THE JOURNAL OF AGRICULTURE
OF THE UNIVERSITY OF PUERTO RICO

Issued by the Agricultural Experiment Station of the University of Puerto Rico, Mayagüez Campus, for the publication of articles and research notes by staff members or others, dealing with scientific agriculture in Puerto Rico and elsewhere in the Caribbean Basin and Latin America.

VOL. 94 JULY AND OCTOBER 2010 No. 3-4

Virulence of *Phakopsora meibomiae* in wild and cultivated legumes in Puerto Rico[1,2]

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J. Agric. Univ. P.R. 94(3-4):211-224 (2010)

ABSTRACT

Soybean rust is caused by two *Phakopsora* species: *Phakopsora meibomiae* (Arthur) Arthur and *P. pachyrhizi* H. Sydow & Sydow. *Phakopsora meibomiae* is found only in limited areas in theWestern hemisphere, and it is not known to cause se vere losses. *Phakopsora meibomiae* was repor ted in Puerto Rico in 1913 infecting *Lablab purpureus* and in 1976 an outbreak produced severe yield losses in so ybeans, common beans, and lima beans in Adjuntas. *Phakopsora meibomiae* infects approximately 60 species of legumes and may represent a potential threat to soybean production in Puerto Rico. This study determined the virulence of one isolate of *P. meibomiae* in fifteen legumes. Eleven of the fifteen species inoculated de veloped rust symptoms, and the identity of the rust fungus was confirmed by Polymerase Chain Reaction (PCR). Symptoms were obser ved first on *Phaseolus vulgaris, P. lunatus*, and *V. unguiculata*. *Phaseolus vulgaris* and *P. lunatus* had the shortest incubation and latent periods. *Phaseolus vulgaris* also pr oduced the highest number of uredinia per individual lesion. *Crotalaria retusa* had the longest incubation period, and *Rhynchosia reticulata* had the longest latent period. *Phakopsora pachyrhizi* was not obser ved infecting legumes in Puerto Rico in areas where it had previously been reported.

Key words: American soybean rust, *Lablab purpureus,* incubation period, latent period, uredinium

[1]Manuscript submitted to Editorial Board 26 May 2009.

[2]Funding for this project w as provided by TSTAR-CSREES award # 2005-34135-16283. We gratefully ac knowledge Dr. Philip Harmon for critically reading the manuscript.

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RESUMEN

Virulencia de *Phakopsora meibomiae* en leguminosas silvestres y cultivadas en Puerto Rico

La roya de la soya es causada por dos especies de *Phakopsora: Phakopsora meibomiae* (Arthur) Arthur y *P. pachyrhizi* H. Sydow & Sydow. *Phakopsora meibomiae* se encuentra limitada en pocas áreas del Hemisferio Occidental y no se conocen de pérdidas severas causadas por este patógeno. *Phakopsora meibomiae* fue reportada en Puerto Rico en 1913 infectando a *Lablab purpureus*; en 1976 produjo pérdidas severas de rendimiento en soya, habichuela, y fréjol lima en Adjuntas. *Phakopsora meibomiae* infecta aproximadamente 60 especies de leguminosas y puede representar una amenaza para la producción de soya en Puerto Rico. El presente estudio determinó la virulencia de un aislado de *P. meibomiae*. Once de quince especies inoculadas desarrollaron síntomas de roya, y su identidad fue confirmada mediante reacción en cadena de la polimerasa (PCR). Los primeros síntomas se observaron en *Phaseolus vulgaris*, *P. lunatus*, y *V. unguiculata*. *Phaseolus vulgaris* y *P. lunatus* presentaron los períodos de incubación y latencia más cortos. *Phaseolus vulgaris* también presentó el mayor número de uredos por lesión individual. *Crotalaria retusa* presentó el período de incubación más largo, y *Rhynchosia reticulata* presentó el período de latencia más largo. No se observó a *P. pachyrhizi* infectando leguminosas en Puerto Rico en áreas donde previamente fue reportada.

