Effects of Temperature and Light Intensity on Telia Development by Puerto Rico and Taiwan Isolates of *Phakopsora pachyrhizi*, the Soybean Rust Fungus

L. A. DUFRESNE and G. A. BEAN, Department of Botany, University of Maryland, College Park 20742; M. R. BONDE, Plant Disease Research Laboratory, USDA, ARS, Frederick, MD 21701; and R. W. GOTH, USDA, ARS, Beltsville, MD 20705

ABSTRACT

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Isolates of *Phakopsora pachyrhizi*, one from Taiwan and one from Puerto Rico, were cultured on the soybean cultivar Williams at two temperatures and three light intensities. 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. At low light intensity (3.9 $\mu E/m^2/sec$), the Taiwan and Puerto Rico isolates produced telia after 29 and 33 days, respectively; at intermediate light intensity (5.3 $\mu E/m^2/sec$) after 26 and 36 days, respectively; and at high light intensity (6.1 $\mu E/m^2/sec$) after 22 and 34 days, respectively. The Taiwan isolate produced larger lesions with a higher percentage of telia than the Puerto Rico isolate.

Phakopsora pachyrhizi Syd., the causal agent of soybean (*Glycine max* (L.) Merr.) rust, has not been found on the U.S. mainland. All U.S. commercial soybean cultivars are susceptible to eastern hemisphere strains of this fungus, and environmental conditions in the United States are favorable for survival of the pathogen and subsequent disease development (3–5,7,11).

Infection by *P. pachyrhizi* causes premature defoliation and a concomitant decrease in yield (2). In susceptible plants, pustules form rapidly, leaf chlorophyll is destroyed, and seeds are malformed and shriveled (10). *P. pachyrhizi* also parasitizes other legume genera found in Puerto Rico and the United States (13,15,16). Only uredinia and telia stages are known (12). Urediniospores can infect soybean plants at any growth stage. Teliospore germination has not been observed (2,3).

According to Bromfield (3) and Bromfield et al (6), soybean cultivars are designated resistant or susceptible to soybean rust on the basis of lesion development. Plants with tan lesions (TAN), each with two to four abundantly sporulating uredinia, were rated susceptible; plants with reddish brown lesions (RB), with zero to two uredinia that sparsely sporulated, were rated resistant; and plants with no lesions to minute flecks were rated immune. On U.S. soybean cultivars tested, isolates of *P. pachyrhizi* from Taiwan are more virulent than those from Brazil and

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Puerto Rico; however, isolates from the western hemisphere cause only a TAN reaction (6). Because these isolates sporulate sparsely, Bromfield (3) suggested they have less epidemic potential and less yield reduction potential.

Several workers have studied the development of uredinia of *P. pachyrhizi*, but only a few have investigated factors affecting telia development. Yeh et al (18) compared the formation of teliospores of *P. pachyrhizi* on leaves of soybeans and other legume hosts at 23–25 C and minimum night temperature for a 12-hr photoperiod (2,060 lux). Hsu and Wu (8) reported teliospore formation of *P. pachyrhizi* on inoculated plants 36 days after being placed in incubators at 15–20 C; no teliospores formed at 30 C. They suggested that "cool" temperatures induce or "warm" temperatures inhibit telia formation. Yeh et al (17) reported that telia formed on detached leaves in incubators after 2 wk at 10 and 15 C and after 3 wk at 20 C but not at 25 C. Under field conditions, no telia were present if the average maximum daily temperature was greater than 29 C. Yeh et al (17) concluded that the absence of low field temperatures may explain the scarcity of *P. pachyrhizi* telia on diseased plants growing in the tropics. Kochman (9) also reported that temperature is the most important factor affecting telia formation by the soybean rust fungus.

The purpose of this study was to compare telia formation by a Puerto Rico isolate and a Taiwan isolate of soybean rust at two temperatures and three light intensities.

MATERIALS AND METHODS

Two *P. pachyrhizi* isolates were used in these studies. The Taiwan isolate was obtained from diseased soybean leaves provided by Lung-chi Wu, AVRDC, Shanhua, Taiwan, and the Puerto Rico isolate was obtained from soybeans in Puerto Rico by N. G. Vakili and K. R. Bromfield (6). The isolates were maintained either on susceptible plants or stored as urediniospores in sealed ampules under liquid nitrogen. The Taiwan isolate, representative of the eastern hemisphere isolates, produced TAN-type lesions on several soybean cultivars. The Puerto Rico isolate, typical of the western hemisphere isolates, produced RB lesions on soybeans.

The soybean cultivar Williams was used. One or two plants per pot were grown in the greenhouse in 10-cm clay pots containing a mixture of soil, sand, peat, and perlite (2:1:1:1, v/v). The growing tips of seedling stems were pinched off to force axillary bud development. This provided each plant with two second trifoliolate leaves for inoculation.

Plants were inoculated at the third- or fourth-trifoliolate-leaf stage of development about 4–6 wk after planting. Only the second trifoliolate leaf was inoculated. Sixty plants were inoculated with each isolate according to a technique adapted from Sackston (14). Ten milligrams of urediniospores was kept suspended in an aqueous Tween 20 solution (0.25%) and painted onto separate labeled leaflets with a camel's-hair brush. Immediately after inoculation, plants were put in a dew chamber in the dark for 16–22 hr at 20 C (4), then removed to a shaded room for a minimum of 1 hr before being returned to the greenhouse bench.

