

## Studies on Asian Soybean Rust (*Phakopsora pachyrhizi*) in Argentina

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### RESUMEN

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La roya asiática causada por *Phakopsora pachyrhizi* fue detectada por primera vez en soja (*Glycine max*) en Argentina en 2002. Desde entonces se ha expandido hacia las regiones del NEA, NOA y Pampeana. Los objetivos de este trabajo fueron cuantificar la infección de *P. pachyrhizi* en campos de soja y en kudzu (*Pueraria lobata*), su principal hospedante alternativo, durante las campañas 2003 y 2004, y caracterizar al patógeno morfológicamente. La incidencia fue 100 % en tres campos de soja evaluados. La severidad fue mayor en el estrato inferior del follaje, tanto en soja como en kudzu. El número de pústulas/cm<sup>2</sup> fue de 72 a 232 en soja y de 42 a 78 en kudzu, y por lesión 1 a 15 en soja y 1 a 10 en kudzu. En todas las muestras de soja observadas se encontraron telios. Los daños no fueron relevantes debido a la aparición tardía de las epidemias, pero la enfermedad representa una amenaza potencial en algunas áreas del país.

### ABSTRACT

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Asian rust caused by *Phakopsora pachyrhizi* was first detected in Argentina infecting soybean (*Glycine max* in 2002). Since then, the disease spread to the Northeast, Northwest and Pampeana regions. The objectives of this study were to characterize the pathogen morphologically and quantify *P. pachyrhizi* infection in soybean fields and kudzu (*Pueraria lobata*), the main alternative host, in the 2003-2004 growing season. Rust incidence was 100 % in the three soybean fields evaluated. Disease severity was higher on the lower canopy both in soybean and kudzu. Number of pustules/cm<sup>2</sup> varied from 72 to 232 in soybean and 42 to 78 in kudzu, and the number of uredinia/lesion from 1 to 15 in soybean and 1 to 10 in kudzu. Telia were found in every soybean sample observed. Damage was not relevant due to the delay of epidemics but the disease represents a potential threat in some areas of the country.

*Additional keywords:* kudzu, telia, uredinia.

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### INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is the most important crop in Argentina with a cultivated area of  $12 \times 10^6$  ha, and  $26 \times 10^6$  tons produced in the 2003 growing season, which represents the most significant source of income for the country. The sown area and the total production have increased significantly during the last 10 years due to international demand and the wide spread of no tillage cultivation and glyphosate resistant cultivars.

The North Pampeana Region (Buenos Aires, Santa Fe and Córdoba provinces) accounts for 82.7 % of the production. The Northeastern Region (Entre Ríos, Chaco, Corrientes, Misiones, Formosa and East of Santiago del Estero) with 11.8 % and Northwestern Region (Tucumán, Salta, Jujuy, Northwest of Santiago del Estero and Southeast of Catamarca) with 5.5 % (14). Argentina is the first exporter country of soybean oil and meal providing 41 % and 36.5 % of the world commerce, respectively (17).

Late season diseases (LSD) are currently factors that mostly reduce soybean yield in no-till soybean monoculture systems. Damages caused by LSD in the Pampeana Region were estimated between 10 and 20 % (4).

During 2002, Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* H. Sydow & Sydow, was detected in Argentina in a limited area in the Northern region of the country (16). *Phakopsora meibomia*, the less aggressive soybean rust species, was not detected during 2002-2005 (19). ASR is the most destructive disease of soybean in many production areas of the world. This disease was first reported in Japan in 1902 (7). In the last ten years it has spread to new regions within Hawaii, South Africa, South America, and more recently in the USA (15). ASR has caused up to 80 % of losses reported from experimental fields in Taiwan (6) and 100 % in individual fields in Southern Africa (1). The disease has emerged as a major constraint to soybean production in South America since 2001 (12). During 2003-2004 growing season, *P. pachyrhizi* extended rapidly throughout soybean growing regions of Northwest and Northeast Argentina and was also found in some areas in Entre Ríos and Santa Fe provinces. During 2004-

2005 the disease also affected Córdoba, Buenos Aires and La Pampa provinces.

