

# Variation of *Phakopsora pachyrhizi* isolates on soybean

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## Abstract

*Phakopsora pachyrhizi* Sydow, the causal fungus of soybean (*Glycine max* (L.) Merrill) rust, occurs in most soybean-growing areas of the world except continental North America. Initial studies on soybean rust isolates from the Western Hemisphere indicated that they were different than isolates from the Eastern Hemisphere. In 1992, the Eastern Hemisphere species, *P. pachyrhizi*, and the Western Hemisphere species, *P. meibomiae*, were established for the soybean rust fungi based on morphological differences. The first molecular differentiation of the two species was reported in 2002. A number of studies have reported the occurrence of race in *P. pachyrhizi* either on soybeans or on other hosts. In 1984, a set of four native Australian *Glycine* species were used to identify six different virulence combinations of *P. pachyrhizi*. Much of the research on differentiating isolates on soybean was completed in a containment facility at in the U.S. Genetic characterization on four plant introductions (PIs) indicated the occurrence of four independently inherited dominant genes. These genes are known to be effective to a limited number of isolates. There are many studies that need to be completed to determine if all isolates respond equally in terms of survival, urediniospore production, telia formation, and host range under different environments. Over the next few years, our understanding of pathogen diversity will increase as more concerted research efforts take place in different parts of the world.

## Introduction

Soybean rust is caused by two fungal species, *Phakopsora meibomiae* and *P. pachyrhizi*. The Asian soybean rust pathogen, *P. pachyrhizi*, is the species of greater concern since it is the more aggressive species and has been identified in new geographical locations beyond Asia. *P. meibomiae*, the less virulent species, has only been found in the Western hemisphere, and it is not known to cause severe yield losses in soybean (Sinclair and Hartman

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1999). Soybean rust, caused by *P. pachyrhizi* is one of the major diseases of soybean in many Asian countries (Sinclair and Hartman 1999) and now in Brazil, since its recent introduction in 2001 (Yorinori et al. 2003). Significant yield losses due to rust have been reported in most soybean-producing countries throughout Asia, where the disease is endemic and may limit soybean production. North America is one of the last major soybean production areas without soybean rust.

On the African continent, the distribution of soybean rust was not well known before 1996, but since then a more expanded view of soybean rust in Africa has been reported indicating that it was found in 1996 in Uganda, Kenya, and Rwanda, in Zambia and Zimbabwe during 1998, Nigeria in 1999, Mozambique in 2000, and South Africa in 2001 (Levy 2003).

The first detection of *P. pachyrhizi* in South America was in Paraguay in the 2000-2001 growing season (Yorinori et al 2003). The disease was found on soybeans grown in the Parana River basin on the eastern border with Brazil in a limited number of fields. Argentina confirmed the occurrence of soybean rust in early 2002 (Rossi 2003). During the 2003 growing season the pathogen was found in most of the soybean growing regions of Brazil and came late in the season for the first report in Bolivia (Yorinori et al. 2003).

The objective of this paper is to provide an overview of what is known about the variation of *Phakopsora pachyrhizi* isolates. This will include a review of some of the studies that confirmed that two species infect soybean, and the variation observed in pathogenicity on soybean and other hosts. Because of the restrictions in length, this overview will highlight some of the refereed papers published and is not meant to be a review of all of the literature on this subject.

#### **Studies on species differentiation**

One of the earliest reports of soybean rust in the Western Hemisphere was when it was found on soybean and other legumes in Puerto Rico (Vakili and Bromfield 1976). Along with soybean, rust was found on *Centrosema pubescens* (butterfly pea), *Dolichos lablab* (hyacinth bean) *Phaseolus coccineus* (scarlet runner bean), *P. lunatus* (lima bean) and *P. vulgaris* (bean). It also was shown that the Puerto Rican cultures were less virulent than three strains from the Eastern Hemisphere on soybean cv. Wayne.

