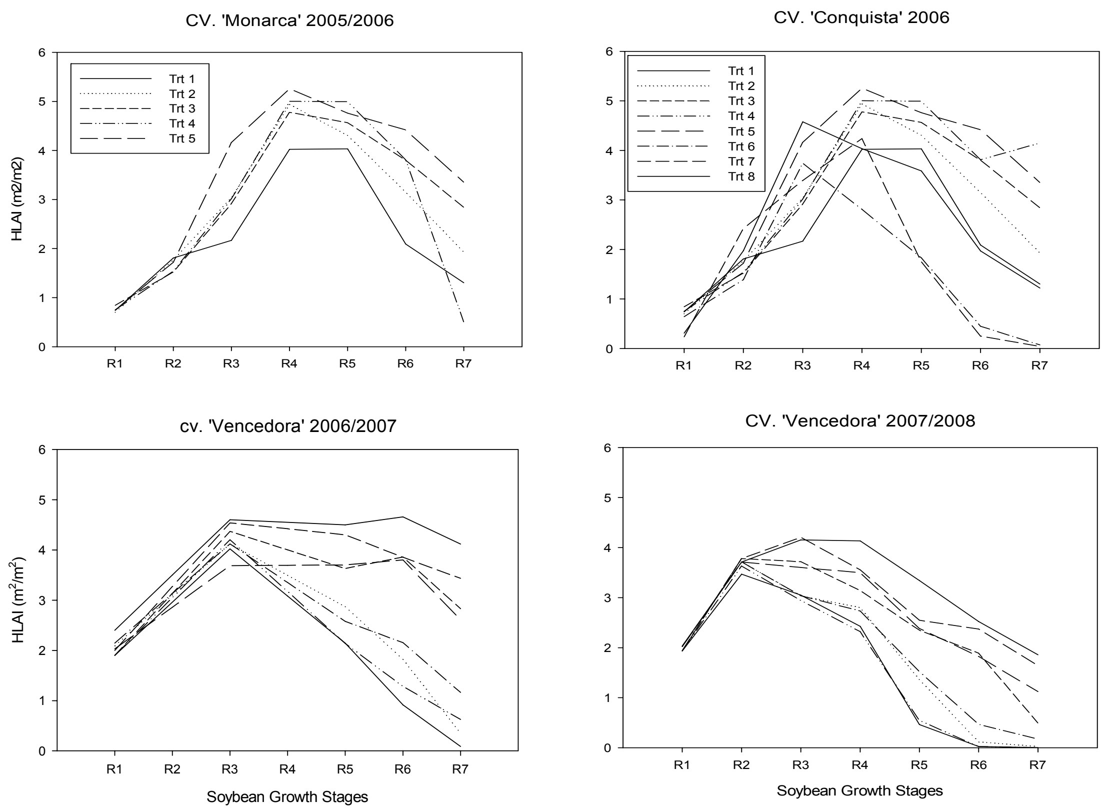

Figure 2 – Healthy Leaf Area Index (HLAI) in different soybean growth stages, for all treatments, in three different soybean cultivars between 2005 and 2008. Viçosa, MG, Brazil. Treatments were obtained with fungicide tebuconazole: ‘Monarca’ = 1: no fungicide spray, the others, three sprays with different concentrations: 2: 0.125 l/ha, 3: 0.25 l/ha; 4: 0.375 l/ha and 5: 0.5 l/ha; to ‘Conquista’ and ‘Vencedora’ = 1: no spray; 2: 0.5 l/ha, in R1; 3: 0.5 l/ha, in R3; 4: 0.5 l/ha, in R5; 5: 0.5 l/ha, two sprays, R1and R3; 6: 0.5 l/ha, two sprays, R1 and R5; 7: 0.5 l/ha, two sprays, R3 and R5; and 0.5 l/ha, three sprays: R1, R3 and R5.

4.4. Yield and yield loss

The maximum winter yield was lower than those obtained in summer, as it is typical for this crop in Brazil, and the yield in all experiments was further lowered by rust epidemics, increasing yield loss (Figure 3).

In each season, the yield increased from the most diseased and no sprayed plots to the less infected and three fungicide sprayed plots, the opposite occurred to yield loss. The maximum values of Yield loss was 58% in the first experiment (‘Monarca’), 53% in the second (‘Conquista’), 58% in the third (‘Vencedora’) and 71% in the fourth (‘Vencedora’).

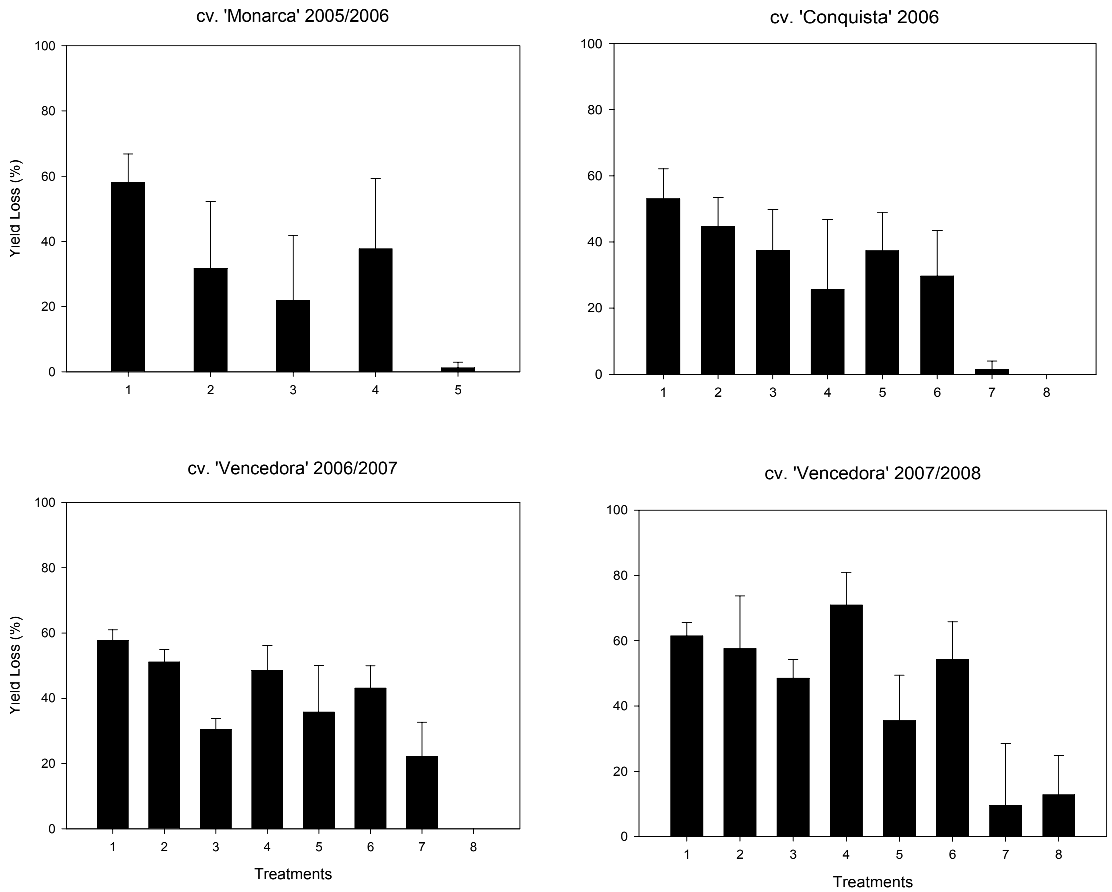

Figure 3 – Yield loss (percentage reduction of grams per plant from treatment 8 - three fungicide sprays in relation to other treatments) in soybean, caused by *Phakopsora pachyrhizi* in three different soybean cultivars in consecutive years. Viçosa, MG, Brazil. Treatments were obtained with fungicide tebuconazole: 'Monarca' = 1: no fungicide spray; 2: 0.125 l/ha; 3: 0.25 l/ha; 4: 0.375 l/ha and 5: 0.5 l/ha; 'Conquista' and 'Vencedora' = 1: no spray; 2: 0.5 l/ha, in R1; 3: 0.5 l/ha, in R3; 4: 0.5 l/ha, in R5; 5: 0.5 l/ha, two sprays, R1and R3; 6: 0.5 l/ha, two sprays, R1 and R5; 7: 0.5 l/ha, two sprays, R3 and R5; and 0.5 l/ha, three sprays: R1, R3 and R5. Vertical bars represent ± standard error.

