Effects of Temperature on Sporulation, Conidial Germination, and Infection of Maize by *Peronosclerospora sorghi* from Different Geographical Areas

M. R. Bonde, G. L. Peterson, and N. B. Duck

Research plant pathologist, biological laboratory technician, and student trainee, respectively, U.S. Department of Agriculture, Agricultural Research Service, Plant Disease Research Laboratory, Ft. Detrick, Building 1301, Frederick, MD 21701.

The authors thank S. S. Bhat, J. Craig, N. Gimenes Fernandes, R. A. Frederiksen, and B. L. Renfro for providing the cultures of *Peronosclerospora sorghi* used in this study, and M. H. Royer for assistance with statistical analysis in this manuscript.

Accepted for publication 30 July 1984.

ABSTRACT

Bonde, M. R., Peterson, G. L., and Duck, N. B. 1985. Effects of temperature on sporulation, conidial germination, and infection of maize by *Peronospora sorghi* from different geographical areas. Phytopathology 75:122-126.

Five isolates of *Peronosclerospora sorghi* (two from the United States, and one each from India, Brazil, and Thailand) were compared for effects of dew-period temperature on sporulation, germination, germ-tube growth, and establishment of infection in maize. Responses of all isolates except that from Thailand were similar at each air temperature. The isolate from Thailand was more tolerant to high temperatures; it germinated optimally from 12 to 32 C, whereas the Indian, Texas, and Brazilian isolates germinated optimally from 12 to 20 C. All isolates sporulated optimally from 15 to 23 C. The Thailand isolate was the only one that produced mature conidia at 26 C. Each isolate following conidial inoculation caused high levels of systemic infection at temperatures from 15 to 32 C, and moderate levels at temperatures as low as 10 C, during the dew period.

Additional key words: corn, epidemiology, sorghum, sorghum downy mildew.

Sorghum downy mildew, which is incited by *Peronosclerospora sorghi* (Weston and Uppal) C. G. Shaw, can cause extensive losses to sorghum and maize in tropical and subtropical areas. The disease was first reported to occur in the United States in Texas in 1961 (9) and it has since spread as far north as southern Indiana and Illinois (5). To date, in the United States sorghum downy mildew has been damaging primarily on sorghum.

Worldwide, there appear to be at least two strains of *P. sorghi*; one is the sorghum-maize strain and the other the maize strain (8). The sorghum-maize strain infects both sorghum and maize and is present in southern India (Karnataka, Maharashtra, and Tamil Nadu), Israel, Mexico, the United States (7), Central and South America, and in some regions of Africa (5).

The maize strain is known in Northwestern India (Rajasthan), Thailand (8), and possibly in Nigeria (4), and it is not pathogenic on sorghums (8). In 1980, however, Siradhana et al (11) designated the maize strain in Rajasthan as a new species, *P. heteropogoni* Siradhana, Dange, Rathore, and Singh sp. nov., on the basis that it attacks the wild grass *Heteropogon contortus* (L.) Beauv. ex Roem. and Schult. and forms oospores in that host. Both oospores and conidiophores were reported to differ morphologically from those of *P. sorghi* and from other known downy mildew species (11). However, host range studies on an isolate of the maize strain from Thailand (M. R. Bonde and G. L. Peterson, *unpublished*) indicate that the pathogen in Thailand is not the same as the pathogen in Rajasthan. Therefore, the pathogen in Thailand still is considered to be *P. sorghi*.

The purpose of this research was to compare effects of temperature during dew periods on production and germination of conidia, and on establishment of systemic infection in maize, by five isolates of *P. sorghi* from four areas of the world. Four isolates were of the sorghum-maize strain and one was of the maize strain.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

No previous reports have compared germination and infection by conidia of *P. sorghi* from different areas of the world under the same set of environmental conditions. Results of a similar study reporting effects of dew-period temperature on conidial germination and systemic infection of maize by one isolate of *P. sorghi* from Texas have been published (2).