In 1980, Bonde and Brown (1980) examined isolates from Australia, India, the Philippines, Taiwan and Puerto Rico on cv. Wayne. Isolates were indistinguishable in their pre- and post-penetration, colonization phases,

and morphology of uredinia. The only observable difference was the appearance of germ pores, in which those of the Puerto Rican isolates were more easily seen suggesting that their spores may have thinner germ pore plugs. A more thorough examination of isolates was reported in 1988 when isozymes of *P. pachyrhizi* from the Eastern and Western Hemispheres were compared (Bonde et al. 1988). No differences in isoenzyme banding patterns were detected among any of the eleven isolates from the Eastern Hemisphere or among those from the Western Hemisphere; however, distinct isoenzyme polymorphisms were observed when comparing isolates from the two hemispheres.

Ono et al. (1992) established two species for the soybean rust fungi. This was based on morphological differences between their anamorphic and teleomorphic stages, and was based primarily on layering of telia and wall thickness of teliospores. *P. pachyrhizi* [uredinal anamorph *Malupa sojae* (syn.: *Uredo sojae*)] included the Eastern Hemisphere populations before its known spread and confirmation in Africa, Hawaii, and South America, and *P. meibomia* [uredinal anamorph *Malupa vignae* (syn.: *Uredo vignae*)] isolates from the Western Hemisphere, before the first report in Hawaii in 1994 (Killgore 1995).

The first molecular differentiation of the two species was reported by Frederick et al. (2002). They showed that the nucleotide sequence of the internal transcribed spacer region had only 80% sequence similarity between the two species. Based on these differences, four sets of polymerase chain reaction primers were designed specifically for *P. pachyrhizi* and two sets for *P. meibomia*. Classical and real-time fluorescent polymerase chain reaction assays were used to identify and differentiate the two species from inoculated and field samples from South America.

#### Variation on hosts other than soybean

One of the earliest reports of *P. pachyrhizi* races is from Lin (1966) in Taiwan. Nine isolates were used to inoculate six soybean genotypes and five leguminous plants. No marked differences in pathogenicity of the isolates were observed on the soybean genotypes; however, the nine isolates were separated into six pathogenic groups differing mainly in their reactions types with and without sporulation or no infection on *Vigna unguiculata* subsp. *sesuquipedalis* (asparagus bean), *P. vulgaris* (kidney bean), and *Pachyrhizus erosus* (short-podded yam bean).

In 1984, Burdon and Speer (1984) established a set of differential *Glycine* hosts for the identification of *P. pachyrhizi* races. They used 257 accessions

of four native Australian species of *Glycine* (*G. canescens*, *G. clandestina*, *G. tabacina* and *G. tomentella*) and eight Australian isolates of *P. pachyrhizi*. Based on differences in infection responses, they distinguished six different virulence combinations. Additional research by Burdon (1987) found nine races *P. pachyrhizi* in two natural populations of *G. canescens*. Genetic analysis showed that the two host populations contained at least 10 and 12 resistance genes with some lines having up to three dominant genes. In another study by Burdon and Lenne (1989), 10 *P. pachyrhizi* isolates from *Kennedia rubicunda* were evaluated on two hosts, *G. canescens* and soybean, known to be susceptible to all tested *P. pachyrhizi* isolates from soybean. Three patterns of were detected with isolates being either avirulent or virulent on both host lines, or being avirulent on *G. canescens* and virulent on soybean. When five isolates from *K. rubicunda* were tested on 10 populations of *K. rubicunda*, significant interactions were detected between particular host population-pathogen isolate combinations.

### Variation on soybean

Much of the research on differentiating isolates on soybean has been done in the containment facility at Fort Detrick, Frederick, Maryland, U.S. Melching et al. (1979) used four isolates to compare their ability to colonize and reproduce on the cv. Wayne. All isolates required a similar time from inoculation to lesion appearance (7 days) and initiation of urediniospore production (9 days). The Indian culture produced more lesions per unit leaf area per inoculum unit than the others. Urediniospores were collected daily 13 to 52 days after inoculation and the calculated total number of spores produced over the life of the lesion was 2028, 3768, 6268, and 6600 for the Australian, Indian, Indonesian, and the Taiwanese isolates, respectively.

