Sporulation of *Peronosclerospora sorghi*, *P. sacchari*, and *P. philippinensis* on Maize

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ABSTRACT

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Nine isolates of *Peronosclerospora sorghi*, three of *P. sacchari*, and four of *P. philippinensis* were compared as to the number of conidiospores produced per square centimeter of surface of infected maize leaves. Systemically infected plants were induced to sporulate 21 days after inoculation by placing them in a dew chamber. All cultures of *P. sorghi* produced less than 3.0×10^3 conidia per square centimeter of infected leaf surface except two cultures from Thailand. The Thailand isolates produced more than 1.5×10^4 conidia per square centimeter of leaf area. Isolates of both *P. sacchari* and *P. philippinensis* produced 4.0×10^3 to 1.0×10^4 spores per square centimeter of infected leaf area. The large number of spores produced by the Thailand strain was atypical for *P. sorghi* and may represent a larger potential threat to maize production than other strains of *P. sorghi*. Isolates of *P. sacchari* and *P. philippinensis* were indistinguishable on the basis of sporulation, and five of six cultures produced significantly more spores per square centimeter of leaf area than the typical isolates of *P. sorghi*.

Additional key words: corn, downy mildews, maize, sorghum, sugarcane.

Downy mildew pathogens of maize, sugarcane, and sorghum are among the most destructive pathogens of these crops throughout the world (1,7). Weston observed that the most prominent feature contributing to the destructiveness of these pathogens is the production of secondary inoculum (= conidia) in huge numbers allowing a single infected plant to pose a threat to an entire maize-growing district (11,12). Although sporulation is an important feature contributing to epidemic severity, few studies have compared different species and strains of downy mildew pathogens based on this epidemiologically significant character (10). The primary reason sporulation has not been compared directly is because of the geographic separation of the pathogens and the threat of causing an epidemic associated with introducing pathogens from different parts of the world into a common location. At the USDA Foreign Disease-Weed Science Research Unit in Frederick, MD, however, pathogens from throughout the world may be studied simultaneously on living plants at one location in specialized plant disease containment facilities.

In this study, sporulation of nine isolates of *Peronosclerospora sorghi* (Weston & Uppal) C. G. Shaw, three isolates of *P. sacchari* (Miyake) Shirai & K. Hara, and four of *P. philippinensis* (Weston) C. G. Shaw from around the world was quantitated and compared.

MATERIALS AND METHODS

Between the years 1972 and 1984, cultures of *P. sorghi* infecting sorghum or maize were obtained from Pergamino, Argentina (E. Teyssendier); Jaboticabal, Brazil (N. G. Fernandes); Bako, Ethiopia (M. Tessera); Mysore, India (S. S. Bhat); and Texas (R. A. Frederiksen). These were transferred to maize by inoculations with oospores collected from leaves of systemically infected sorghum (*Sorghum bicolor* (L.) Moench). In 1975 and 1985, two cultures of the maize strain of *P. sorghi* were sent to Frederick in living maize plants from Pak Chang, Thailand, by B. L. Renfro and C. DeLeon. Between the years 1975 and 1984, cultures of *P. philippinensis* from Los Banos, Philippines (O. Exconde and F. R. Husmillo), and cultures of *P. sacchari* from Tainan, Taiwan (S. C. Chang), were transferred to maize by conidia collected from systemically infected sugarcane sets sent to Frederick. In 1975 a culture of *P. philippinensis* was collected from living maize plants in Los Banos, Philippines. All of the above cultures have been maintained on maize since their initial transfer.