MATERIALS AND METHODS

Isolates of *P. sorghi* were obtained from Texas, United States (R. A. Frederiksen and J. Craig) in 1972 and 1978; from Thailand (B. L. Renfro) in 1975; from Mysore, India (S. S. Bhat) in 1980; and from Jaboticabal, Brazil (N. Gimenes Fernandes) in 1982. All cultures except the one from Thailand were of the sorghum-maize strain. The cultures were maintained in the greenhouse on maize. In all experiments, freshly harvested conidia were used as inocula and studies were conducted in a quarantine containment facility with permission from the appropriate state and federal officials.

Preparation of inoculum. Conidia were collected from infected "donor" plants of *Zea mays* L. 'Pioneer 3369A' or 'DeKalb XL55a' previously inoculated and maintained in a greenhouse for 3–5 wk. Prior to spore collection, the donors were exposed to supplemental light from 1,000-W Metalarc high-intensity lamps (Sylvania Lighting Center, Danvers, MA) for approximately 16 hr (from 1600 hours to 0800 hours) and then placed in a dark dew chamber at 20 C for 5–6 hr to induce sporulation. Conidia were collected by washing the spores from the surface of maize leaves with a fine stream of cold (approximately 5 C) distilled water delivered by an atomizer at approximately 3,500 kg/m^2 (5 lb/in^2) air-line pressure. The spore suspension was filtered immediately through a 150-μm (100-mesh) screen, quantified by counts with a hemocytometer, and adjusted to the desired concentrations by dilution with cold distilled water.

Systemic infection studies. A series of experiments was conducted to determine the relationship of air temperature during dew periods and incidence of systemic infection in inoculated plants.

Pioneer 3369A or DeKalb XL55a maize seedlings in the two-leaf stage (two per 10-cm-diameter clay pot) were placed in dew chambers at selected near-constant air temperatures ranging from 8

to 36 C. After temperature equilibration, the seedlings were sprayed with conidia at $1-20 \times 10^3$ conidia per milliliter, 0.5–1.0 ml per pot. In each experiment, all inoculated treatments were sprayed at the same conidial concentration and volume of inoculum. Inoculated plants were incubated in the dew chambers at the desired air temperatures for 18 hr. Sixteen to 32 plants were used for each temperature-dew period treatment in each experiment. The experiment was conducted four to eight times with the Texas 1972, Texas 1978, and India 1980 isolates, once for the Brazil isolate, and 12 times for the isolate from Thailand. When several isolates were compared simultaneously, conidia were applied to plants in separate chambers to avoid cross contamination before plants were combined in the same chamber. Plants sprayed with distilled water were incubated at a favorable temperature (20 C) for infection, and these served as controls.

During the dew period, air temperatures in each chamber were monitored continuously with a thermocouple and recorder and were always within ±2 C of the stated means except temporarily (≤18 min) when the chamber door was opened during inoculation. The temporary change was ≤3.6 C at all temperatures.

After the dew period, inoculated and control seedlings were placed in the greenhouse. All plants were examined for symptoms of systemic infection 4 wk after inoculation. Normally the air temperature in the greenhouse fluctuated from 21 to 28 C; however, occasionally during summer months it approached 34 C shortly after noon and returned to the normal temperature range within 3 hr.

Conidial germination and germ tube growth. To compare conidial germination and germ tube growth at specific temperatures, conidia of Texas 1972, Texas 1978, India 1980, Brazil 1982, or Thailand 1975 isolates were sprayed onto 1.5% water agar in plastic petri plates (35 × 10 mm). In each experiment, three to five replicate plates were incubated at each temperature for each incubation period; the spore density on the agar surface was from 1.6 to 12 spores per square millimeter. Agar-plate temperatures were equilibrated with the chamber air temperatures prior to seeding plates with spores. In most experiments, air temperatures in chambers were monitored throughout incubation with thermocouples and a recorder. Prior tests with calibrated mercury-bulb thermometers showed that the mean temperature of the agar surface was always within ±1.2 C of the stated mean temperature, even when the chamber door was open during seeding of plates.

