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Older leaf tissues in younger plants are more susceptible to soybean rust

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ABSTRACT. The aim of this study was to clarify the relationship between soybean leaf/plant age and the susceptibility to infection by *Phakopsora pachyrhizi*. Two studies were conducted in the greenhouse during the 2009/2010 season using the BRS 232 cultivar. The experimental design for Study 1 was fully randomized in a 2 x 3 factorial arrangement (phenological stage x age of the 4th, 5th and 6th trifoliate at the time of inoculation), and in Study 2, the experimental design was fully randomized with five treatments (T) and four replicates. The variables assessed were disease severity, number of lesions, uredinia per lesion, and viable spores. In Study 1, it was observed that disease severity was lower when the plant was in the growth stage V6 (11.4%), compared to the reproductive stage R4 (16.9%). The regression for disease severity and leaf age at the time of inoculation showed that older trifoliates on the plants in the reproductive stage exhibited higher severity. However, in Study 2, for trifoliates of the same age on plants of different ages, the trifoliates of younger plants were more susceptible to the disease. It was concluded that soybean plant susceptibility to soybean rust was directly proportional to the age of the trifoliate on a given plant and the phenological stage of the plant at the time of inoculation.

Keywords: *Phakopsora pachyrhizi*, phenological stage, age of trifoliates.

Tecidos de folha velha em plantas mais jovens são mais suscetíveis à ferrugem da soja

RESUMO. O objetivo do estudo foi esclarecer a relação entre a idade da folha/planta e susceptibilidade à infecção de *Phakopsora pachyrhizi*. Foram conduzidos dois estudos em cada de vegetação durante a safra 2009/2010 utilizando a cultivar BRS 232. O delineamento experimental do Estudo 1 foi inteiramente casualizado em arranjo fatorial 2 x 3 (Estágio fenológico x idade do 4th, 5th e 6th trifólio no momento da inoculação), e Estudo 2, delineamento inteiramente casualizado com cinco tratamentos (T) e quatro repetições. As variáveis avaliadas foram severidade da doença, numero de lesões, urédias por lesão e esporos viáveis. No Estudo 1 foi observado que severidade da doença foi menor quando as plantas estavam no estágio de crescimento V6 (11,4 %), comparado com estágio reprodutivo R4 (16,9%). A regressão para severidade da doença e idade da folha no momento de inoculação mostrou que trifólios mais velhos em plantas no estágio reprodutivo exibiram maior severidade. Contudo, no estudo 2, para trifólios com a mesma idade em plantas de diferentes idades, os trifólios de plantas jovens foram mais susceptíveis à doença. Conclui-se que a suscetibilidade de plantas a ferrugem asiática foi diretamente proporcional à idade do trifólio em uma dada planta e o estágio fenológico no momento de inoculação.

Palavras-chave: *Phakopsora pachyrhizi*, estágio fenológico, idade do trifólio.

Introduction

Plant disease are important causes of reduced crop yield. A plant's phenological stage can directly affect disease development and host tissue susceptibility, which may increase or decrease as the plant develops, depending on the disease (Furtado, Alves, Carneiro, Godoy & Massola Júnior, 2009). Among the soybean diseases, Asian soybean rust caused by *Phakopsora pachyrhizi* Syd & P. Syd. is the principal disease of soybeans in Brazil. Preventative chemical control is the primary technique used for its control. Thus, information regarding the susceptibility of plants to this pathogen can help in identifying the best times to spray and can contribute to developing efficient approaches for disease control management.

Many studies have shown the influence of growth stage on susceptibility to *P. pachyrhizi* (Melching, Dowler, Koogle, & Royer, 1988; Yujun, 1991; Furtado et al., 2009). Melching et al. (1988) reported that younger leaves were more susceptible to *P. pachyrhizi*, and that susceptibility dropped as

leaf tissue aged. However, studies conducted by Yujun (1991) showed that soybean plants were more susceptible to *P. pachyrhizi* during more advanced phenological stages in comparison to the vegetative stages. Furtado et al. (2009) also reported results corresponding with those of Melching et al. (1988). However, these authors worked with either trifoliates of different ages or plants at different growth stages, but did not consider the relationship between them.

