Uredial Development of Phakopsora pachyrhizi in Soybeans

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ABSTRACT

Uredial primordia were evident in leaflets of 'Lee 68' soybeans 5-7 days after inoculation with uredospores of *Phakopsora pachyrhizi*. Sporulation from the first uredia started 9 days after inoculation, and continued for about 3 weeks. New uredia continued to form until about 4 weeks after inoculation. In contrast, uredia in P.I. 200492, a soybean accession with field resistance to rust, were about a day behind in development and senesced 2-4 days sooner than in Lee 68; new uredia continued to form in P.I. 200492 for about 3 weeks after inoculation.

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This paper describes the development of uredia of *Phakopsora pachyrhizi* Syd. in leaves of soybean [*Glycine max* (L.) Merr.] cultivar Lee 68, and compares uredial development in Lee 68 with that in P.I. 200492, a soybean accession showing field resistance to soybean rust in Taiwan and used as a parent by soybean breeders there (1, 2).

P. pachyrhizi is a member of the Melampsoraceae. The uredial and telial stages occur on soybeans, and the uredial stage has been reported on other leguminous species (3, 5, 6). The function of the telial stage has not been shown, and the pycnial and aecial stages are unknown. According to a study by Kitani and Inoue (5), uredia mature at 22 C in 6-7 days after inoculation, and measure 83 × 309-365 μm. No extensive study of uredial development of *P. pachyrhizi* has been recorded in the literature.

Soybean rust occurs in the Far East, from Korea and Japan south to Australia and in China, India, and southeast Asia (5, 8). Annual losses of 20-30% have been reported in Taiwan (7). However, soybean rust has not been reported in the western hemisphere. This histological study was part of an investigation being conducted in the containment facilities of the Plant Disease Research Laboratory, U.S. Department of Agriculture, Frederick, MD, to evaluate the epidemiological potential of soybean rust on soybeans grown in North America.

MATERIALS AND METHODS.—Four 6-week-old greenhouse-grown plants each of Lee 68 and P.I. 200492 were inoculated simultaneously in September, 1972, with 40 mg of freshly harvested uredospores of a Taiwanese strain of *P. pachyrhizi* dispersed forcibly in a settling tower. The inoculated plants were incubated in 25 C dew chambers for 20 hours. Afterwards, the infected plants were held in a greenhouse with diurnal temperature fluctuation between 20 and 30 C. The plants were not exposed again to dew, thereby precluding secondary infection. Inoculated leaves were sampled at 20 hours and at 3, 5, 7, 9, 10, 12, 17, 21, 27, and 37 days after inoculation. Two leaflets of each variety were removed, cut into 0.5-cm squares, and placed in small bottles of standard FAA killing fluid. The bottles were evacuated in a vacuum desiccator until all leaf pieces sank in the FAA. Later, leaf pieces were dehydrated in *t*-butanol (4), inbedded in paraffin, and sectioned at 10 μm on a rotary microtome. Sectioned material was stained with safranin-fast green and mounted in synthetic resin.

RESULTS AND DISCUSSION.—*Uredial development of Lee 68 soybean.*—Uredospores germinated to produce appressoria attached by short germ tubes, usually less than 20 μm. Penetration of the host was direct and usually resulted in necrosis of only one epidermal cell. Hyphae were observed in the intercellular spaces of the palisade and spongy mesophyll of leaflets processed 20 hours after inoculation. The pathogen continued to colonize the mesophyll, and 5-7 days after inoculation, the first uredial primordia became evident as loosely woven to compact masses of mycelium in the palisade or spongy mesophyll (Fig. 1). Later, most new uredia developed in the spongy mesophyll.

In 7-9 days, uredospores were differentiated (Fig. 2), and by the 9th day, uredia had erupted through the epidermis, spores being liberated through a pore lined with clavate paraphyses (Fig. 3, 4). Until then, the epidermal cells appeared intact and turgid. Uredia that formed in the palisade layer erupted through the upper epidermis. They usually were between 75 and 120 μm in diameter and tended to remain submerged in the palisade layer and to retain a cup shape (Fig. 3). In contrast, uredia that formed in the spongy mesophyll erupted through the lower epidermis, usually ranged from 110-145 μm across and tended to become saucer-shaped, thereby exposing more sporogenous tissue (Fig. 4).

New uredia continued to form for about 4 weeks after inoculation. Diseased tissue collected 27 days after inoculation revealed only occasional uredial primorida. Virtually all of these primordia were in the spongy mesophyll.

In specimens collected 27 days after inoculation, uredia were seen that appeared senescent (Fig. 5). They contained numerous clavate paraphysoid cells, but no developing uredospores. Senescent uredia were not observed in 21-day-old, or younger, specimens. Apparently uredia continued to produce uredospores for 3 weeks after sporulation began, but by 27 days, they had ceased active spore production.