4.5. Integral variables related to yield

4.5.1. Area Under Disease Progress Curve (AUDPC)

The relation between AUDPC and yield was found in all growing seasons; it was negative and linear, but the slopes were different among growing seasons, cultivars (Table 2) and growth stages (Figure 4).

Table 2 – Parameters of regression models (intercept and slope) , probability and coefficient of determination (R^2) between soybean yield (g/m^{-2}) and Area Under Disease Progress Curve (AUDPC), Leaf Area Index (LAI – m^{-2}/m^{-2}), Healthy Leaf Area Index (HLAI - m^{-2}/m^{-2}), Leaf Area Duration (LAD - days), Healthy Area Duration (HAD - days), Healthy Area Absorption (HAA – MJm^{-2}), Intercepted Radiation (IR - MJm^{-2}) and HRI (Radiation Intercepted by the Healthy leaf area - MJm^{-2}) for cultivars 'Monarca', 'Conquista' and 'Vencedora', in consecutive years (2005-2008). Viçosa, MG, Brazil. (n=2181)

Cultivar	Variable	Intercept	Slope	Std error	P-value	R^2
'Monarca'	AUDPC	1633.32	-110.84	0.08	0.0001	25.20
	LAI	1435.63	104.52	8.65	0.0001	43.20
	HLAI	1434.77	95.74	8.60	0.0001	46.60
	LAD	84.50	8.60	44.51	0.0057	45.70
	HAD	76.35	7.79	49.89	0.0132	49.20
	HAA	352.58	1573.00	49.51	0.0005	50.30
	RI	1415.50	27.95	3.40	0.0016	44.80
	HRI	1327.18	26.82	2.46	0.0001	49.00
'Conquista'	AUDPC	901.69	-58.98	0.37	0.0001	51.80
	LAI	795.78	80.98	7.67	0.0215	52.10
	HLAI	764.09	107.03	8.03	0.0001	52.20
	LAD	84.50	8.60	44.51	0.0057	47.50
	HAD	76.35	7.79	49.89	0.0132	47.60
	HAA	352.58	1573.00	49.51	0.0005	43.80
	RI	771.70	23.85	2.85	0.0203	50.40
	HRI	758.97	18.51	2.38	0.0004	50.50
'Vencedora'	AUDPC	747.13	-39.25	0.04	0.0001	22.30
	LAI	487.67	63.34	6.42	0.0001	46.70
	HLAI	494.67	64.03	8.18	0.0001	10.70
	LAD	84.50	8.60	43.94	0.0057	42.90
	HAD	76.35	7.79	49.89	0.0132	26.30
	HAA	352.58	1573.00	49.51	0.0005	51.80
	RI	423.44	17.21	1.89	0.0001	56.00
	HRI	535.01	10.08	1.43	0.0001	52.80

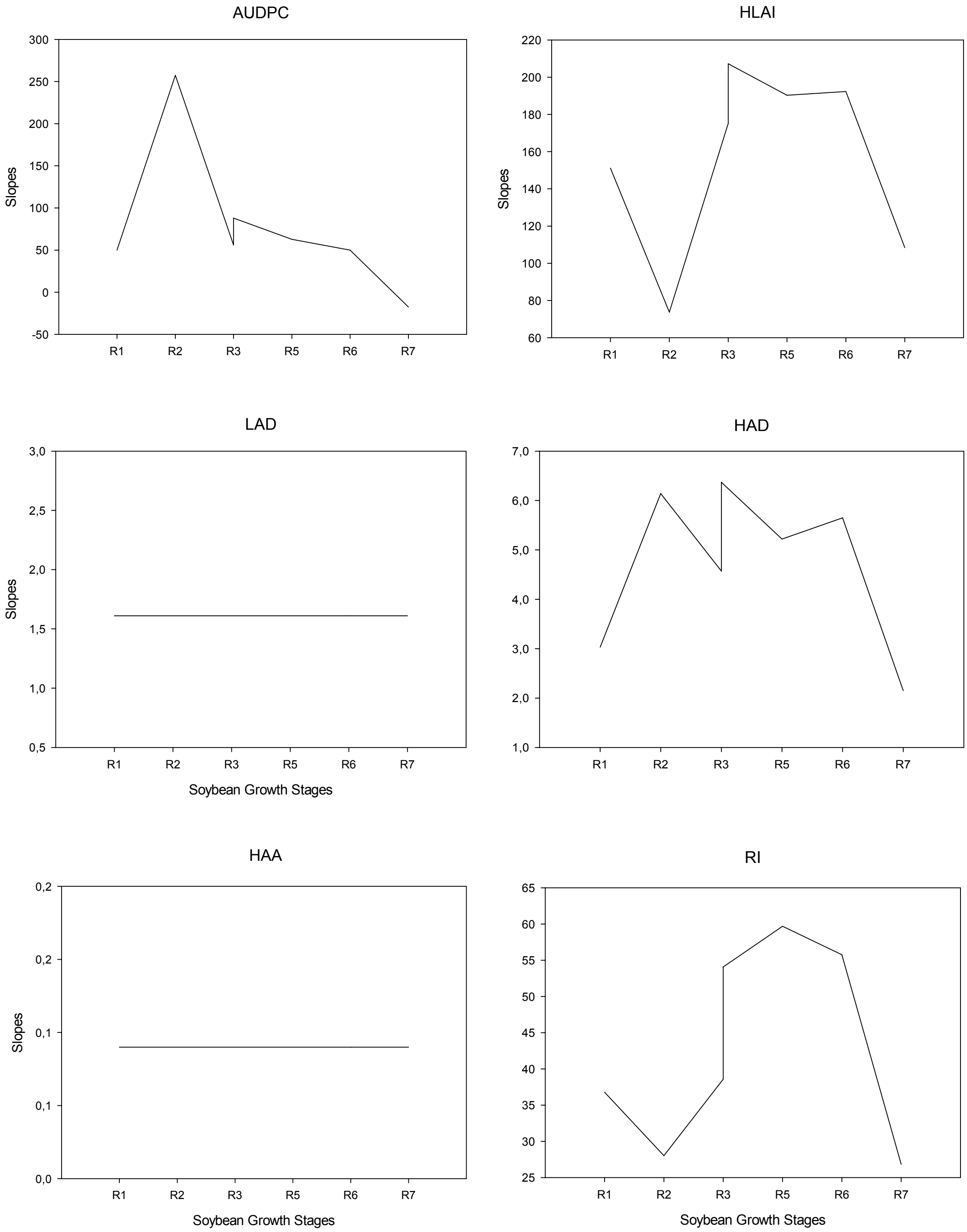

Figure 4 – Slopes of regression models (P<0.01) between soybean yield (g/m^{-2}) and Area Under Disease Progress Curve (AUDPC), Healthy Leaf Area Index (HLAI - m^{-2}/m^{-2}), Leaf Area Duration (LAD - days), Healthy Leaf Area Duration (HAD - days), Healthy Area Absorption (HAA – MJm^{-2}) and Radiation intercepted (RI - MJm^{-2}) for different reproductive stages in cultivars ‘Monarca’, ‘Conquista’ and ‘Vencedora’, in consecutive years (2005-2008). Viçosa, MG, Brazil. (n=2181).

4.5.2. Leaf Area Index (LAI) and Healthy Leaf Area Index (HLAI)

The maximum values for LAI and HLAI were 6.73 and 6.70 to ‘Monarca’, 7.03 and 6.92 to ‘Conquista’ and 7.30 and 7.06 to ‘Vencedora’. The relation between LAI and HLAI with disease severity was negative.

Thew Healthy Leaf Area was clearly different between the most and least diseased plants throughout growing seasons and cultivars (Figure 2). Summed to the smallest leaf area per plant in the unprotected plants (and those with just only one spray), LAI decreased even more because of the defoliation caused by the pathogen, especially in the third and fourth experiments where epidemic reached higher levels (Figure 1).