Palabras clave: Roya americana de la soya, *Lablab purpureus,* período de incubación, período de latencia, uredo

INTRODUCTION

Asian Soybean Rust (ASR), caused by *Phakopsora pachyrhizi* H. Sydow & Sydow, is the most destructive foliar disease of soybean. This disease causes from 10 to 80% yield losses (Isard et al., 2006; Levy, 2005). American soybean rust (AmSR), caused by *Phakopsora meibomiae* (Arthur) Arthur, is also an important disease of soybean that has not been studied as extensively as ASR (Frederick et al., 2002; Posada-Buitrago and Frederick, 2005; Pivonia and Yang, 2004; Villavicencio et al., 2007; Yorinori et al., 2005; Rossi, 2003; Schneider et al., 2005). The new world rust, AmSR, is less virulent; it is endemic to Central and South America and the Caribbean (Bonde et al.,2006; Ono et al., 1992). The 1,000 ha of soybean (*Glycine max* [L.] Merrill) planted in Puerto Rico in winter nurseries has an annual crop value of $30 million (Estévez de Jensen et al., 2009). The first report of *P. meibomiae* in cultivated legumes in Puerto Rico was published in April 1976, when it was discovered in scarlet runner bean (*Phaseolus coccineus* [L.]) and soybean. In August of the same year, 200 common bean lines (*Phaseolus vulgaris* [L.]) were severely infected with rust in the municipality of Adjuntas (Limaní Valley) at 590 m altitude (V akili and Bromfield, 1976).

Earlier studies of rust infection in legumes in the Caribbean region reported *Uredo concors* Arthur and *Physopella concors* Arthur as the causal agents of the disease (Arthur, 1915; Arthur, 1917). In the Americas, AmSR was described in 1917 in Mexico , in 1940 in Guatemala, and later in 1976 in Costa Rica infecting wild legumes (Cummins , 1943; Bromfield, 1984). *Phakopsora meibomiae* was reported in 15 wild legume species of the subfamily Papilionoidea (Ono et al., 1992).

Phakopsora meibomiae naturally infects 42 species of legumes in 19 genera. Under controlled conditions, 18 additional species in 12 genera are reported to be infected by the pathogen (Frederick et al., 2002; Ono et al., 1992). The wide range of species infected by *P. meibomiae* could affect the production of grain legumes, including soybeans and dry beans, in Puerto Rico. The objective of this study was to determine the virulence of one isolate of *P. meibomiae* collected in Adjuntas, by inoculating fifteen different legumes in order to determine incubation, latent period, and uredinium size . Knowledge of these parameters will contribute to the understanding of the virulence of*P. meibomiae* in the wild and in cultivated legumes growing in Puerto Rico.

MATERIALS AND METHODS

Sowing. Fifteen legumes previously reported as hosts of *P. meibomiae* were selected for the study. Seeds of*Crotalaria brevidens, C. juncea, C. retusa, Macroptilium lathyroides, Pueraria phaseoloides, Rhynchosia reticulata,* and *Vigna luteola* were sown in the screen house at the Agricultural Experiment Station, Juana Díaz. After scarification with abrasive paper #80 (Norton Abrasives, Worcester, MA) [5] to promote germination, seeds were placed in pots containing artificial growing media (PRO-MIX, Bx, Premier Horticulture, Québec, Canada). Seeds of *Canavalia gladiata, Glycine max* (cv. Williams and line 2053 A), *Lablab purpureus, Mucuna pruriens, Pachyrhizus erosus, P. lunatus, P. vulgaris* (cv. Verano), and *Vigna unguiculata* (cv. Gorda) were superficially sterilized with 2.5% sodium hypochlorite for three minutes and planted without scarification. All legumes were planted in 2.5-liter pots 19 November 2007. A completely random design with five to seven replications per legume species was used in the experiment. The number of plants per pot varied because of seed availability and viability, and ranged between two and 37 plants per species tested. Depending on the legume species, either primary or cotyledonary leaves were inoculated 21 days after germination.