Inoculation of host plants. Maize plants (*Zea mays* L. Pioneer 3369A) infected with *P. sorghi*, *P. sacchari*, or *P. philippinensis*, maintained in temperature-controlled greenhouses, were exposed to supplemental light supplied by Sylvania 1,000-W Metalarc high-intensity lamps for about 15 hr (1600–0700 hours). The plants then were placed in dark temperature-calibrated dew chambers (20 ± 2 C) for about 6 hr or until maximum sporulation occurred. At the time of maximum sporulation, conidia were harvested by spraying a fine stream of distilled water (5 C) at 3,500 kg/m^2 (5 lb/in.^{2}) air-line pressure onto the leaf surface of the donor plants. The conidial suspension was then filtered through a 150-μm (100-mesh) screen and the spore concentration for each culture was determined by counting conidia in a hemacytometer. The spore concentration was adjusted to 1.0×10^4 spores per milliliter with cold (about 5 C) distilled water and 1 ml was sprayed onto two seedlings at the two-leaf stage in a 10-cm-diameter clay pot. There were 30 pots of seedlings for each culture. These seedlings were incubated overnight in dark temperature-calibrated (20 ± 2 C) dew chambers and then moved to a greenhouse with a temperature fluctuation of 20–28 C. On occasion the temperature peaked as high as 34 C shortly after noon but returned to 28 C or less within 3 hr. All cultures were inoculated in separate dew chambers and maintained on separate benches in the greenhouse to ensure that the cultures did not become mixed.

Comparison of sporulation. A time course study was conducted

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to determine when maximum sporulation occurs on Pioneer 3369A maize plants after inoculation. Sets of 32 plants were inoculated with 1.0×10^4 conidia per milliliter with either the Thailand 1975 culture or the Texas 1972 culture. These represented the extremes for sporulation capacity. At 17, 21, 24, and 31 days after inoculation, eight nonsporulating plants for each culture were placed in dew chambers (20 ± 2 C) after receiving supplemental light from Sylvania 1,000-W high-intensity lamps the previous night. At maximum sporulation between 5 1/2 to 6 hr after placement in dew when 85–95% of all conidia were fully grown, conidia were harvested as described above. The infected portion of each leaf was cut from the uninfected portion and the total infected leaf surface area (top and bottom of leaves) of each plant was measured with an electronic leaf area meter (Licor, Lambda Instrument Corp., Lincoln, NE).

The spore suspension collected from each plant was filtered through a 150-μm (100-mesh) screen. One milliliter was diluted with 9 ml of Isoton II electrolyte solution (Coulter Diagnostics, Hialeah, FL), and the number of spores and volume for each spore in a 800-μl spore suspension was determined by using a calibrated 80 XY Electrozone Celloscope Particle Counter (Particle Data Inc., Elmhurst, IL) and accompanying computerized terminal. The experiment was repeated once.

After determining that 21 days was well within the range for each of the two isolates tested in which maximum sporulation occurred after inoculation in the dew chamber, all isolates were induced to sporulate at 21 days after inoculation. Numbers of conidia per square centimeter of infected leaf area for each of eight systemically infected plants for each of 15 isolates were determined. In one instance, where there were so few conidia produced ($< 1.0 \times 10^3$ spores per square centimeter), hemacytometer counts were used because meaningful counts could not be achieved with the particle counter.

RESULTS AND DISCUSSION

At 21 days after inoculation, plants inoculated with the Texas 1972 and Thailand 1975 cultures of *P. sorghi*, representing the extremes of sporulating capacity, did not differ significantly (Student's t test, $P = 0.05$) in leaf area displaying systemic symptoms and were well within the range in which maximum sporulation occurred after inoculation in the dew chamber. At 28–31 days after inoculation, the culture from Thailand had significantly stunted the growth of Pioneer 3369A, yet the plants maintained a high capacity to support sporulation. In contrast, the overall size and infected surface area of the host plant of the Texas culture was still expanding while it produced fewer spores per square centimeter of infected leaf surface (Figs. 1 and 2).

When cultures of *P. sorghi* were compared on the basis of spore production at 21 days, we determined that four of the six cultures produced less than 3.0×10^3 conidia per square centimeter of infected leaf surface, sporulating primarily on the lower leaf surface. However, two cultures from Thailand each produced more than 1.5×10^4 conidia per square centimeter of infected leaf surface, sporulating heavily on both the upper and lower leaf surfaces (Fig. 3). The mean spore volume was 4.2×10^3 μm^3 for cultures of *P. sorghi* from countries other than Thailand. This differed significantly (Student's t test, $P = 0.05$) from the mean spore volume determined for the two Thailand cultures of *P. sorghi*, which was 2.9×10^3 μm^3. These results complement other studies in which differences were observed in host range, symptoms, and morphology between the Thailand cultures and other cultures of *P. sorghi*, and indicate that the Thailand cultures are markedly different from the other cultures in respect to both morphology and physiology (1,4). These striking differences bring into question the current classification of the pathogen in Thailand as *P. sorghi*.

