Virulence and Aggressiveness of *Phakopsora pachyrhizi* Isolates Causing Soybean Rust

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ABSTRACT

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Isolates of *Phakopsora pachyrhizi* (the soybean rust pathogen) from Australia, India, Puerto Rico, and Taiwan differed in virulence on an array of soybean (*Glycine max*) genotypes. Three distinct infection types were recognized: TAN, RB, and 0. Type TAN, a tan lesion about 0.4 mm^2, usually with 2–5 uredia on the abaxial surface of the leaf 2 wk after inoculation, indicated host susceptibility. Type RB, a reddish-brown lesion about 0.4 mm^2, usually with 0–2 uredia on the abaxial surface of the leaf 2 wk after inoculation, indicated host resistance associated with hypersensitivity. Type 0, the absence of macroscopically visible evidence of rust, indicated host immunity or near immunity. With increasing time after inoculation, lesion area and number of uredia per lesion increased for both TAN and RB, but the rates of increase were slower for RB. Culture Australia-72-1 induced both TAN and RB on each of eight accessions; it may be composed of at least two physiologic races. Culture Taiwan-72-1 induced TAN on 13 accessions. Culture India-73-1 induced either RB (on eight) or 0 (on five). On 20 soybean cultivars, culture PR-Comp from Puerto Rico induced RB only, but the other cultures induced TAN. All four cultures induced only RB on PI 230970 and PI 230971. Cultures Australia-72-1, India-73-1, and Taiwan-72-1 induced TAN on soybean cultivar Wayne. The rate of lesion enlargement and the rate of increase in number of uredia per lesion on the lower surface of leaves of Wayne were about equal for India-73-1 and Taiwan-72-1, but were lower for Australia-72-1, which indicates that Australia-72-1 was less aggressive on Wayne than the other two under the test conditions used. Culture Taiwan-72-1 consistently produced more uredia per lesion at a given time on the upper surface of leaves of Wayne than did India-73-1.

Additional key words: disease resistance, pathotypes, physiologic specialization.

Cultures of the soybean rust fungus, *Phakopsora pachyrhizi* Sydow, from widely separated geographical regions were compared as part of a study aimed at assessing the threat of soybean rust to soybean (*Glycine max* [L.] Merr.) crops in the United States. Isolates from Australia, India, Taiwan, and Puerto Rico were cultured on soybean accessions in the U.S. Department Plant Disease Research Laboratory (PDRL), Frederick, MD, and observed for virulence and aggressiveness.

MATERIALS AND METHODS

Two lines of investigation were pursued. In the first, the four rust fungus isolates were cultured on young plants of soybean cultivar Wayne. At weekly intervals after inoculation, the plants were observed for three parameters: area of rust lesion, number of uredia per lesion on the upper (adaxial) surface of host leaves, and number of uredia per lesion on the lower (abaxial) surface of host leaves. In the second, soybean accessions from a variety of sources were inoculated with the four isolates, and each isolate-host combination was observed to evaluate virulence of the pathogen and reaction of the host.

Cultures and inoculum. The rust cultures were established from uredospore inoculum airmailed to PDRL on dried rusted leaves or cotton swabs that had been rubbed over sporulating rust lesions. Spore-bearing donor leaves or swabs were positioned above plants of Wayne and gently tapped to dislodge and deposit uredospores on the upper surface of recipient leaves. The inoculated plants were kept in a dew chamber at 20 C for 18 hr and then moved to a greenhouse.

Culture Australia-72-1 was established 9 June 1972, from uredial material collected from soybeans at Redland Bay, Queensland, Australia, by D. E. Byth. Culture Taiwan-72-1 was established 15 June 1972, with uredospores on cotton swabs comprising a collection made in Taiwan by Lung-Chi Wu. Culture India-73-1 was established 15 January 1973, from uredial material collected from soybean cultivar Lee at Pantnagar, India, by P. N. Thapliyal. Culture PR-Comp is a composite of uredospores collected from plants of Wayne that had been inoculated individually with isolates obtained initially by N. G. Vakili and K. R. Bromfield from hyacinth bean (*Dolichos lablab* L.), common bean (*Phaseolus vulgaris* L.), lima bean (*P. lunatus* L.), scarlet runner bean (*P. coccineus* L.), and soybean in Puerto Rico in August, 1976 (9). The composite was increased through several uredial generations on Wayne, as were the other cultures, before it was used as inoculum in the tests reported here.

