

# XI. APPENDIX I: INVITED PAPERS

## I - A. Research at the USDA, ARS Containment Facility on Soybean Rust and its Causal Agent

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Soybean rust research in the U.S. began in 1971 at the U.S. Department of Agriculture (USDA) in Frederick, Maryland. In 1971, the research unit had just been transferred from the Department of Defense to the USDA. The new mission of the unit was to study foreign diseases threatening the major U.S. crops (maize, soybeans, and wheat). It was believed that research information prior to appearance of a disease could be instrumental in lessening its impact when and if it arrived.

Since 1971, six plant pathologists including K. R. Bromfield, J. S. Melching, M. A. Marchetti, M. R. Bonde, M. H. Royer, and X. B. Yang have worked on soybean rust at Frederick. The first five were permanent USDA employees, the last a postdoctoral student.

Besides in-house research on soybean rust, the laboratory has had several research contracts and informal cooperative arrangements with other laboratories. Many of these cooperative endeavors were in foreign countries where the disease exists, however this presentation will cover mainly research conducted in the disease containment facility at Frederick. It must be recognized that the research at Frederick was not done in a vacuum. The scientists at Frederick have traveled extensively and are in direct contact with scientists throughout the world who have similar interests.

### THE CONTAINMENT FACILITY

The Foreign Disease-Weed Science Research (FDWSR), the current name of the unit, operates a plant disease containment facility in Frederick, Maryland where foreign plant pathogens of quarantine significance are studied without danger of escape (8). The facility is a brick-faced 41 x 176-foot concrete building with five attached 25 x 60-foot greenhouses. Each greenhouse has large

double-layered glass panels supported by a steel superstructure. At this facility, with the permission of state and federal regulatory officials, plant pathogens from anywhere in the world, and suitable host material, can be imported for research.

The containment facility operates under a slightly negative air pressure to prevent air leakage to the outside. All refuse and equipment leaving the containment portion of the building are sterilized by heat or gas. Water is sterilized by Fort Detrick personnel by repeated heating, and air exiting passes through several filter systems that remove all particles greater than 0.5 mm diameter. Furthermore, all personnel wear special work clothes and remove these clothes and shower thoroughly before exiting containment. Once through the shower, they put on their street clothes in the nondisease containment portion of the building.

The ability to conduct research experiments under strict containment conditions provides an opportunity not available elsewhere. It is an opportunity for critical studies to be conducted that provides information to assess the threat of a disease to U.S. agriculture and prepare for the possibility of its eventual appearance.

### HISTORY OF THE SOYBEAN RUST PROGRAM

Early years of research. Soybean rust was chosen to be in the first round of diseases to be studied because of: (i) the demonstrated ability of the pathogen, *Phakopsora pachyrhizi*, to cause major yield losses in Australasia, (ii) the lack of disease resistance to soybean rust in U.S. commercial cultivars and germ plasm, and (iii) the

scarcity of published information on the pathogen and disease (4). Also, there was a widely-held belief that rust diseases in general, due to their frequent ability to spread quickly and cause large losses, posed a greater threat than most other diseases.

The soybean rust program began in 1971 with the accession of several cultures of *P. pachyrhizi* from Asia. Comparative studies were conducted in the plant disease containment facility to determine the general susceptibility of the U.S. soybean crop and whether races existed among the cultures. Studies also were conducted to compare the cultures to determine the effects of temperature, dew period duration, and other environmental factors on spore germination, penetration, establishment of infection, and sporulation. Mycological and histological studies were conducted to better understand the pathogen.

The research of Marchetti *et al.* (6), Meiching *et al.* (7,9), and Bonde *et al.* (2) established with precision the effects of temperature and dew duration on urediniospore germination, and establishment of infection by cultures of the pathogen from Australia, India, Indonesia, and Taiwan. This was a first step at determining whether *P. pachyrhizi* could thrive in the continental U.S. From the results it appeared that temperature requirements for infection would not preclude the establishment of soybean rust in the major soybean growing areas of the U.S. (6).