[5]Company and trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute a w arranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

Inoculation. One isolate of *P. meibomiae* infecting *L. purpureus* (collected in the municipality of Adjuntas, N 18°10.855', W 066°46.123' at 561-m altitude), was used as an inoculum source. Inoculum consisted of a 1.5 cm^2 piece of *L. purpureus* leaf infected with *P. meibomiae* (average of 3.5×10^3 urediniospores per cm^2). The lower surface of the donor leaf was placed in contact with the upper surface of the recipient leaflet and secured with a staple (Vakili, 1979). Inoculated plants were placed in a screen house (50% shade); then followed 48 hours of sprinkler irrigation (16.26 mm/experiment) at 24 ± 3° C. After inoculation, plants were maintained in the screen house with an average air temperature of 24.5° C for a period of 30 days.

Infection and histological observations. After inoculation, plants were observed daily for first symptoms of rust and response to *P. meibomiae* infection. Two parameters were evaluated: i) incubation period (number of days from inoculation to first symptoms); and ii) latent period (number of days from inoculation to the first pustule erupted) (Van Der Plank, 1963). Twenty days after inoculation, leaflets of each infected legume were removed from the plants. Leaf sections of approximately 1 × 1 cm were placed in 70% ethyl alcohol for three hours to remove plant pigments from the tissue. Leaf tissue was stained with lactophenol with cotton blue. Uredinium size and number of uredinia per individual lesion were evaluated with an optical microscope (Olympus Cx31) and documented with a digital camera (Olympus DP, Olympus America Inc., Center Valley, PA) at 20, 40 and 200X.

Rust infection reactions were c lassified as previously described: i) immune (lack of visible symptoms), corresponding to an incompatible reaction; ii) RB, characterized by reddish-brown lesions with zero to two uredinia per lesion and scarce sporulation (semi compatible reaction); and iii) tan, characterized by tan lesions with two to five uredinia per lesion and abundant sporulation (compatible reaction) (Bromfield, 1984; Bromfield and Hartwig, 1980).

DNA extraction and polymerase chain reaction. DNA extraction and PCR was conducted at the Plant Disease Clinic at the Juana Díaz Substation. For total DNA extraction, the Extract-N-Amp plant PCR kit (Sigma-Aldrich, St. Louis, MO) was used according to the manufacturer's instructions. DNA was amplified by using *P. meibomiae*-specific primers: Pme1 (5'-GAAGTTTTTGGGCAAATCAC-3') and Pme2 (5'-GCACTCAAAATCCAACATGC-3') in a total volume of 20 µl (Frederick et al., 2002; Lamour et al., 2006). The PCR reaction contained REDExtract-N-Amp PCR ready mix according to the manufacturer's directions, each primer at a concentration of 20 pmol, and 5 to 50 ng of extracted template DNA. Amplification of extracted DNA was performed with a thermocycler model T 3000 (Biometra, Goettingen, Germany) under the following conditions: 94° C for 2 min initial denaturation, followed by 35 cycles of 94° C for 30 s, 65° C for 30 s, and 72° C for 30 s, and a final extension for 10 min at 72° C. The amplification products were visualized with UV light after electrophoresis in a 1.5% agarose (Sigma-Aldrich, St. Louis, MO) gel stained with ethidium bromide.

Scanning electron microscopy. Leaf sections of 7 to 8 mm with visible rust pustules were placed in 4% glutaraldehyde with 0.1 M phosphate buffer (pH 7.2) for 24 h at 4° C. The leaf tissue was rinsed three times in a 0.1 M phosphate buffer, pH 7.2, for 15 min. Dehydration was conducted in ethyl alcohol (5% to 100% each time) for 15 min at each concentration. Critical point drying was done for two hours (Electron Microscopy Sciences, Washington, PA). Samples were coated with gold for 10 min; tissue was examined with a scanning electron microscope model JSM-5410 LV (Jeol Ltd., Tokyo, Japan).