The objectives of this study were to quantify *P. pachyrhizi* infection in soybean fields and on the main alternative host kudzu *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & S. M. Almeida and to characterize the pathogen morphologically. Kudzu is a perennial weed that grows spontaneously in Northeast Argentina, Paraguay and Brazil, and the main primary source of inoculum of the disease as well as early soybean crops. Preliminary work has been already reported (2,3).

### MATERIALS AND METHODS

#### Pathometric and morphologic characterization of *Phakopsora pachyrhizi* on commercial soybean fields

Three fields of 100, 90, and 60 ha, with ASR symptomatic plants were selected in Santiago del Estero, Chaco, and Salta provinces respectively, in April, 2004. Symptoms consisted of chlorosis and dark lesions on basal leaflets with erumpent uredinia on the undersides observed in the field using a 20x magnifier, with abundant production of urediniospores. The causal fungus had been previously detected in the area by PCR assay (19). Incidence (percentage of plants affected) and severity (percentage of diseased leaf area affected, including chlorosis) were visually estimated on ten plants arbitrarily collected in April, 2004, from each of three fields located in Charata (Chacabuco Department), Chaco province (24-28° S, Sample 1); La Paloma, (Moreno Department), Santiago del Estero province (25° 35' - 30° 41' S, Sample 2) and Tolloche (Anta Department), Salta province (22° - 26° 23' S, Sample 3). Phenological stages according to Fehr & Caviness scale (5), were: R6 for Sample 1, R6-R7 for Sample 2 and R5.5 for Sample 3. Disease incidence was estimated for the lower, middle, and upper canopy in fifteen leaves from each plant. Number of pustules/cm<sup>2</sup> was recorded in laboratory on abaxial surfaces of 5 central leaflets from the lower nodes of 10 plants of each field and number of uredinia/lesion was counted on 3 lesions of each leaflet observed.

In order to characterize the pathogen, transversal sections of leaves with uredinial and telial structures were stained with Floxine or Cotton Blue (9) and observed under Zeiss



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Axioscop microscope. One hundred urediniospores and 30 teliospores from each location (Charata, Tolloche and La Paloma) were measured. Structures and spores in fresh tissue samples were also examined under Environmental Scanning Microscopy (Electroscan 2020 Wilmington, MA) at CITEFA (Instituto de Investigaciones Científicas y Técnicas para la Defensa).

### Patometry and morphological characterization of *P. pachyrhizi* on kudzu

Severely infected kudzu plants were sampled on January 2005 in Cerro Azul (Misiones province, 29° 29' S), in order to confirm pathogen survival in this host and to characterize the infection. Number of lesions, pustules/cm<sup>2</sup> and uredinia/lesion were recorded from the abaxial surfaces of 40 leaflets. Size and shape of 100 urediniospores were examined under light microscopy.

## RESULTS

### Pathometric and morphologic characterization of *P. pachyrhizi* on commercial soybean fields

Symptoms were expressed late in the season (growth stages R5.5 to R7) in all sites. Incidence of affected plants was 100 % in all fields and severity was always higher on the lower canopy (Table 1). The lesions were polygonal with one to several uredinia. The number of pustules/cm<sup>2</sup> varied from 72 to 232 and the number of uredinia/lesion from 1 to 15, depending on the origin of the samples (Table 2).

Uredinia were amphigenous, mostly hypophyllous, pulverulent, with incurved

paraphyses from peridioid pseudoparenchyma and a central opening (Figures 1C, 1D and 1E). Urediniospores were sessile, obovoid, and densely echinulate (Figures 1A and 1B), released through a circular ostiole (Figure 1C) and measured 18.5 (16-22) µm x 27 (25 to 30) µm (mean). Telia were found on infected leaves mixed with uredinia in every sample (Figures 1E, 1F and 1G). They were brown to dark-brown, subepidermic or erumpent and crust-like. Teliospores were single-celled, subglobose oblong to ellipsoid, 9 (8 to 11) µm wide x 23.8 (19 to 27) µm long. They were irregularly arranged in 2 to 7 layers (Figure 1G).