Thus, the aim of this study was to clarify the relationship between leaf or plant age and their susceptibility to infection by *P. pachyrhizi*.

Material and methods

The studies were conducted in a greenhouse during the 2009/2010 season, using the BRS 232 soybean cultivar sown in 10-liter plastic pots containing a mixture of clay soil, topsoil and sand in proportions of 2:2:1, and fertilized with 30 g of NPK 0-20-20. The temperature inside the greenhouse was similar to the external temperature, and there was no supplementary light.

Sowing

The soybean plants were sown on different dates to obtain plants at different stages at the time of inoculation. Sowing in Study 1 occurred over an interval of 30 days, on Nov 13 and Dec 13, 2009. In Study 2, the plants were sown on Nov 3, 2009, and subsequently on every 10th day (Nov. 13, Nov. 23, Dec. 3, and Dec. 13 2009). In both studies, two soybean plants were grown in each pot.

Experimental design

Two different experiments were conducted in an effort to obtain two different levels of assessment. The first (Study 1) was established using a fully randomized design in a 2 x 3 factorial arrangement (phenological stage x age of the 4, 5 and 6th trifoliates leaves at the time of inoculation), for a total of six treatments and 10 replicates per treatment. The second experiment (Study 2) was established using a fully randomized design with five treatments (T) and four replicates.

Inoculation

The plants were inoculated with spores collected from soybean plants that were previously inoculated with *P. pachyrhizi*, which were obtained from the Phytopathology Section of the EMBRAPA Soybean Research Center in Londrina (State of Paraná, Brazil). The spores were collected from plants that were inoculated on 12 Jan 2010. The plants were inoculated with a 4 x 10^5 spores mL^{-1} suspension. The suspension was prepared by adding eight drops of a polyoxyethylene sorbitan spreader (Tween 20) per liter of distilled water. The suspension was sprayed onto all plant trifoliates to the runoff point. The plants were then wrapped in transparent, moistened plastic bags for 24 hours to obtain the soaking conditions necessary for infection. During Study 1, the plants were inoculated at two growth stages, R4 (Treatments 1, 2, and 3) and V6 (Treatments 4, 5 and 6). For Study 2, the plants were inoculated at stages R5, R4, R1, V6, and V4, totaling five treatments.

Parameters evaluated

The parameters evaluated were the disease severity of trifoliates, the number of lesions, the number of uredinia per lesion and the number of viable spores. The age of the evaluated trifoliates was determined based on the node above the cotyledonary node, from base to apex. Leaves were marked as the 4, 5, and 6th trifoliate, depending on when the trifoliates fully expanded.

During Study 1, the parameters were evaluated when the trifoliates were of different ages. For the plants inoculated in stage R4, the trifoliates in treatments T1, T2, and T3 were 41, 38 and 34 days old, respectively, at the time of inoculation, and for the plants inoculated in stage V6, the trifoliates in treatments T4, T5, and T6, were 11, 7 and, 4 days old, respectively, at the time of inoculation.

During Study 2, the 4th trifoliate of the plants inoculated in stages R5, R4, R1, and V6 (corresponding to T1, T2, T3, and T4) was 41, 31, 21, and 11 days old, respectively, at the time of inoculation. Additionally, for the plants inoculated in stage V4, the 3rd trifoliate was 5 days old at the time of inoculation, because the 4th trifoliate had not fully expanded.

Leaf disease severity was estimated at seven and 13 days after inoculation (DAI) in Study 1, and at eight and 14 DAI in Study 2. This evaluation was carried out on all trifoliates of the plant inoculated. Soybean rust severity was estimated using the diagrammatic scale proposed by Godoy, Koga, and Canteri (2006).

At 15 DAI, the 4, 5, and 6th trifoliates from each plant in Study 1, and at 14 DAI, the 4th trifoliate of each plant in Study 2 were collected and evaluated to estimate the number of lesions, the number of uredinia per lesion and the percentage of viable spores.