In some tests, inoculum was freshly harvested uredospores; in others, it was uredospores that had been stored in sealed glass ampules in liquid nitrogen. Sealed ampules removed from liquid nitrogen were held at 21 to 27 C for at least 30 min before removal of spores. Previous tests had shown that disease initiation and development were similar when either freshly harvested or stored spores were used as inoculum.

Test plants. Individual soybean plants were grown in 10-cm-diameter clay pots containing a potting mixture of soil, sand, peat, and vermiculite (2:1:1:1, v/v). Plants were inoculated at the three-

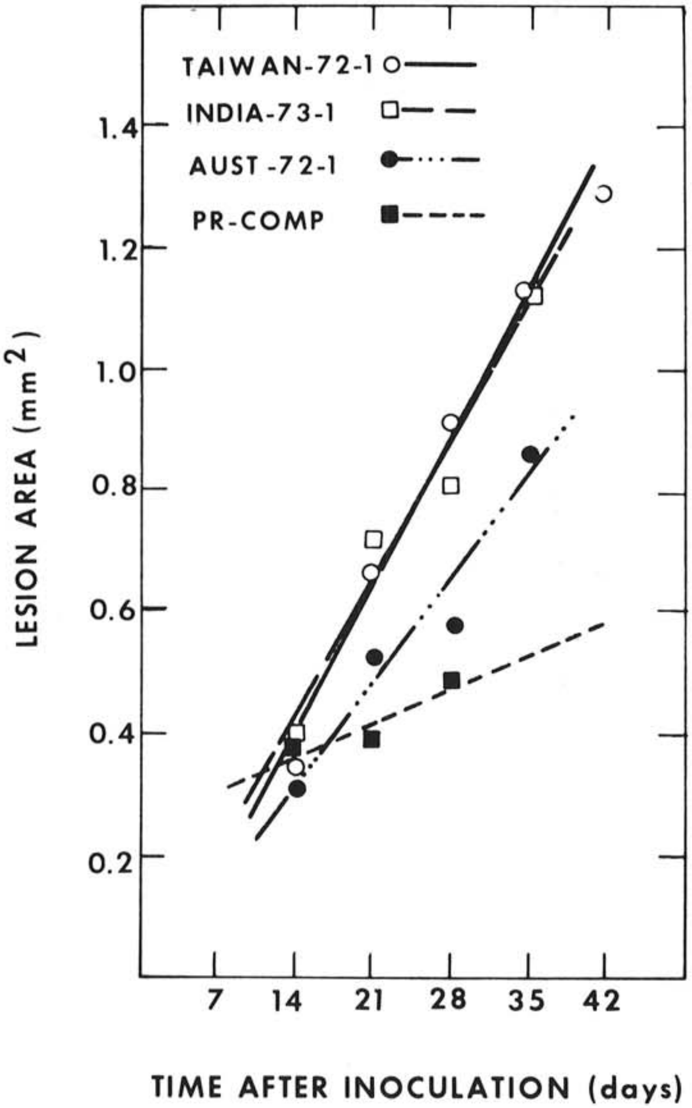

Fig. 1. Mean area of individual lesions produced by each of four cultures of *Phakopsora pachyrhizi* on the lower (abaxial) surface of Wayne soybean.

or four-trifoliolate leaf stage, 4–6 wk after seeding. Seed of cultivar Wayne was obtained from Wilkin Seed Grains Co., Pontiac, IL. Accessions tested for rust reaction were provided primarily by E. E. Hartwig, USDA, Stoneville, MS; R. L. Bernard, USDA, Urbana, IL; S. Shanmugasundaram, Asian Vegetable Research and Development Center (AVRDC), Taiwan; D. E. Byth, University of Queensland, Brisbane, Australia; and B. B. Singh, G. B. Pant University, Pantnagar, India.