Based on research by Bromfield et al. (1980), isolates from Australia, India, Puerto Rico and Taiwan differed in virulence and three infection types were observed. A susceptible reaction or tan (TAN) lesion with 2-5 uredia per lesion, a resistant reaction or a reddish-brown (RB) lesion with 0-2 uredia, and an immune reaction or Type 0 with no macroscopically visible evidence of rust. Isolate Australia-72-1 induced both TAN and RB on each of eight accessions. Taiwan-72-1 induced TAN on 13 accessions. The rates of lesion enlargement and increase in uredinia per lesion on the lower surface of leaves of Wayne were about equal for India-73-1 and Taiwan-72-1, but were lower for Australia-72-1 indicating that Australia-72-1 was less aggressive. Taiwan-72-1 consistently produced more uredinia per lesion at a given time on the upper surface of leaves of Wayne than did India-73-1.

Specific resistance to *P. pachyrhizi* has been described as four single dominant genes (*Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4*). Hartwig (1995) summarized the finding of the reports on resistance. These four genes condition resistance to a limited set of rust isolates. The *Rpp1* was described as

having an immune reaction when inoculated with a few isolates, including India 73-1. Inoculation of most rust isolates on *Rpp1* or the other genes produces a resistant red-brown (RB) lesion with no or sparsely sporulating uredinia. There are numerous examples of single gene resistance not being durable. Only Bing Nan, the source of the *Rpp4* gene, has not been reported to be defeated in the field, although observations both in the field in Paraguay and greenhouse inoculation tests indicate that it is susceptible to at least some isolates (Miles et al, pers. comm.).

Based on the studies mentioned in this paper and many others in the literature, it appears that *P. pachyrhizi* possess multiple genes for virulence in most isolates and/or field populations are a complex mixture of many races with possible multiple virulent factors. In recent studies done at Fort Detrick, isolates of from southern Africa and South America were significantly more virulent on soybean cultivars containing the single resistance genes when compared to the Asian isolates from the 1970s (Frederick et al, pers. comm.). The most virulent isolate was collected from Zimbabwe in 2001.

### Variation due to environment

There are only a few papers that have reported on the interaction of isolates to differing environmental conditions. Marchetti et al. (1976) compared the effects of temperature and dew period on germination and infection by urediniospores of four isolates. Spores germination and infection occurred over similar temperature ranges with the maximum infection of cv. Wayne soybean leaves occurring at 20-25° C with 10-12 hours dew and at 15-17.5° C with 16-18 hours dew. The minimum period for infection was 6 h at 20-25° C and 8-10 hour at 15-17.5° C. Infection did not occur above 27.5° C. Dufresne et al. (1987) studied the effects of temperature and light intensity on telia development by Puerto Rico and Taiwan isolates. The Taiwan isolate produced telia after 21 and 30 days and the Puerto Rico isolate produced telia after 34 and 35 days at 10 and 15° C, respectively. The Taiwan isolate produced larger lesions with more telia. Light intensities did not greatly alter the time it took for telia to form.

### Conclusion

The literature indicates without a doubt that there are two species that cause soybean rust. The geographic range of *P. meibomia* is restricted compared to that of *P. pachyrhizi*, which has been on the move in last few years and now occurs in all major soybean growing areas except North America. More research is needed to better define the geographical limitations of *P. meibomia*. As more work is done in the taxonomic area using new molecular tools, it may not be surprising to find another rust species that may infect soybean as well. It is also clear from the literature

that *P. meibomia* and *P. pachyrhizi* have effective pathogenicity genes for infecting many leguminous plants. *P. pachyrhizi* in particular also has many virulence genes, as isolates are known that overcome any dominant resistance genes in soybean. Additional research in this area will surely uncover many more resistance genes in soybean and other hosts, and many more races of *P. pachyrhizi*. There is less information about effects of environment on isolates. For example, it is not known if urediniospores from India survive better in high temperatures than those collected from Taiwan. Many more studies are needed to determine if all isolates respond equally in terms of survival, urediniospore production, telia formation, host range under different environments. Over the next few years, our understanding of the diversity of this pathogen will expand as more concerted research efforts develop throughout the world.

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