Infection, Colonization, and Uredospore Production on Wayne Soybean by Four Cultures of *Phakopsora pachyrhizi*, the Cause of Soybean Rust

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We express appreciation to David Koogle for excellent technical assistance during these studies.

Accepted for publication 30 May 1979.

ABSTRACT

MELCHING, J. S., K. R. BROMFIELD, and C. H. KINGSOLVER. 1979. Infection, colonization, and uredospore production on Wayne soybean by four cultures of *Phakopsora pachyrhizi*, the cause of soybean rust. Phytopathology 69: 1262-1265.

Under greenhouse conditions, cultures of *Phakopsora pachyrhizi* from Taiwan, India, Australia, and Indonesia were compared for quantitative characteristics related to their ability to colonize and reproduce on plants of soybean cultivar Wayne. All cultures required similar time periods from inoculation until lesion appearance (7 days) and initiation of secondary uredospore production (9 days). The Indian culture produced more lesions per unit leaf area per unit of inoculum than the other cultures. The mean lesion areas at 2 wk after inoculation on both upper and lower leaf surfaces were similar for the Indian, Taiwanese, and Indonesian cultures (range 0.61–0.77 mm^2), but for the Australian culture the mean lesion areas were smaller (0.30 mm^2 on upper surface and 0.42 mm^2 on lower surface). The mean number of uredia per lesion at 2 wk on the upper leaf surface was 1.0 for the Australian culture and 2.0–3.5 for the other three; little increase with time occurred with any culture. On the lower surface, however, new uredia continued to form in lesions induced by all cultures. By 7 wk there were eight uredia per lesion for the Australian and 12.6–14 for the other cultures. Uredospores were collected daily from 13–52 days after inoculation of plants on which numbers of lesions and leaf areas had been determined. The mean mass (fresh weight) of spores produced per lesion each day and the calculated total number of spores produced over the life of the lesion were: Australian, 0.13 μg and 2,028; Indian, 0.24 μg and 3,768; Indonesian, 0.40 μg and 6,268; and Taiwanese, 0.42 μg and 6,600. Uredospores of all cultures were similar in length and width. No consistent differences in germination potential were found in uredospores tested from each culture at each harvest.

Additional key words: Sporulation, pathogen aggressiveness, *Glycine max*.

Rust of soybean (*Glycine max* [L.] Merr.) is caused by the fungus *Phakopsora pachyrhizi* Sydow. The disease has been known in the Orient since 1914 (1), but it was not reported on soybeans in the Western Hemisphere until 1976 when it was observed in Puerto Rico (11). There are no confirmed reports of soybean rust within the continental United States. The world soybean rust situation has been reviewed recently (2,12).

To our knowledge, isolates of *P. pachyrhizi* within a given region or country, or between regions or countries, have not been compared for parameters other than physiologic specialization (virulence). Quantitative data on many characteristics of epidemiological significance are presently scarce or nonexistent for the pathogen. The tests reported here were initiated to investigate some of the characteristics that collectively contribute to a pathogen's aggressiveness and fitness and to identify those parameters that could be effectively utilized to characterize populations of *P. pachyrhizi*. The work was conducted within the pathogen containment facilities of the Plant Disease Research Laboratory as part of a program to assess the vulnerability of the U.S. soybean crop to nonendemic pathogens. Preliminary findings were reported earlier (9).

MATERIALS AND METHODS

Inoculum. The four cultures of the fungus were established in our laboratory from uredospores on cotton swabs and on rusted leaves collected from diseased plants in India, Australia, Indonesia, and Taiwan. After increase on soybean in the containment greenhouse, uredospores of each culture were stored in liquid nitrogen. Inocula used in these studies were uredospores either taken directly from storage or freshly harvested from sporulating uredia on soybean plants (cultivar Wayne).

Suscept plants. Wayne was selected because it is an important commercial cultivar in the United States, it grows well under our greenhouse conditions, it is susceptible to the pathogen cultures, and it has been used for many other studies in our laboratory which permitted some comparisons with other observations.

Soybeans were grown in the greenhouse in 1.1-L plastic pots. Plants were thinned to one per pot when the second trifoliolate leaf appeared. Day/night greenhouse air temperatures and relative humidities were 25–29/20–24 C and 45–56/52–67%, respectively. Illumination was natural daylight for the area of Frederick, MD, during the experimental period (February–June). Air flow through the greenhouse was about 7m/min.