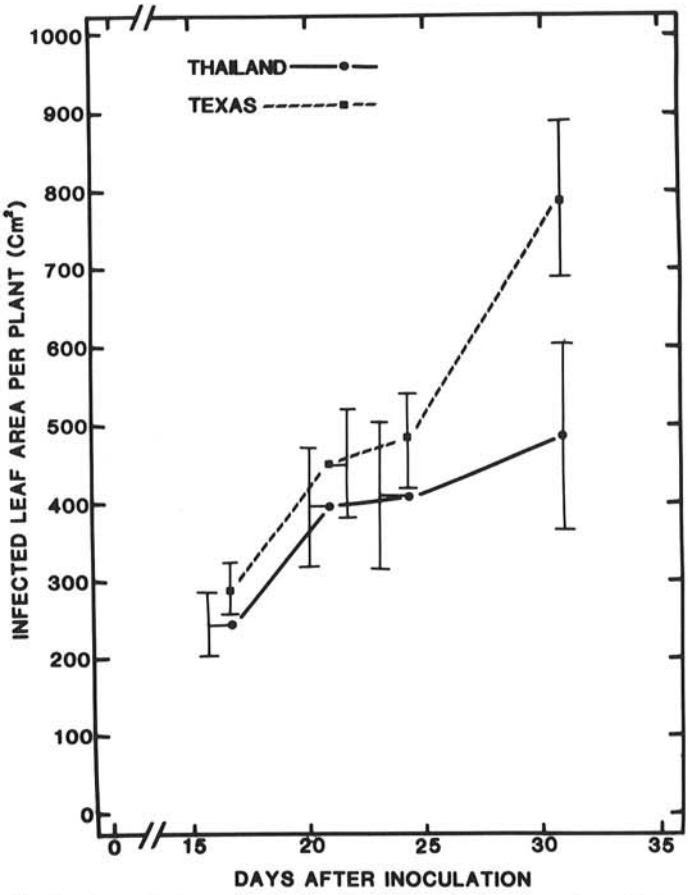

Fig. 1. Infected leaf area (square centimeters) per plant for maize cultivar Pioneer 3369A inoculated at the two-leaf stage with the Thailand 1975 culture or the Texas 1972 culture of *Peronosclerospora sorghi* and examined after specific time intervals following inoculation (95% confidence limits determined using Student's t test).

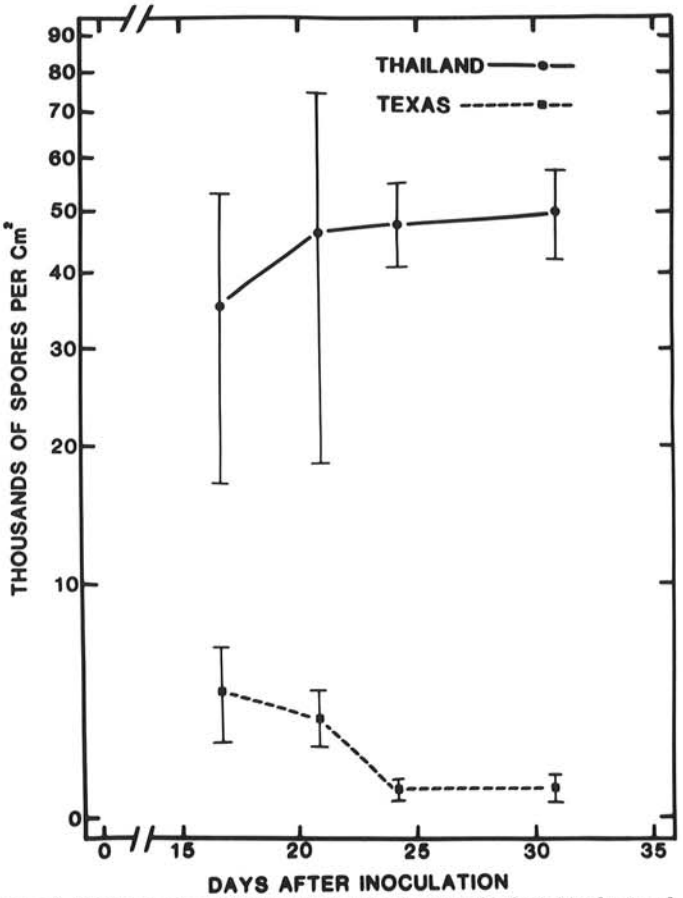

Fig. 2. Numbers of conidia per square centimeter of infected leaf area of systemically infected plants at specific time intervals after inoculation of maize seedlings with the Thailand 1975 culture or the Texas 1972 culture of *Peronosclerospora sorghi* (95% confidence limits determined using Student's t test).