To count the number of lesions and uredinia per lesion, 10 random readings were taken under a stereoscopic microscope, using five fields on each side of the midrib on the lower (abaxial) side only.

To quantify the percentage of viable spores, a small brush was used to transfer the spores from the relevant trifoliate of each plant to a 9 cm Petri dish containing water agar (2%) culture medium. The dishes were kept in an incubator at 20°C and in total darkness. After six hours of incubation, the percentage of spores that germinated out of 100 spores that were selected at random under an optical microscope at x100 magnification per replicate was quantified. A spore was considered to have germinated when the length of the germination tube exceeded the spore diameter.

In Study 2, we calculated the rate of increase in severity between the two evaluations using the logistic model below:

$$r = (1/t)\ (\ln (x/ (1-x)) - \ln (x_0/ (1- x_0)))$$

where x is the proportion of disease leaf tissue at time t, x_0 is the proportion of disease at time t_0 and t is the time in days between one evaluation and another.

Data analysis

In Study 1, the data were evaluated for normality using the Shapiro-Wilk test and for homogeneity of variance using Bartlett's test. The data were then analyzed using analysis of variance with the F-test. The values for number of lesions, number of uredinia per lesion and spore germination were transformed by $\sqrt{x+k}$, where $k = 0.01$. The differences between the mean values were compared using the Tukey test with a significance value of $p < 0.10\%$.

In Study 2, the data were analyzed and compared using Duncan's mean separation tests at a significance level of 10%. An analysis of variance on the regressions was run using the Sisvar program (Ferreira, 1998). The Statistica program was used for the Shapiro-Wilk test of normality and to obtain its significance value.

Results

Soybean leaf age vs. susceptibility to *Phakopsora pachyrhizi* infection

The trifoliates exhibited a higher severity of soybean rust on plants inoculated at a later date in both Study 1 (Figure 1) and Study 2 (Table 1).

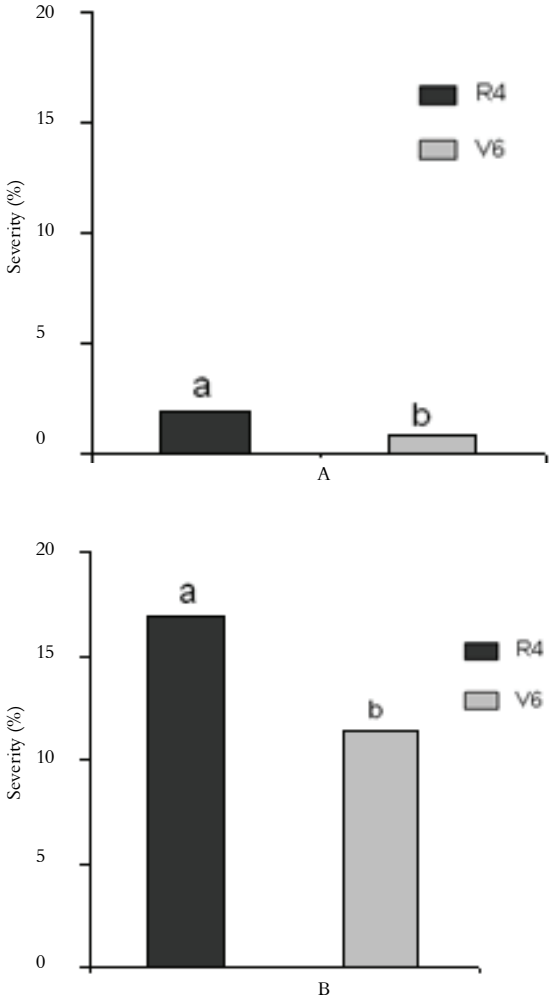

Figure 1. Average soybean rust severity for the 4, 5 and 6th trifoliates at 7 (A) and 13 (B) days after inoculation in soybean plants inoculated at stages R4 (black bars) and V6 (gray bars) in Study 1. Treatments with different letters differed statistically according to the Tukey test ($p < 010$).

Table 1. Variables related to soybean rust as a function of inoculation at different phenological stages (R5, R4, R1, V6, and V4) in soybean plants in Study 2.