RESULTS

Eleven of the fifteen species inoculated were infected with *P. meibomiae* and showed symptoms of AmSR. The first symptoms were observed on *P. vulgaris*, *P. lunatus*, and *V. unguiculata*, 3.2, 3.3, and 3.3 d after inoculation, respectively (Table 1). Symptoms appeared as small reddish spots surrounded by chlorotic rings. *Vigna unguiculata* had an incubation period similar to that of *P. vulgaris* and of *P. lunatus*. The longest incubation periods were found in *C. retusa* (6.2 d), followed by *R. reticulata* with 6 d, and by *P. erosus* with 5.9 d. In *G. max*, the incubation period was 4.5 d for cv. Williams and 4.9 d for line 2053 A (Table 1).

The shortest latent period was observed in *P. vulgaris* (6.2 d) and in *P. lunatus* (6.4 d), followed by *M. lathyroides* (6.8 d) and *L. purpureus* (6.9 d). The longest latent period was observed in *R. reticulata* with 11 d, followed by *V. unguiculata* and soybeans (line 2053 A). Two of the longest latent periods observed in soybean were 10.3 d (cv. Williams) and 10.9 d (line 2053 A) (Table 1). Symptoms varied among species. In *L. purpureus*, *P. lunatus*, *P. vulgaris*, and *G. max*, small reddish-brown spots were observed as the initial symptoms, whereas reddish flecks were observed in *V. luteola*. In *C. retusa* the first symptoms were small necrotic spots with a dear brown region in the center and a dark brown border. Symptoms observed in *V. unguiculata* were different from those of the other legume species and appeared as small tan to reddish-brown spots visible at the beginning of the infection (three days after inoculation). Four to five days after inoculation, small dark brown lesions developed and the center of the lesions coalesced.

TABLE 1.—*Mean values of incubation and latent periods in legumes inoculated with* Phakopsora meibomiae.

		Incubation period (days)			Latent period (days)	
Legumes	n[1]	Average	S.D.[2]	n[1]	Average	S.D.[2]
Canavalia gladiata	21	—	—	21	—	—
Crotalaria brevidens	15	—	—	15	—	—
Crotalaria juncea	31	3.65	0.91	9	10.44	1.59
Crotalaria retusa	9	6.22	1.20	5	9.60	0.55
Glycine max 2053 A	13	4.92	0.49	13	10.92	0.76
Glycine max cv. Williams	21	4.52	0.51	18	10.39	1.50
Lablab purpureus	21	3.52	0.51	20	6.95	0.94
Macroptilium lathyroides	31	4.52	0.77	28	6.86	0.80
Mucuna pruriens	22	—	—	22	—	—
Pachyrhizus erosus	17	5.94	1.03	8	10.75	1.39
Phaseolus lunatus	21	3.29	0.46	20	6.40	0.60
Phaseolus vulgaris	22	3.23	0.53	22	6.27	0.55
Pueraria phaseoloides	5	—	—	5	—	—
Rhynchosia reticulata	2	6.00	0	2	11.00	0
Vigna luteola	37	3.38	0.64	34	7.76	1.56
Vigna unguiculata	21	3.33	0.48	20	10.95	1.05

[1]n = number of plants evaluated.
[2]S.D. = standard deviation.

Significant differences (P < 0.01) were found between legumes for the number of uredinia per individual lesion. *Phaseolus vulgaris* produced the highest number of uredinia per individual lesion, with a mean of 5.7, followed by *P. lunatus* (4.7). *Lablab purpureus* produced 60% fewer uredinia than *P. vulgaris* (mean of 2.3). *Glycine max* cv. Williams produced more uredinia than line 2053A (1.8 uredinia per lesion versus 1.4 uredinia per lesion). *Pachyrhizus erosus* produced statistically the least uredinia per individual lesion (mean of 0.6) (Table 2 and Figure 1C). The number of uredinia per individual lesion in *P. vulgaris* and in *P. lunatus* had the highest standard deviation (data not shown). In *P. vulgaris* the maximum value of uredinia per individual lesion was 14; the minimum was 2 (Table 2).