4.5.3. Leaf Area Duration (LAD) and Healthy leaf area Duration (HAD)

The relation between LAD and HAD with yield was positive and linear ($P<0.01$), indicating that ASR affects leaf retention over time.

Regression lines for all four experiments (growing seasons) and cultivars did not differ significantly for LAD and HAD (Table 2), and considering host growth stages, a linear model fitted different slopes (Figure 4).

4.5.4. Radiation Interception (RI) and Radiation Interception by the Healthy tissue (HRI)

RI and HRI were positive and linear ($P<0.01$) with severity when related to yield, which means that *P. pachyrhizi* has a depressing effect on radiation interception from the amount of tissue and the healthy parts of the tissues.

The regression lines for all four experiments (growing seasons) and cultivars as well as host growth stages, had the same result as the previous variables (Tables 2 and 3).

4.5.5. Healthy leaf Area Absorption (HAA) and Radiation Use Efficiency (RUE)

The relation between HAA and RUE with yield was positive and linear (P<0.01). The highest values of HAA were 1470, 1118 and 1573 MJ/m^2 to 'Monarca', 'Conquista' and 'Vencedora', respectively. The reduction in HAA was 15.81% in 'Monarca', 32.28% in 'Conquista' and 33.45% in 'Vencedora' and RUE dropped 52.94, 66.38 and 58.54% respectively (Table 3).

Table 3 – Radiation Use Efficiency (RUE – g/MJ) in control plots (no fungicide spray) and the treatment with 3 sprays, cultivars 'Monarca', 'Conquista' and 'Vencedora'. Viçosa, MG, Brazil

Cultivar / year	RUE (g/MJ)	
	Control ± StErr	Treat 8 ± StErr
'Monarca'	0.81 ± 0.06	1.69 ± 0.34
'Conquista'	0.78 ± 0.10	1.18 ± 0.12
'Vencedora'	0.57 ± 0.09	0.97 ± 0.05

When related to yield, HAA had the same result as LAD, which means, throughout the experiments, that regression lines were not significantly different (Table 2); the same occurred between the host growth stages (Figure 4).

5. DISCUSSION

All the four cultivars used in this work have a determinate growth habit and it was given special attention to the recommended sowing date, because of the isignificance of the day length and sun radiation for soybean. When a late cultivar is sown in the summer, for example, its cycle is shortened and it starts to bloom before the right time and yield reduction can be halved; in contrary, an early cultivar sown during winter does not have enough water and sun radiation as it is required for its development because of the dry season and shorter days, thus impairing growth and crop yield. Temperature, for example, is the second limiting environmental factor for soybean production in China, influencing its distribution, growth, yield and quality; the threshold temperature from 15 days before flowering is17°C and 15°C during seed filling (Liu *et al.*, 2008). In Brazil, although there is no severe (freeze) winters, delayed plant development was evident (table 1), from emergence to V3 in 'Conquista', associated with the lower temperatures that occurred in August.

The soybean maximum yield is largely governed by the optimum plant capacity to intercept solar radiation and accumulate dry mass during vegetative and reproductive stages; in addition, the other most important environmental factors influencing soybean growth and seed yield are temperature and water supply (Costa & Santos, 2000; Mathew *et al.* 2000; Heiffig *et al.* 2006; Liu *et al.* 2008). Thus, the smaller yield obtained by

‘Conquista’, when compared to the similar disease severity in ‘Vencedora’, was probably due to environmental conditions, because during winter, when ‘Conquista’ was sown, the lowest temperatures and solar radiation occurred, summed to the driest season, leading to plant water deficit. Winter season (2006 – cultivar ‘Conquista’) had effect not only on the yield, but on LAI, HLAI, HAD, RI, HRI and HAA. The maximum values obtained in this season were lower than in the other three trials. Jesus Junior *et al.* (2001) observed the same season effect on bean growth and yield. The authors highlighted that if yield varies in healthy plants according to seasons, a disease-crop-loss model has even more chances to fail.

In all trials, different disease and yield levels were successfully established, although in the first one, the ASR severity values were lower than in the others, including the second one that was conducted during the winter, when weather conditions – colder and dryer - were more adverse to *P. pachyrhizi* (Kawuki *et al.*, 2003) (Table 1). This mainly occurred because the soybean was grown for the first time in that experimental area and the region is not a soybean producer. As a consequence, the initial inoculum was probably low. These disease and yield levels were crucial in this work, because to study crop losses, yield and yield loss response to epidemic development must be characterized, which is made by obtaining data from a range of yield and disease values (Cooke, 2007). Also considering the first trial in contrast to the others, attempting to get better separation among treatments, in the other 3 experiments, treatments were changed from 5 to 8, which promoted more disease levels, but kept the same trend between the variables considered (Tables 2 and 3).

The yield loss was similar among the first three trials (Figures 1 and 2). Based on the fact that ‘Monarca’ presented a yield loss similar to the others, despite the lower disease levels, it can be assumed that this cultivar is more affected by ASR than ‘Conquista’ and ‘Vencedora’. In the fourth trial, although visual disease severity was similar to the second and third ones, defoliation was more severe and the yield loss was 71%, proving its importance in epidemics.

ASR progresses from the bottom to the upper part of plants and when host leaves are severely attacked. One of the main symptoms of the pathogen action is the premature defoliation (Synclair & Hartman, 1999). When disease is assessed, there is a need for accounting defoliation, otherwise visual disease severity would seem lower than it really is. In this case, disease values would be evaluated not only lower than they really are, but it is even possible that instead of increasing with epidemics progress, disease severity decreases (Waggoner & Berger, 1987).

In this work, a better adjustment was found between disease severity and yield when defoliation was considered, with higher values of R^2 ($P<0.01$) (data not shown). Certainly, this increase occurred, among other things, because it considered the lower light interception by plants, which is important for soybean as highlighted previously. The same results were found by Hartman & Sinclair (1996), Silva et al (1998) and Gianasi (2002).

Taking into consideration other soybean diseases, such as red leaf blotch, defoliation is an important component of disease syndrome as well as for ASR and its quantification is critical to obtain more reliable methods to assess disease and, in a further understanding, the relationship of disease severity parameters to yield components (Hartman & Sinclair, 1995).

Regardless of its importance for some diseases, defoliation can be hard to measure, especially because of the indeterminate habit of host growth, but it is believed that if it were quantified, the relation between disease and yield would be found (Bergamin Filho *et al.*, 1997; Jesus Junior *et al.* 2001; Bassanezi *et al.* 2001).

Bearing in mind that defoliation includes a lot of extra work and that it is hard to be measured, it is not always important to be accounted for, as in the case of diseases in which it is not a component of the epidemics. Ferrandino & Elmer (1992) did not find significant reduction in tomato yield caused by defoliation by Septoria leaf spot, unless until 75% of the leaves were removed.

In this work, it was found defoliation of around 61%, caused by ASR ($P<0.01$). Unlike other diseases caused by the fungi *Septoria glycines* and bacteria *Pseudomonas glycinea,* only 12% was reported to defoliation (Williams & Nyvall, 1980). Figure 1 presents the difference between the visual disease severity and disease severity corrected by considering defoliation.

Plants in plots which are unprotected or with only one spray reached physiological maturity about 15 days before the protected plants. This is explained by the decrease in host growth and lower LAI, once that dry matter is a LAI function (Watson 1947; Setiyono *et al.* 2007) and with smaller leaf area in size and number, the capacity to produce assimilates is compromised. Considering that pod formation occurs on branches in soybean and seed filling depends specially on the close leaves to them (Ogle at al., 1979; Larcher, 2004), with decreased assimilate production, this is another way that rust leads to yield loss. These effects are in agreement with those described by Yang *et al.* (1991) in ASR-soybean and by Gaunt (1987), in general pathosystems.

Still, defoliation explains the decrease in LAI, HLAI, LAD and HAD values, especially in the later growth stages, when disease severity was higher. As Watson (1947) showed in different crops, yield is largely related to leaf area (LAI) and its duration (LAD), because it is in the leaves where photosynthesis occurs. When hosts drop their leaves because of the action of pathogens, it is not only smaller LAI and LAD, but RI too, due to smaller remain leaf area. Considering that the ASR symptoms are not only defoliation, but include leaf spots, clorosis, necrosis and shriveling. Variables considering disease severity, such as HLAI, HAD and HRI, also have their values reduced (Waggoner & Berger, 1987), similarly to HAA and RUE (Johnson, 1987). Because of the better adjustment reached in this work, from now on, only the data accounting defoliation is going to be considered.