Inoculation and incubation. Test plants were inoculated in groups of eight per culture in a turntable settling tower with 10 mg of uredospores pneumatically discharged into the tower. Immediately after inoculation, the plants were placed in a dew chamber at 20 C in darkness for 16 to 22 hr. They were then moved to a greenhouse at 24–30 C during the day and 24 C at night with about 50% relative humidity and natural light. With these procedures, generally five to 25 rust lesions developed per square centimeter of leaf area. Leaves were not wetted after the initial dew period. This procedure precluded secondary infection and confined observations to lesions of a single infection cycle.

Observations. Observations for record were made on first, second, and third trifoliolate leaves.

Rust development was observed on Wayne at weekly intervals beginning at 2 wk and continuing for up to 6 wk after inoculation. At each observation, one leaflet from each plant of a group was removed and examined at ×30 magnification under a stereomicroscope. The area of 10 randomly selected discrete, individual lesions on the lower surface of the leaflet was measured with a calibrated eyepiece graticule. For each of the 10 lesions for which the area was determined, the number of uredia per lesion also was determined. The number of uredia per lesion was counted also for each of 10 randomly selected lesions on the upper surface of the leaflet.

Rust development was observed on plants inoculated at various times over a 3-year period. A total of 34 plants infected with Australia-72-1, 46 with India-73-1, 104 with Taiwan-72-1, and 17 with PR-Comp were observed.

Rust reaction was observed at 2, 3, and 4 wk after inoculation. At each observation, the macroscopic characteristics of each culture-accession interaction was recorded. A total of 110 different soybean accessions was challenged with Taiwan-72-1, 87 with Australia-72-1, 85 with India-73-1, and 23 with PR-Comp over a period of 2 yr. Accessions included cultivars, breeding lines, and selections.

RESULTS

Lesion development on Wayne. The mean area of macroscopic lesions produced on the lower surface of the leaf by each of the four different cultures was ~0.3–0.4 mm^2 at 2 wk after inoculation. Lesion area increased linearly with time. The rate of lesion area increase was greatest (and almost identical) for Taiwan-72-1 and India-73-1, least for PR-Comp, and intermediate for Australia-72-1 (Fig. 1) (7).

Uredia formation on Wayne. With all cultures the number of uredia per lesion on the lower surface of the leaf was greater than that on the upper surface at each observation time. The number of uredia per lesion on the lower surface increased markedly with time. The rate of increase was greatest (and about equal) for Taiwan-72-1 and India-73-1, somewhat less for Australia-72-1, and least for PR-Comp (Fig. 2) (7).

The number of uredia per lesion on the upper surface increased slowly or not at all with time. Very few, if any, uredia developed on the upper surface of leaves infected with PR-Comp. An average of one or few uredia per lesion developed on the upper surface of leaves infected with Australia-72-1. Culture India-73-1 produced an average of about one uredium per lesion, with no appreciable increase with time, but culture Taiwan increased from one and a half uredia per lesion at 2 wk after inoculation to about three uredia per lesion at 6 wk after inoculation (7).

Although they were not measured, the uredia of all cultures appeared to be smaller on the upper surface than on the lower surface.

Pathogen-host interactions. The interactions among the various pathogen-host combinations were characterized by one of three infection types: TAN, RB, or 0. The majority of interactions produced type TAN (Fig. 3A). At 2 wk after inoculation, this type on the lower surface of the leaf was characterized by a tan lesion about 0.4 mm^2 in area in which two to five uredia were present and sporulation was abundant. Both lesion area and the number of uredia per lesion increased with time.

Infection type RB on the lower surface of the leaf at 2 wk after inoculation was characterized by a reddish-brown lesion about 0.4 mm^2 in area, in which usually zero, one, or two uredia were present and sporulation was sparse (Fig. 3B). The lesion area and number of uredia per lesion increased with time, but not as rapidly as for TAN.