Treat	Sev. 8DAI (%)		Sev. 14DAI (%)		Rate of increase in severity		Disease severity (%)		Number of lesions		Number of uredinia per lesion		Viable spores (%)	
$T1_{R5}$	0.24	b	15.57	a	0.72	a	26.75	a	7.63	b	27.38	b	76.00	B
$T2_{R4}$	1.02	a	15.14	a	0.48	b	25.50	ab	14.25	a	65.58	a	95.00	A
$T3_{R1}$	0.97	ab	10.89	a	0.42	bc	21.00	ab	10.95	ab	57.30	ab	93.00	A
$T4_{V6}$	0.83	ab	9.96	a	0.43	bc	19.75	ab	8.60	ab	41.20	ab	88.00	A
$T5_{V4}$	1.59	a	7.05	a	0.26	c	12.25	b	8.23	ab	45.25	ab	76.50	B
CV%	74.5		55.0		22.6		47.5		43.4		48.1		8.9	

Averages followed by the same letter within a single column did not differ statistically according to Duncan's tests (p>10 %). Treat: treatment. DAI: days after inoculation. Sev.: severity. CV%: Coefficient of variation

In Study 1, there was a significant difference in disease severity between the 4, 5 and 6th marked trifoliates at 7 DAI ($p < 0.10$) and at 13 DAI ($p < 0.10$) (Figure 1). However, there was no observed significant interaction between leaf age and developmental stage ($p < 0.1$). It was observed that as the disease and the plant developed, older leaves exhibited greater susceptibility compared to younger leaves (Figure 2), although leaf age did not significantly influence disease severity ($p < 0.10$).

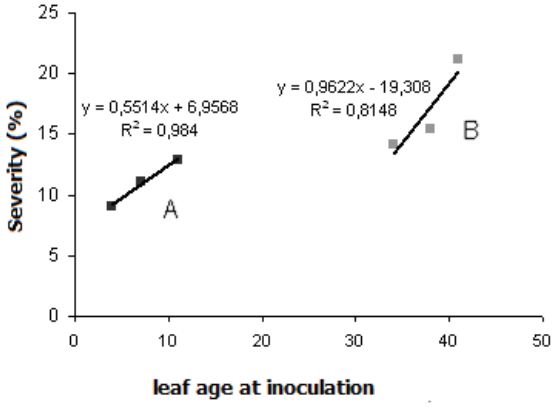

Figure 2. Average severity of soybean rust at 13 days after inoculation in soybean trifoliates of different ages in plants inoculated at stage V6 (A) and plants inoculated at stage R4 (B) in Study 1.

In Study 2, in terms of the comparison of disease severity for the 4th trifoliate at the various phenological stages at which inoculation took place (Table 1), disease severity was higher in soybean plants inoculated at an advanced phenological stage ($p < 0.10$), increasing from 12% in plants inoculated at stage V4, to 25 and 27% in plants inoculated in stages R4 and R5, respectively, and there was a positive correlation between age and severity ($R^2 = 0.80$). However, in trifoliates of the same age at the time of inoculation on soybean plants of different ages, we observed that plants in the initial phenological stages were more susceptible to infection by *P. pachyrhizi* (Figure 3).

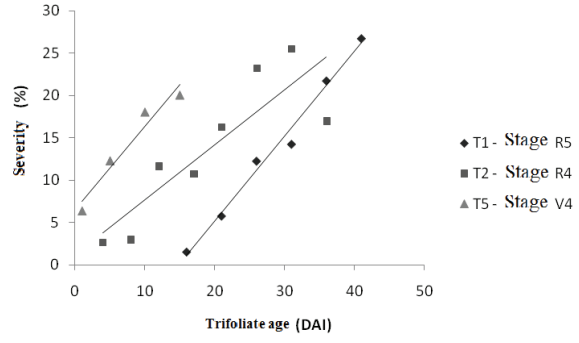

Figure 3. Soybean rust severity on soybean plants assessed at 14 days after inoculation as a function of trifoliate age and plant phenological stage at the time of inoculation in Study 2. T1 - inoculation at stage R5, T2 - inoculation at stage R4, T5 - inoculation at stage V4.

