Comparative Susceptibility of Kudzu Accessions from the Southeastern United States to Infection by *Phakopsora pachyrhizi*

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ABSTRACT

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Soybean rust, caused by *Phakopsora pachyrhizi*, was first discovered in the continental United States in the fall of 2004. The potential for economic loss in the United States hinges largely on whether or not the pathogen can survive winters in the absence of soybean. Kudzu (*Pueraria lobata*) is known to be a host for *P. pachyrhizi* in Asia and South America and is widely distributed in the southern United States. This study examined reactions of kudzu collected from several areas of the southeastern United States to three isolates of *P. pachyrhizi*, one each from Alabama, Louisiana, and Brazil. Susceptible tan (TAN) lesions, resistant reddish-brown (RB) lesions, and immune (IM) response, previously described on soybean, were produced on kudzu based on the evaluation of 125 plants. However, in contrast to soybean, the RB response on kudzu was common, with approximately 50% frequency. IM responses to at least one isolate were observed on five individual plants, and two plants were immune to all three pathogen isolates used in the test. TAN lesions averaged 3.2 uredinia per lesion with an average diameter per uredinium of 121 μ m. In contrast, RB lesions had an average of 0.3 uredinia per lesion with an average uredinial diameter of 77 μ m. In 25 of 39 (64%) instances in which multiple plants were tested from a site, each reacted the same to the individual pathogen isolates. This suggested a tendency for plants at specific sites to be genetically identical with respect to rust reaction. Only 19 of 125 (15%) individual plants produced a different reaction to one isolate than to the other two isolates. When four kudzu plants previously shown to produce only TAN lesions to *P. pachyrhizi* isolates Alabama 04-1, Brazil 01-1, and Louisiana 04-1 were inoculated with eight additional isolates from several areas of the world, all 11 isolates produced only TAN lesions. Likewise, when five other plants previously shown to produce only RB lesions when inoculated with the three isolates were inoculated with the 11 isolates, all produced only RB lesions. These results suggest that susceptibility or resistance to *P. pachyrhizi* in individual kudzu plants often is broad, extending over a wide range of *P. pachyrhizi* isolates.

Soybean rust, incited by *Phakopsora pachyrhizi* Syd., was discovered for the first time in the continental United States in 2004 (11), but as of 2008 it has caused little damage. Research on the disease at the United States Department of Agriculture (USDA), Foreign Disease-Weed Science Research Unit began in 1972, and

until recently it was conducted only in the USDA Biosafety Level-3 plant disease containment facility at Fort Detrick, MD (9) or in foreign countries where the disease existed in the field (5).

During early studies at USDA, Melching et al. (8) and Bromfield (5) recognized the importance of legumes, other than soybean, as a possible bridge on which *P. pachyrhizi* could survive winters in certain areas of the United States. Bromfield (5), and more recently Bonde et al. (3), suggested that the prolific leguminous weed kudzu (*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Sanjappa & Pradeep), in areas where leaves did not freeze, might be that bridge. Bonde et al. (3) showed that at least some kudzu accessions from the United States were highly susceptible to soybean rust and produced copious amounts of urediniospores.

Kudzu, a perennial semi-woody, climbing vine, is a leguminous plant belonging to the tribe Phaseoleae Benth., subtribe Glycininae Benth. (according to International Legume Database & Information

Service Web Site: <http://www.ildis.org/LegumeWeb>), and was first introduced into the United States in 1876 as an ornamental plant (4). Its nearest relative in the continental United States is soybean (*Glycine max* (L.) Merr.) (4). *P. phaseoloides* (Roxb.) Benth., although a closer relative, is established in Hawaii and Puerto Rico, and not in the continental United States. By the 1930s and 1940s, kudzu became highly promoted by agriculturalists in Alabama, Georgia, and Mississippi as a means of decreasing soil erosion (4). The Federal Soil Erosion Service eventually paid farmers to plant kudzu on more than 1.2 million acres, and kudzu became promoted as a drought-resistant, high-nitrogen forage crop. But kudzu had several major problems, including the difficulty that farmers had in baling the hay and the tendency for the crop to grow so rapidly and extensively that it covered all other vegetation. As a result, in 1998 Congress placed kudzu on the federal list of noxious weeds. Today, it is estimated to infest between 2 and 7 million acres, primarily in the southeastern United States (4).