Five to 6 wk after inoculation, TAN and RB were still distinguishable on leaves that remained alive and green, although there was a tendency for differences to become less obvious with increasing time. The lesion color of TAN often darkened and the numbers of uredia per lesion in RB sometimes equalled those observed in young lesions of TAN.

A third type of pathogen-host interaction exhibited no macroscopic evidence of rust. This was designated type 0.

Culture PR-Comp induced RB on all 23 accessions, including the 20 most extensively grown U.S. cultivars.

India-73-1 interacted with PI 200492, Tainung 3, Tainung 4, PI 368039, and selection X74-853001 (PI 200492 × Ogden) to produce type 0. It induced RB on accessions Ankur, PK-71-39, PK-73-84, PI-73-94, UPSL-18, UPSL-85, UPSM-91, and UPSM-168 from India, and on PI 230970 and PI 230971. The Indian accessions, all from G. B. Pant University, may represent closely related genotypes. TAN developed on the other 70 accessions inoculated with the Indian culture.

Australia-72-1 induced both RB and TAN on the same leaflets of the eight accessions from G. B. Pant University. Only RB was observed on PI 230970 and PI 230971. TAN was induced on the other 77 accessions inoculated.

Taiwan-72-1 induced only RB on accessions PI 230970 and PI 230971. TAN developed on the other 108 accessions tested.

On the basis of differential host reactions observed, three physiologic races were identified among the four cultures examined. The culture from Puerto Rico represents one race. It can be differentiated readily by its limited virulence. The race comprising culture Taiwan-72-1 can be differentiated from that comprising India-73-1 on eight accessions from India, PI 200492, and four other accessions with PI 200492 in their parentage. A simplified scheme for the identification of these three races is shown in Table 1.

Australia-72-1 may be composed of a mixture of at least two races. No attempts were made to establish subcultures by isolating from the two different infection types, and as yet no cultures of two races identified in Queensland, Australia, have been obtained for comparison.

DISCUSSION

The reaction of certain cultivars inoculated with the four isolates revealed differences in pathogen virulence and development.

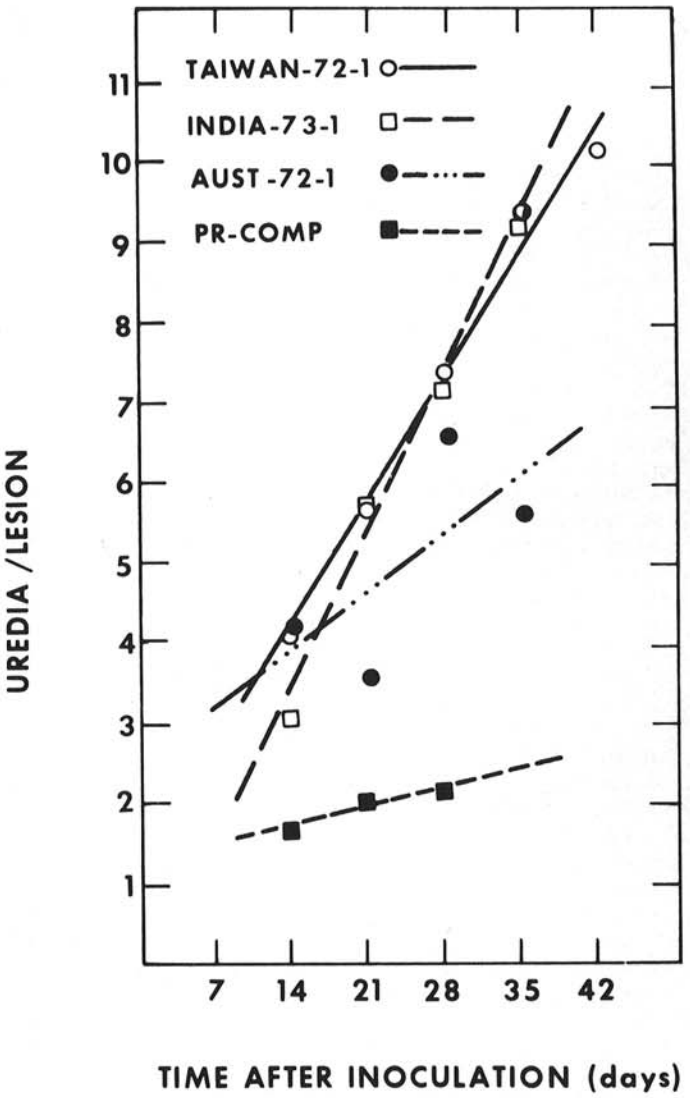

Fig. 2. Mean number of uredia per lesion produced by each of four cultures of *Phakopsora pachryhizi* on the lower (abaxial) surface of Wayne soybean.