The same was observed for trifoliates that were 20 and 30 days old at the time of inoculation, where once again, the trifoliates of younger plants (inoculated at stage R4) exhibited higher disease severity compared to the trifoliates of older plants (inoculated at stage R5).

In Study 1, in terms of soybean trifoliate susceptibility to infection by *P. pachyrhizi*, there was a significant difference in disease severity among trifoliates of different ages in the assessment conducted at 7 DAI for both phenological stages. The average severity of soybean rust on the trifoliates was 2, 1 and 0.6% for the 4, 5, and 6th trifoliates, respectively. The values recorded were higher for trifoliates that were older (the 4th trifoliate) compared to trifoliates that were younger (5 and 6th) at the time of inoculation (Figure 4).

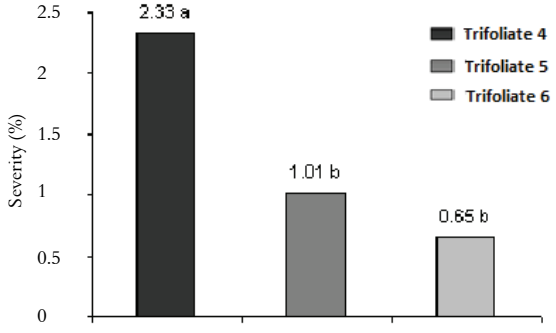

Figure 4. Average severity of soybean rust in soybean at two inoculation stages (R4 and V6) at 7 days after inoculation for trifoliates in the 4th, 5th and 6th positions in Study 1. Treatments with different letters differed statistically according to the Tukey test ($p < 0.10$).

In the assessment conducted at 13 DAI, the highest severity values were found on the oldest trifoliate for both phenological stages (Figure 2). The average severity of soybean rust on the 4, 5 and 6th trifoliates was 21, 15 and 14% for stage R4 and 13, 11 and 9% for stage V6 (Table 2).

However, we did not find significant differences in disease severity as a function of trifoliate age at the time of inoculation. The average severity for trifoliates inoculated in plants that were at stage V6 was lower than for plants at stage R4 at the time of inoculation (Figure 1), but when analyzing the regression for stage V6 (Figure 2A), which relates disease severity and leaf age at the time of inoculation, it was observed that when trifoliates were older, disease severity was higher than that observed for plants at stage R4 (Figure 2B). This can be calculated (Table 2) using the regression equation for trifoliates inoculated when the plants were at stage V6 (Figure 2A). The straight regression line in Figure 2B can be used to determine that the

susceptibility of these trifoliates will be almost zero if the plants inoculated at R4 have trifoliates of the same age as those inoculated at V6, which were 11, 7 and 4 days old. This suggests that, if considering the effect of trifoliate age on severity, the vegetative phenological stage (V6) was more susceptible to infection by *P. pachyrhizi* than the reproductive stage (R4).

Table 2. Observed severity (Actual Sev.) and estimated severity (Est. Sev.) of soybean rust in soybean trifoliates of varying ages on plants inoculated at stage R4 in Study 2.

	R4	
Leaf age	Actual Sev. (%)	Est. Sev. (%)
41	21.2	29.6
38	15.4	27.9
34	14.2	25.7

Est. Sev. = Severity estimated using the regression equation for plants inoculated at stage V6 (severity= (0.551) leaf age + 6.956).

Plant age vs. susceptibility to infection by *Phakopsora pachyrhizi*

The disease severity on leaves increased from the first to the second assessment in both studies. In Study 1, the average observed severity at 7 and 13 DAI was 1% ± 1 and 15% ± 6, respectively, and in Study 2 the severity at 8 and 14 DAI was 1% ± 0.5 and 12% ±, respectively. The data showed that there was a significant difference in soybean rust severity at 7 and 13 DAI between the inoculations at phenological stages V6 and R4. This indicates that in the initial stages of infection, soybean rust development in soybean plants was influenced by both plant age and trifoliate age (Figures 1 and 4).