The Taiwanese culture was used further in a series of complex experiments to determine the effects of various combinations of specific dew durations, dew frequency, and temperature on soybean rust (10). For example, on leaves in the dark at 20°C, urediniospores of *P. pachyrhizi* began germinating 1.5 hours after dew was provided and reached a maximum level after 6 to 7 hours. After 8 hours of dew at 18° to 26.5°C, lesion intensities were 10-fold higher than those at 6 hours at the corresponding temperatures. Spores on leaves exposed to 4- or 6- hours dew followed by drying for up to 4 days were able to infect when a 12-hour dew period was provided, but were 50% or

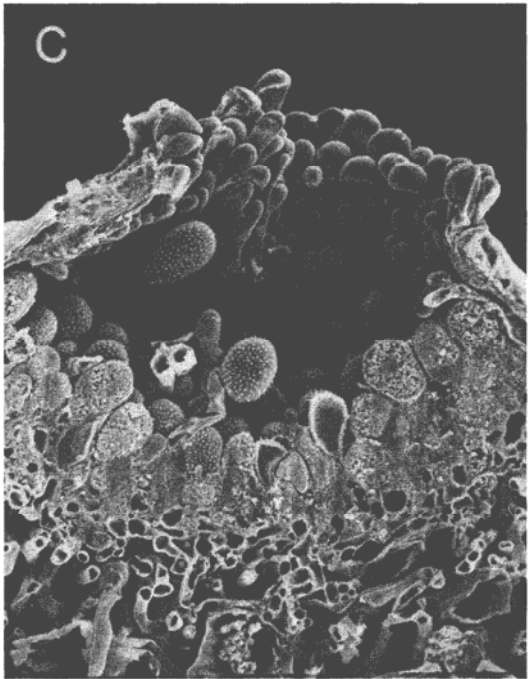
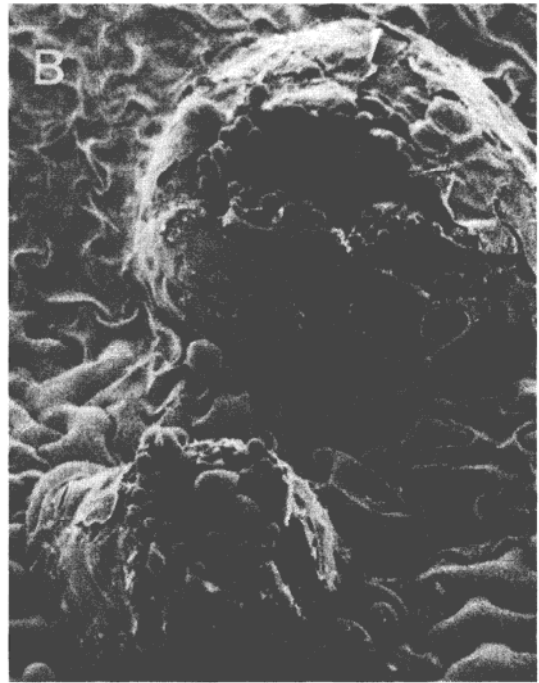
less as infectious as similar spores that had not been exposed to brief initial wetting. Rust reduced the number of filled pods, number of seeds per pod, and mean seed weight (10).

Under greenhouse conditions in containment, cultures of *P. pachyrhizi* from Australia, India, Indonesia, and Taiwan required similar time periods from inoculation until lesion appearance (7 days) and the initiation of secondary urediniospore production (9 days)(7). Daily quantitative measurements of the number of lesions, size of lesions, and number of lesions for 52 days after inoculation revealed large differences in the cultures. For example, the Australian culture produced a total of 2,028 µg spores/plant, and the Taiwanese culture a total of 6,600 µg urediniospores/plant when spores were collected during the life of the lesions.

In order to determine whether the pathogen might be able to overwinter in continental U.S., host range studies were conducted using plant species, including legumes, present in the continental U.S. The results agreed with those of other workers showing that *P. pachyrhizi* has a broad host range (12). The results also suggested that the pathogen might be able to overwinter in the southern part of the continental U.S., Cuba, or Mexico and act as a source of inoculum to infect a new soybean crop.

Discovery of soybean rust in the New World. In 1976, a rust on soybeans was discovered in Puerto Rico by USDA-ARS plant pathologist N. G. Vakili while examining experimental plantings of legumes at the Adjuntas Agricultural Substation in the Limani Valley (14). This was a historic event since it was the first record of a rust on soybeans in the Western Hemisphere. Vakili conducted a series of surveys on the island to determine the geographical and seasonal distribution of the disease and hosts of the pathogen in Puerto Rico (13).