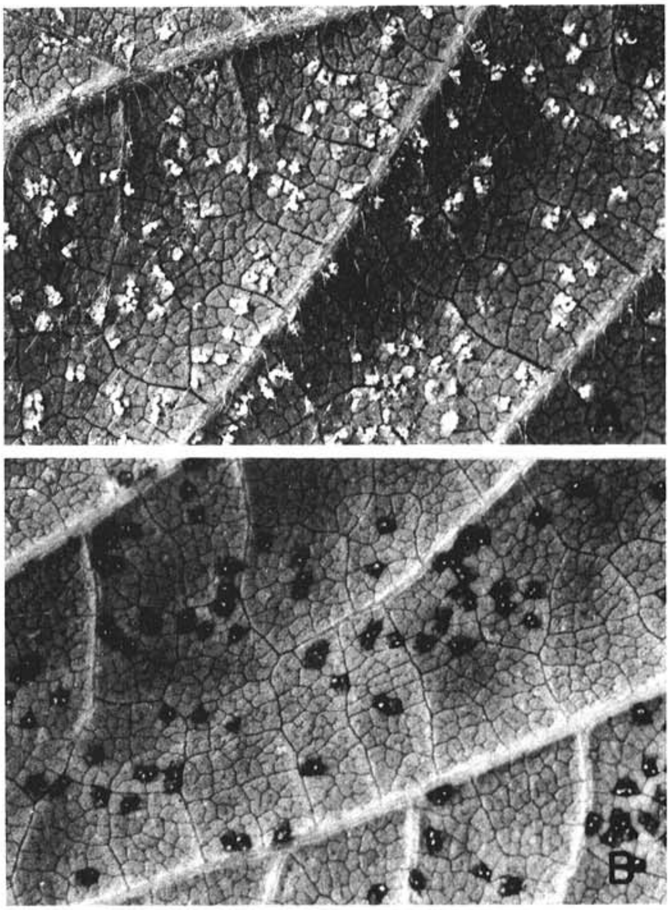

Fig. 3. Appearance of soybean rust infection types on the lower (abaxial) surface of soybean leaves 14 days after inoculation. **A,** Infection type TAN. **B,** Infection type RB.

TABLE 1. Rust infection types produced by three cultures of *Phakopsora pachyrhizi* on three accessions of soybean

Culture	Physiologic race[a]	Infection type[b] on differential host		
		Wayne	Ankur	PI 200492
Taiwan-72-1	PDRL-1	TAN	TAN	TAN
India-73-1	PDRL-2	TAN	RB	0
PR-Comp	PDRL-3	RB	RB	RB

[a] Physiologic race designations are temporarily used, pending adoption of a universal convention for the designation of races of *Phakopsora pachyrhizi*.
[b] TAN = tan lesion, ~ 0.4 mm^2, usually with 2–5 uredia per lesion; RB = reddish-brown lesion, ~ 0.4 mm^2, usually with 0–2 uredia per lesion; 0 = absence of macroscopically visible signs or symptoms. Abaxial surface of leaves were observed 2 wk following inoculation.

Three infection types define three classes of host reaction. TAN indicates host susceptibility, RB indicates resistance, and 0 indicates host immunity or near-immunity. Further research may reveal additional reaction types, or variations of those described. Additional categories of infection type would permit definition of more classes of host reaction and thus permit assignment of a range of degrees of resistance or susceptibility.