After incubation, the plates were opened and placed over 38% formaldehyde in a desiccator jar to kill the spores quickly. Germination percentages were determined by microscopic observation of 100 spores per plate. A spore was considered germinated if the length of the longest germ tube exceeded the width of the spore. The germ tube lengths of each of 20 randomly selected germinated spores per petri plate were measured at ×100. If a spore had more than one germ tube, only the longest was measured.

Sporulation. Infected Pioneer 3369A or DeKalb XL55a plants (3–5 wk after inoculation) were incubated in the dark in dew chambers at near-constant air temperatures (12, 15, 18, 21, 23, and 26 C) for up to 7.5 hr. Between 4 and 7.5 hr, leaf pieces were excised at half-hour intervals from areas of leaves displaying systemic symptoms and immediately fixed in a mixture of absolute ethanol

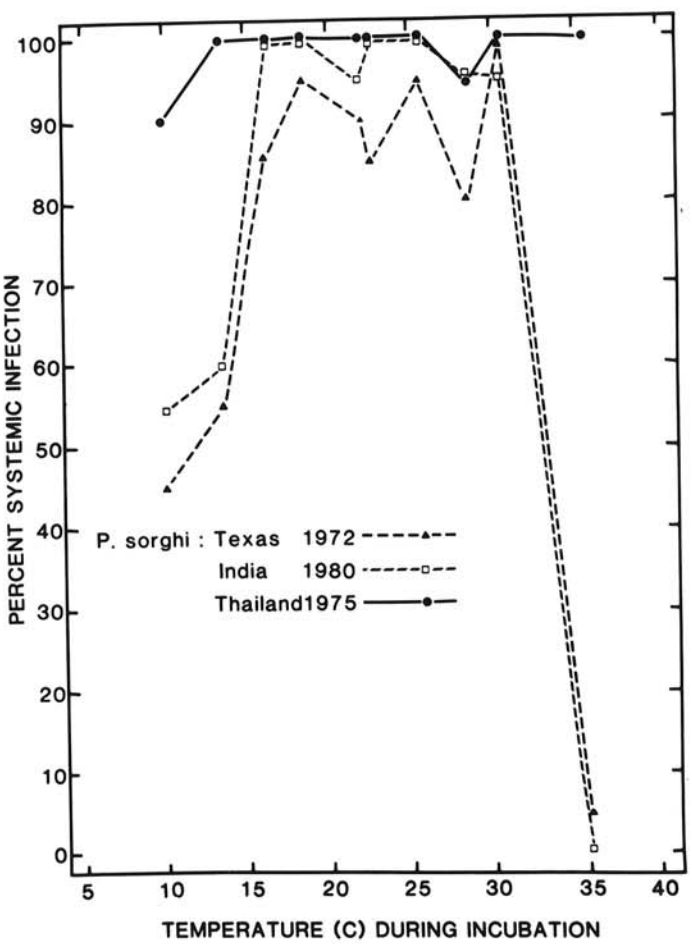

Fig. 1. Percent DeKalb XL55a maize plants with symptoms of systemic infection in a representative experiment after inoculation of seedlings at the two-leaf stage with conidia of the Texas 1972, India 1980, or Thailand 1975 isolates of *Peronosclerospora sorghi*. Plants were incubated for 18 hr in dew chambers at indicated temperatures and then grown in a greenhouse for 28 days. Each point represents 20 plants.