To study the influence of light and temperature, inoculated plants were incubated for 14 days in the greenhouse until lesions developed and then placed in growth chambers at 10 or 15 C at varying light intensities. Reduced light intensities were achieved by draping cheesecloth over inoculated plants. Four layers of cheesecloth caused a 36% light reduction to 3.9 $\mu E/m^2/sec$, and two layers reduced light by 13% to 5.3 $\mu E/m^2/sec$. A photometer model I L150 (International Light, Inc., Newberryport, MA) was used to measure intensity at the second trifoliolate leaf. Intensities of red (600–700 nm) and blue (400–500 nm) wavelengths were measured and converted into Einstein units. Temperature of inoculated leaves was measured with a Yellow Springs telethermometer, model 44 TD (Yellow Springs Instrument Co., Yellow Springs, OH); thermometer probes were attached to adaxial leaflet surfaces with cellophane tape. For each treatment, five leaflets were examined, each on a different plant growing in separate pots. The date of the first appearance of telia after inoculation was recorded along with the percentage of lesions with telia in four 1-cm^2 leaf areas on each leaflet.

Telia development also was studied in two growth chambers regulated for 12 hr of fluorescent light (6.1 $\mu E/m^2/sec$), 82–99% RH, and a temperature of 10 C. A treatment consisted of a rust isolate in a chamber. At weekly intervals, three leaflets were collected at random from separate plants for each treatment. Leaflets were cut into 2-cm^2 sections, cleared in glacial acetic acid and ethanol (1:2, v/v), stained with 0.1% cotton blue in lactophenol, and examined for presence of lesions and telia (1).

Leaf tissue was examined with a light microscope (15–30×) to determine the date of appearance of telia and also the percentage and number of lesions with telia and the size of telia on each leaflet at each sampling time. The measurement of telial dimensions and the count of the telia within a sampled tissue were made at 400× with a bright-field compound microscope. Telia both inside and outside the necrotic zone were counted for each of six randomly chosen telia-bearing lesions of each culture. Telial length × width was used as an index of telial area. To compare number of telia per lesion and also telial areas, a two-way analysis of variance was used at each sampling time ($P = 0.05$). Each trial consisted of six replicates for the number of telia per lesion and 10 replicates for telial area.

To compare telial areas over sampling times, a split-plot design was used, with incubation intervals as main plots and rust isolates as subplots. A logarithmic transformation was used for the data on telial area. An *F*-test was performed on the ratio of the mean square of A × B to the mean square (MS) within groups to determine when sample variances were homogenous; if they were, the MS within was used as the error term, otherwise a pooled MS was used as the best available estimate of sample variance.

RESULTS AND DISCUSSION

Effects of temperature and light on telia formation. All leaves of the cultivar Williams inoculated with the Puerto Rico isolate developed typical RB-type lesions, and leaves inoculated with the Taiwan isolate developed typical TAN-type lesions. The color differences were detected macroscopically. When these lesions were observed at 150×, the necrotic cells in leaves with the Puerto Rico isolate induced RB-type lesions and the attendent leaf veins had a deeper mahogany-brown pigmentation than leaves inoculated with the TAN Taiwan isolate. The influence of temperature and light intensity on time required for *P. pachyrhizi* to produce telia is summarized in Table 1. On the average, the Taiwan isolate produced telia more rapidly than the Puerto Rico isolate under similar temperatures and light conditions. For example, at low light intensity (3.9

Table 1. Comparison of the effects of temperature and light on the rate of telia production by two isolates of *Phakopsora pachyrhizi* on Williams soybean[z]

Light intensity ($\mu E/m^2/sec$)	Taiwan isolate (temp. [C])			Puerto Rico isolate (temp. [C])		
	10	15	Mean	10	15	Mean
Low (3.9)	23	35	29 b	38	28	33 c
Medium (5.3)	23	28	26 b	35	38	36 c
High (6.1)	16	28	22 a	30	38	34 c
Mean	21 a	30 b		34 c	35 c	

[z] Minimum number of days for lesions with telia to form on five leaflets; numbers followed by the same letter are not significantly different ($P = 0.05$).

$\mu E/m^2/sec$) and 10 C, the Taiwan isolate produced telia after 23 days, but it was 38 days before the Puerto Rico isolate produced telia. The Puerto Rico isolate, however, produced telia more rapidly at 15 C and low light intensity. The Taiwan isolate was more temperature and light sensitive than the Puerto Rico isolate. Time required for telia production for the Puerto Rico isolate under all light and temperature conditions ranged from 33 to 36 days, whereas time required for telia production by the Taiwan isolate varied from 21 to 30 days.

Telia development by *P. pachyrhizi* isolates. In these experiments, telia development by the two isolates of *P. pachyrhizi* were compared under similar light, RH, and temperature conditions. As in the previous experiments comparing the time required for telia formation, the Taiwan isolate developed mature telia more rapidly than the Puerto Rico isolate. Telia first appeared 21 days after inoculation with the Taiwan isolate and 29 days after inoculation with the Puerto Rico isolate. There were also other differences between the two isolates. For example, the Taiwan isolate had a higher ($P = 0.05$) percentage of lesions with telia than the Puerto Rico isolate (5 vs. 34%). The number of telia per lesion, however, was the same for the two isolates (5 vs. 6). Telia produced by the Taiwan isolate also were larger ($P = 0.05$) than those produced by the Puerto Rico isolate (0.050 vs. 0.096 mm^2, respectively). There was no significant change in telial size with time (32 vs. 63 days) with either isolate.

This is the first comparison of telia formation by isolates of *P. pachyrhizi* from the western and eastern hemispheres. The response of the Taiwan isolate to temperature was similar to the results reported by Yeh et al (17). The Puerto Rico isolate was affected little by either temperature or light except at low light intensity. Additional studies using a greater number of temperatures and light intensities and using a number of isolates of *P. pachyrhizi* from diverse geographical areas should be done to better understand the effect of light and temperature on the formation of telia.

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