McLean (5) in Australia also identified three infection types, designated 1, 2, and 3, and used them to define three classes of host reaction. Type 1, described as "sporulating uredia surrounded by necrotic tissue" appears to correspond to TAN. Hosts producing type 1 were classified as susceptible. Type 2 "consisted of necrotic lesions with no uredia." This may correspond to RB, although McLean does not discuss lesion color and the precision of the statement "no uredia" is not known. In our tests with Australia-72-1, India-73-1, and Taiwan-72-1, and various hosts that reacted by producing RB lesions, about 51% of the RB lesions lacked uredia at 2 wk after inoculation, 26% had one uredium per lesion, and 23% had two or more uredia per lesion. At 4 wk after inoculation, however, only 10% of the lesions lacked uredia, 13% had one uredium, and 77% had two or more uredia per lesion. Type 2 of McLean and RB both are used to designate resistant hosts.

Type 3 was described as having "no visible symptoms of infection." McLean rated hosts with infection type 3 as "highly resistant." Type 3 corresponds to our type 0.

Singh et al (8) also designated lines as "resistant" or "moderately resistant" on the basis of infection type produced. Those found to be free of rust were rated "resistant;" we would designate such lines as immune or near-immune (infection type 0). Lines rated "moderately resistant" by Singh et al (8) "developed a pink spot at the point of contact with fungal spores indicating a hypersensitive reaction. There was very little or no further development of the fungus on these spots." We assume that these "pink spots" correspond to our RB.

Our results may help to dispel some of the confusion surrounding the "resistance" of PI 200492 and its potential value in soybean breeding programs aimed at incorporating resistance to soybean rust. In the early 1960s the U.S. Department of Agriculture sent seed of its soybean germplasm holdings to Taiwan for planting in field nurseries. These plantings were severely attacked by soybean rust. Among the accessions, only PI 200490 and PI 200492 were reported to have "resistance" to rust. Field notes made in Taiwan in 1965 and 1966 show that most of the lines observed had rust severities of 50% or greater but that the rust severities on PI 200492 and selections with PI 200492 parentage were much below 50% (notes accompanying letter from K. L. Chan to R. L. Bernard). No mention of infection type was made.

Because of its "rust resistance," PI 200492 was used as a parent in Taiwan in the development of Tainung 3, which was released in 1968 (3), and in Tainung 4, which was released in 1971 (2). The tests reported here show that culture Taiwan-72-1, received from Taiwan in 1972, is virulent on PI 200492, Tainung 3, and Tainung 4. Reports from AVRDC indicate that PI 200492 and lines and cultivars with PI 200492 parentage now become severely rusted in the field in Taiwan (10). Apparently components of the pathogen population capable of attacking PI 200492 have increased in prevalence in Taiwan.

In 1971, D. E. Byth at the University of Queensland began using PI 200492 in crosses aimed at incorporating rust resistance into locally adapted soybean cultivars. Initially PI 200492 was immune to the rust pathogens at Redland Bay, Australia, but subsequently rust lesions appeared on it. The presence of two rust races at Redland Bay, one avirulent and the other virulent on PI 200492, has been confirmed (6). Whether the race attacking PI 200492 in Australia is the same race as that attacking PI 200492 in Taiwan is not yet known.

Singh et al (8) reported PI 200492 to be immune to the rust pathogens at Pantnagar, India (8). We also observed that PI 200492 and selections with that accession in their parentage are immune to India-73-1, which was obtained from Pantnagar. Whether the race avirulent on PI 200492 at Pantnagar is the same race which is avirulent on PI 200492 at Redland Bay is not yet known.

Accessions PI 230970 and PI 230971, both originally obtained from Japan, may be of special value to breeders because of their demonstrated resistance (infection type RB) to all four cultures used in this study. They are the only accessions of 110 tested that are resistant to Taiwan-72-1.

The early race identification work of Lin (4) in Taiwan, C. Y. Yang (1) at AVRDC, and McLean and Byth (5,6) in Queensland, and the results reported here, are first steps in the exploration of races within populations of *P. pachyrhizi*. Detailed information on pathogen-host interactions is urgently needed from all countries in which soybean rust is a present or potential hazard to soybean growing. Also required is a broadening of the approach to investigate the "strength of vertical genes conferring resistance" (*sensu* Vanderplank), the mechanisms by which new races arise, and the survival fitness of the races that do arise.