Cultures of *P. philippinensis* and *P. sacchari* were indistinguishable from one another on the basis of amounts of sporulation, both producing $4.0\times10^3-1.0\times10^4$ spores per square centimeter of infected leaf area and both sporulating on the upper and lower leaf surfaces. The sporulation capacity of *P. philippinensis* and *P. sacchari* was intermediate between *P. sorghi* from Thailand and cultures of *P. sorghi* from countries other than Thailand (Fig. 3).

Furthermore, the mean spore volume for both species was 5.6 $\times10^3\ \mu m^3$ and did not differ significantly (Student's *t* test, $P=0.05$). In a previous experiment (2), no morphological character was detected that could differentiate *P. sacchari* from *P. philippinensis*. The similarity in spore and conidiophore morphology was first recognized by Weston (11) who described the "very close resemblance in the size, the form, and even the minor structural characteristics of the conidiophores . . . [and] conidia." The original basis for classifying *P. sacchari* and *P. philippinensis* as separate species was their "virulence on various hosts" (11). Weston originally reported that *P. philippinensis* did not infect sugarcane, whereas *P. sacchari* did. However, in more recent experimental host range studies (3,6), this difference was never detected. Furthermore, it is not valid to differentiate species based solely on physiological characteristics such as differences in host range. Consequently, the similarity between *P. philippinensis* and *P. sacchari* in sporulating capacity, concomitant with their almost identical host ranges (3,6) and morphologies (2), is additional information suggesting that the classification of *P. philippinensis* and *P. sacchari* as separate species should be reevaluated.

We observed in this and other studies that the length of time a culture has been maintained on maize may influence the number of spores produced. Parasitic fitness related to maintenance on a susceptible host has been reported with another maize pathogen (9). Our study suggests that when downy mildew organisms were transferred to maize from another host on which they had previously been established, the sporulation was initially low. As the pathogen becomes adapted to its new maize host through selection, mutation, or by activation of previously inactive portions of its genome (actual mechanism unknown), sporulation, and vigor with which the host apparently is colonized usually increases. This phenomenon of increasing sporulation is demonstrated by the relatively high sporulation of cultures of *P. sacchari*, *P. philippinensis*, and *P. sorghi* established on maize in the 1970s as compared with the relatively low sporulation of the cultures established in 1983 and 1984 (Fig. 3). In fact, an Ethiopian 1984 culture and a Nigerian 1985 culture of *P. sorghi* produced so few conidia when initially transferred from sorghum to maize that they were extremely difficult to maintain on maize. These cultures are excellent examples of what may be a 'sorghum strain' of *P. sorghi*, which readily infects and produces conidia on sorghum, whereas infection and degree of sporulation on maize are minimal (8). Whether the amounts of sporulation of the Ethiopian and Nigerian cultures eventually will increase on maize is not known. However, typically cultures of *P. sorghi* that we have obtained from sorghum initially sporulated poorly on maize but gradually increased in sporulating capacity. The Argentina 1984 culture (obtained from sorghum) is the only culture that has shown relatively high sporulation since it was first established on maize. Cultures of *P. sacchari* and *P. philippinensis* that were isolated from sugarcane initially sporulated relatively poorly on maize; however, sporulation increased with successive transfers. This phenomenon has been observed with several additional cultures of *Peronosclerospora* not included in this study.

The apparent vigor of mycelial colonization of maize as a new host for a culture increases with the time the culture has been maintained on that host, as indicated by a marked increase in intensity and uniformity of chlorotic streaks on leaves. Figure 4 depicts a culture of *P. sacchari* that has been on maize for less than 1 yr as compared with another culture after several years on maize.