In this work, considering that the soybean cultivars presented a determinate growth habit, it was found a linear, negative and significant relation between AUDPC and yield, as reported by previous studies in the pathosystem rust - soybean (Yang *et al.* 1991; Koga *et al.*, 2007). It occurred in all experiments, either for cultivars or growth stages (Tables 2 and 3), but values of determination coefficient varied and were low in three out of four experiments. These results are in accordance with those found by Koga et al (2007), that had R^2= 25.4% and Yang *et al.* (1991), who obtained R^2 ranging from 13 to 71% (mean = 44.7%). Thus, despite the same relation was found in Brazil and China for the same pathosystem, the low and varying values of R^2, summed to slopes that did not stabilize in any cultivars or growth stages

(Tables 2 and 3), led to the conclusion that AUDPC is not an appropriated variable to be used to estimate crop loss for ASR.

In opposition to the results presented, this relation between different AUDPC diseases and yield was not found in many works, including those with beans, when defoliation was not considered (Bergamin Filho *et al.*, 1997; Silva *et al.*, 1998; Jesus Junior *et al.* 2001; Bassanezi *et al.*, 2001; Díaz *et al.*, 2001; Jesus Junior *et al.*, 2003), potato (Rotem *et al.*, 1983), wheat (McKirdy *et al.*, 2002) and *Populus* spp. (May-De Mio *et al.* 2006). Gianasi (2002), working with beans, but accounting defoliation in his evaluations, was able to achieve a 17 - 60% relation between these two variables. In crops where there is a determinate leaf number, the relation between disease and yield has been successfully demonstrated, especially in cereals (Amorim *et al.*, 1995). For tomato (Ferrandino & Elmer, 1992), sunflower (Leite *et al.*, 2006) and soybeans (Lim, 1980; Hartman & Sinclair, 1996; Godoy & Canteri, 2004), including ASR (Yang *et al.* 1991; Koga *et al.*, 2007), a negative and significant relation between AUDPC and yield was found.

The regression analysis was significant for LAI and for its disease-associated counterpart (HLAI), which means that ASR affected leaf area, especially due to defoliation, which has already been discussed. In addition, LAI reduction occurred by other means, by the pathogen causing shriveling and by the host producing less leaf area (data not shown), which was also described for bean rust (Mersha & Hau, 2008). Liu *et al.* (2008) reported that other authors in China found a positive correlation of LAI and grain yield and that the maximum LAI values depend on the cultivar, light intensity, leaf shape, leaf angle and other factors.

The relation between LAI and HLAI was linear, negative and significant ($P<0.01$) (Figure 3) to disease severity, but slopes did not stabilized, meaning that their behavior was different in all trials for cultivars and growth stages. Both LAI and HLAI had better values of R^2 than AUDPC when used to explain yield loss, but even with this improvement in values, due to the variation in slopes among cultivars and growth stages, this variable may not be recommended to quantify and predict yield.

In relation to leaf persistence, it was found a significant and positive relation with yield (Tables 2 and 3) to LAD and HAD, and the same slope among cultivars to HAD (Table 2). This result of the same trend obtained across cultivars is in disagreement with Aquino *et al.* (1992), which informed that the relation between yield and HAD is a characteristic of cultivars. In beans, peculiarities such as pod formation in the base of the leaves, and the indeterminate habit of growth help to elucidate this linearity (Gianasi, 2002). Soybeans have the same pod formation described in beans, and this can be the reason for the same linear relation found in our data analysis. Slopes between HAD and yield stabilized only among cultivars, but not growth stages (Tables 2 and 3) and between LAD and yield stabilized in different cultivars and growth stages, demonstrating to be a good variable to be used in damage quantification models for this pathosystem.

The linear relation between HAD and yield have been found in other works with sunflower (Leite *et al.*, 2006), tomato (Ferrandino & Elmer, 1992), beans (Bassanezi *et al.*, 2001; Diaz *et al.*, 2001; Gianasi, 2002; Silva *et al.*, 1998; Bergamin Filho *et al.*, 1997), potatoes (Shah *et al.*, 2004), barley (Carver & Griffiths, 1981) and even in soybeans with ASR (Koga *et al.* 2007). This linear relation was different from that suggested by Waggoner & Berger (1987), to peanut-*Cercosporidium*.

Taking into consideration the radiation interception for ASR, neither RI nor HRI stabilized slopes among soybean cultivars or growth stages, but values of R^2 were around 50%, with better relation of HRI than RI with yield (Table 2). Leite *et al.* (2005) presented similar results, but with high coefficients of determination in HRI, although the slopes did not stabilize in any growth stage. The explanation was that HRI was influenced by the sowing date in two seasons, which led them to consider this variable inadequate for alternaria leaf spot management. The same decision was taken in relation to this variable in this work. In beans infected with *Colletotrichum*, there was a tendency of the slopes to stabilize only between R5 and R8 (Gianasi, 2002).

Shah *et al.* (2004) highlighted the fact that RI needs to be corrected when measured by canopy analyzers, because the necrotic parts of the leaves also intercept radiation, but it is not going to be used by the plant and, according to them, this is the reason why a correction is necessary to avoid overestimation in the further calculus in the other variables. In soybean, light intercepted during and after seed initiation is a major determinant of yield (Liu *et al.* 2008).

The relation between HAA and yield in this research was significant, linear and positive, which agrees with Koga *et al.* (2007). The difference was that they could not find better relation to HAA than in HAD, as observed in this work, and as suggested by other authors (Gianasi, 2002; Bryson *et al.*, 1997; Bergamin Filho *et al.* 1997; Waggoner & Berger, 1987). During the experiments, cultivars and growth stages, HAA was the variable associated to the host that presented the best relation with yield. Even considering the lower value of R^2 obtained for 'Vencedora' and higher values for R^2 in 'Monarca' and 'Conquista', different cultivars, seasons and growth stages were characterized by the same slopes in all cultivars (Tables 2 and 3); the same result was obtained in different seasons and locations in beans (Bergamin Filho *et al.*, 1997), using HAA.

Monteith (1977) and Waggoner & Berger (1997) describe the relation between HAA and yield as linear, but according to Bergamin Filho *et al.* (1997), the curve depends on HLAI and k value, which in turn depends on the crop. For example, Silva *et al.* (1998) achieved the best adjustment in beans with an exponential curve, although other authors found it as a linear relation in the same cultivars (Gianasi, 2002; Bergamin Filho *et al.*, 1997).

To evaluate radiation use by host under ASR interference, RUE values were obtained; they ranged from 0.51 g/MJ to 1.69 g/MJ, and were lower for the most diseased and most healthy plants, respectively (Table 3). The average RUE values acquired in the less diseased plants are in accordance with those between 0.50 to 1.20 g/MJ (and can reach until 1.80 g/MJ) and for the control plots, with no treatment, of 0.41 g/MJ (Casaroli *et al.*, 2007; Koga et al., 2007). Considering that defoliation was computed by counting the scars on the stems, it was unable, in this work, to accurately quantify the RUE value for all plants. An estimate (Table 3) was based on previous results of Bergamin Filho *et al.* (1997), where they had the same problem and based their estimative of RUE values using the yields obtained and the respective HAA values. The reduction in RUE values achieved in this work were 52, 34 and 40%, pointing out that *Phakopsora pachyrhizi* affects the photosynthetic efficiency of soybean plants, as showed by Johnson in other pathosystems (1987).

The results presented in this work lead to conclude that *P. pachyrhizi* affects leaf functioning (acting as a sink and by yellowing and shriveling) and persistence (by defoliation), thus affecting the radiation interception, absorption and use. This work data are in accordance with those obtained by Kumudini *et al*. (2008) in the same pathosystem, for the common variables studied. In barley, rust shifts the limited sink (carbohydrate accumulation sites) to limited source (carbohydrate production limitation) (Lim & Gaunt, 1986) and the same probably occurs in soybean with rust.

