

## The Effects of Temperature and Dew Period on Germination and Infection by Uredospores of *Phakopsora pachyrhizi*

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### ABSTRACT

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Uredospores of *Phakopsora pachyrhizi* germinated between 10 C and 28.5 C, with a broad optimum in the range 15-25 C. Spores of isolates from Australia, India, Indonesia, and Taiwan germinated over similar temperature ranges, but the optimal temperature range for the Indian isolate was narrower than that for the other isolates. Maximal infection of Wayne soybean leaves occurred at 20-25 C with 10-12

hours of dew and at 15-17.5 C with 16-18 hours of dew. The minimal dew period for infection was 6 hours at 20-25 C, and 8-10 hours at 15-17.5 C. Infection did not occur above 27.5 C. It appears that temperature-moisture requirements for infection of soybeans by uredospores of *P. pachyrhizi* would not preclude the establishment of the soybean rust fungus in major soybean growing areas of the U. S.

*Additional key words:* soybean rust, *Glycine max*, soybean, epidemiology, epiphytology, etiology.

Soybean rust, caused by *Phakopsora pachyrhizi* Syd., is not known to be present in the Americas. This disease is being studied within containment facilities of the Plant Disease Research Laboratory as part of a program to assess the vulnerability of U.S. soybean crops to nonendemic pathogens.

Most of the research on soybean rust has been conducted in the Orient. The English summary of an extensive study by Kitani and Inoue (3) stated that uredospores of *P. pachyrhizi* germinated at temperatures from 8-32 C, 25 C being optimal, and that penetration could occur within 6 hours at 24 C. In a later study in Taiwan, Hsu and Wu (2) reported that germination occurred at temperatures between 9 and 26 C (21 C optimal) and that penetration occurred within 5 hours at optimal temperature. Free moisture was required for germination and infection (2, 3, 4). In a histological study, Marchetti et al. (4) found hyphae in soybean mesophyll 20 hours after inoculation with uredospores of *P. pachyrhizi* and frequently observed direct penetration from appressoria formed at the end of short germ tubes, usually less than 20  $\mu$ m long. We report here, for uredospores of *P. pachyrhizi*, effects of temperature on germination and effects of temperature-dew period combinations on infection.

### MATERIALS AND METHODS

**Germination.**—Uredospores from freshly sporulating pustules of a strain of *P. pachyrhizi* from Taiwan were collected by tapping infected soybean leaves over glassine paper. The spores then were deposited on the inverted covers of 35-mm disposable petri dishes exposed in a

small settling tower. Then the spore-laden covers were placed over 1.25% water agar (Difco Bacto) in 35-mm diameter plastic petri dishes that were previously positioned and equilibrated on a temperature gradient plate. The tops were tapped sharply to deposit the spores on the agar surfaces. The spores were incubated in darkness at discrete temperatures in the range 5.5 to 31  $\pm$  0.2 C. Germination percentages were determined for incubation periods of 4 and 22 hours.

Another experiment was conducted in similar fashion to compare the germination-temperature responses of four isolates of *P. pachyrhizi*, one each from Taiwan, Australia, Indonesia, and India. Uredospores of the Taiwan, Indonesia, and India isolates were harvested from infected soybean plants within 3 hours before deposition on the agar plates. Uredospores of the Australia isolate were removed from cryogenic storage, heat-shocked at 41 C for 6 minutes, and hydrated for 1 hour before deposition on agar (1). Average percentages were determined after an incubation of 6 hours, based on observations of 200 or more spores for each temperature.

**Infection.**—The effects of various temperatures and dew periods upon initial infection of soybeans by uredospores were compared. Wayne soybeans were grown in the greenhouse in 1.1-liter plastic pots, two plants per pot. Plants were thinned to one per pot when the second trifoliolates were expanding and inoculated when the second trifoliolates were fully expanded. The period between planting and inoculation lengthened gradually from 20 days to 32 days as the natural photoperiod in the greenhouse became shorter.

Test plants in groups of eight were exposed to 10-mg spore releases in a large turntable tower (5). Inoculum was

freshly harvested uredospores of the Taiwan isolate. All plants from a given inoculation were placed in dew chambers at the same time immediately following inoculation. For each inoculation, plants were incubated at 20 C and at one other temperature.

Plants were incubated at temperatures of 15, 17.5, 20, 22.5, and 27.5 C for dew periods of 4 to 12 hours or longer, in 2-hour increments. Six replicate plants were used for each temperature-time combination. As plants were removed from the dew chambers, they were allowed to dry, and then placed in a greenhouse with diurnal fluctuations in the range 19-30 C.

Pustules were counted 12-14 days after inoculation. A small plastic petri dish lid on which three 1-cm<sup>2</sup> circles had been scribed was superimposed on the upper surface of each leaflet and the number of lesions within each circle was recorded. Counts were obtained from each leaflet of the first and second trifoliolates of each plant; i.e., a total of 18 cm<sup>2</sup> of leaf surface per plant was sampled.