Although the existence of some races of *P. pachyrhizi* has been demonstrated and we can expect others to be found, it is premature to suggest a standard set of differentials for race identification. Decisions on designation of specific differential cultivars and lines should be deferred. More information is needed on genes conveying resistance in the host, genes conveying virulence in the pathogen, and the effect of environmental factors on the expression of pathogen-host interactions.

It is also premature to suggest a universal convention for the designation of races of *P. pachyrhizi*. A number of race nomenclature schemes are currently in use for various pathogen-host combinations. These range from the sequential numbering of races in the chronological order in which they are identified to those in which the designation provides information on genes for virulence in the pathogen and genes for resistance in the host. Additional research with a broader spectrum of soybean genotypes and pathogen isolates will provide data needed to formulate a scheme suited to the needs of soybean pathologists and breeders.

Patterns of infection types resulting from the differential interactions of various culture-host combinations serve to identify a widely recognized, qualitative kind of diversity among cultures of the soybean rust fungus. As it does for other diseases, exploration of these patterns provides information of value to breeders seeking to incorporate specific rust resistance into soybean cultivars, geneticists studying the frequencies of virulence genes in pathogen populations, and pathologists seeking to understand and manage soybean rust. Pathogen diversity of another sort is demonstrated by quantitative differences in pathogen development among cultures on a given susceptible host. Information on cultural differences of this kind is especially useful to epidemiologists and to breeders endeavoring to enhance general resistance in cultivars. Research on the comparative dynamics of pathogen development in hosts lacking strong specific resistance will provide needed information on relative aggressiveness, epidemiological potential, and survival fitness of isolates of *P. pachyrhizi* (7).

The culture of *P. pachyrhizi* from Puerto Rico, although morphologically similar to those from the Orient, lacks virulence on soybean cultivars. It may represent a distinct forma specialis. At present it appears not to pose a threat to soybean crops. If, however, soybeans become more widely grown in areas within the current range of the fungus, variants that may arise with greater virulence on soybean will have enhanced opportunity for being "selected out," increased, and disseminated. If this occurs on a sufficiently large scale, rust pathogens from that source eventually may become a factor in soybean production in the Americas.

LITERATURE CITED

1. ASIAN VEGETABLE RESEARCH AND DEVELOPMENT CENTER. 1975. Annual Report for 1974. Shanhua, Taiwan, Republic of China. 142 pp.
2. CHAN, K. L., Y. B. CHENG, and S. H. CHEN. 1971. The breeding of "Tainung 4" soybean. J. Taiwan Agric. Res. 20:1-5.
3. CHENG, Y. W., and K. L. CHAN. 1968. The breeding of rust resistant soybean "Tainung 3." J. Taiwan Agric. Res. 17:1-5.
4. LIN, S. Y. 1966. Studies on the physiologic races of soybean rust fungus, *Phakopsora pachyrhizi* Syd. J. Taiwan Agric. Res. 15:24-38.
5. McLEAN, R. J. 1979. Histological studies of resistance to soybean rust, *Phakopsora pachyrhizi* Syd. Aust. J. Agric. Res. 30:77-84.
6. McLEAN, R., and D. E. BYTH. 1976. Resistance of soybean to rust in Australia. Aust. Plant Pathol. Soc. Newsl. 5:34-36.
7. 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.
8. SINGH, B. B., S. C. GUPTA, and B. D. SINGH. 1974. Sources of field resistance to rust and yellow mosaic diseases of soybean. Indian J. Genet. Plant Breed. 34:400-404.
9. VAKILI, N. G., and K. R. BROMFIELD. 1976. Phakopsora rust on soybean and other legumes in Puerto Rico. Plant Dis. Rep. 60:996-999.
10. YANG, C. Y. 1977. Past and present studies of soybean rust incited by *Phakopsora pachyrhizi* Syd. Bull. Inst. Trop. Agric., Kyushu Univ., Jpn. 2:78-94.