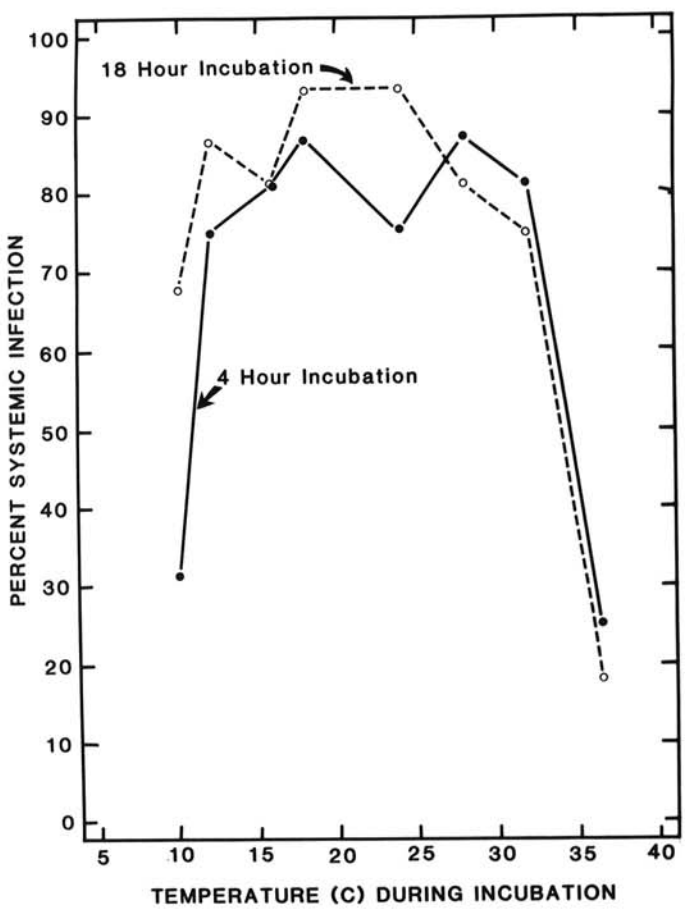

Fig. 2. Percent Pioneer 3369A maize plants with symptoms of systemic infection in a representative experiment after inoculation of seedlings at the two-leaf stage with conidia of the Thailand isolate of *Peronosclerospora sorghi* at 1/10 the concentration used in Fig. 1. Plants were incubated for 4 or 18 hr in dew chambers over a range of constant temperatures and then grown in a greenhouse for 24 days. Each point represents 16 plants.

and acetic acid (2:1, v/v). After 12 hr in the fixative, the leaf pieces were stained with 0.1% aniline blue in lactophenol and observed microscopically for development of conidiophores and conidia.

RESULTS

Systemic infection from conidia. All isolates of *P. sorghi* caused high levels of systemic infection at air temperatures ranging from 15 to 30 C during the dew period (Fig. 1). Isolates Texas 1978 and Brazil 1982 behaved similar to Texas 1972 and India 1980 as shown in Fig. 1. The Thailand isolate (Fig. 1) caused higher levels of systemic infection at the temperature extremes than the other isolates. This was because the Thailand isolate was more virulent. When the inoculum concentration was reduced to one-tenth the original, the curve depicting temperature versus systemic infection was the same as that for each of the other isolates (Fig. 2).

Tests with selected isolates (Texas 1972 and Thailand 1975) demonstrated that there was little difference in numbers of plants subsequently developing systemic infection with either 4- or 18-hr dew periods except in a few instances at the extremes of the temperature range (Fig. 2).

Conidial germination and germ tube growth. Of the five isolates of *P. sorghi* tested, the Thailand isolate had by far the broadest optimum temperature range (about 12–32 C) for conidial germination (Fig. 3). The Indian, two Texas, and Brazilian isolates had relatively narrow optimum ranges (about 12–20 C).

Two-hour and 5-hr incubation periods gave similar germination percentages. With the longer incubation period, however. slightly higher germination frequently occurred at one or both ends of the temperature range.

To demonstrate the difference in effect of temperature on conidial germination between typical isolates of *P. sorghi* and the Thailand isolate, data from all experiments were combined and tested using the full- versus reduced-model approach (6) at $P<0.05$ (Fig. 4). The regression solution to the quartic reponse model of the typical isolates of *P. sorghi* differed significantly from the regression solution of the Thailand isolate, verifying that typical *P. sorghi* differed from the Thailand isolate in its germination response to temperature. Differences also occurred between some of the typical isolates; but although significant, the magnitude of the differences was small. The quartic response model more accurately described the effect of temperature on germination for the typical isolates of *P. sorghi* than for the Thailand isolate.