The research presented here expands upon a previous study in which we compared several leguminous species as hosts for *P. pachyrhizi* (3). Here we compare reactions of 125 kudzu plants, most from Mississippi, to infection by three pathogen isolates, one each from Alabama, Louisiana, and Brazil. The results should help determine the relative frequencies of resistance and susceptibility to *P. pachyrhizi* in kudzu and the possible role this host might play in the over wintering of *P. pachyrhizi* in the southeastern United States.

MATERIALS AND METHODS

Pathogen isolates. Three isolates of *P. pachyrhizi* were used to inoculate each kudzu plant in the study. Brazil 01-1 was a field collection of urediniospores collected in Brazil in 2001 by J. T. Yorinori, EM-BRAPA SOJA, Londrina, Brazil, and sent to R. Frederick at the FDWSRU on infected leaves under an appropriate APHIS permit. Alabama 04-1 was collected in 2004 by R. D. Frederick and M. R. Bonde in Mobile County, AL, and Louisiana 04-1 was collected in 2004 by R. Schneider (Louisiana State University) from the Ben Hur location, site of the first discovery of soybean rust in the continental United

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States, Baton Rouge, LA. The Alabama and Louisiana isolates, following collection, were purified by repeated serial passage as single pustules on soybean cv. Williams prior to this study.

In an experiment to better understand the breadth of resistance, or susceptibility, to soybean rust in individual kudzu plants, eight *P. pachyrhizi* isolates were used to inoculate a set of four plants that previously had produced only tan (TAN) lesions with Alabama 04-1, Brazil 01-1, and Louisiana 04-1. A second set of four plants that had produced only reddish-brown (RB) lesions with the same three isolates likewise was inoculated with the eight additional *P. pachyrhizi* isolates. Due to the death of one plant during the experiment, a replacement plant was added to the experiment and inoculated with some of the isolates.

All isolates were maintained on cv. Williams soybean. Periodically, urediniospores were collected from infected plants by means of a vacuum spore collection device (7) and stored in a liquid nitrogen refrigerator for later use in experiments or for long-term preservation. All pathogen isolates used in this study currently are being maintained in liquid nitrogen storage at FDWSRU.

Kudzu accessions. Pods from kudzu plants at each of 56 geographically distinct locations in the southeastern United States (Table 1) were collected and sent to the laboratory at Fort Detrick, where seeds were removed from the pods. Twenty-two collections were made in 2005 and are referred to in this publication as "Set #1", and 34 were made in 2006 and referred to as "Set #2". Geographic coordinates were recorded for all 51 locations in Mississippi.

Kudzu seeds were soaked in concentrated sulfuric acid for 2 h, rinsed 3 to 4 min with tap water, and incubated on wet filter paper in petri dishes at 30°C (13). Following germination, seedlings were transplanted into soil in 10.2-cm-diameter clay pots and placed in a greenhouse for approximately 3 months to allow sufficient plant growth for use in experiments.

Inoculation and maintenance of plants. One day prior to inoculation, urediniospores of the three *P. pachyrhizi* isolates were removed separately from liquid nitrogen storage, heat shocked while dry for 5 min at 40°C, and then hydrated for 16 h at 100% RH by placement over water in a closed glass petri dish at room temperature (2). Hydrated urediniospores were suspended in distilled water containing one drop of Tween 20 per 100 ml, enumerated by means of a hemacytometer, and adjusted to a final concentration of 2.5 to 3.5×10^4 urediniospores/ml. Each of three trifoliolate leaves on each plant in an experiment was inoculated by painting with a suspension of urediniospores of a single isolate using a 1-in.-wide bristle brush.

Table 1. Lesion types for specific kudzu (*Pueraria montana* var. *lobata*) plants grown from seeds obtained from 56 field locations in southeastern United States inoculated separately with three isolates of *Phakopsora pachyrhizi*^a