Despite a relation between disease severity (AUDPC) with yield was found, the results of the four trials demonstrated that the variables LAD, HAD and HAA, especially the last one, showed better association with yield losses. This is in accordance with Aquino *et al.* (1992), Ferrandino & Elmer (1992), Hartman &Sinclair (1996), Gianasi (2002) and Koga *et al.* (2007), who found a significant relation with yield and AUDPC, but HAD and HAA presented better results. Therefore, the results obtained in this research clearly show the importance of considering not only the disease, but also the remarkable role of host physiology in a model to quantify yield loss. Using variables as LAD, HAD and HAA, data obtained from different regions, cultivars and seasons generate more consistent data that can be applied even in other countries, which make them more useful.

The use of a main variable that is stable and transportable in a system of disease management is crucial. Lim & Gaunt (1981) mentioned that yield loss models need to use physiological analyses where the emphasis is placed on the host rather than pathogen characteristic. In this context, these results with HAA in different cultivars, growing seasons and growth stages, showed promising results to be used in the establishment of a threshold for ASR.

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IMPACT OF ASIAN SOYBEAN RUST ON SOYBEAN PHYSIOLOGY: EFFECTS ON PHOTOSYNTHESIS AND YIELD

1. ABSTRACT

The relationship between photosynthetic rate of soybean leaves and severity of Asian Soybean Rust (ASR) and their relationship with yield were investigated by gas-exchange analysis in three field experiments with soybean cultivars Monarca, Vencedora and Conquista. The relationship between visual and virtual lesion was used to quantify the reduction in photosynthetic efficiency of the green leaf tissue surrounding lesions. The β value was used to recalculate values previously obtained for area under visual and virtual disease progress curve (AUDPC), healthy leaf area duration (HAD), effective leaf area duration (ELAD), healthy leaf area absorption (HAA) and effective leaf area absorption (ELAA). In order to obtain a wide range of disease severity, treatments were applied using the fungicide tebuconazole at growth stages R1, R3, and R5 in different spray combinations. β value obtained (0.99, R^2=95%) was close to one, indicating that visual and virtual severity were identical and the effect of *Phakopsora pachyrhizi* on the green leaf area was nominal. As a result, variables that considered effective leaf area did not present improvement in the values obtained for AUDPC, HAD and HAA.

2. INTRODUCTION

Among the diseases affecting soybeans worldwide, Asian Soybean Rust (ASR) is known to cause great yield losses. Symptoms initially appear on the lower leaves and progress upward in the canopy (Sinclair & Hartman, 1999). The effect on yield varies, depending on the disease onset and severity relative to the host growth stage and can reduce yield as much as 80% (Ogle et al., 1979; Hartman et al., 1991). When ASR occurs early during reproductive growth, it causes intense defoliation that cannot be overcome by the plant (Yorinory & Paiva, 2002).

Pathogen effects can be grouped into two main categories, those that affect radiation interception (RI) and those that reduce radiation use efficiency (RUE) (Johnson, 1987). ASR effects were attributed to reduction in leaf area and, consequently, RI and RUE, as demonstrated by Kumudini *et al.* (2008).

Many attempts to find useful predictors to relate crop loss and disease have failed (Waggoner & Berger, 1987). This includes the use of Area Under Disease Progress Curve (AUDPC), which is the most widely used variable, although in many pathosystems, its relationship with yield is weak (Jesus Junior et *al.*, 2001).

In an attempt to find better relationships with yield, it was proposed the use of other variables that consider attributes related to the host (Waggoner & Berger, 1987). Watson (1947) have already been shown that

Leaf Area Index (LAI) was related to yield and that not only leaf size and quantity were important, but leaf persistence was also, leading to the concept of Leaf Area Duration (LAD).

Waggoner & Berger (1987), based on Watson`s work (1947) recommended the use of two new variables, the Healthy Leaf Area Duration (HAD), as that is the same as LAD, but considers the persistence of healthy foliage, and the Leaf Area Absorption (HAA), which represents the quantity of radiation intercepted by leaves green portion.

Waggoner & Berger (1987) findings had a huge repercussion on the scientific community because the healthy parts on the crops are better associated with yield than disease. The use of HAD and HAA had better results than AUDPC, even when the relationship between the latter variable and yield was found (Jesus Junior *et al.*, 2001; Diaz *et al*., 2001; Bassanezi *et al*., 2001; Jesus Junior *et al*., 2003; Shah *et al*., 2004; May-De-Mio *et al*.; 2006; Bergamin Filho *et al*., 1997; Carneiro *et al*., 1997).

Nevertheless, the relationship between HAD and HAA was not always as strong as it was expected to be, because the effect of disease on the healthy parts still were not considered. Johnson (1987) argued that not only RI contributes to biomass production, RUE should also be considered. In this case, the plant capacity to photosynthesize is reduced when compared to healthy individuals.

Pathogens can reduce not only the photosynthetic efficiency of diseased areas, but also symptomless areas on diseased plants can be affected in a region larger than is visible. Bastiaans (1991) proposed a model to quantify pathogens effect on apparently healthy green tissue. The virtual lesion concept is that a pathogen can affect photosynthesis on remaining green leaf tissue, and the relation is expressed by a "β" value. When β equals 1, both the real affected area and the area visible diseased are the same. This means that the effect on green tissue photosynthesis is small; values higher than 1 indicate that virtual lesion is bigger than the diseased and the higher the value, the larger is the area compromised.

Based on virtual lesion, the integral variables HAD and HAA can be corrected to account for the invisible effect of disease. The concepts of effective leaf area duration (ELAD) and effective leaf area absorption (ELAA) may be useful (Jesus Junior et al., 2003) in trials to improve relationships between disease and yield loss in pathosystems where virtual lesion has a large effect.

This work aimed to analyze the effect of ASR on soybean physiology through the relationship between disease severity and yield focusing on the photosynthetic efficiency of soybean; and to test the usefulness of the virtual lesion concept for this pathosystem.

3. MATERIAL AND METHODS

3.1. Field experiments

Field experiments were conducted on a Cambic Podzol, in Viçosa (20°45'S, 42°15'W, 650 m a.s.l.), southeastern Brazil. Three independent trials were conducted in the rainy and dry seasons: from November 2005 to March 2006 (rainy), October 2007 to January 2008 (rainy) and April to August 2008 (dry) with soybean cv. ‘Monarca’ in the first experiment, cv. ‘Vencedora’ in the second and cv. ‘Conquista’ for third. All cultivars are susceptible to rust and present determinate growth habit. The soil was prepared following traditional practices, with dressing based on chemical soil analysis. Before sowing, seeds were treated with fungicide (Thiram) and inoculated with *Bradyrhizobium japonicum*.

All trials were in a randomized complete block design with five treatments and five replications in the first experiment, and eight treatments and three replications in the other two experiments. Each plot (14 m^2) consisted of 4-m-long rows, spaced 0.5 m apart. There was 1.5 m between adjacent plots. Eighteen seeds were sown, and 15 plants were allowed to grow per linear meter of row. The plots were maintained with conventional cultural practices used in commercial fields, including, topdressing with fertilizer, insecticide sprays, weeding, and irrigation.

Plants were naturally infected by *P. pachyrhizi* and irrigated to enhance infection. In order to obtain a range of disease levels, plants in different plots were sprayed with a fungicide (Tebuconazole) with a manual applicator until the running point, at different stages of plant growth (before, during and after flowering). The treatments were, for the first experiment: (1) no spray = control; (2) 0.125 l/ha, (3) 0.25 l/ha, (4) 0.375 l/ha and (5) 0.5 l/ha; for experiments 2 to 4: (1) spray = control; (2) one spray in R1; (3) one spray in R3; (4) one spray in R5; (5) two sprays, in R1 and R3; (6) two sprays, one in R1 and other in R5; (7) two sprays, one in R3 and other in R5; and (8) three sprays: R1, R3 and R5.