The optimum temperature range for germ tube growth was broader for the Thailand isolate than for any of the other four isolates (Fig. 5). The Brazil 1982 and Texas 1978 isolates were similar to the Texas 1972 and India 1980 isolates as shown in Fig. 5. The high end of the optimum temperature range for the Thailand isolate was about 5–8 C higher than for all the other isolates (Fig. 5). Germ tubes of the Thailand isolate, but not those of any others, grew well at 32 C.

The most dramatic difference among isolates with respect to germ tube growth was that the Thailand isolate produced much longer germ tubes than did those of the other isolates over a broad range of temperatures (Fig. 5). By 5 hr, germ tubes of the Thailand isolate frequently averaged two or more times the lengths of those for other isolates grown at the same temperatures (Fig. 5).

To demonstrate this dramatic difference in germ tube growth between typical isolates of *P. sorghi* and the Thailand isolate, data from all experiments were again combined and tested by using the

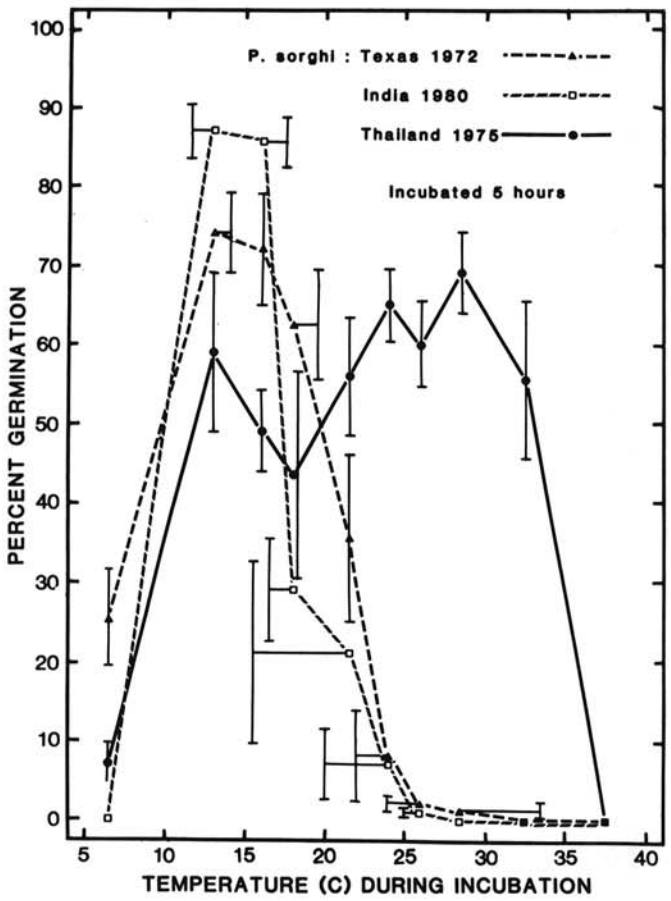

Fig. 3. Germination percentages from a representative experiment using conidia of Texas 1972, India 1980, and Thailand 1975 isolates of *Peronosclerospora sorghi* after 5 hr of incubation on 1.5% water agar at the specified temperatures. Each point represents the mean percent germination of 100 conidia on each of five replicate plates. Confidence limits (95%) are indicated.