Analyzing the epidemiological variables after inoculation with *P. pachyrhizi* in Study 2, we observed that disease severity for the 4th trifoliate at 8 DAI, the number of lesions, the number of uredinia per lesion and the percentage of viable spores all exhibited significant differences at 10% significance among the development stages at the time of inoculation (Table 1). However, there were no significant differences among stages in terms of severity at 14 DAI. The number of lesions per leaf was significantly different for the different inoculation stages in Studies 1 and 2 (Table 3).

The soybean rust severity at 8 DAI differed significantly ($p < 0.10$) for inoculations at stages R5 and V4. Inoculations at stages R1 and V6 exhibited similar severity levels ($p < 0.10$), but these were higher than the severity in plants inoculated at stage R5, and lower than the severity in plants inoculated at stages R4 and V4 (Table 1). In the assessment at 14 DAI, the plants inoculated at different stages did not exhibit any significant differences ($p < 0.10$) in terms of disease severity (Table 1).

In this study, because of the tendency for plants inoculated at more advanced stages to exhibit greater disease severity, a regression analysis was carried out on plant severity data at 14 DAI as a function of plant age at the time of inoculation. We observed a linear correlation between plant age at the time of inoculation and soybean rust severity ($R^2 = 0.94$) (Figure 5).

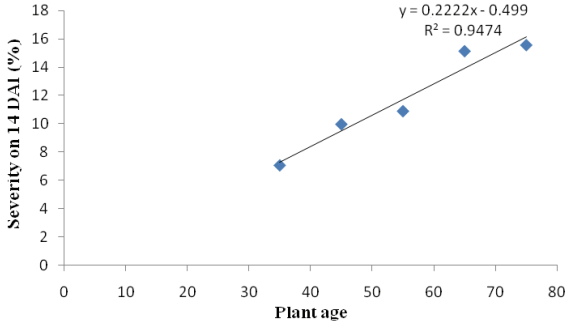

Figure 5. Relationship between soybean rust severity on soybean plants at 14 days after inoculation (DAI) and plant age at the time of inoculation in Study 2.

Table 3. Number of lesions, number of uredinia/lesion, and percentage of viable spores of *P. pachyrhizi* as a function of phenological stage (R4 and V6) and trifoliate position on soybean plants in Study 1.

Position of	Number of lesions		Number of uredinia/lesion		Viable spores (%)	
trifoliate leaf	R4	V6	R4	V6	R4	V6
4th	13.47	9.95	66.70	48.50	88.60	89.90
5th	12.12	7.14	55.00	37.50	87.40	88.80
6th	11.38	6.62	45.20	32.00	85.50	88.30
Average	12.32 a	7.09 b	55.63 a	39.33 a	87.17 a	89.00 a
CV	24.5		33.7		3.1	

Averages followed by the same letter did not differ statistically according to the Tukey test at $p < 0.10$.

There was a greater number of lesions and a greater number of uredinia per lesion on plants that were older at the time of inoculation. In Study 1, we observed a significant difference ($p < 0.10$) in the number of lesions between the two phenological stages at the time of inoculation, with the highest value found in plants inoculated at the reproductive stage (R4). The average number of lesions was 12.3 and 7.9, respectively, for stages R4 and V6 (Table 3). In Study 2, inoculating the plants at stage R4 resulted in a number of lesions and number of uredinia per lesion that was statistically higher ($p < 0.10$) than that occurring as a result of inoculation at other growth stages (Table 1). However, there was no correlation between the number of lesions or the number of uredinia per lesion in relation to the age of trifoliates at the time of inoculation: $R^2 = 0.05$ (number of lesions) and $R^2 = 0.02$ (number of uredinia per lesion).

For percentage of viable spores, inoculation at the R5 and V4 stages resulted in values that were

statistically lower than those of the other phenological stages analyzed ($p < 0.10$) (Table 1).

When the disease became established at 8 DAI, the severity of infection by *P. pachyrhizi* was lower in plants inoculated at advanced growth stages (Table 1). Plants inoculated at stage R5 exhibited lower disease severity. However, at 14 DAI, when the parasitic relationships had set in and were expressed, there was a greater susceptibility to infection in plants inoculated at later growth stages. Inoculation at R5 exhibited the highest disease severity, whereas inoculation at V4 exhibited the lowest severity (Figure 5).