Year/location ^c	Plant identity	Pathogen isolate ^b		
		Alabama 04-1	Brazil 01-1	Louisiana 04-1
2005				
1	A ^d	RB ^e	RB	TAN ^f
1	B	TAN	TAN	TAN
2	A	RB	RB	TAN
3	A	TAN	TAN	TAN
3	B	TAN	TAN	TAN
3	C	TAN	TAN	TAN
4	A	RB	TAN	TAN
5	A	TAN	TAN	TAN
5	B	TAN	TAN	TAN
5	C	TAN	TAN	TAN
6	A	RB	RB	RB
6	B	RB	RB	RB
7	A	RB	RB	TAN
7	B	RB	TAN\RB ^g	TAN
8	A	TAN	TAN	TAN
8	B	TAN	TAN	TAN
9	A	RB	RB	RB
10	A	TAN	TAN	TAN
10	B	TAN	TAN	TAN
11	A	TAN	TAN	TAN
11	B	TAN	TAN	TAN
12	A	RB	RB	RB
12	B	RB	RB	RB
12	C	RB	RB	RB
13	A	TAN	TAN	TAN
13	B	TAN	TAN	TAN
13	C	TAN	— ^h	TAN
14	A	TAN	TAN	TAN
14	B	RB	RB	RB
14	C	RB	RB	RB
14	D	RB	RB	RB
14	E	RB	RB	RB
14	F	TAN	TAN	TAN
14	G	TAN	TAN	TAN
15	A	TAN	TAN	TAN
16	A	RB	RB	RB
16	B	RB	TAN/RB	RB
16	C	RB (IR) ⁱ	RB (IR)	RB (IR)
17	A	TAN	TAN	TAN
17	B	RB	RB	RB
18	A	TAN	TAN	TAN
18	B	TAN	TAN	TAN
19	A	TAN	TAN	TAN
20	B	TAN	TAN	TAN
21	B	RB	RB	RB
22	B	RB	RB	RB
2006				
23	A	RB	RB	RB
23	B	RB	RB	RB
23	C	RB	RB	RB
24	A	RB	RB	RB
24	B	RB	RB	TAN
24	C	TAN	TAN	TAN

(Continued on next page)

^a Kudzu plants were inoculated by painting each of three leaves separately with a urediniospore suspension at 2.5 to 3.5×10^4 urediniospores/ml distilled water containing 1 drop Tween 20 per 100 ml. Inoculated plants were incubated overnight in dew chambers at 20°C, removed from the chambers the next morning, allowed to dry, and then placed in the greenhouse at 20 to 25°C for disease development. Two weeks after inoculation, plants were examined for symptoms.

^b Alabama 04-1 is a purified isolate from a field collection made in Alabama in 2004; Brazil 01-1 is a nonpurified field isolate from Londrina, Brazil; and Louisiana 04-1 is a purified isolate from a field collection from the Ben Hur Field Station, LA, initial site of discovery of soybean rust in the United States in 2004.

^c Plant locations 1 through 17 and 23 through 56 were in Mississippi. Plant locations 18 through 20 were in Louisiana, 21 in Kentucky, and 22 in North Carolina.

^d Each plant, with letter designation, is from the location immediately to the left.

^e RB = reddish-brown lesions, characterized by few or no urediniospores.

^f TAN = tan-colored lesions, characterized by numerous tan-colored urediniospores.

^g TAN\RB = a mixture of TAN and RB lesions on the inoculated leaf.

^h — Indicates missing data.

ⁱ RB (IR) indicates reddish-brown lesions in first experiment and no symptoms in second experiment.

Table 1. (Continued from previous page)