The data for sporulation suggest a strong relation between light and/or discontinuous symptoms and the low sporulation of these cultures on maize. In one instance, the sporulation of a new culture of *P. sacchari* obtained from sugarcane in Taiwan in 1984 could not be quantitated on maize in relation to leaf areas with systemic symptoms because the discontinuous (broken) streaking on leaves rendered measurement of infected leaf areas impossible with our present techniques. Visual examination, as well as the comparison of the number of spores produced per plant, indicated that this culture sporulated to a lower extent than other cultures of *P. sacchari* that had been maintained on maize for about 1 yr. After 1–2 yr on maize, cultures of *P. sacchari* usually become

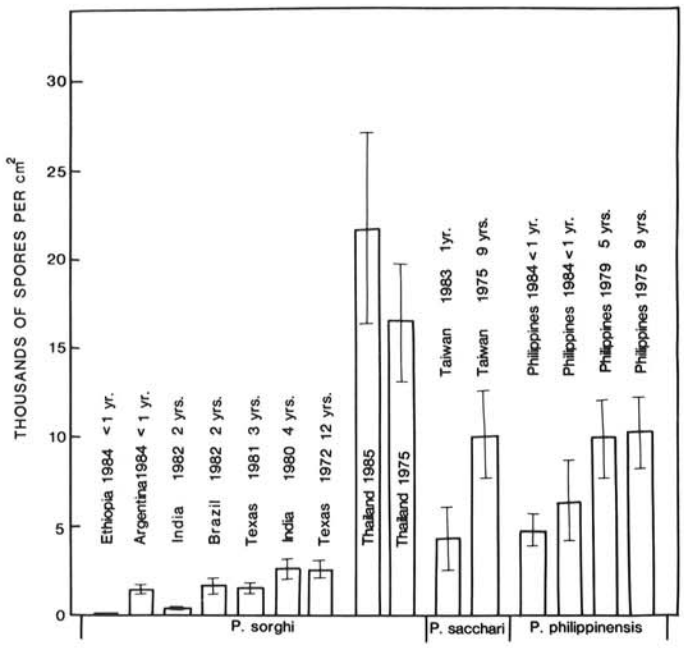

Fig. 3. Numbers of conidia per square centimeter of infected leaf area at 21 days following inoculation of maize seedlings with specific cultures of *Peronosclerospora sorghi*, *P. sacchari*, and *P. philippinensis*. Duration of maintenance on maize indicated except for the cultures that were obtained from maize in Thailand (95% confidence limits determined using Student's *t* test).

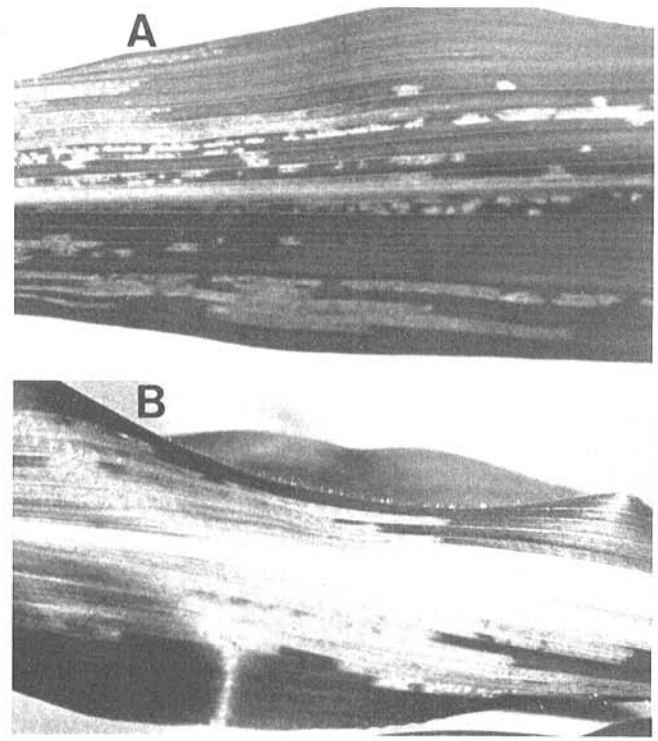

Fig. 4. Typical symptoms of **A,** A culture of *P. sacchari* maintained on Pioneer 3369A for less than 1 yr, as compared with **B,** A culture of *P. sacchari* maintained on Pioneer 3369A for over 5 yr.

indistinguishable from *P. philippinensis* on the basis of sporulation and symptoms.