3.2. Gas exchange measurements

The net photosynthetic rate, stomatal conductance to water vapor (g_s), and internal-to-ambient CO_2 concentration ratio (C_i/C_a) were measured on cloudless days using a portable open-flow gas exchange system (LI-6400, LiCor Biosciences, Lincoln, NE, USA). All measurements were taken between 08 A.M. and 12 P.M. (rainy, warm season) or between 10 A.M. and 13 P.M. (dry, cool season). Measurements were conducted under ambient CO_2 (~370 μmol mol^{-1}), 900 μmol m^{-2} s^{-1} photosyntheticaly active radiation (saturating light conditions for field-grown soybean) and 20-25 ^{0}C leaf temperature. A 6 cm^2 leaf area, from the middle leaflet of the eighth leaves, was taken for measurements, avoiding the main vain and necrotic areas.

Plants were chosen randomly in each plot, considering plants and leaves which received similar sun radiation, because shaded plants present normally smaller photosynthetic rate. Each time, one leaf in each plot was evaluated for photosynthesis and the disease severity in that same area was recorded using a diagrammatic scale (Godoy et al., 2006). The assessments were made from vegetative stage until the plants in the control plots (most severe disease) were completely defoliated and could no longer be used for comparative purposes with the other treatments.

3.3. Crop growth, disease severity, defoliation and yield

Every measurement was taken in three plants in each plot, performing a total of 75 plants in each of the first trial and 96 plants in the other trials. Leaf area, used as an estimate of crop growth, was measured on all leaves of the harvested plants with a Leaf Area Meter (LI-3100C, LiCor Biosciences, Lincoln, NE, USA). Plants were weekly harvested at random.

Disease severity was accessed using a diagrammatic scale (Godoy et al., 2006) on every leaf of three plants sampled weekly (n=96), starting with the appearance of the first disease symptoms. These total readings were divided by the total number of leaves to give an average rust area for each plant (in percentage).

Defoliation was determined in each evaluation. The values were obtained by counting the marks on the stem left by the fallen leaves in relation to the total number of leaves. Defoliation data were used to correct disease severity values according to Aquino et al (1992):

$$y_t = [(1 - d) y_v] + d$$

in which y_t represents total disease severity, y_v is the proportion of visible disease, and d is the proportion of defoliation.

Yield (Y) was determined at harvest maturity (growth stage R8). Ten relatively uniform plants were removed randomly from the five center rows in each plot to determinate the yield components. The yield components considered were, per plant (directly counted): the number of filled and unfilled (shriveled) pods per plant, grains per pod, 100-seed weight, total seed weight (gram per plant and per plot), and total yield (gram per meter square). The weight was always determined after drying the seeds to correct moisture to 12%.

3.4. Integral variables

The area under disease progress curve (AUDPC) was calculated by trapezoidal integration (Shaner and Finney, 1977). Healthy Leaf Area

Duration (HAD, in days) and Healthy leaf Area Absorption (HAA, in MJ m^{-2}) were calculated for each plant according to Waggoner and Berger (1997).

Beta (β) value estimation

Values of disease severity obtained in each leaf were related to the net photosynthesis rate according to Bastiaans (1991):

$$P_x/P_0 = (1-x)^{\beta}$$

in which: P_x = photosynthesis of a leaf with disease severity x, P_0 = photosynthesis of a healthy leaf and x = disease severity.

3.5. Laboratory work

In addition to field work, chlorophyll content and electrolyte leakage were determined in leaves in all 32 plots with different disease severity levels. Chlorophyll content was determined according to Lichtenthaler (1987) and electrolyte leakage was estimated using a conductivity meter according to Lima *et al.* (2002).

3.6. Use of β in estimates of AUDPC, HAD and HAA

Virtual disease severity ($y = 1 – (1- x)^{\beta}$; Bassanezi et al., 2001) was used to correct the integral variables. In this equation, y is the proportion of the leaf area with virtual lesion and x is the proportion of leaf area with visual lesion.

Values obtained for virtual disease and β were used to correct the integral variables substituting *x* by *y* in the original equations. Then, AUDPC = AUDPC*v*; HAD (Healthy Area Duration) = ELAD (Effective Leaf Area Duration) and HAA (Healthy Area Absorption) = ELAA (Effective Leaf Area Absorption).

Calculations were made according to Jesus Junior *et al.* (2003)

$$ELAD = \sum_{i=1}^{n-1} \left(\frac{LAI_i\,(1 - Y_i) + LAI_{i+1}\,(1 - Y_{i+1})}{2} \right) (t_{i+1} - t_i)$$

in which: LAI= leaf area index and t = time (in days).

$$ELAA = \left(\frac{RI_i + RI_{i+1}}{2} \right) (t_{i+1} - t_i)$$

in which: RI= radiation intercepted (MJm^{-2})and t = time (in days).

3.7. Data analysis

Calculations of the integral variables were made using the Excel software (Microsoft Office, 2007). All gas-exchange data were analyzed by covariance using SAS system (SAS Institute, Cary, NC) to determine if they could be analyzed combined. The β value was estimated using non-linear regression analysis. All regression analyses were performed using the Statistica software version 7.0 (Statsoft, Tulsa, USA).

4. RESULTS

The successful fungicide spraying produced different disease levels of ASR in all experiments, irrespective of the experiment and cultivar ($P<0.01$). Visual disease severity and yield loss, as well as defoliation increased faster in control plots (unprotected) than in the other treatments. Plants located in control plots reached stages R7 and R8 about 10 -15 days earlier, compared to the protected individuals in all experiments, regardless of the cultivar.

The maximum disease severities were 54% in the cv. ‘Monarca’ (2005/2006 experiment, rainy season), 100% in ‘Vencedora’ (2007/2008 experiment, rainy season) and 100% in ‘Conquista’ (2008 experiment, dry season). During the first experiment, despite the optimal conditions for *P. pachyrhizi*, the maximum disease severity was lower, compared to the other trials, probably due to the low inoculum in the field. It should be mentioned that an ASR experiment was conducted for the first time in the experimental area. This can be noticed while disease levels in the first experiment in summer are contrasted to the third one, conducted during winter, when weather conditions were colder and dryer.

Regardless of the growing seasons, AUDPC correlated negatively with yield ($P<0.01$), with different slopes among cultivars and growth stages. The maximum values for Leaf Area Index (LAI) were 6.73 for ‘Monarca’, 7.30 for ‘Vencedora’ and 7.01 for ‘Conquista’. LAI values decreased due to the pathogen effect on the host growth and premature defoliation, especially in the second and third experiments, in which the epidemics was more severe. The relations between HAD and yield and HAA and RUE with yield were positive and linear (P<0.01), indicating that ASR affects not only the radiation absorption due to defoliation, but also remarkably compromises radiation use by healthy green tissue. The reduction in HAA was 15.8% in 'Monarca', 32.3% in 'Conquista' and 33.5% in 'Vencedora'; the reductions in RUE were 52.9%, 66.4% and 58.5%, respectively, for the three cultivars.

The total chlorophyll content decreased markedly at the beginning of the disease progress, until about 15%, showing an exponential curve (R^2=0.55) and, then, reducing more slowly (Figure 1). The electrolyte leakage (EL) was significantly different when control and treated plots are compared, but only after disease severity was higher than 15% in the control than in the other treatments; the estimated equation was EL = 23.046 + (1,491 * Disease Severity), with R^2=0.32, P<0.001 (n=52). Stomatal conductance and the Ci/Ca ratio were negatively and significantly (P<0.02) related to the disease severity increase (Table 1), as well as photosynthetic rates (Table 2).