Eleven of the fifteen legumes evaluated were susceptible to *P. meibomiae*, showing RB and tan lesions, whereas four had an immune reaction. The identity of the rust fungus w as confirmed as *P. meibomiae* by PCR (Figure 2). In a susceptible reaction, scanning electron microscopy showed dispersion of urediniospores from the uredinia as well as the uredinia distribution in the host tissue . In *P. vulgaris* the great variability in uredinium size and urediniospore production was evident (Figures 3A and 3B). On this host, both the uredinium pore size and the

TABLE 2.—*Number of uredinia per individual lesion in legumes inoculated with* Phakopsora meibomiae.

Legumes	n[1]	Number of uredinia per individual lesion		
		Mean[2]	Minimum	Maximum
Canavalia gladiata	—	—	—	—
Crotalaria brevidens	—	—	—	—
Crotalaria juncea	36	1.86 cd	0	5
Crotalaria retusa	8	1.50 cd	0	4
Glycine max 2053 A	100	1.42 de	0	3
Glycine max Williams	100	1.80 cd	0	4
Lablab purpureus	100	2.32 c	1	6
Macroptilium lathyroides	100	1.54 cd	0	4
Mucuna pruriens	—	—	—	—
Pachyrhizus erosus	44	0.64 e	0	2
Phaseolus lunatus	100	4.74 b	1	11
Phaseolus vulgaris	100	5.79 a	2	14
Pueraria phaseoloides	—	—	—	—
Rhynchosia reticulata	—	—	—	—
Vigna luteola	100	1.93 cd	0	6
Vigna unguiculata	100	1.71 cd	0	6

[1]n = number of uredinia evaluated.

[2]Mean separation by the Tukey test, P ≤ 0.05.

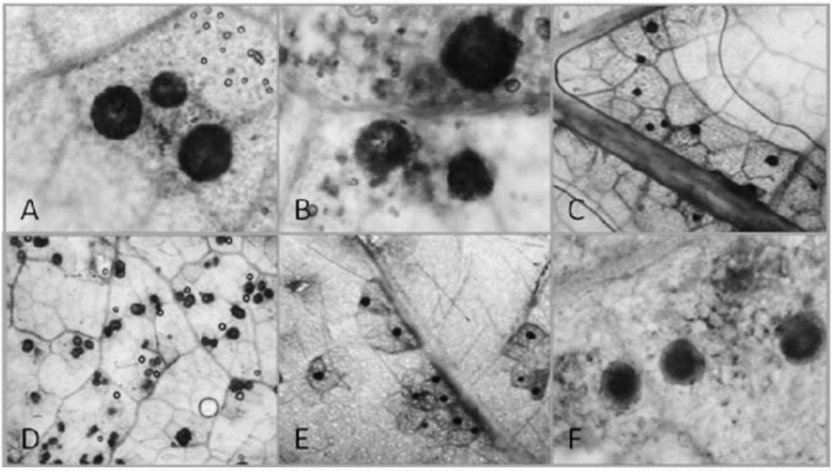

FIGURE 1. Uredinia of *Phakopsora meibomiae* in leaf tissue stained with cotton blue in different hosts. A, *Lablab purpureus* (200 X); B, *Phaseolus lunatus* (200 X); C, *Pachyrhizus erosus* (40 X); D, *Macroptilium lathyroides* (40 X); E, *Glycine max* cv. Williams (40 X); and F, *Vigna luteola* (200 X).

paraphyses production tended to be bigger and more developed than that in the other legumes evaluated.

Significant differences (P < 0.01) were found between legumes for the uredinium diameter. Mean uredinium size in *P. vulgaris* was 133.18 µm, with a maximum and minimum value of 220 µm and 47.30 µm, respectively (Table 3). *Macroptilium lathyroides* produced fewer urediniospores per uredinium than *P. vulgaris* or *P. lunatus*, as well as strong paraphyses The average size of the uredinium was 127.17 µm (Table 3 and Figure 3D).

Glycine max produced uredinia with a small pore, and the release of urediniospores was limited, whereas the paraphyses were well formed around the uredinium pore (Figures 3E and 3F). Uredinia production on soybean leaves produced abrupt fissures through the epidermal tissue after erupting. Uredinium average size in cv. Williams was 108.75 µm, whereas in line 2053 A it was 113.95 µm (Table 3). The highest average size of uredinium was found in *C. retusa*, 161.29 µm in diameter, whereas the lowest value was observed in *P. erosus* (38% less than the size of uredinium formed on leaves of *C. retusa*) (Table 3).