Within each test, the data were converted to an index using the maximum number of lesions recorded after 12 hours or more of dew at 20 C as unity. This permitted comparison among results from all temperature-time combinations. Raw data could not be compared validly among runs because of day-to-day differences in the infectivity of inoculum and other factors that were not controlled. Maximum infection among individual tests at 20 C varied from 0.3 to 3.2 lesions/cm<sup>2</sup> of leaf per mg of inoculum.

## RESULTS AND DISCUSSION

**Germination.**—After 4 hours of incubation, germination of uredospores of *P. pachyrhizi* had occurred in the range 12 to 28.5 C; after 22 hours, in the range 10 to 28.5 C. Germination declined rapidly between 25 and 28.5 C and ceased between 28.5 and 31 C. Temperatures in the range 15-25 C were favorable for germination at both 4 and 22 hours of incubation (Fig. 1).

In the comparative test of four isolates, no germination occurred below 7 C or above 28 C. Temperatures between 14 C and 24 C were favorable for germination of all four isolates. The isolate from India seemed to have a somewhat narrower optimum range than the other three, but it still showed appreciable capacity to germinate over a wide range of temperature (Fig. 2).

**Infection.**—At 20 to 25 C, about 6 hours was the minimum dew period that permitted infection; at 10 to 12 hours, infection was maximum and was not increased by extending the dew period. At 17.5 and 15 C, the minimum dew periods that permitted infection were 8 and 10 hours, respectively. At these temperatures, increasing the length of the dew period up to 18 hours resulted in increased infection. Plants incubated at 27.5 C were free of infection regardless of the length of dew period (Fig. 3). Over the range 15-25 C, the temperature requirements for germination and infection appear essentially similar.

We conclude that the temperature-moisture conditions in the major soybean-growing areas of the U. S. would be favorable for infection of soybeans by uredospores of *P. pachyrhizi*.

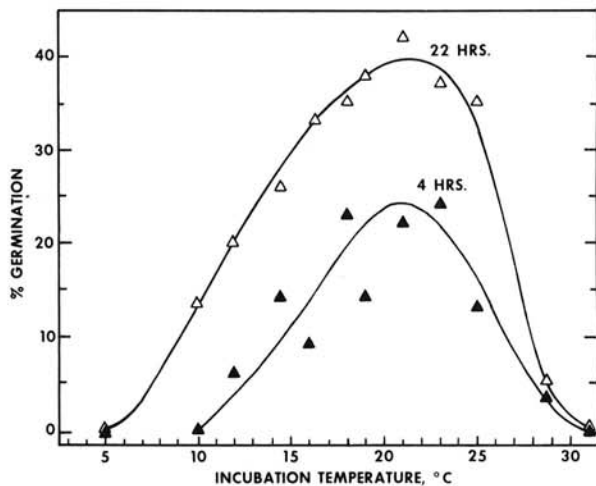


Fig. 1. Germination of uredospores of *Phakopsora pachyrhizi* (Taiwan isolate) after 4 hours and 22 hours in darkness on 1.25% water agar at various temperatures.

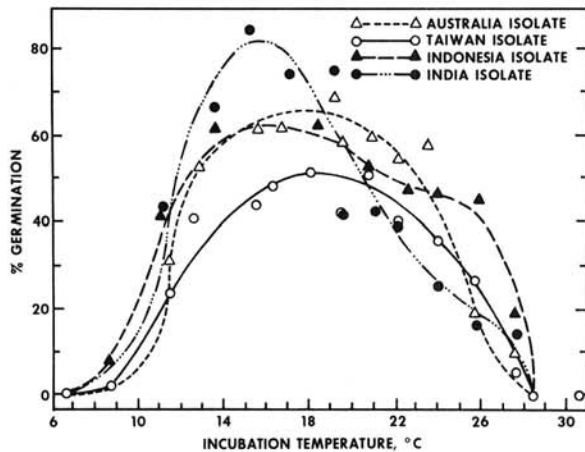


Fig. 2. Germination of uredospores of four strains of *Phakopsora pachyrhizi* after 6 hours in darkness on 1.25% water agar at various temperatures.

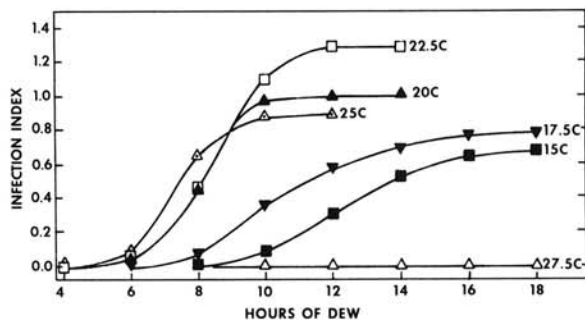


Fig. 3. Effects of dew period and temperature on infection of Wayne soybean leaves by *Phakopsora pachyrhizi* (Taiwan isolate). The infection index is the number of pustules per square centimeter at each specific temperature-dew period divided by the number of pustules per square centimeter at 20 C-12-hour dew period.

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