Inoculation and disease assessment. When the fourth trifoliolate leaves were fully expanded, test plants (eight per culture in each experiment) were inoculated and agar plates (four per inoculation) were seeded with "dry" uredospores in a turntable tower (8). Both plants and plates (covered) were incubated in dew chambers (6) at 19.5 ± 1 C in the dark for 16 hr. Following incubation, plants were placed in the greenhouse under the conditions previously described; germinability of the spores on the plates was determined. Just before inoculation areas of all leaflets were estimated by the formula length × width × 0.76. The number of spores deposited on suscept leaves was estimated by direct microscopic observation of sample leaflets from each leaf position in each inoculation. Lesions were counted on all leaflets at 10 days after inoculation with ×3 magnification.

Lesion areas on the upper and lower surfaces of sample leaflets were measured with a calibrated ocular grid in a dissecting microscope at 2, 3, 4, 5, 6, and 7 wk after inoculation; the numbers of uredia per lesion also were determined.

Spore production assessment. Thirteen days after inoculation, and each day thereafter until death of the tissue, spores were harvested by tapping each leaf individually over a large petri dish. An attempt was made to remove and collect all spores that could be dislodged from each leaflet; undoubtedly some escaped collection. Harvesting was done each day between 1030 and 1230 hours. Immediately after collection, the fresh weight of spores from each culture was determined. A sample from each culture was seeded on 1.25% water agar (Difco Bacto) plates, incubated at 22 ± 1 C in the dark for 20 hr, then placed in a formaldehyde-saturated atmosphere until each was evaluated for spore viability.

Data evaluation. The statistical significance of the differences among the various treatment means was tested by analysis of variance. When an overall F-test result was significant, Duncan's multiple range test was employed to compare the individual means within the study in question.

RESULTS

Primary disease establishment. Three separate studies were done to determine the relative abilities of the four cultures to initiate primary disease (Table 1). The Indian culture produced significantly more lesions per unit of inoculum than did the other cultures. No differences between the Taiwanese and the Indonesian cultures were noted. The Australian culture produced the fewest lesions except in Experiment 1, in which both its viability ($P = 0.01$) and infectiousness ($P = 0.05$) were significantly higher than those of the Taiwanese and Indonesian cultures and significantly lower ($P = 0.01$) than those of the Indian culture.

There was no difference among plants inoculated with uredospores of the four cultures in the time that elapsed between inoculation and the appearance of incipient lesions; this was 7 days in each of the three experiments.

On the upper leaf surface, the Australian culture produced significantly fewer ($P = 0.01$) uredia per lesion than did the other three cultures (Fig. 1). The Taiwanese culture produced the most (3–4 per lesion), followed closely by the Indonesian and Indian cultures, respectively. Little increase in uredia production with time was noted with any of the cultures.

On the lower leaf surface at 2 wk after incoulation, the number of uredia for each culture was about the same as that on the upper leaf surface (Fig. 1). With time, however, new uredia continued to appear in all cultures. At 7 wk after inoculation, there were 4–6 times as many uredia per lesion than at 2 wk. The Australian culture again produced the fewest uredia, eight per lesion at the end of 7 wk compared to a range of 12.6–14.0 per lesion for the other three cultures.

The mean lesion areas on both leaf surfaces 2 wk after inoculation were very similar (0.61 mm^2–0.77 mm^2) for all cultures except the Australian, which averaged 0.30 mm^2 (upper surface) and 0.43 mm^2 (lower surface). At 7 wk after inoculation, the upper/lower average lesion areas (mm^2) for the cultures were: Indian, 1.81/1.76; Indonesian, 1.70/1.89; Taiwanese, 1.75/2.01; and Australian, 1.43/1.45.

Microscopic measurements of freshly harvested uredospores (100 of each culture) resulted in the following estimates of average widths and lengths (μm): Indian, 14.6 × 20.5; Indonesian, 15.9 × 21.9; Taiwanese, 15.2 × 21.6; Australian, 15.6 × 21.6. These were not significantly different.

The number of uredospores per gram was determined from counts of spores in liquid suspension in a hemacytometer. The average value was 4×10^8.

Spore production. Nine days after inoculation, very sparse sporulation was evident on plants inoculated with any of the four cultures. At this time there were no obvious differences in sporulation between upper and lower leaf surfaces or among cultures.