### Patometry and morphological characterization of *P. pachyrhizi* on kudzu

The average number of lesions per cm<sup>2</sup> counted on was 14 (4 to 22). The number of uredinia per lesion and cm<sup>2</sup> counted on twenty leaflets from the lower canopy with higher infection were: 3 (1-10) and 55 (42-78) respectively. Urediniospores were slightly smaller: 12.5 to 22.5 µm (mean 18.4) X 17.5 to 26.3 µm (mean 22.7). Telia were not found.

## DISCUSSION

The rapid spread of *P. pachyrhizi* in Argentina makes soybean rust the most important disease currently. Severity was high in every sample analysed in this study. Nevertheless, damages were not relevant due to the delayed outbreak of epidemics. According to current distribution of ASR in Argentina (19) and due to the fact that the pathogen survives in a wide range of temperature conditions (10), the disease represents a potential threat in some areas of the country where favourable environmental conditions for the pathogen are expected in the near future. Wind dissemination from overwintering areas of Brazil,

**Table 1.** Disease severity (percentage of leaf surface affected including chlorosis) of Asian soybean rust (*Phakopsora pachyrhizi*) in the lower, middle and upper part of soybean (*Glycine max*) plants in three samples from Chaco (1), Santiago del Estero (2) and Salta (3) provinces, Argentina.

Samples	Leaf surface affected (%)		
	Lower	Middle	Upper
1. Chaco	45	20	10
2. Santiago del Estero	60	40	25
3. Salta	25	15	10

**Table 2.** Number of uredinia per lesion and cm<sup>2</sup> from the lower canopy of soybean.

Geographic location	Uredinia/cm <sup>2</sup> <sup>(1)</sup>	Uredinia/lesion <sup>(2)</sup>
Charata	156 (88 to 200)	6 (1 to 15)
La Paloma	172 (128 to 232)	5 (1 to 13)
Tolloche	120 (72 to 212)	3 (1 to 5)

(1) Average of number of pustules Range of values shown in brackets.

(2) Uredinia/lesion: average uredinia Range of values shown in brackets.



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Paraguay, Bolivia, and northeastern Argentina either from earlier soybean crops or from kudzu can be expected.

Urediniospores and teliospores of *P. pachyrhizi* coincided morphologically with those described by Ono (13).

Under field conditions, *P. pachyrhizi* was able

to produce the sexual stage on soybean in every site sampled. During 2005 telia were also found on samples from Vedia (province of Chaco) and Villaguay (province of Entre Rios) reaching 31° S. Germination of teliospores and production of basidiospores were induced *in vitro* (18), but the identity of the hosts that they are capable of infecting has not been elucidated yet (13). Therefore, the epidemiologic importance of

