

Asian soybean rust (*Phakopsora pachyrhizi*): quantification for disease progress evaluation in the field

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INTRODUCTION

Asian soybean rust quantification (ASR) (*Phakopsora pachyrhizi* Syd. & P. Syd.) in the field is very difficult, because of the need to use 20X or greater magnification to observe the diagnostic characteristics. It is especially important when trying to determine precise levels of the disease in nondestructive disease progress evaluations on fields (Raid *et al.*, 1988; Campbell & Madden, 1990; Bergamin Filho & Amorim, 1996). The appearance of bacterial pustule (*Xanthomonas axonopodis* pv. *glycines*) and brown spot (*Septoria glycines*) (Formento *et al.*, 2004) (Fig. 1) complicates diagnosis and leads to problems of overestimation of disease levels.

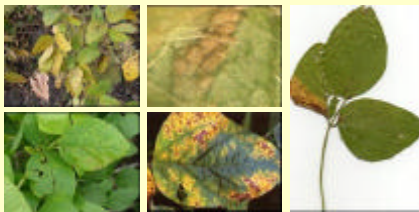
Feces of thrips and small wasps (Hymenoptera) eclosed puparium in the foliar tissue also lead to confusion (Fig. 2). Sunburn damage and drought act to mask the ASR symptoms. Although several authors have developed different Standard Disease Diagrams for estimate Severity (Canteri & Godoy, 2003), in this case, their use is difficult because of the presence of other diseases and for their low sensivity in the detection low diseases levels. Therefore they can underestimate the low ASR levels which appear at the beginning of epidemics or when some another factor was limiting for the disease. This research was carried out to develop a precise method for ASR measurements for epidemiological studies on the field.

Violations of assumptions for regression models were detected by an examination of residual plots as part of the PROC UNIVARIATE output, and determining the potential influence of particular observation with option INFLUENCE of PROC REG. For inferences about model parameter in model under consideration F test was used to test H₀: with PROC REG of SAS statistical package (SAS, 1999).



Fig. 3. A frame of 1cm² placed in four equidistant positions over the leaflets. The density of leaflet 34 has 10,903 pustules (mean = 2.44) and the 39 has 102 (mean = 0.75).

Fig. 1. Symptoms of asian soybean rust (*Phakopsora pachyrhizi*), bacterial pustule (*Xanthomonas axonopodis* pv. *glycines*) bacterial blight (*Pseudomonas syringae* pv. *glycines*) and brown spot (*Septoria glycines*). (Photos by J.C. Velázquez, INTA EEA Paraná).



MATERIALS AND METHODS

Forty five leaflets of soybean leaves (*Glycine max* (L.) Merr.) were collected which showed approximately 100 or more uredinia (Anamorph: *Malvula sojae* (P. Hennings) Ono, Butrica & Hennings) per leaflet. Because uredinia are found predominantly on the abaxial surface of the leaflet (Melching *et al.*, 1979), counts were performed only on this surface.

The leaflets were collected from the inferior, middle and upper levels of the crop, and from different cultivars. Counting of erumpent uredinia was carried out with a stereoscopic microscope of 20-40X. To determine the disease level, the following values were obtained:

Actual Value: Direct counts of uredinia per leaflet (density).

Estimated Value: Over the same leaflets a frame of 1cm² was placed in four equidistant positions (Fig. 3) and uredinia were counted inside these areas. Then the leaflet area were determined using the ASSESS program (Lamari, 2002). This average value and the leaflet area were used to estimate uredinia per leaflet (density).

A simple linear regression model was fitted between the actual number of uredinia (independent variable) and estimated number of uredinia (dependent variable) and the line was being forced through the origin.

RESULTS AND DISCUSSION

The linear model was highly significant (P<0.0001) in the analysis of variance for regression (Table 1). The model fitted is $y = 0.86403(x)$ for 100 or more uredinia observed. The coefficient of determination (R²) obtained for the 45 leaflets sampled was 0.97 and indicated a strong linear relation between the two variables (Fig. 4). The model explains the 97% of the observed variability.

Table 1. Analysis of Variance and Parameter Estimates.

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	54379	54379	1307.65	<.0001
Error	44	1829.75573	41.58536		
Corrected Total	45	56209			
Root MSE					6.44867
R-Square					0.9674
Dependent Mean					21.36756
Adj R-Sq					0.9667
Coeff Var					29.89987
Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > t
actual	1	0.86403	0.02389	36.16	<.0001

The model fit adequately to the interval including approximate values between 100 and 12000 uredinia per leaflet. For ASR measurement we propose the direct uredinia counts are made where densities do not exceed 100 uredinia per leaflet. When densities exceed approximately 100 uredinia per leaflet, the ASR density may be estimated by the average of 1cm² area method at four leaflet equidistant positions.

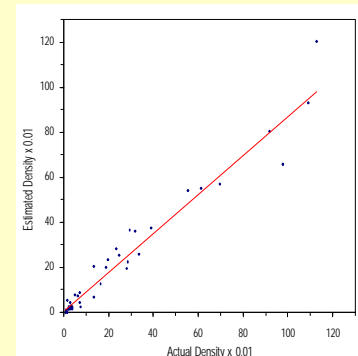


Fig. 4. Estimated vs. actual number of pustules per leaflet. R² 0.97; $y = 0.86403(x)$ (n = 45).



Fig. 2. Small wasps (Hymenoptera) and small wasps eclosed puparium. (Photos by J.C. Velázquez, INTA EEA Paraná & M. Cragochna, INTA EEA Reconquista).

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