DISCUSSION

This is the first study of the incubation and latent periods of *P. meibomiae* inoculation of wild and cultivated legumes in Puerto Rico *Pha-*

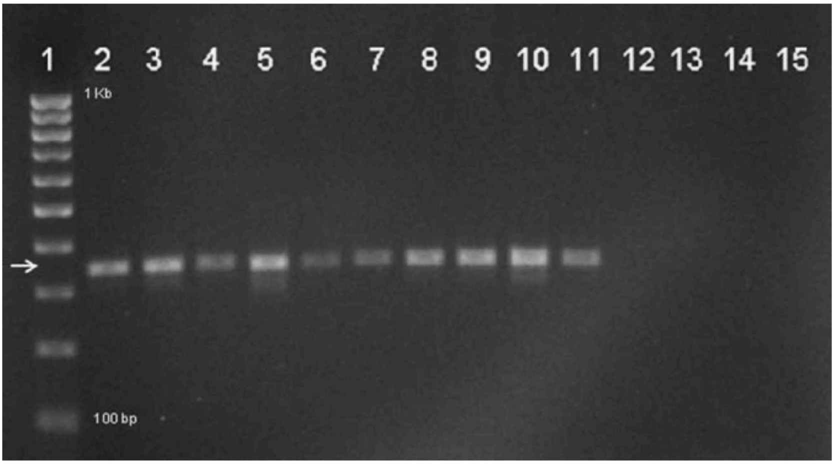

FIGURE 2. Agarose gel with polymerase chain reaction (PCR) products produced with specific primers Pme1/Pme2 and DNA extractions from legumes inoculated with *Phakopsora meibomiae*. 1 = molecular weight marker (100-bp ladder); 2 = *Lablab purpureus*; 3 = *Phaseolus lunatus*; 4 = *Glycine max*; 5 = *Phaseolus vulgaris*; 6 = *Vigna luteola*; 7 = *Pachyrhizus erosus*; 8 = *Macroptilium lathyroides*; 9 = *Vigna unguiculata*; 10 = *Crotalaria retusa*; 11 = *Crotalaria juncea*; 12 = *Pueraria phaseoloides*; 13 = *Mucuna pruriens*; 14 = *Canavalia gladiata*; and 15 = *Crotalaria brevidens*. PCR products at 338 bp are indicated by arrows.

kopsora meibomiae was described for the first time in soybean in the western hemisphere, in Puerto Rico in 1976. Host range studies conducted elsewhere provided the description of infection types produced in several wild legumes (Bromfield, 1984; Vakili, 1979). The incubation and latent periods are two of the most important epidemiological factors evaluated after pathogen infection to identify the resistance level in legume hosts inoculated with a rust pathogen (described as the type of lesion produced during the infection). *Phakopsora meibomiae* infection and establishment in different legumes w as successful with the use of sprinkler irrigation during the 48 hours after inoculation. The screen house with 50% shade w as ideal for the study of the infection process. Typically, tan and RB reactions have been described as susceptible in compatible and semi compatible hosts , respectively. The immune reaction has been produced by resistant hosts in an incompatible host-pathogen interaction during the soybean rust infection (Bromfield, 1984; Bromfield and Hartwig, 1980).

Phaseolus vulgaris cv. Verano and *P. lunatus* were susceptible to *P. meibomiae* because of greater numbers of uredinia per lesion and a large uredinium diameter, all of which results in faster spread of the