The rate of severity increase, calculated for the plants in Study 2 using the logistic model, showed that severity was significantly higher ($p < 0.10$) for inoculation at the most advanced stage (R5) in comparison to other inoculated growth stages (Table 1). This indicates that symptoms take longer to be expressed in older plants, but once the initial symptoms have appeared, they progress more rapidly. Regression analysis of the rate of increase in severity as a function of plant age at the time of inoculation (Figure 6) showed that the rate of increase in severity ($R^2 = 0.84$) was directly proportional to plant age.

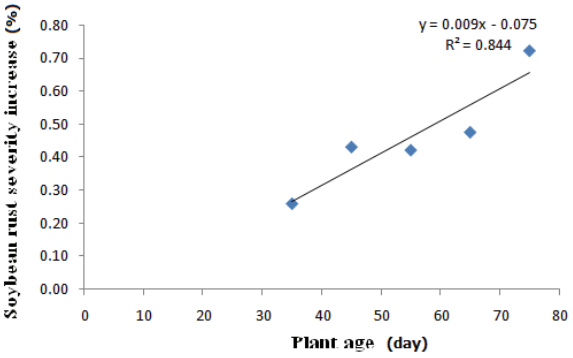

Figure 6. Rate of soybean rust severity increase in soybean plants as a function of plant age at the time of inoculation, expressed in days after inoculation (DAI).

Discussion

Soybean leaf age vs. susceptibility to infection by *Phakopsora pachyrhizi*

In these studies, it was found that older plants at the time of inoculation experienced greater soybean rust severity. According to Koga, Canteri, and Godoy (2007), for many soybean cultivars, the expression of resistance drops as the plant ages. This is why plants seem to be more susceptible when the rust epidemic begins later in the growing season. This response is associated with changes in the plant's defense mechanisms because the flowering and seed-filling phases impose a high demand for photoassimilates and nutrients to fill the seeds, which can impair the plant's capacity to prevent the rapid development of disease.

Melching et al. (1988) observed a difference in susceptibility to soybean rust between trifoliates that were 15 and 42 days old at the time of inoculation. The younger plants were more sensitive to infection, and susceptibility dropped as the tissue aged from 20.5 lesions/ cm^2 for the younger trifoliate (plants that were 15 days old) to 0.4 lesions cm^{-2} in plants that were 42 days old.

Similar results were seen by Broers (1989) in wheat, showing that younger plants were more susceptible to leaf rust. Buiel and Parlevliet (1996) also reported a significant reduction in the incidence of the peanut bud necrosis virus (PBNV) as infected plants aged. The incidence of diseased plants in the genotypes tested dropped significantly as the plants aged. They concluded that only young plant tissue was susceptible to the disease. This observed resistance in peanut plants at advanced developmental stages is in line with the results reported by Savary (1987) for peanut rust.

Welty and Barker (1992) compared the susceptibility of perennial barley to infection by *Puccinia graminis* Pers subsp. *graminicola* and observed that younger plants were more susceptible than older plants. However, common bean plants exhibited a higher susceptibility to *Uromyces phaseoli* G. Winter and tobacco mosaic virus (TMV) in more developed leaves compared to less developed leaves (Schein, 1965).

Yujun (1991) showed that soybean plants were more susceptible to *P. pachyrhizi* during more advanced phenological stages, such as the flowering and reproductive stages, compared to the vegetative stages, whereas Melching et al. (1988) reported that younger soybean plants (vegetative stages) were more susceptible to *P. pachyrhizi.* The data obtained from our study are in line with the results of both of the above authors because we observed that soybean plant susceptibility to *P. pachyrhizi* is proportional to both the age of the trifoliate on a given plant and the phenological stage of the plant at the time of inoculation (Figure 3), and that trifoliates of the same age are more susceptible to the disease on plants that are younger (Figure 3).