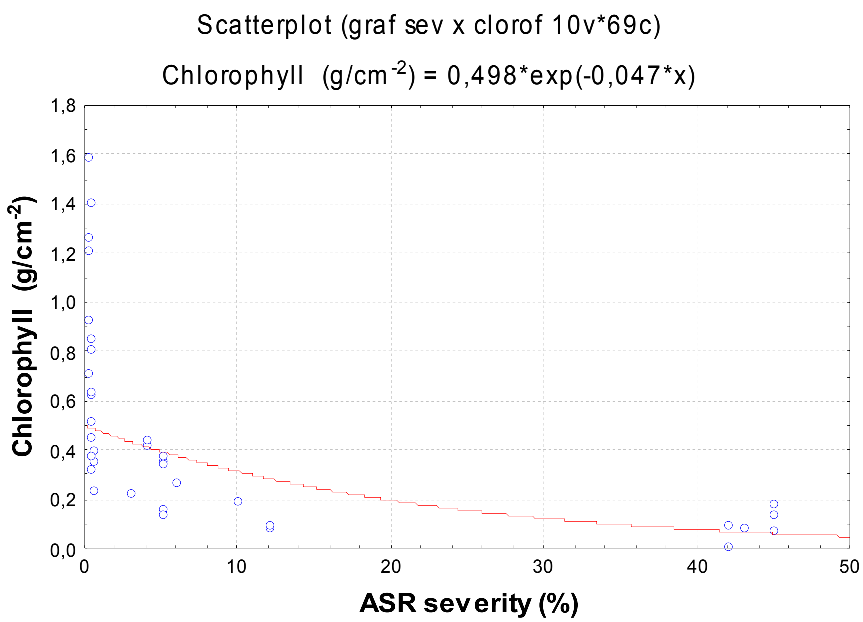

Figure 1 – Total chlorophyll content (mg cm^{-2}) in soybean leaves infected by *Phakopsora pachyrhizi* regressed against disease severity. Viçosa, MG, Brazil.

Table 1 – Pearson`s correlation analysis between Asian Soybean Rust (ASR) (*Phakopsora pachyrhizi* Sidow & Sidow), stomatal conductance (mol m^{-2} s^{-1}) and internal-to-ambient CO_2 concentration ratio (C_i/C_a) correlation in cultivars 'Monarca', 'Vencedora' and 'Conquista'. *n= 295*. Viçosa, MG, Brazil. r= coefficient of correlation, R^2=coefficient of determination, SE= Standard Error.

Variable	r value	Intercept	Slope	R^2 value	Probability	SE
Conductance	-0.14	0.918	0.004	0.18	0.02	0.81
Ci/Ca	-0.44	0.833	0.001	0.20	<0.001	0.09

Table 2 – Net photosynthetic rate (± SE; n = 344) in soybean (*Glycine max* Merrill) leaves with different severities of Asian Soybean Rust (ASR) (*Phakopsora pachyrhizi* Sidow & Sidow) in cultivars Monarca, Vencedora and Conquista. Viçosa, MG, Brazil. Sever= severity (%), N°= number of sampled leaves in which measurements were taken, P_N= photosynthetic rate (µmol CO^2 $m^{-2}s^{-1}$).

ASR sever.	*Exp 1 – cv. Monarca*		*Exp 2 – cv. Vencedora*		*Exp 3 – cv. Conquista*	
	N°	P_N	N°	P_N	N°	P_N
0	15	20.0 ± 1.5	11	26.5 ± 1.3	6	28.9 ± 1.8
0.1-1.0	53	18.2 ± 1.4	5	25.8 ± 0.2	3	26.9 ± 0.3
1.1-5.0	3	16.0 ± 1.1	10	23.4 ± 0.9	14	23.8 ± 1.0
5.1 – 10	3	13.8 ± 0.7	9	22.1 ± 3.2	0	-
10.1-20	10	11.6 ± 1.8	10	20.8 ± 1.7	4	20.4 ± 1.0
20.1-30	7	13.7 ± 3.0	9	19.4 ± 1.8	0	-
30.1-40	3	11.7 ± 2.2	10	15.6 ± 1.9	2	14.8 ± 1.2
40.1-50	3	9.7 ± 1.1	10	16.4 ± 3.6	3	11.9 ± 1.5
50.1-60	9	9.0 ± 1.5	10	15.6 ± 3.1	0	-
60.1-70	6	7.6 ± 0.6	18	10.6 ± 1.7	4	11.3 ± 0.3
70.1-80	0	-	6	7.8 ± 0.5	8	8.9 ± 0.8
>80.1	16	4.7 ± 1.9	24	4.6 ± 2.2	42	2.8 ± 0.3

Average net photosynthetic rates in the healthy leaves were 20 µmol m^{-2} s^{-1} in 'Monarca', 26 µmol m^{-2} s^{-1} in 'Vencedora' and 28.9 µmol m^{-2} s^{-1} in 'Conquista'. These rates decreased just after infection was detected, even before ASR reached 5% severity (Table 2). In all experiments, photosynthetic rates were reduced to about 4.0 µmol m^{-2} s^{-1} when disease severity reached 80% (Table 2) and approached zero (0.6-2.0 µmol m^{-2} s^{-1}), with more than 90% disease severity (data not shown). Disease-related decreases in net photosynthetic rates were 76% in 'Monarca', 82% in 'Vencedora' and 90% in 'Conquista' in the most diseased plants.

Considering all experiments, the nonlinear model proposed by Bastiaans (1991) fitted well to the data, with coefficient of determination of 95%, and as the estimated β value of 0.991 (Figure 2). This value close to 1 indicates that the virtual and visual lesions have similar sizes for ASR, when all cultivars are considered together.

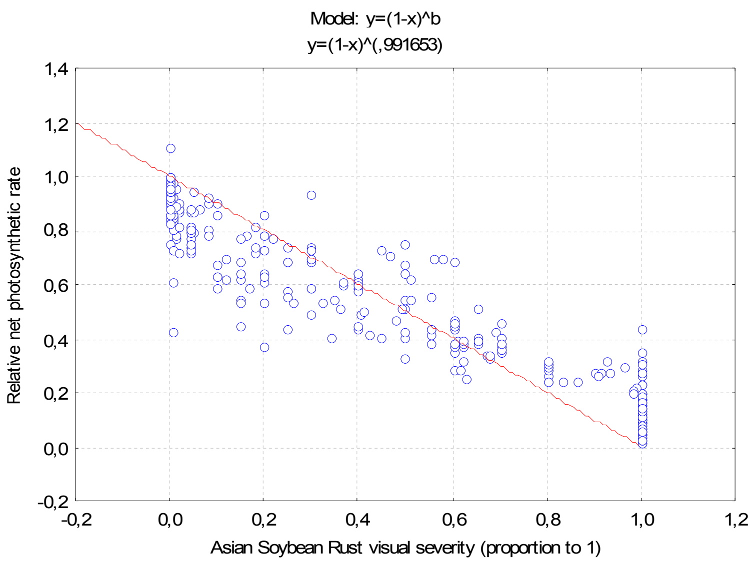

Figure 2 – Relative net photosynthetic rate of leaves of soybean infected by *Phakopsora pachyrhizi* related to disease severity (P<0.01, R^2= 0.9). Relative photosynthetic rate = photosynthesis rate on a diseased leaf divided by the healthy one. Viçosa, MG, Brazil.

5. DISCUSSION

In all experiments, different disease levels were successfully obtained, which is necessary to study a pathogen's effect on the leaves on its host (Bastiaans, 1991; Lopes and Berger, 2001). Bassanezi et al. (2001) explain that this allow the quantification of the impact of the disease on leaf physiology.

Leaf physiology is a complex system that involves many different processes. For example, the stress caused by a pathogen attack to tissues in the host leads to cell disturbances that provoke, among other things, electrolyte leakage; a disturbance that can be detected since the beginning of infection (Campos et al, 2003). Based on the trophic relation between rusts and their hosts, the result obtained in this study for electrolyte leakage measurements was expected. Because *P. pachyrhizi* is a biotrophic pathogen, it does not kill the infected cells until the disease severity is high, thereby avoiding the breakage of cell membranes.

The greater reduction in chlorophyll content in the beginning of the epidemics was expected too, considering that the first symptoms of ASR are a formation of little spots lighter than the leaf, that can be perceived putting it against the light. In bean rust, the chlorophyll content decreased latter on epidemics, only when disease severity is higher than 30% (Lopes and Berger, 2001). Kumudini *et al.* (2008), studying ASR impact on photosynthesis, found results similar toi those of this study, that the pathogen causes loss in chlorophyll content in leaves, which reduces the ability of soybean leaves to absorb solar energy and photosynthesize.

Pathogens affect more than the host capacity for RI. As it was proven in several studies with HAD and HAA, they could also affect the host capacity to use radiation, thus decreasing RUE (Johnson, 1987). In the previous chapter, it was found that ASR affects not only RI by early defoliation and shortened the leaf lifespan, but also affected RUE by decreasing healthy leaf area and the conversion of radiation into yield. Similar results were reported by Kumudini et al. (2008) in the same pathosystem.