Spore production of each culture was plotted as the mass of fresh uredospores collected per lesion per day (Fig. 2). Actually, the first

TABLE 1. Percentage germination[a] of uredospores of four cultures of *Phakopsora pachyrhizi* and primary disease[b] produced on leaves of Wayne soybean 10 days after inoculation

Culture	Experiment number					
	1		2		3	
	Germination (%)	Lesion/cm^2/mg	Germination (%)	Lesion/cm^2/ml	Germination (%)	Lesion/cm^2/mg
Indian	85[c]	1.87	12	1.05	16	1.14
Indonesian	32	.25	20	.84	27	.94
Taiwanese	27	.24	18	.76	26	.91
Australian	63	.44	22	.66	33	.84

[a] Each value is the mean of counts of 100 spores on each of four plates for each culture; germination substrate was 1.25% water agar.

[b] Figures represent number of lesions per square centimeter of inoculated leaf per milligram of inoculum. Each value is the mean of 88 observations (two unifoliolate leaves plus nine leaflets on the first three trifoliolate leaves of eight replicate plants).

[c] Data were processed by analysis of variance and Duncan's multiple range test. Comparable means within each column with no borderlining in common on the left side are significantly different, $P = 0.01$; those with no borderlining in common on the right side are significantly different, $P = 0.05$.

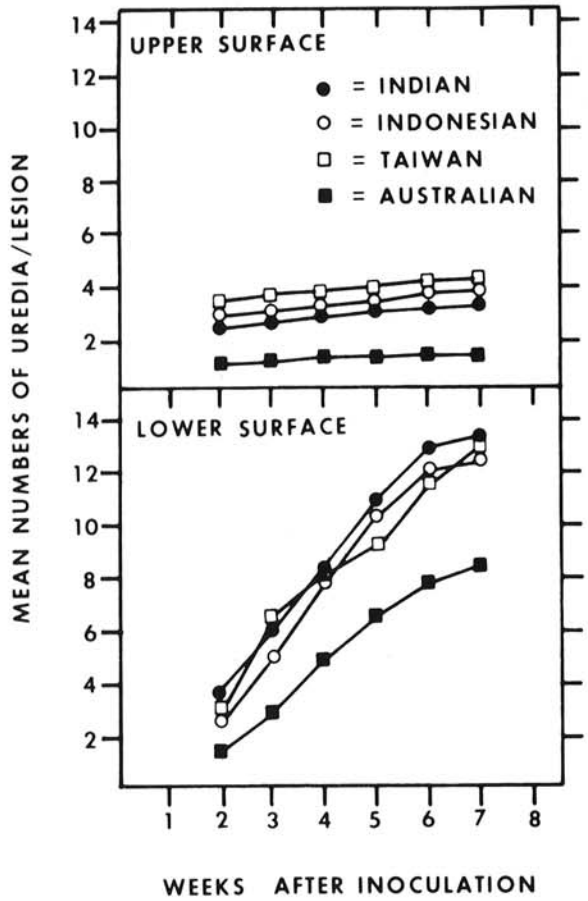

Fig. 1. Mean numbers of uredia per lesion produced on upper and lower surfaces of leaves of Wayne soybean inoculated with four cultures of *Phakopsora pachyrhizi*. Each point is the mean of 80 lesions observed, 20 on each of four replicate leaflets. Postinoculation dew period was at 19.5 ± 1 C for 16 hr in the dark.

harvest was of spores produced from the initiation of sporulation (about 9–10 days after inoculation) until the time of the first collection (13 days after inoculation). The fresh-weight figures can be converted approximately to the corresponding numbers of uredospores with the formula 1 μg = 400 uredospores. About 24 days after inoculation, leaflets began to be lost on plants in all groups by defoliation, so that subsequent harvests on such plants were from fewer lesions.

Initially, the Australian culture produced about 0.1 μg of spores per lesion, peaked at about 0.5 μg on the 3rd collection date, and by the 6th harvest had dropped to a fluctuating rate of 0.15–0.25 μg/day that continued for the next 3 wk. About 33 days after the first harvest, a steady decrease in sporulation began. During the period when sporulation was sufficient to permit quantitative collection of spores (39 days), production averaged 0.13 μg per lesion per day—this would correspond to 2,028 spores during the life of the lesion.

The Indian culture produced 0.65 μg per lesion per day on the first and second collecting dates and reached a maximum of 0.90 μg on the 6th day. Production then decreased and from 2 wk after the first harvest onward was similar to that of the Australian culture. An average of 0.24 μg per lesion per day over a 39-day period was collected, or 3,768 spores during the life of the lesion.