The cultures of *P. sorghi* from Thailand, however, always showed vigorous symptoms on maize ever since they were obtained from Thailand in 1975 and in 1985, respectively. They were imported on living maize plants and have always sporulated profusely. They represent what has been termed the 'maize strain' (8). In Thailand, this strain has been observed to infect only maize, but one sorghum species (*Sorghum nitidum* (Vahl.) Pers.) was infected in an experimental host range study (5). Unlike all other cultures of *P. sorghi* tested, this strain does not produce oospores in any known host (5; Bonde, Peterson, and Duck, *unpublished*).

Whereas it seems likely that *P. sorghi* in Thailand became adapted to maize from wild grasses, the cultures we obtained from Thailand probably had been on maize for several years before our acquisitions, and therefore were fully adapted to this host. This would account for their initial high sporulation on maize when they arrived at our laboratory in 1975 and 1985.

The development of more prominent symptoms and increased sporulation with time seems to be very gradual. However, as sporulation increases, there appears to be a maximum level of sporulation on maize cultivar 3369A associated with each strain, and it is reached after a maximum of about 5 yr on that host. Consequently, we believe the differences seen between the Thailand cultures of *P. sorghi* (maize strain) and all other cultures of *P. sorghi* are of a sufficient magnitude to be considered inherent differences of the pathogens rather than differential host preferences caused by having been maintained on maize for different lengths of time.

The results of this study compare an important factor that contributes to the potential threat of various species and strains of downy mildew pathogens to agriculture. The greater capacity a pathogen or strain of pathogen has for sporulation, the greater the threat of epidemic increase assuming all other factors are equal. Based on sporulation on maize, the maize strain of *P. sorghi* from Thailand has the greatest potential threat to susceptible maize varieties such as Pioneer 3369A. Our observations over the years also suggest that cultures of these pathogens can adapt to become significantly better sporulators on maize.

LITERATURE CITED

1. Bonde, M. R. 1982. Epidemiology of downy mildew diseases of maize, sorghum and pearl millet. Trop. Pest Manage. 28:49-60.
2. Bonde, M. R., Duck, N. B., and Peterson, G. L. 1984. Morphological comparison of *Peronosclerospora sacchari* from Taiwan with *P. philippinensis* from the Philippines. (Abstr.) Phytopathology 74:755.
3. Bonde, M. R., and Peterson, G. L. 1983. Comparison of host ranges of *Peronosclerospora philippinensis* and *P. sacchari*. Phytopathology 73:875-878.
4. Bonde, M. R., Peterson, G. L., and Duck, N. B. 1983. Comparison of *Peronosclerospora sorghi* from different areas of the world. (Abstr.) Phytopathology 73:795-796.
5. Bonman, J. M., Paisooksantivatana, Y., and Pitipornchai, P. 1983. Host range of *Peronosclerospora sorghi* in Thailand. Plant Dis. 67:630-632.
6. Exconde, O. R., Elec, J. V., and Advincula, B. A. 1968. Host range of *Sclerospora philippinensis* Weston in the Philippines. Philipp. Agric. 52:175-188.
7. Frederiksen, R. A., and Renfro, B. L. 1977. Global status of maize downy mildew. Annu. Rev. Phytopathol. 15:249-275.
8. Payak, M. M. 1975. Downy mildews of maize in India. Trop. Agric. Res. 8:13-18.
9. Royer, M. H., and Nelson, R. R. 1981. The effect of host resistance on relative parasitic fitness of *Helminthosporium maydis* race T. Phytopathology 71:351-354.
10. Safeeulla, K. M. 1976. Biology and Control of the Downy Mildews of Pearl Millet, Sorghum and Finger Millet. Wesley Press, Mysore, India. 304 pp.
11. Weston, W. H., Jr. 1920. Philippine downy mildew of maize. J. Agric. Res. 19:97-122.
12. Weston, W. H., Jr. 1923. Philippine Sclerosporas of maize. J. Agric. Res. 23:239-278.