Year/location ^c	Plant identity	Pathogen isolate ^b		
		Alabama 04-1	Brazil 01-1	Louisiana 04-1
25	A	RB	RB	RB
26	A	RB	RB	RB
27	A	RB	RB	RB
28	A	RB	RB	RB
28	B	RB	RB	RB
28	C	RB	RB	RB
29	A	TAN	TAN	TAN
29	B	TAN	TAN	TAN
29	C	TAN	TAN	TAN
30	A	RB	RB	RB
30	B	RB	RB	RB
31	A	TAN	TAN	TAN
32	A	RB	RB	RB
32	B	RB	RB	RB
32	C	TAN	TAN	TAN
33	A	TAN	TAN	TAN
33	B	TAN	TAN	TAN
33	C	TAN	TAN	TAN
34	A	TAN	TAN	TAN
34	B	TAN	TAN	TAN
34	C	TAN	TAN	TAN
35	A	RB	RB	RB
35	B	RB	RB	RB
35	C	RB	RB	RB
36	A	RB	RB	RB
36	B	RB	RB	RB
36	C	RB	RB	RB
37	A	RB	RB	RB
38	A	RB	RB	RB
39	A	RB	RB	RB
40	A	RB	RB	RB
40	B	TAN	TAN	TAN
40	C	RB	RB	RB
41	A	RB	RB	RB
41	B	IM ⁱ	IM	IM
41	C	IM	IM	IM
42	A	RB	RB	RB
42	B	RB	RB	RB
42	C	RB	RB	RB
43	A	TAN	TAN	TAN
43	B	TAN	TAN	TAN
43	C	TAN	TAN	TAN
44	A	RB	RB	RB
44	B	RB	RB	RB
44	C	RB	RB	RB
45	A	RB	TAN	TAN
45	B	RB	TAN	TAN
46	A	TAN	TAN	TAN
46	B	TAN	TAN	TAN
46	C	TAN	TAN	TAN
47	A	RB	RB	RB
47	B	RB	RB	TAN
48	A	TAN	TAN	TAN
48	B	TAN	TAN	TAN
49	A	TAN	TAN	TAN
49	B	TAN	TAN	TAN
49	C	TAN	TAN	TAN
50	A	RB	RB	TAN
50	B	TAN	TAN	TAN
50	C	RB	RB	TAN
51	A	TAN	RB	TAN
52	A	TAN	TAN	TAN
52	B	TAN	RB	TAN
52	C	TAN	TAN	TAN
53	A	IM	TAN	TAN
54	A	RB	RB	RB
54	B	RB	RB	RB
55	A	RB	RB	TAN
55	B	TAN	TAN	TAN
55	C	RB	RB	TAN
56	A	TAN	TAN	TAN
56	B	IM	TAN	TAN
56	C	IM	RB	TAN
Soybean (control)	A-K	TAN	TAN	TAN

Leaves of soybean plants of the susceptible cv. Williams were inoculated similarly with each of the three pathogen isolates to serve as controls. Following inoculation, all plants were incubated overnight in dew chambers at 20°C, removed from the chambers, allowed to dry, and then placed in a greenhouse at 20 to 25°C for disease development.

After the first experiment was completed, the plants were allowed to regrow until sufficient new leaves were present for repeating the experiment using the same pathogen isolates.

Lesion type, density, and sporulation ratings. Two weeks after inoculation, data were collected for lesion types, relative lesion densities, and proportion of lesions that had sporulating uredinia. Lesion reactions, recorded 2 weeks after inoculation, were classified as TAN, RB, or IM, as described on soybean (2,6). TAN indicated lesions were tan-colored, RB that lesions were reddish to dark chocolate brown, and IM that there were no apparent symptoms. TAN and RB designations were assigned solely on lesion color; however, TAN reactions usually had much higher proportions of lesions with sporulating uredinia than RB reactions. Mixtures of TAN and RB lesions were noted. Photographs at $\times 7$ and $\times 25$ were taken of the surface of leaves with a Nikon Digital Sight DS-L1 digital camera mounted on an Olympus SZX12 dissecting microscope at the time of scoring.

Lesion densities were rated on a 5-point scale as described (3) in which 1 = no lesions, 2 = a few lesions, 3 = a light lesion density, 4 = a moderate lesion density, and 5 = a heavy lesion density, the last comparable to that of infection on the soybean control included in each experiment. The proportion of lesions with sporulating uredinia (referred to as sporulating lesions) for each of three pathogen isolates per plant was rated from 0 to 5, where 0 = no lesions sporulating, 1 = 20% lesions sporulating, 2 = 40% lesions sporulating, 3 = 60% lesions sporulating, 4 = 80% lesions sporulating, and 5 = 100% lesions with uredinia sporulating (10).

Number of uredinia per lesion and uredinium diameters. For kudzu Set #1, after collecting data for lesion density, sporulation, and reaction type, selected leaflets with symptoms were excised from the plants and fixed in Farmer's solution (2:1 absolute ethanol:acetic acid) for at least 24 h (1). The leaflets then were submerged for 24 h in lactophenol to remove pigments and stained overnight in lactophenol containing 0.1% cotton blue (1). Excess stain was removed from the surface of the leaflets by rinsing them in clear lactophenol.

The stained leaflets were mounted by placing them abaxial side up on a layer of lactophenol in a plastic petri dish lid, placing drops of lactophenol on the surface of

the leaflets, and inserting a petri dish bottom into the top so that the leaflets were sandwiched between two pieces of clear plastic. They were examined as whole-leaf mounts at $\times 15$ magnification to determine numbers of uredinia per lesion and at $\times 90$ magnification to measure uredinium diameters by means of an ocular micrometer.