In general, the Taiwanese and Indonesian cultures were similar; during the first 5 days of collecting, however, the Taiwanese culture consistently produced more spores, reaching a maximum of 1.1 μg per lesion per day on the 4th and 5th days. Both cultures continued to produce from about 0.4 to 0.75 μg per lesion per day until 3–4 wk after the first collection. The Taiwanese culture produced an average of 0.42 μg per lesion per day over a 39-day period, or 6,600 spores during the life of the lesion. The corresponding figures for the Indonesian culture were 0.40 μg per lesion per day and 6,268 spores.

Peaks in spore production common to all or most of the cultures occurred at 16–17, 24–26, 31, and 36 days after inoculation (Fig. 2). These peaks followed 2–3 successive days of sunny weather; however, increased spore production did not always follow 2 or more days of bright sunshine. Some lesions on plants inoculated with each culture continued to produce a few spores until about 55 days after inoculation. No marked differences in viability of spores were noted among the cultures, with germination percentages

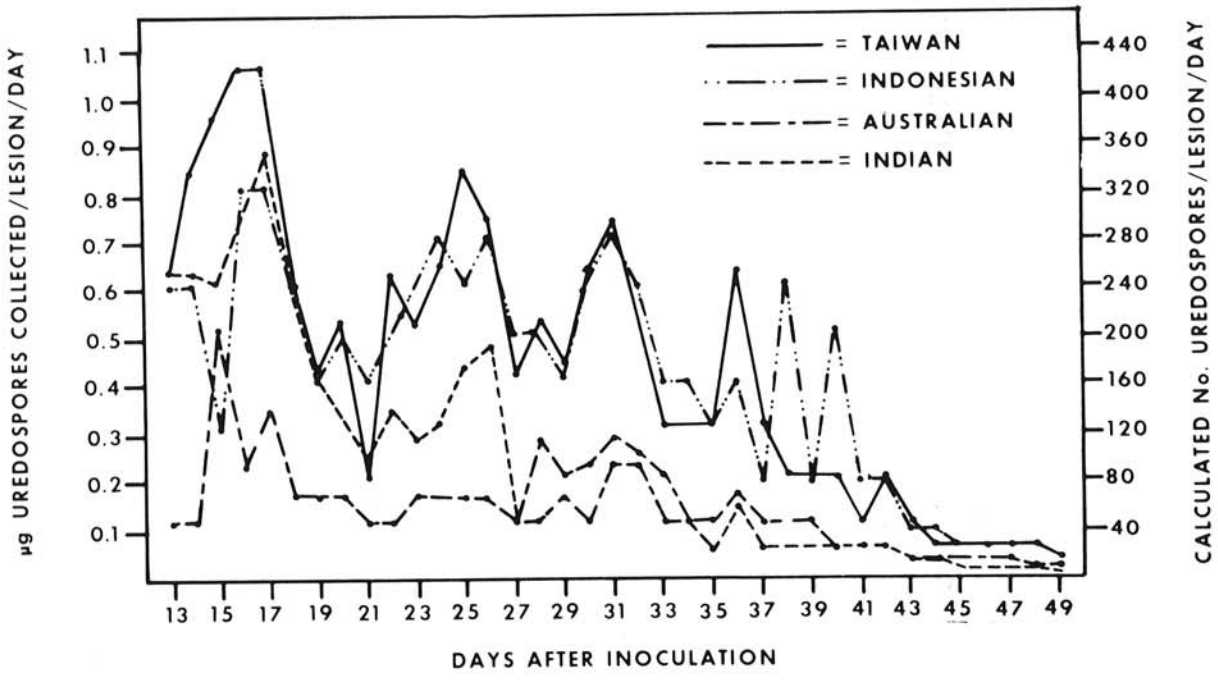

Fig. 2. Mass (fresh weight) and calculated number of uredospores collected daily from Wayne soybean plants inoculated with four cultures of *Phakopsora pachyrhizi*.

ranging from 10–42% until the last few days, when values below 8% were observed.

DISCUSSION

The four cultures were similar in uredospore size, the germinability of fresh uredospores on water agar, the time required to induce macroscopic symptoms (lesions), the time required to begin sporulation, and the duration of infectious periods. Significant differences among cultures occurred with the other characteristics that were studied. Whether these differences can be related to "ecologic races" or perhaps to different formae speciales remains to be determined.