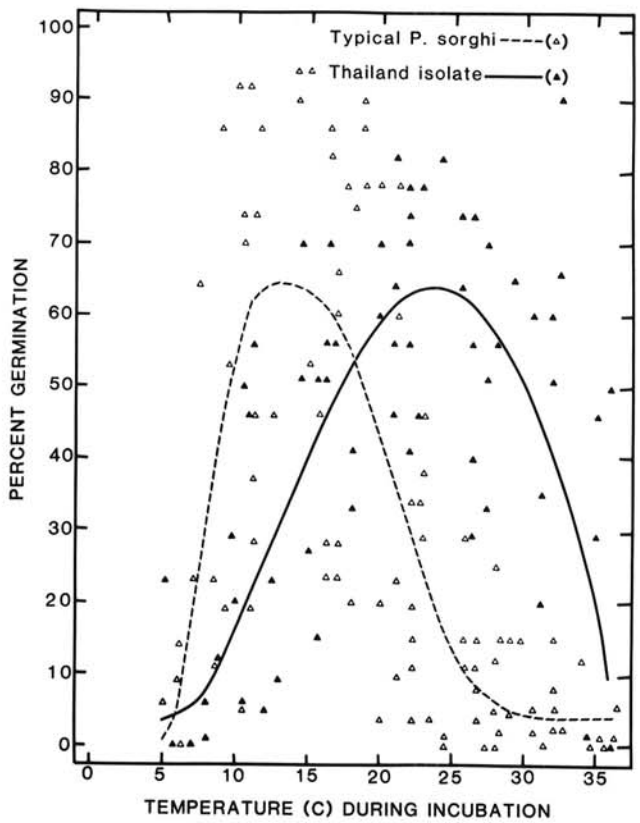

Fig. 4. The regression solutions to the quartic response models for germination versus temperature for the typical isolates of *Peronosclerospora sorghi* [(arcsine $\sqrt{(Y)} = -2.003 + 0.56\ T - 0.036\ T^2 + 8.0 \times 10^{-4}\ T^3 - 6.8 \times 10^{-6}\ T^4$), in which Y = proportion of conidia germinated and T = temperature (C), $R^2 = 0.58$] and for the Thailand isolate [(arcsine $\sqrt{(Y)} = -0.086 + 0.034\ T + 0.0029\ T^2 - 1.0 \times 10^{-4}\ T^3 + 7.2\ T^4$, $R^2 = 0.47$)]. The regression solution for typical *P. sorghi* was significantly different from that of the Thailand isolate at $P<0.05$ using the full- versus reduced-model approach (6).

full- versus reduced-model approach at $P<0.05$ (Fig. 6). A quartic response model was fitted to the data. The regression solution of the typical isolates of *P. sorghi* was significantly different from the regression solution of the Thailand isolate. Although significant differences existed among the typical isolates of *P. sorghi* at $P<0.05$, the magnitude of the differences was always very small relative to the typical isolates versus the Thailand isolate (Fig. 5).

Sporulation. Sporulation by the Texas 1972, India 1980, Brazil 1982, and Thailand 1975 isolates was optimum at 15–23 C based on time required to produce mature conidia and relative numbers of conidia. All isolates usually required 5–6 hr to form mature conidia. The Thailand isolate was the only one that produced mature conidia at 26 C. At that temperature, the other isolates produced immature conidiophores which frequently were deformed.

DISCUSSION

In early experiments in which we tested the effect of specific temperatures on the initiation of infection, only a single isolate was used per experiment. Inoculations were conducted with little germination occurring prior to completion of the final treatment. However, in later experiments in which several isolates were compared, we doubled the conidial concentrations and halved inoculum volumes as compared to previous studies. This allowed inoculations still to be conducted in a manageable length of time with little or no germination as long as the inoculum was maintained at a low temperature (about 5 C) prior to inoculation.

Different inoculum concentrations were used among experiments because the India 1980 isolate required more conidia per plant and the Thailand isolate fewer conidia to cause the same levels of systemic infection as the other isolates. Inoculum concentrations were adjusted depending on isolates to be used in a given experiment; in each experiment, however, conidial concentrations for all isolates were the same.

The Thailand isolate performed the process of sporulation, conidial germination, germ tube growth, and establishment of infection at higher temperatures than did isolates from Texas, India, or Brazil. Furthermore, conidial germ tubes of the Thailand isolate grew about twice as fast as germ tubes of the other four isolates over much of the temperature range tested. It appears to be epidemiologically significant that each isolate caused high levels of systemic infection from 15 to 32 C, and moderate levels at temperatures as low as 10 C, during the dew period.

All isolates other than the one from Thailand were similar with respect to effects of temperature on sporulation, germination, germ tube growth, and initiation of infection, although there were small differences in germination and germ tube growth responses among some of the typical isolates of *P. sorghi*. For instance, we observed that the India 1980 isolate exhibited a slightly narrower optimum temperature range for germination (Fig. 3) than did the isolates from Texas and Brazil; however, this difference did not occur in every experiment. When it did occur, germ tube growth also was decreased at the higher temperatures (Fig. 5). Safeeulla et al (10) reported that 21–25 C was optimum for germination of *P. sorghi* in southern India, but we found a lower optimum for the isolate we used from the same geographical area.