Data analyses. Each leaf (consisting of three leaflets) was treated as a separate unit and represented a pathogen isolate/plant combination when determining lesion densities and sporulation ratings. The mean lesion density rating was calculated for all TAN and for all RB reactions, respectively, for each pathogen isolate in each of the two experiments, and results of the three isolates were averaged within experiments. Similarly, the mean sporulation rating was calculated for all TAN reactions and all RB reactions, respectively, for each pathogen isolate in each of the two experiments, and results of the three isolates were averaged within experiments.

In a single experiment, the mean number of uredinia per lesion ($n = 25$) for each leaf on each of 27 kudzu plants (from locations 1 to 13 of Set #1) was determined, and the data within isolates were averaged for TAN and RB lesions, respectively (Table 2).

Similarly, the mean uredinium diameter ($n = 25$, fewer if 25 not available) for each pathogen isolate/plant combination was determined, and data for all TAN and for all RB lesions, respectively, were averaged within isolates (Table 2).

RESULTS

Lesion type, density, and proportion of lesions with sporulating uredinia.

Each of the 125 plants, from a total of 56

locations, 51 of which were in Mississippi (Table 1), produced the same reaction type in each of the two experiments to the respective *P. pachyrhizi* isolates, confirming reproducibility of results. In the few instances in which symptoms were borderline between TAN and RB, final assignment of reaction type was based on a histological examination of fixed, cleared, and stained leaf tissue. Typical symptoms on living leaves and fungal development within leaves as revealed by histological treatment are shown in Figure 1.

Of the 125 inoculated plants, 19 (15%) developed a different reaction to one isolate as compared to the other two isolates (Table 1). Fifty-four plants produced only TAN lesions, 50 only RB lesions, and two had no symptoms when inoculated with the three pathogen isolates. Two plants (7B and 16B) gave mixtures of TAN and RB lesions when inoculated with isolate Brazil 01-1, which was not unexpected since this isolate is known to be a mixture of "races" (2). When four individual plants (12B, 17C, 18B, and 18C) previously shown to produce only TAN lesions to the three isolates were inoculated with eight additional *P. pachyrhizi* isolates, collected from different parts of the world over three decades, each produced only TAN lesions (Table 3). Similarly, when five plants (1B, 14B, 16B, 19A, and 25C), one a replacement partway through the experiment due to death of a plant, that previously had produced only RB lesions to the three isolates were inoculated with the eight additional isolates, all 11 isolates produced only RB lesions (Table 3). These results show a strong tendency for individual kudzu plants to have resistance or susceptibility to a very broad range of *P. pachyrhizi* isolates.

All of the plants from 25 of 39 (64%) sites from which multiple plants were tested with Alabama 04-1, Brazil 01-1, and Louisiana 04-1 reacted the same to the respective isolates (Table 1). For example, for location #12, all three plants produced RB lesions in response to the three pathogen isolates, and for location #45, both plants produced RB lesions to Alabama 04-1 and TAN lesions to the other two isolates. The distribution of reaction types associated with field locations was random (Fig. 2).

Average lesion densities always were significantly higher on plants with TAN reactions than on plants with RB reactions. In experiment #1, average lesion density ratings ($n = 3$) for the three rust isolates were (mean $\pm$ conf. int., $P = 0.05$) 4.2 (± 0.1) and 3.4 (± 0.1) for plants with TAN and RB reactions, respectively. In experiment #2, average lesion density ratings ($n = 3$) were 4.8 (± 0.1) and 3.9 (± 0.2) for plants with TAN and RB lesions, respectively. In all six instances (includes experiments #1 and #2) in which a given isolate was used to inoculate a set of kudzu accessions, ratings for lesion densities were higher for TAN than for RB reactions.

TAN reactions also produced significantly higher proportions of lesions with sporulating uredinia than RB reactions. TAN reactions almost always were rated 5 (100% of lesions with sporulating uredinia), and RB reactions rated 0 to 2 (0 to 40% of lesions with sporulating uredinia). Average sporulation ratings ($n = 3$) for the three rust isolates were 4.9 (± 0.1) and 1.0 (± 0.3) for TAN and RB lesions, respectively, in experiment #1. In experiment #2 ($n = 3$), they were 4.9 (± 0.1) and 0.8 (± 0.2) for TAN and RB reactions, respectively.