Phakopsora pachyrhizi had the effect on host described by Bastiaans (1993), which provoked decreased photosynthesis rate and reduced leaf area formation, in addition to early defoliation, which doubly compromises the host, with lower formation of LAI and early loss of leaves and because of them, reduction on the radiation absorbed by this crop. Another work will be prepared to discuss the shift in the assimilate translocation in this pathosystem, which is probably the same that occurred in Livne and Daly's (1966) research. They used $^{14}CO_2$ and showed that bean rust was responsible for retaining assimilates of the source instead of reallocating them. They demonstrated that all sinks receive less intake than they would on a healthy plant, especially the young growing parts that grow less, which leads to smaller roots and leaf area produced and, therefore, lower LAI.

Researchers have described many kinds of effects of rust on their host physiology, including decreased photosynthetic rates, increased respiration, and altered assimilate translocation (Livne, 1963; Lopes & Berger, 2001). Another example of decreased photosynthetic rate is the leaf blast, which reduced carbohydrate production in rice. The pathogen's effect was on leaf photosynthesis and respiration, associated with reduction in crop growth rate and leaf area formation (Bastiaans, 1993).

Still considering the changes, under lower levels of infection, a compensatory effect on photosynthesis of diseased leaves can occur by the healthy ones in some crops, as in beans infected by rusts (Livne and Daly, 1966). The decrease found in the photosynthetic rates since the beginning of infection (less than 5% of severity) in all cultivars (Table 2) suggests no compensatory effect occurring in the pathosystem ASR-soybean.

The stomatal opening/closure, which can be assessed by stomatal conductance (g_s), largely governs the incoming CO_2 inside the mesophyll, and transpiration rate. The Ci/Ca ratio describes this gas diffusion and is related to mesophyll photosynthetic capacity (Lopes & Berger, 2001). The decrease in Ci/Ca ratio combined with stomatal closure reveals that once the stomata closes, the internal CO_2 is consumed in the chloroplasts. This can be interpreted as the main reason why photosynthesis rate starts to drop with the beginning of disease symptoms; once stomata closes, internal CO_2 is consumed and not enough of it enters to keep the regular process going on. As a result, the relation between gas exchange and disease severity found in the three experiments revealed that this pathogen significantly alters stomata functioning, which means that *P. pachyrhizi* somehow causes stomata to close. In opposition to these results, Kumudini *et al*. (2008) reported in the same pathosystem that this disease causes a weak reduction in leaf carbon exchange rate. Similar results to to those of this study were obtained for $g_{s,}$ but the opposite was found for Ci/Ca ratio in another pathosystem involving rust and *Uromyces appendiculatus* and *Phaseolus vulgaris* (Lopes & Berger, 2001).

Lopes & Berger (2001) explain all the necessary conditions to obtain reliability in the estimates of photosynthetic rates to properly reach the beta value; among these conditions, sampling leaves of similar physiological age or position in the canopy are crucial. In physiological studies, measurements of photosynthesis are usually performed on the youngest expanded leaf due to the fact that older leaves lose their full photosynthetic capacity with aging, in addition to being shaded by upper leaves in the canopy. Considering that in the studied pathosystem, ASR comes from the bottom to upper leaves, it was decided to use the eighth leaf in all sampled plants in all assessments, as a fix leaf where measurements were taken; otherwise, diseased leaves would be considered in the measurements just at the end of the disease cycle. Although photosynthetic rates naturally started to drop in the lower leaves as compared with the newer ones, the differences obtained between treatments were due to the disease effect, since every measurement was taken in the same conditions, just with different severity levels.

Another point to be considered relates to cultivar differences in the photosynthetic rates obtained in healthy plants. These differences were already expected since the net photosynthetic rate varies from one cultivar to another and there is no experimental evidence that higher yields are related to greater photosynthetic rates (Pereira, 1989). This points out the necessity to use in this study the relative photosynthetic rate (RPR), which considers the maximum value obtained in a healthy leaf against the other values in diseased leaves of the same cultivar, in order to compare the RPRs cultivars response to ASR.

After Bastiaans' (1991) study, many different pathosystems have been examined. Generally, when necrotrophic microorganisms are involved, their mode of action, using toxins that diffuse in the tissue results in larger values of β, being similar to hemibiotrophic pathogens. Biotrophic microorganisms, which are less destructive to the tissue because of their mode of living, usually induce values of β equal or close to one (Bassanezi *et al.*, 2001). Lopes & Berger, working with bean rust, showed a beta value that was not significantly different from zero in the major part of epidemics. Bastiaans (1991) found, in the wheat-rust pathosystem, a β value of 1.2, with similar values for bean-rust (Bassanezi *et al*., 2001). While the values were different from 1, they were not as large as those obtained with hemi-biotrophic and necrotrophic pathogens, for example, a value around 7 for anthracnose.

In this study, there was no difference in the beta value among cultivars; a similar result was obtained by Bastiaans & Roumen (1993) in *Pyricularia grisea*-rice, Diaz *et al.* (2001) in common bacterial blight-bean and Bassanezi *et al*. (2001) in *Uromyces appendiculatus* and *Colletrotrichum lindemuthianum*-bean. This small value obtained for beta means that the reduction in the photosynthetic rate was limited to an area close to the lesions, but this did not deny the strong negative effect that ASR has on soybean photosynthesis. In the studies with brown rust of wheat (Bastiaans, 1991) and bean rust (Bassanezi *et al*., 2001; Lopes & Berger, 2001), beta value differing from one was not found.

In general, the values of beta when a biotrophic pathogen is involved are not different from one and hemi-biotrophic and necrotrophic pathogens have bigger values (Bassanezi *et al*., 2001; Lopes & Berger, 2001; Diaz *et al.*, 2001; Bastiaans and Roumen, 1993). However, one needs to consider the fact that every pathosystem is different and the pathogen effect on green leaf tissue vary and can present different results (McGrath & Pennypacker, 1990; Elings *et al.*, 1999; Robert *et al.*, 2005).

The small effect of *P. pachyrhizi* on photosynthetic rate of green tissues in soybean were proportional to the amount of tissue seen as diseased (virtual lesion = visual lesion). Thus, the relations between ELAD and ELAA and yield did not improve the relations between HAD and HAA and yield, suggesting that there is no need to incorporate beta value in this pathosystem. Jesus Junior *et al.* (2003), working with angular leaf spot in beans, found better relations between HAD and HAA with yield when a beta value equal to 3.81 was used. Hence, ELAD and ELAA proved to be better variables than HAD and HAA for the estimation of yield loss.

The strong decrease in leaf photosynthesis was related to ASR severity, although beta values did not indicate that the photosynthetic impairment extended beyond the visible damaged soybean leaf tissue. This drop off in RPR appears to be a result of the stoma closure, electrolyte leakage and disruption of the internal cell structure.

The quantitative determination of disease effects on the physiology of individual leaves is the first step toward a broader understanding of crop losses and this is vital to provide trustworthy information to enable producers to decide when and which measures to apply for disease control.

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5. GENERAL CONCLUSIONS

1) In this study a significant, negative relationship between AUDPC and yield was observed. This is somewhat contrary to what is common and is probably due to the fact that defoliation was considered and the determinate growth habit of the soybean cultivars used in Brazil.

2) Although a relationship between disease severity and yield was found, some variables considering host physiology (HAD and HAA) provided better relationship with yield than AUDPC, with higher coefficient of determination and similar slopes independent of cultivar, season and growth stage.

3) Asian Soybean Rust causes different effects on soybean physiology and this explains why this disease is so aggressive and causes such big yield losses. The main effects on the host can be summarized as:

a) Reduction in the green leaf area due to the area covered with lesions;
b) Decrease in the radiation intercepted by the canopy and the persistence of healthy leaves, provoked by early defoliation;
c) Negative interference in the amount of radiation absorpted, decreasing plant capacity to convert ilight into dry matter.

4) In addition, P. pachyrhizi interferes with the photosynthetic competence of soybean leaves. Even though the effect is in an area close to the lesions, this pathogen is responsible for a drop in photosynthetic rate of more than 85%.