When soybean rust was discovered in 1976, K.R. Bromfield went to Puerto Rico to examine the pathogen and disease with Vakili. Vakili and Bromfield (14) published a paper in the Plant Disease Reporter titled *Phakopsora Rust*



*Soybean and Other Legumes in Puerto Rico.* Because of the seriousness of the discovery, special arrangements had been made to have the paper published quickly. This is a perfect example of a rapid response to a perceived threat to U.S. agriculture.

Subsequent to the discovery of soybean rust in Puerto Rico, Bromfield *et al.* (5) conducted virulence studies in the plant disease containment facility on cultures of *P. pachyrhizi* in which the Puerto Rican organism was included. These studies showed that the pathogen in Puerto Rico was pathogenically different from the pathogen in Asia and Australia. On all soybean cultivars and germ plasm, the Puerto Rican organism produced what was called a “resistant reaction”. Bromfield *et al.* (5) referred to the type of reaction as an “RB reaction” because of a distinct reddish brown coloration of the lesions. In contrast, the Asian and Australian cultures produced a susceptible (TAN), resistant, or immune reaction, depending on soybean genotype. A susceptible reaction was referred to as “TAN” because of the tan color of the lesions. The differential reactions of the Asian isolate on the

Figure 1A-C. Development of *Phakopsora pachyrhizi* on cv. Wayne soybean leaves: A, scanning electron micrograph (SEM) of echinulated germinated urediniospore with germ tube and large appressorium. (X1000); B, SEM of two uredinia of the pathogen on leaf surface. (X300); C, SEM of a cross section of uredinium with paraphyses. (X500).