Table 2. Mean number of uredinia per lesion and mean uredinia diameters for isolates Alabama 04-1, Brazil 01-1, and Louisiana 04-1 of *Phakopsora pachyrhizi* following inoculation of kudzu or soybean plants^a

Plant/reaction type	Mean number of uredinia/lesion ^b			Mean uredinia diameter (μm) ^c		
	AL04-1	BZ01-1	LA04-1	AL04-1	BZ01-1	LA04-1
Kudzu TAN	2.9 (16) (± 0.3)	3.2 (16) (± 0.2)	3.4 (21) (± 0.3)	122 (16) (± 3)	124 (16) (± 3)	116 (21) (± 3)
Kudzu RB	0.4 (11) (± 0.3)	0.3 (9) (± 0.1)	0.2 (6) (± 0.2)	72 (6) (± 12)	80 (6) (± 3)	79 (3) (± 7)
Soybean TAN	4.1 (2) (± 0.3)	4.5 (1) (± 0.0)	5.4 (2) (± 0.0)	131 (2) (± 0)	125 (1) (± 0)	130 (2) (± 8)

^a Kudzu and soybean plants were inoculated by painting each of three leaves per plant with a separate urediniospore suspension at 2.5 to 3.5×10^4 urediniospores/ml distilled water containing 1 drop Tween 20 per 100 ml. Inoculated plants were incubated overnight in dew chambers at 20°C , removed from the chambers the following morning, allowed to dry, and then placed in the greenhouse at 20 to 25°C for disease development. Two weeks after inoculation, plants were examined for symptoms and leaves collected for histology. AL04-1 = isolate Alabama 04-1, BZ01-1 = isolate Brazil 01-1, and LA04-1 = isolate Louisiana 04-1.

^b Mean number of uredinia per lesion was determined for each plant/pathogen isolate combination from 25 randomly selected uredinia; overall means for each specific isolate were determined by averaging data from all plants with TAN or RB reactions, respectively. Upper numbers in parentheses are the numbers of plants developing the specific lesion type, and lower numbers in parentheses one standard error of the means based on variation among plants (in upper parentheses).

^c Mean uredinia diameters were based on 25 randomly selected uredinia per plant/pathogen isolate combination, and overall means were determined for each specific pathogen isolate. Upper numbers in parentheses are numbers of plants sampled. Lower numbers in parentheses are one standard error of the means based on variation among plants (upper parentheses). Note that some plants produced no uredinia within RB lesions, and therefore contributed no data toward overall means for uredinia diameters.

Average numbers of uredinia per lesion and average uredinium diameters. The average numbers of uredinia per lesion and average uredinium diameters for TAN and RB lesions, respectively, for each of the three pathogen isolates on 27 plants (from Set #1) are presented in Table 2. For example, isolate Alabama 04-1 produced a mean of 2.9 uredinia per lesion on leaves with TAN reactions and 0.4 uredinia per lesion on leaves with RB reactions. Mean uredinia diameters were 122 μm for TAN reactions and 72 μm for RB reactions. Furthermore, for all pathogen isolates, 49 of 53 interactions in which TAN lesions were produced had an average of 1.8 or more uredinia per lesion, whereas 24 of 26 interactions in which RB lesions were produced had an average of 1.0 or fewer uredinia per lesion.

DISCUSSION

Reaction types produced on kudzu in this study were similar to those previously reported on soybean (2,5), that is TAN, RB, and IM. However, in contrast to soybean, the RB reaction was very common. In a total of 374 interactions between host and pathogen, 177 (47%) were RB. IM reactions to at least one isolate were produced on only five kudzu plants; however, plants #41B and #41C were immune to all three pathogen isolates.

The tendency for kudzu plants to react similarly at individual sites is in line with results of a genetic diversity study conducted by Sun et al. (12) in which they used inter-simple sequence repeat analysis

Fig. 2. Locations of sites in Mississippi from which kudzu plants were obtained and their reactions to *Phakopsora pachyrhizi* isolates based on inoculations in the greenhouse. Mixed reactions indicate that both susceptible tan-colored (TAN) and resistant reddish-brown (RB) lesions developed either on the same plant or on separate plants from the respective locations.