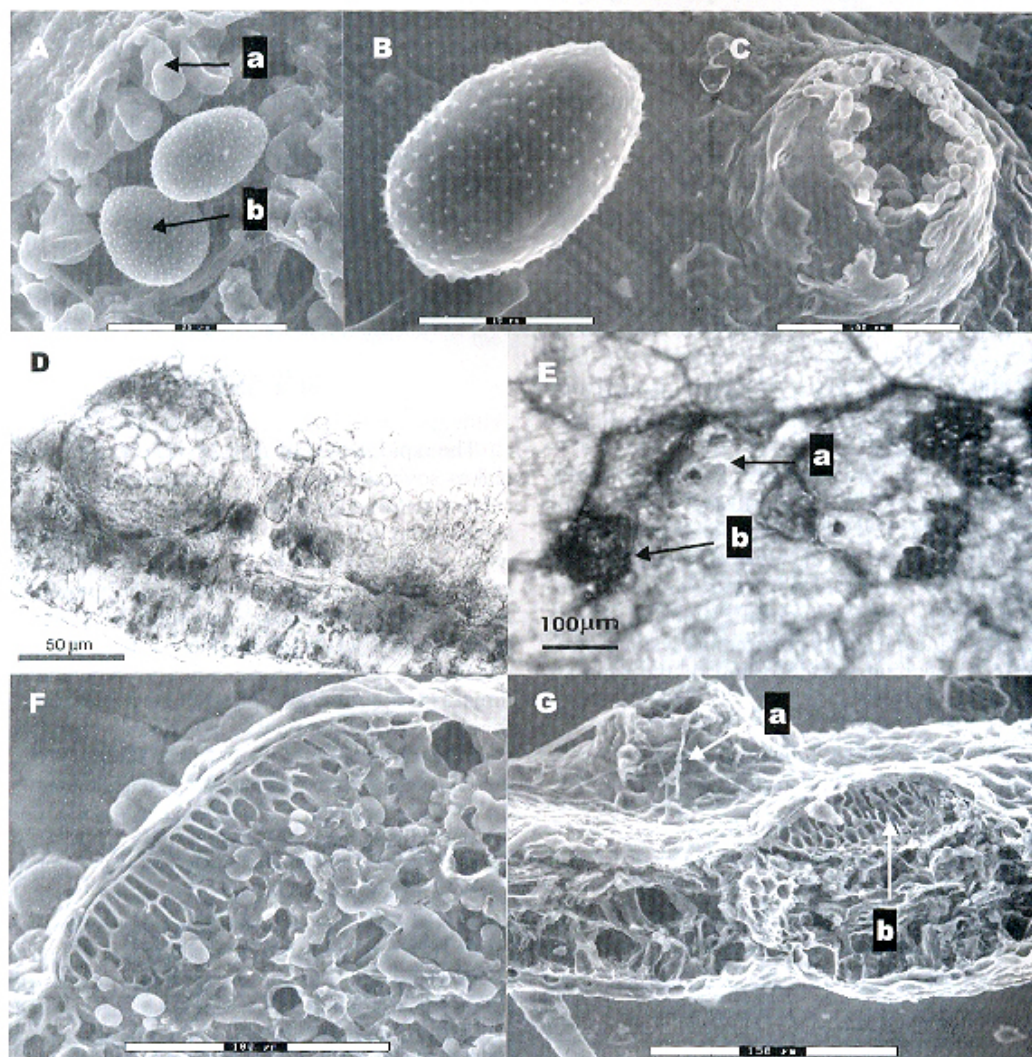


Fig. 1. (A) Incurved paraphysis (a) around sorus opening and urediniospores (b) ESEM; (B) Urediniospore, detail of equiculated wall. ESEM; (C) Sorus erumpent through epidermis (arrows) with emergent paraphysis ESEM; (D) Closed uredinia with peridioid pseudoparenchyma (left) and another opened (right) with emerging paraphysis. Transversal section seen at MO; (E) Uredinia (a) and telia (b) on abaxial surface of leaf; (F) Subepidermal telia and teliospores disposed in layers; (G) Leaf section of soybean with an open uredinia and subepidermal telia (b).



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teliospores and their function is still unknown.

*P. pachyrhizi* is frequently observed in plants of kudzu in Cerro Azul (8, 19). The symptoms of soybean rust observed on kudzu were similar to those found in soybean leaves collected from commercial soybean fields. The causal fungus had been previously detected on kudzu in the area by molecular methods (8). Since soybean is not grown from June to September in Argentina, kudzu, a very susceptible secondary host of *P. pachyrhizi* as confirmed in this study, must be considered an important source of inoculum as it is severely infected earlier than soybean commercial crops, and its perennial character determines a constant source of inoculum throughout the year. ASR was also detected on volunteer soybean plants, but they generally die because of occurrence of frosts. However, in 2005 the pathogen was found by several researchers on volunteer soybean plants from July to October in some provinces of the country (19).

Since most soybean cultivars are susceptible to the disease, the main strategy to control it is the use of fungicides. The timing of application is critical and should be supported by a systematic field monitoring program.

This is believed to be the first report of the telial stage of *P. pachyrhizi* in South America (2). Further research is needed to determine the significance of the telial stage and relevance of secondary hosts.

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