In addition to temperature responses, the Thailand isolate differed from all other isolates in numbers of conidia produced on systemically infected plants. At 3–4 wk after inoculation, the Thailand isolate characteristically produced about 20 times as

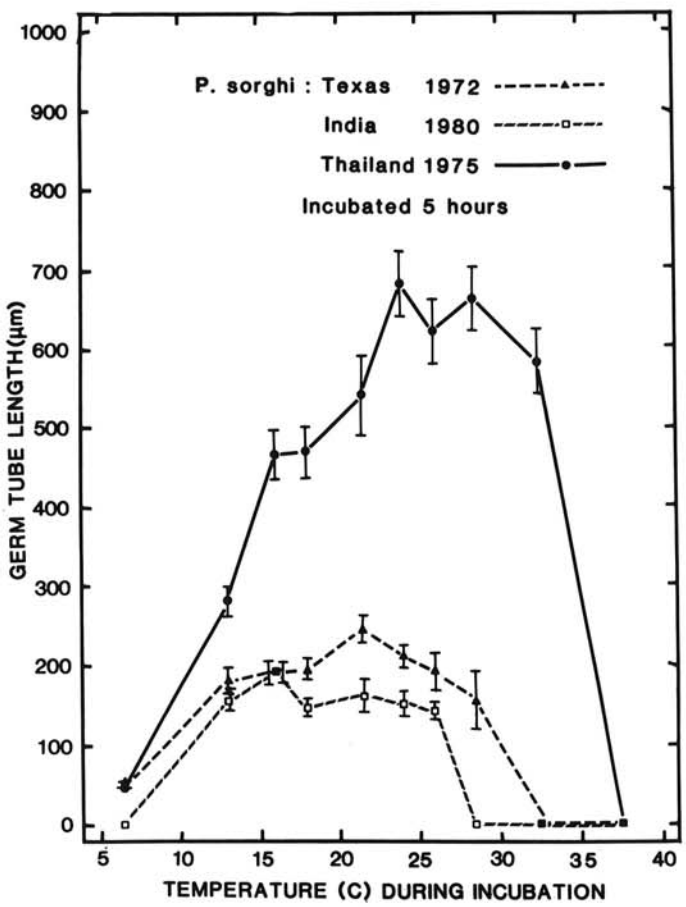

Fig. 5. Germ tube lengths from a representative experiment after 5 hr incubation of conidia of Texas 1972, India 1980, or Thailand 1975 isolates of *Peronosclerospora sorghi* on 1.5% water agar at specified temperatures. Each point represents the mean germ tube length of 20 germinated spores on each of five plates. Confidence limits (95%) are indicated.