Fig. 1. Susceptible tan-colored (TAN) lesions and resistant reddish-brown (RB) lesions on the same kudzu plant. Upper row shows typical symptoms on leaves, which differentiate biotypes of *Phakopsora pachyrhizi*. **A**, TAN lesions with large proportion of lesions sporulating, **B**, RB lesions with little or no sporulation, and **C**, RB lesions with water-soaking around individual lesions. Lower row shows typical fungal development within fixed, cleared, and stained leaf tissue from the leaf shown immediately above. **D**, Uredinia clumps within TAN lesions, **E**, RB lesions with a few small uredinia, and **F**, RB lesions with few uredinia in water-soaked lesions. The 2-mm bar applies to the entire figure.

Table 3. Lesion type reactions on kudzu (*Pueraria montana* var. *lobata*) plants inoculated separately with urediniospores of 11 isolates of *Phakopsora pachyrhizi*^a

Isolate	Plant/ reaction type								
	12B	17C	18B	18C	1B	14B	16B	19A	25C
Alabama 04-1	TAN	TAN	TAN	TAN	RB	RB	RB	RB	RB
Brazil 01-1	TAN	TAN	TAN	TAN	RB	RB	RB	RB	RB
Louisiana 04-1	TAN	TAN	TAN	TAN	RB	RB	RB	RB	RB
Thailand 01-1	TAN	TAN	TAN	TAN	IM	— ^b	RB	RB	RB
Paraguay 01-2	TAN	TAN	TAN	TAN	RB	—	RB	RB	RB
Zimbabwe01-1	TAN	TAN	TAN	TAN	IM	—	RB	RB	RB
Taiwan 72-1	TAN	TAN	— ^c	TAN	IM	RB	RB	— ^c	RB
Taiwan 80-2	TAN	TAN	—	TAN	IM	RB	RB	—	RB
Vietnam 05-1	TAN	TAN	—	TAN	IM	RB	RB	—	RB
Columbia 04-1	TAN	TAN	—	TAN	IM	RB	RB	—	RB
India 73-1	TAN	TAN	—	TAN	IM	RB	RB	—	RB

^a Plants selected for this study had either all tan-colored (TAN) or all reddish-brown (RB) lesions when inoculated with Alabama 04-1, Brazil 01-1, and Louisiana 04-1 in previous tests. Each isolate was collected from the location and in the year indicated by its designation. (For example, Thailand 01-1 was collected in Thailand in 2001, and was the first isolate of a series. Taiwan 80-2 was collected in Taiwan in 1980, and was the second isolate of a series.)

^b Plant 14B replaced 19A partway through experiment, and thus was not inoculated with all isolates.

^c Plants 18B and 19A died partway through the experiment, and therefore were not inoculated with all isolates.

to determine genetic diversity in species of *Pueraria*. They found high genetic diversity among populations but low diversity within populations of *P. lobata* in the United States, and suggested that this might be caused by a combination of multiple introductions of kudzu from Asia followed by high levels of inbreeding within populations.

Although it was not within the scope of our present research to conduct an exhaustive genetic population study, our results did demonstrate the existence of biotypes within the U.S. kudzu population and support observations of Yamaoka et al. (14) that physiological biotypes of *P. pachyrhizi* can be differentiated on kudzu. It is possible that physiological specialization, concomitant with a tendency for plants at some sites to react similarly to all *P. pachyrhizi* isolates, could explain why soybean rust is common on kudzu in some areas and absent in others (W. F. Moore and T. W. Allen, *personal observation*). Where kudzu can survive winters, the biotypes of *P. pachyrhizi* on kudzu possibly could influence the disease on soybean. Because many plants inoculated with Alabama 04-1 (41%), Brazil 01-1 (33%), or Louisiana 04-1 (31%) produced no or extremely low numbers of uredinia, and approximately 25% produced no or extremely low numbers of uredinia for all three isolates, it was apparent that there are situations in which kudzu might not contribute to disease increase or spread.

Average numbers of uredinia per lesion and average uredinium diameters are quantitative measurements that correlate with the ability of *P. pachyrhizi* to sporulate on a host (2). As such, they could be used to measure susceptibility or resistance to soybean rust. Although lesion densities on kudzu leaves with TAN reactions were significantly higher than on leaves with RB reactions, this observation could have been an artifact related to the subjective manner (i.e., ratings) in which lesion densities

were determined. In order to determine with certainty any association of TAN reactions with lesion density, individual lesions would have to be counted on plants.