Since new uredia continue to develop for up to 4 weeks after the initial infection, and continue to produce uredospores for up to 3 weeks after spore production begins, the inoculum potential of this fungus is appreciable. Spores produced as long as 8 weeks after initial infection could still be considered first-generation offspring of that original infection. Under field conditions favoring infection and disease development, soybean rust could cause defoliation by multiple secondary infections

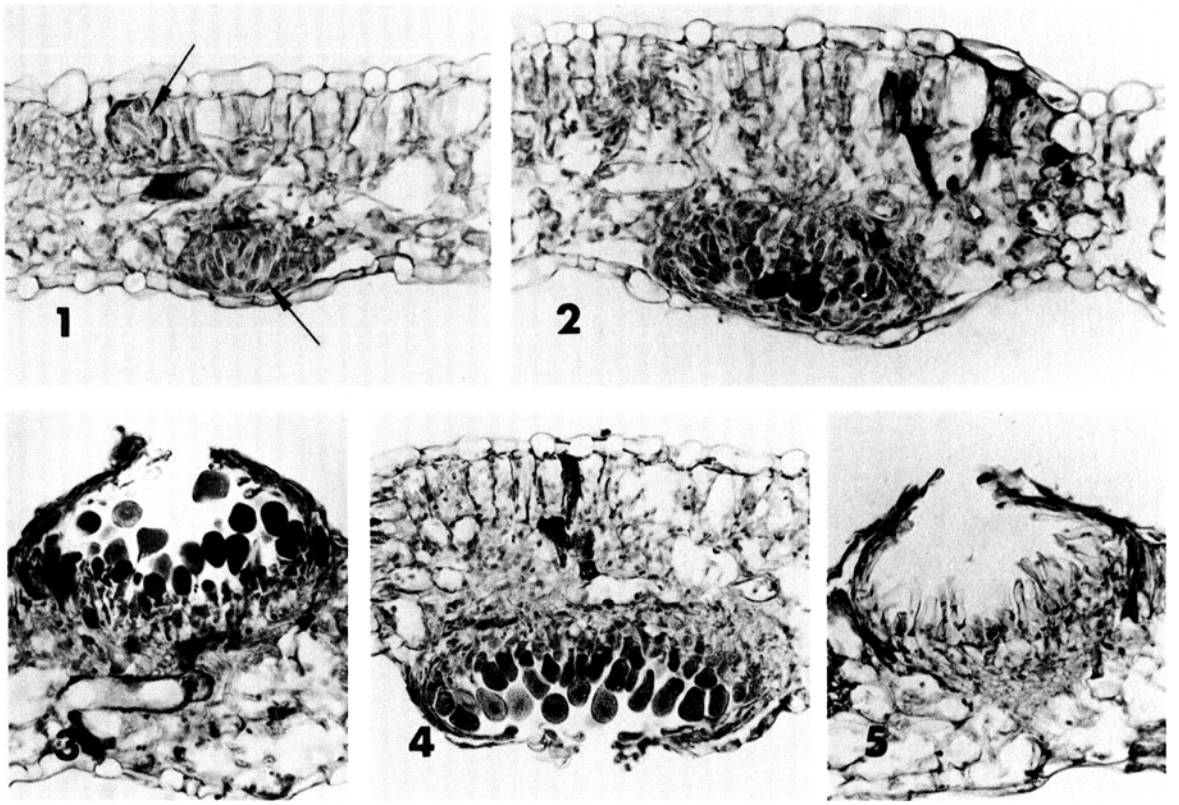

Fig. 1-5. Sections through uredia of *Phakopsora pachyrhizi* on leaves of *Glycine max* 'Lee 68'. **1)** Young uredium developing in palisade layer (upper arrows; off median), more advanced uredium developing in spongy mesophyll (lower arrow; median). **2)** Uredium with spores developing in spongy mesophyll layer. **3)** Mature uredium in palisade layer. **4)** Mature uredium in spongy mesophyll layer. **5)** Senescent uredium, no longer producing uredospores.

within a few weeks. In this situation, the capability for continued production of uredospores over an extended period becomes academic. However, when conditions favor reinfection by uredospores only sporadically during prolonged unfavorable periods, the capability for extended sporulation would allow the fungus to persist and remain a threat.

The period during which new uredia are formed within established lesions changes seasonally within cultivars, as well as among cultivars. The numbers of uredia on P.I. 200492 increased up to 8 weeks after a single inoculation in early December. Also, uredial primordia were present in sections of 'Wayne' leaf tissue collected 7 weeks after inoculation in mid-December.

Comparative development of uredia in Lee 68 and P.I. 200492 soybean leaves.—Soybean accession P.I. 200492 has shown field resistance to soybean rust (1, 2), even though it is infected easily in a greenhouse. We tried to determine histologically if differences in the rate of uredial development and sporulation contributed to its field resistance. The number of initial infections on the two cultivars did not differ noticeably. Initial uredial formation in leaves of P.I. 200492, however, lagged about a day behind than in Lee 68. The period from inoculation to cessation of uredial formation was about 6 days shorter in P.I. 200492 than in Lee 68; 21-day-old material of P.I. 200492 was comparable to 27-day-old material of Lee 68 in terms of the paucity of uredial primordia. Finally, although senescent uredia were absent in 21-day-old material of both cultivars, they were observed more frequently in 27-day-old material of P.I. 200492 than of Lee 68, suggesting that uredia senesced 2-4 days sooner in P.I. 200492. These factors–slower uredial development, shorter period during which new uredia form, and earlier senescence of uredia–contribute to the reduction in the amount of secondary inoculum, thereby diminishing the potential for spread of the pathogen in P.I. 200492 in the field.

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