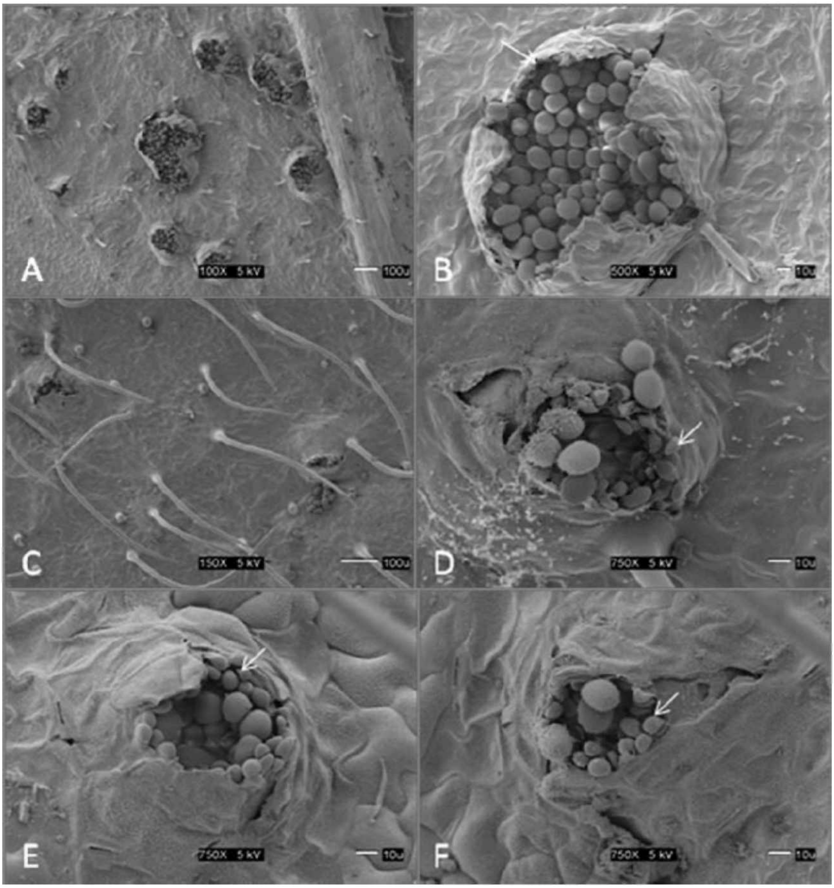

FIGURE 3. Scanning electron microscopy of *Phakopsora meibomiae*. A, uredinia distribution on *Phaseolus vulgaris* (100X); B, urediniospores releasing through uredinium erupted on *P. vulgaris* (500X); C, pubescence and uredinia distribution on *Macroptilium lathyroides* (150X); D, uredinium structure and urediniospores releasing on *M. lathyroides* (750X); E, uredinium structure and urediniospores in *Glycine max* cv. Williams (750X); and F, epidermis rupture and urediniospores releasing in *G. max* cv. Williams (750X). The presence of paraphyses is indicated by arrows.

fungus. *Phaseolus lunatus* and *P. vulgaris* are native to Central America; thus *P. meibomiae* may be more virulent to these legumes. Common bean was reported to be more susceptible to *P. meibomiae* (isolates originated in Brazil and Puerto Rico) than to a *P. pachyrhizi* isolate from Taiwan (Miles et al., 2007) *Phaseolus* has been used to increase inoculum of *P. meibomiae* because of its high rate of uredinia production

TABLE 3.—*Uredinium diameter in legumes inoculated with* Phakopsora meibomiae.

Legumes	n[1]	Uredinium diameter (µm)		
		Mean[2]	Minimum	Maximum
Canavalia gladiata	—	—	—	—
Crotalaria brevidens	—	—	—	—
Crotalaria juncea	50	137.35 b	66.00	203.20
Crotalaria retusa	12	161.29 a	113.50	217.80
Glycine max 2053 A	50	113.95 cde	78.80	152.20
Glycine max Williams	50	108.75 de	64.20	142.60
Lablab purpureus	50	127.69 bcd	79.60	196.70
Macroptilium lathyroides	50	127.17 bcd	84.00	203.20
Mucuna pruriens	—	—	—	—
Pachyrhizus erosus	34	100.48 e	50.20	141.70
Phaseolus lunatus	50	136.60 b	59.80	224.00
Phaseolus vulgaris	50	133.18 bc	47.30	220.00
Pueraria phaseoloides	—	—	—	—
Rhynchosia reticulata	—	—	—	—
Vigna luteola	50	109.63 de	59.40	198.90
Vigna unguiculata	50	114.27 cde	48.00	167.60

[1]n = number of uredinia evaluated.
[2]Mean separation by the Tukey test, $P \leq 0.05$.