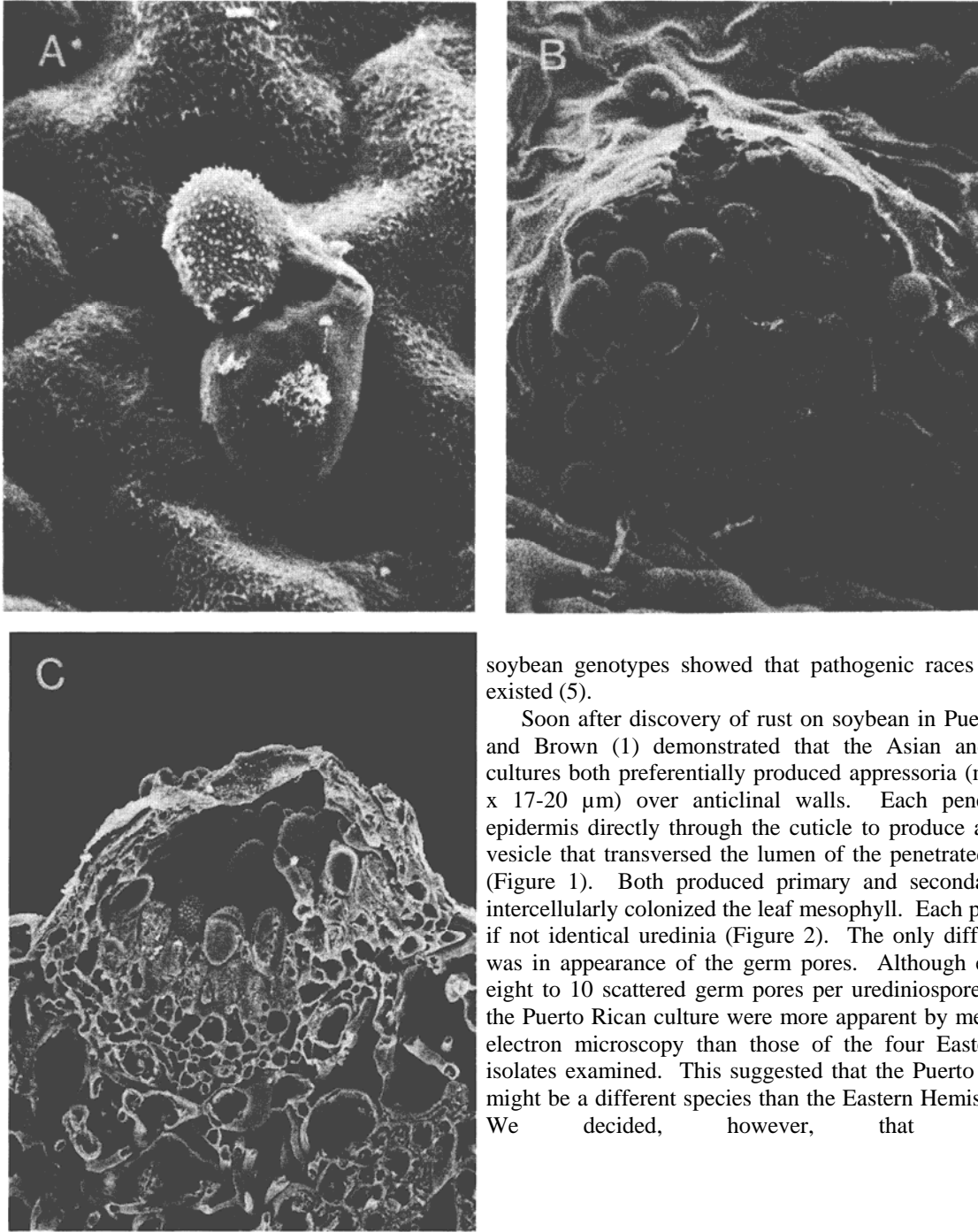


Figure 2A-C. Development of *Phakopsora meibomiae* on cv. Wayne soybean leaves: A, scanning electron micrograph of germinated urediniospore with germ tube and appressorium. (X1500); B, uredinium of pathogen on leaf surface. (X500); C, a cross section of uredinium with peripheral para physes which can be seen beneath the strip of host epidermis. (X500).

soybean genotypes showed that pathogenic races of *P. pachyrizi* existed (5).

Soon after discovery of rust on soybean in Puerto Rico, Bonde and Brown (1) demonstrated that the Asian and Puerto Rican cultures both preferentially produced appressoria (measuring 20-24 x 17-20  $\mu\text{m}$ ) over anticlinal walls. Each penetrated the leaf epidermis directly through the cuticle to produce a transepidermal vesicle that transversed the lumen of the penetrated epidermal cell (Figure 1). Both produced primary and secondary hyphae that intercellularly colonized the leaf mesophyll. Each produced similar, if not identical uredinia (Figure 2). The only difference observed was in appearance of the germ pores. Although each isolate had eight to 10 scattered germ pores per urediniospore, germ pores of the Puerto Rican culture were more apparent by means of scanning electron microscopy than those of the four Eastern Hemisphere isolates examined. This suggested that the Puerto Rican organism might be a different species than the Eastern Hemisphere pathogen. We decided, however, that this minor

difference by itself was not sufficient to warrant separate species status (1).

In 1988, however, Bonde *et al.* (3) compared the isozymes of cultures of *P. pachyrhizi* from Asia and Australia with cultures from Puerto Rico and Brazil. No isozyme variation was detected within 11 cultures of *P. pachyrhizi* from widely separated areas of Asia and Australia, nor was variation detected in four cultures from the Western Hemisphere. However, when comparing the two groups, there was only a 7% genetic similarity in isozyme coding alleles. This low frequency of common alleles between the two groups indicated that two populations, with distinct isozyme polymorphisms, were involved in causing rust on soybean, one in Asia and Australia, the second in the Western Hemisphere. The identical isozymes in the Brazilian and Puerto Rican isolates confirmed that the same, or nearly the same, organism caused rust on soybeans in Brazil and Puerto Rico. They suggested at least two species, and perhaps more, may be involved in causing rust on soybean. Long-term geographical separation of the pathogen in the two hemispheres might have resulted in specialization of the pathogen.

Bonde *et al.* (3) found the lack of isozyme variation within the Asian *P. pachyrhizi* might be due to the lack of an effective sexual cycle. Although teliospores of the pathogen have been observed, germination may be rare or absent (3). The lack of isozyme variation has been related to a lack of an effective sexual cycle in other fungi (3).

To complete the story on identification of the soybean rust pathogen in the Western Hemisphere, Ono, Buritica, and Hennen (11) published a comprehensive paper on the morphology of the phakopsoroid fungi on legumes and proposed a unified classification for these taxa. In their paper, the authors identified the rust pathogen on soybeans in the Western Hemisphere as *Phakopsora meibomia* (Arthur) Arthur.