Based on reports during the last several years of soybean rust on kudzu in the southern United States during winter and early spring months, it is probable that *P. pachyrhizi* survives in isolated pockets (USDA Public PIPE Website, www.sbrusa.net/). In these areas, and areas where the pathogen subsequently moves, the susceptibility of the kudzu vegetation, and that of other possible hosts, to the prevalent biotypes of *P. pachyrhizi* could influence disease development on soybean.

In addition to the ability of the pathogen to survive winters on kudzu and other hosts, economic losses to soybean will depend on the suitability of the environment during the growing season for rust buildup and spread. Consequently, we are conducting research to determine how temperature and moisture affect soybean rust development in the United States and anticipate that when all information is assimilated, we will have a better understanding of the future of soybean rust in the United States.

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LITERATURE CITED

1. Bonde, M. R., Melching, J. S., and Bromfield, K. R. 1976. Histology of the suspect-pathogen relationship between *Glycine max* and *Phakopsora pachyrhizi*, the cause of soybean rust. *Phytopathology* 66:1290-1294.
2. Bonde, M. R., Nester, S. E., Austin, C. N., Stone, C. L., Frederick, R. D., Hartman, G. L., and Miles, M. R. 2006. Evaluation of virulence of *Phakopsora pachyrhizi* and *P. meibomia* isolates. *Plant Dis.* 90:708-716.
3. Bonde, M. R., Nester, S. E., Berner, D. K.,

Frederick, R. D., Moore, W. F., and Little, S. 2008. Comparative susceptibilities of legume species to infection by *Phakopsora pachyrhizi*. *Plant Dis.* 92:30-36.

4. Britton, K. O., Orr, D., and Sun, J. 2002. Kudzu. In: *Biological Control of Invasive Plants in the Eastern United States*. R. van Driesche, B. Blossey, M. Hoddle, S. Lyon, and R. Reardon, eds. USDA Forest Service, publ. no. FHTET-2002-04.
5. Bromfield, K. R. 1984. Soybean Rust. Monogr. No. 11. American Phytopathological Society, St. Paul, MN.
6. Bromfield, K. R., Melching, J. S., and Kingsolver, C. H. 1980. Virulence and aggressiveness of *Phakopsora pachyrhizi* isolates causing soybean rust. *Phytopathology* 70:17-21.
7. Cherry, E., and Peet, C. E. 1966. An efficient device for the rapid collection of fungal spores from infected plants. *Phytopathology* 56:1102-1103.
8. Melching, J. S., Bromfield, K. R., and Kingsolver, C. H. 1979. Infection, colonization, and urediniospore production on Wayne soybean by four cultures of *Phakopsora pachyrhizi*, the cause of soybean rust. *Phytopathology* 69:1262-1265.
9. Melching, J. S., Bromfield, K. R., and Kingsolver, C. H. 1983. The pathogen containment facility at Frederick, Maryland. *Plant Dis.* 67:717-722.
10. Melching, J. S., Dowler, W. M., Koogler, D. L., and Royer, M. H. 1989. Effects of duration, frequency, and temperature of leaf wetness periods on soybean rust. *Plant Dis.* 73:117-122.
11. Schneider, R. W., Hollier, C. A., Whitam, H. K., Palm, M. E., McKemy, J. M., Hernandez, J. R., Levy, L., and DeVries-Paterson, R. 2005. First report of soybean rust caused by *Phakopsora pachyrhizi* in the continental United States. *Plant Dis.* 89:774.
12. Sun, J. H., Li, Z.-C., Jewett, D. K., Britton, K. O., Ye, W. H., and Ge, X.-J. 2005. Genetic diversity of *Pueraria lobata* (kudzu) and closely related taxa as revealed by inter-simple sequence repeat analysis. *Eur. Weed Res. Soc. Weed Res.* 45:255-260.
13. Susko, D. J., Mueller, J. P., and Spears, J. F. 2001. An evaluation of methods for breaking seed dormancy in kudzu (*Pueraria lobata*). *Can. J. Bot.* 79:197-203.
14. Yamaoka, Y., Fujiwara, Y., Kakishima, M., Katsuya, K., Yamada, K., and Hagiwara, H. 2002. Pathogenic races of *Phakopsora pachyrhizi* on soybean and wild host plants collected in Japan. *J. Gen. Plant Pathol.* 68:52-56.