The average severity of soybean rust symptoms on trifoliates was statistically higher for older trifoliates than younger trifoliates at the time of inoculation (Figure 4). Furtado et al. (2009) also observed that the oldest trifoliate exhibited the highest disease severity.

According to the data obtained in this study, older trifoliates are more susceptible to infection by *P. pachyrhizi*, regardless of the plant phenological stage, and plants inoculated at the reproductive stages exhibit higher disease severity than plants inoculated during the vegetative stages. However, when comparing plants at different stages, the plants inoculated during the reproductive stages had older, and therefore more susceptible, leaves. In terms of the effect of trifoliate age on severity, it was observed that plants were more susceptible to infection by *P. pachyrhizi* at the vegetative stages, rather than the reproductive stages. These results are in line with Melching et al. (1988), who found that infection by *P. pachyrhizi* was more effective in younger soybean plants compared to older plants.

Plant age vs. susceptibility to infection by *Phakopsora pachyrhizi*

Soybean rust severity at 8 DAI was higher in plants inoculated at stages R4 and V4 than plants inoculated at stages R5, R1, and V6 (Table 1). These results are in line with the studies conducted by Melching et al. (1988), who observed a greater severity of *P. pachyrhizi* infection in assessments made at 12 DAI in younger soybean plants compared to older plants. The authors reported that the percentages of infection leaf area in plants that were 15 and 42 days old at the time of inoculation were 5 and 1%, respectively.

There was no significant difference in the severity assessment conducted at 14 DAI among plants inoculated at different developmental stages. Similar results were observed at 8 and 14 DAI by Furtado et al. (2009), in which soybean plants inoculated at stage R5 exhibited severity that was statistically similar to that observed for plants inoculated at V3 and R1 when assessed at 14 DAI. However, in the assessment at 8 DAI, the severity of plants inoculated at stage R5 was lower than the severity of plants inoculated at V3 and R1.

Melching et al. (1988) found that the number of uredinia that had developed at 12 DAI was greater in younger plants than in older plants, and the same was observed for younger trifoliates compared to older trifoliates. According to Melching et al. (1988) and Bergamin Filho (2006), the growth of the lesions caused by soybean rust is due to increased numbers of uredinia. Thus, based on the data obtained in this study, it is thought that the higher number of lesions observed on older plants is due to the greater number of uredinia observed in plants inoculated at the reproductive stage (R4). There was also a positive correlation between the number of lesions and the number of uredinia per lesion ($R^2 = 0.83$), indicating that these variables are closely linked.

The severity assessment at 8 DAI (Table 1) showed a significant difference ($p < 0.10$) between inoculation during the vegetative stage (V4) compared to inoculation during the reproductive stage (R5), supporting the idea that the latency period in younger plants is shorter. This is corroborated by the work of Melching et al. (1988), in which a shorter average latency period (ALP) was found for soybean rust in plants that were between 15 and 20 days old at the time of inoculation (ALP of 8 days), compared to plants inoculated at 37 and 42 days old (ALP of 10 days).

The latency period in younger plants at the time of inoculation was shorter, but a higher severity was observed in plants inoculated at more advanced stages of development. Therefore, the older leaves on younger plants were the most susceptible to infection by the pathogen. This result, combined with the sterilizing effect of solar radiation on spores (Isard et al., 2006), could explain why, in a commercial soybean growing area, the initial symptoms show up on the leaves occurring within the lower third of the plants.

The implications of this observation for breeding strategies suggest that it would be more efficient to select genotypes that senesce old, shaded leaves during the early cropping stages because these leaves serve as a medium for pathogen reproduction. The implications for chemical control suggest that protecting older leaves on younger plants from infection would be most effective, i.e., protecting the leaves on the lower third of plants that are approaching the reproductive stage.

Conclusion

The older the trifoliate on a given plant, and the more advanced the plant developmental stage at the time of inoculation, the more susceptible the plant becomes to *P. pachyrhizi*. However, given identical trifoliate ages on plants of different ages, the trifoliates of younger plants were more susceptible to the disease. In other words, younger soybean plants are more susceptible to the disease than older plants, and older trifoliates exhibited the highest disease severity.

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