Development of a pest risk analysis for *P. pachyrhizi* to U.S.

soybeans. In 1989, X. B. Yang came to Frederick to work with W.M. Dowber and M.H. Royer to develop a soybean rust disease loss model that would provide a risk analysis of soybean rust to U.S. agriculture. Yang, Royer, and Dowber cooperated with A.T. Tschanz, B.Y. Tsai, and T.C. Wang of the Asian Vegetable Research and Development Center (AVRDC), Taiwan to analyze and quantify soybean rust epidemic data generated at AVRDC and develop yield loss models for *P. pachyrhizi* (16,17). As a result of the effort, Yang *et al.* (15) were able to use the models to make a soybean rust risk analysis and hypothesize on the potential impact of soybean rust to the American soybean crop. The results are presented in detail by X.B. Yang (15). In this presentation we only present the broad conclusions.

Yang *et al.* (15) concluded that (i) it is possible to predict quantitatively the impact of an exotic pathogen before the pathogen arrives, (ii) the Asian rust poses a greater threat to the continental U.S. than does the Western Hemisphere organism, and (iii) if the Asian organism were to become introduced into continental U.S., significant yield losses (>10%) could occur in nearly all soybean growing areas. They further estimated that up to 50% losses could occur in the Mississippi delta and southern coastal areas. These are only predictions, but they were developed using the best information available at the time.

## CURRENT RESEARCH ON SOYBEAN RUST AT FREDERICK

With the discovery in 1994 of soybean rust in Hawaii, research was resumed at Frederick. On 25 May 1994 rusted soybean leaves arrived at FDWSR, sent by Animal and Plant Health Inspection Service (APHIS) personnel in Hawaii. The pathogen was transferred to soybean plants of the cultivar Williams.

Subsequent to increase of the pathogen, a comparison was made of the isozymes of Hawaiian, Puerto Rican, and Taiwanese cultures. The isozyme banding patterns indicated that the pathogen in Hawaii is nearly identical to the one in Taiwan but

different from the Western Hemisphere organism. Further studies are necessary to pinpoint the exact degree of relatedness.

Studies are in progress to compare the virulence of isolates from Australia, Hawaii, India, Puerto Rico, and Taiwan on soybean genotypes and other legumes. Preliminary results show that the pathogen in Hawaii is the same pathogen as in Asia, with the possible exception of being a different race. These studies will continue with an accurate determination of the identification of the pathogen. Results also show that the Hawaiian organism sporulates profusely and that soybean rust resistant cultivars being developed by U.S. breeders are susceptible to the isolate from Hawaii.

## FUTURE STUDIES

There are many types of studies that could be conducted. However, we must be selective as to which ones we undertake. The most important, possibly, are those that would allow an evaluation of the likelihood of *P. pachyrhizi* being inadvertently introduced into the continental U.S. The most probable mode of entry is in the form of urediniospores on seeds. At what level can we detect these spores in seed lots? Can we increase our sensitivity of detection? Can we prevent entry of the pathogen, or pathogens?

The frequency of transmission of soybean rust pathogens on soybean germ plasm and commercial grain can be determined. Such studies have been conducted with many other pathogens.

The development of highly sensitive pathogen detection and identification methods for the soybean rust organisms can be readily accomplished. Some of these methods would involve molecular biology. For example, the development of polymerase chain reaction-based species-specific DNA primers for *P. pachyrhizi* would increase greatly our ability to detect the pathogen in seed samples. These techniques are being developed for the identification of other plant pathogens, including fungi. We are using them in our

laboratory for several foreign pathogens, including *Tilletia indica*, the causal agent of karnal bunt of wheat.

These are only a few of the things that could be done. It is the job of the scientific and business communities to decide the research that is to be conducted.

## DISCUSSION

Soybean rust is a serious disease in parts of Asia. Some people contend that soybean rust will not occur at serious levels outside a belt roughly delimited by the 30th parallel. They believe the disease is confined to the tropics and immediate subtropics because of certain environmental requirements of the pathogen. As evidence, they cite the lack of serious disease in places such as the northeastern provinces of the Peoples' Republic of China where the organism is present and climate analogous to the south-eastern states of the U.S. (4).

I believe this reasoning may be incorrect. For example, the weather in the northeastern Provinces is not similar to that in the U.S. The northeast provinces are next to the Gobi Desert and greatly influenced by the desert.

Most of the research on soybean rust at Frederick was done when rust was not an immediate threat to U.S. agriculture. The pathogen discovered in Puerto Rico was shown within a few months to be of little concern. For that reason, little precaution had to be taken. Discovery of the Asian soybean rust causing pathogen in Hawaii is of much greater concern. Should we be taking greater measures to prevent its introduction into the continental U.S.?

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