In terms of disease during a single pathogen life cycle: the number of lesions produced per unit of leaf tissue per unit of inoculum; the time required from penetration until lesions appeared; and the area of the lesions at given times after penetration are all important criteria for assessing pathogen aggressiveness. Based on these criteria we can separate the Indian from the other cultures as being most aggressive and the Australian from the others as the least aggressive on Wayne soybean; the Taiwanese and Indonesian cultures cannot be dintinguished from each other by these criteria.

In terms of disease over the entire developmental cycle of the suscept plant or crop, however, there is superimposed on the parameters given above the factor of secondary inoculum production, with such considerations as quantity, quality, and hardiness (differential responses to the important environmental factors affecting inoculum were not considered here). In quantity of spores produced, the Taiwanese and Indonesian cultures were nearly equal and more prolific than the Indian—a difference that should tend to compensate for the differences in inoculum effieciency reported earlier. The Australian culture produced substantially fewer spores, perhaps because of its fewer uredia per lesion (from 3–7 wk after inoculation it had only about 40–56% as many as the other cultures).

Although the lesion has been used here as the unit of disease, it is the uredium that is the unit of spore production. Because the numbers of uredia increase 4–8 times from 3–7 wk while spore production remains at the same level or decreases, it might be assumed that the number of spores produced per uredium decreases drastically with time. There is histological evidence, however, that an individual uredium only produced spores for about 3 wk (7). The number of *active* uredia per lesion on the lower surface, therefore, may remain fairly constant from about 4 wk after inoculation.

When the sporulating capacity of *P. pachyrhizi* was compared with that reported for some other rust-causing organisms on the basis of cumulative numbers of spores produced per lesion, we found: soybean rust, 0.7×10^4 (Taiwanese culture); leaf rust of wheat, 2.8×10^4 (4); southern corn rust, 4×10^4 (3); stem rust of wheat, 10×10^4 (10); and bean rust, 7×10^4 (13). The other organisms produced 4–16 times as many uredospores per lesion as did *P. pachyrhizi*. In soybean rust only a small portion ($< 15\%$) of the lesion area is comprised of sporogenous tissue, whereas with the other rusts nearly all of the obviously affected area produces spores. Also, the soybean rust lesion is rarely as large as 1.75 mm^2; most of the rusts mentioned above have mature pustules ranging from about 1.8 mm^2 (southern corn rust) to perhaps 20 mm^2 (stem rust of wheat).

The rate at which uredospores of *P. pachyrhizi* were formed was relatively slow for a rust pathogen. For example, at 14 days after inoculation *Uromyces phaseoli* on Pinto bean had produced 0.8×10^4 to 9×10^4 spores per pustule, depending on pustule density (13), whereas *P. pachyrhizi* (Taiwanese culture) on Wayne soybean had produced $\sim 0.06 \times 10^4$. By 20 days, most bean rust pustules had completed sporulation, and pustules at densities of 6–15/cm^2 (comparable with the lesion density in the soybean rust studies) had produced $5.3–13.3 \times 10^4$ uredospores per pustule, whereas *P. pachyrhizi* (Taiwanese culture) had produced 0.25×10^4, about 39% of its eventual 39-day total. The estimated number of spores produced by *P. pachyrhizi* is somewhat lower than the actual number of spores produced, because some of the spores escaped collection. We believe this percentage was small, however; the uredospores of *P. pachyrhizi* tend to clump and stick together in the lesion, a characteristic that may have decreased the number carried away by air currents during the harvesting operation (the infectious units in nature are usually probably clumps of six or more spores).

Soybean rust differs in many ways from what we have come to expect from our familiarity with the well-known rust diseases on other important crops. In soybean, *P. pachyrhizi* causes extensive necrosis of tissue in and around the penetration site (5); it is later—sometimes weeks later—that productive uredia appear within this necrotic zone. This is not typical of the majority of rust fungi. In soybean rust, the living hyphae must connect these uredia to food and water sources in living cells at distances up to perhaps 1 mm.

Among the four cultures of *P. pachyrhizi*, differences were found in some of the factors affecting multiplication of the fungus and possibly the rate of increase of the rust in nature. Reproduction in even the most prolific of the cultures was less than that found in some commonly studied rust-causing organisms. Although these studies were done in the greenhouse, there is no reason to believe that the characteristics would not be expressed in the field. The data should advance our understanding of the epidemiology of soybean rust, but an analysis of the threat of rust to soybeans in the United States must await the results of other studies involving the potential host range and pathogen survival. It is a matter of record that soybean rust can increase and spread rapidly enough to cause substantial yield losses (2,9,12).

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