(66% higher than that of *L. purpureus*) and seed availability (Frederick et al., 2002; Bonde et al., 2006). However, only one genotype of common bean and lima bean were evaluated in the study. Both *Phaseolus* species evaluated, *P. lunatus* and *P. vulgaris,* showed low incubation (3.2 and 3.2 d) and latent (6.4 and 6.2 d) periods. In contrast, *C. retusa* had an incubation period of 6.2 d, probably because of leaf pubescence and the thickness of the cuticle layer. Furthermore, the abundant pubescence observed on *M. lathyroides* leaves is probably a natural barrier to avoid the pathogen establishment (Mmbaga et al., 1994; Niks and Rubiales, 2002). During the formation of the uredinium, the epidermal leaf rupture was pronounced (Figures 3C and 3D).

In soybean, the average number of uredinia per lesion varied from 1.42 (line 2053 A) to 1.80 (cultivar Williams). Similar results were obtained in 2006 when nine cultivars of soybean inoculated with *P. meibomiae,* isolates Brazil 82-1 and Puerto Rico 76, produced an average number of 1.4 to 2.2 uredinia per lesion (Bonde et al.,2006). Soybean cv. Dare, inoculated with an Australian isolate of *P. pachyrhizi,* produced both longer incubation (6.0 days) and latent (13.7 d) periods than soybean cv. Williams inoculated with *P. meibomiae* (4.52 and 10.39 days, respectively) (Burdon and Marshall, 1981a; Burdon and Marshall, 1981b). These results indicate the potential of *P. meibomiae* to infect soybeans and develop an epidemic, as was observed in 1976 in Adjuntas, if environmental conditions are favorable for infection.

Pueraria phaseoloides, a broadly dispersed legume in Puerto Rico, showed an immune reaction to *P. meibomiae;* therefore, there is a high probability that it should not be host to *P. meibomiae* on the island. *Pueraria lobata,* dispersed in North and South America and not found in the surveyed areas, is an overwintering host to*P. pachyrhizi* throughout Florida, Alabama, Georgia, Louisiana, Mississippi, and other states in the United States (Carmona et al., 2005; Harmon et al., 2006; Isakeit et al., 2006; Koenning et al., 2007; Sikora and Hershman, 2008).

Phakospsora meibomiae infection in the legumes evaluated is common in the central mountainous area of Puerto Rico. In a survey conducted by Vakili (1979), *P. meibomiae* occurred at sites with average annual temperatures between 17 and 23° C and precipitation of 170 to 260 mm. However, in the coastal area where soybeans are grown, no symptoms or signs of rust in legumes were observed. Soybean nurseries in Puerto Rico are located in the southern coastal plains that have much drier and warmer climatic conditions than the mountainous region, where *P. meibomiae* is widespread. Therefore, a possible factor involved in the absence of rust in soybeans is the environmental conditions in the area. Solar irradiation in the mountains is lower than in the coastal area; also, higher rainfall and air temperatures (17 to 23° C) make the mountain areas ideal for rust infection (Vakili, 1979; Vakili, 1981; Vakili and Bromfield, 1976; Vega and Estévez de Jensen, 2008). The soybean production areas, located in the northwest and southwest of Puerto Rico, are characterized by high solar radiation and air temperature. These are probably the most important factors that limit rust infection in soybeans on the island. In 2007, the average solar radiation in Juana Díaz (southwest) was 388 W/m^2, with 787 mm of precipitation, 26.4° C and 72.9% relative humidity, whereas in Isabela (northwest) the average solar radiation was 391 W/m^2, with 1,626 mm of precipitation, 24.6° C and 81.7% relative humidity (www.wcc.nrcs.usda.gov/scan/). Therefore, it is not anticipated that American soybean rust will present a threat to soybean or dry bean production on the island as reported previously (Estévez de Jensen et al., 2009).

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