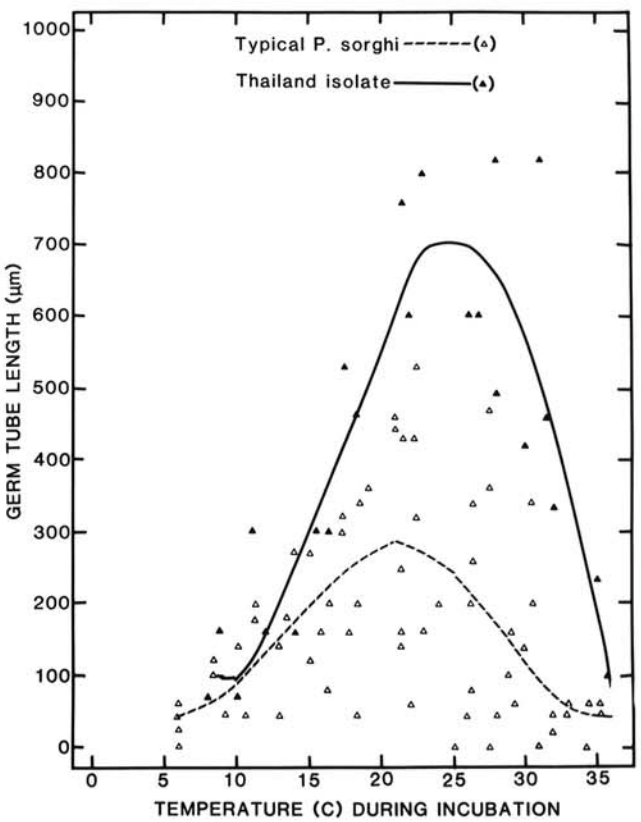

Fig. 6. The regression solutions to the quartic response models for germ tube lengths versus temperature for the typical *Peronosclerospora sorghi* isolates [$(Y=269.02-85.20\ T+10.12\ T^2-0.39\ T^3+0.005\ T^4)$, in which Y = germ-tube length, T = temperature (C), and $R^2 = 0.34$] and for the Thailand isolate [$(Y=1{,}540.29-377.61\ T+32.26\ T^2-0.98\ T^3+0.0096\ T^4)$, $R^2 = 0.77$]. The regression solution for typical *P. sorghi* was significantly different from that of the Thailand isolate at $P<0.05$ using the full- versus reduced-model approach (6).

many conidia per plant or square centimeter of infected leaf area.

Results obtained in this study show that the isolate of *P. sorghi* from Thailand responded quite differently to temperature than did the other isolates of *P. sorghi*. Although few morphological features distinguish this isolate from Thailand (1), differences in host range (3), symptoms (M. R. Bonde and G. L. Peterson, *unpublished*), and in reaction to temperature (as shown here) suggest that the pathogen in Thailand is different from other isolates of *P. sorghi*. It is possible that the pathogen in Thailand may be a different species, and this points out the need for better methods of identification. More isolates from Thailand should be compared to determine the amount of variation of *P. sorghi* in Thailand, and these isolates should be compared with isolates from other areas of the world.

LITERATURE CITED

1. 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.
2. Bonde, M. R., Schmitt, C. G., and Dapper, R. W. 1978. Effects of dew-period temperature on germination of conidia and systemic infection of maize by *Sclerospora sorghi*. Phytopathology 68:219-222.
3. Bonman, J. M., Paisooksantivatana, Y., and Pitipornchai; P. 1983. Host range of *Peronosclerospora sorghi* in Thailand. Plant Dis. 67:630-632.
4. Fajemisin, J. M. 1980. Downy mildew of maize in Nigeria. Pages 120-134 in: Part II International Conference on the Graminaceous Downy Mildew Diseases, Bellagio, Italy, 1979. Strategies for Control of Graminaceous Downy Mildew. Rockefeller Foundation, New York. 197 pp.
5. Frederiksen, R. A., and Renfro, B. L. 1977. Global status of maize downy mildew. Annu. Rev. Phytopathol. 15:249-275.
6. Neter, J., and Wasserman, W. 1974. Applied Linear Statistical Models. Richard D. Irwin, Inc., Homewood, IL. 842 pp.
7. Payak, M. M. 1975. Downy mildews of maize in India. Trop. Agric. Res. 8:13-18.
8. Payak, M. M. 1975. Epidemiology of maize downy mildews with special reference to those occurring in Asia. Trop. Agric. Res. 8:81-91.
9. Reyes, L., Rosenow, D. T., Berry, R. W., and Futrell, M. C. 1964. Downy mildew and head smut diseases of sorghum in Texas. Plant Dis. Rep. 48:249-253.
10. Safeeulla, K. M., Shetty, H. S., and Rao, N. G. P. 1974. Sorghum downy mildew. In Research Report, Department of Post-Graduate Studies and Research in Botany, Manasagangothri, Mysore, India, 1973–1974. 49 pp.
11. Siradhana, B. S., Dange, S. R. S., Rathore, R. S., and Singh, S. D. 1980. A new downy mildew on maize in Rajasthan, India. Curr. Sci. 49:316-317.