Temperature Effects on Germination and Comparative Morphology of Conidia for Thai and USA Isolates of *Cercosporidium personatum*[1,2]

Tharmmasak Sommartya and M. K. Beute*[3]

ABSTRACT

Ten isolates of *Cercosporidium personatum* (Cp) were collected from seven geographical areas in Thailand and the USA. Four USA and 6 Thai isolates were cultured on a susceptible peanut genotype, NC 3033, to produce conidia for all studies. Conidial germination was determined after 12, 24, and 48 h at 16-36 C. Percent germination of conidia for all populations were greatest at 16-20 C. At 30 and 32 C, 58 and 22% of conidia from Thai isolates germinated, respectively. Only 33 and 6% of conidia from USA isolates germinated at 30 and 32 C. Only Thai isolates germinated at 36 C. No differences were observed among isolates for conidial length or number of septa per conidium. Conidia of all isolates, however, were longer and had more septa than previously reported. Conidial length in this study ranged from 16-90 um and number of septa per conidium ranged from 1-11. Conidia with furcate branching were observed with both Thai and USA isolates. Forked conidia produced normal germtubes either intercalary or terminally and all three terminal cells produced germ tubes.

Key Words: *Arachis hypogaea*, late leafspot, spore germination.

Woodroof described late leafspot disease of peanut *Arachis hypogaea L.*) caused by *Cercosporidium personatum* (Berk. & Cur.) Deighton (13). Although peanut leafspot diseases caused by *Cercospora arachidicola* Hori. (early leafspot) and *C. personatum* (Cp) occur wherever peanuts are grown, Woodroof suggested that Cp was better adapted to wet climates and caused greater damage to peanut than *C. arachidicola.* Gibbons (3) in 1966 recognized the destructive potential of Cp on peanut and noted that most epidemics occurred late in the peanut growing season. Gibbons also suggested that geographically isolated populations of Cp may be adapted to local growing conditions. Adaptations would include environmental and host specificity factors which could influence pathogenecity of Cp on local peanut cultivars. Adaptation

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[3]Visiting Assistant Professor and Professor, respectively, Department of Plant Pathology, Kasetsart University, Bangkok, Thailand, and Department of Plant Pathology, N C State University, Raleigh, NC 27695-7616.

of Cp to warm climatic conditions was recently reported from Florida (4) and Thailand (12).

Das (1) in 1951 reported minimum, optimum and maximum temperatures for germination of Cp conidia from Texas as 23, 27 and 32 C. Miller (7) in 1953 reported that optimum temperature for germination of Cp was between 25-30 C. Conidial dimensions of 17-70 μm x 3-11 μm have been reported for Cp (6,8,9,11,13). Conidia of Cp were reported as having 1-9 septa with 3-4 septa being commonly observed (11). Sommartya *et al.* (12) recently observed that conidia of Cp collected from local peanut cultivars in Thailand were 20-100 μm in length. Under similar conditions conidia of *C. arachidicola* were 61-153 μm X 5-10 μm (12).

Peanut genotypes were introduced to Africa and Asia from South America in about 1565 (5). Adapted genotypes were introduced to North America from Africa by slave traders during 1707-1725 (5). Thai and USA populations of Cp have had opportunity to adapt over several hundred years to fit local climate and host genetics. Knowledge of changes in pathogen behavior is essential to the current development and exchange of peanut cultivars between Thailand and USA. The purpose of this study was to determine whether populations of Cp in Thailand and USA have evolved different ecotypes and/or morphological characteristics. Pathogenic specialization of Cp isolates and differential response of 14 peanut genotypes from Thailand and USA was evaluated concurrently in a separate study.

Materials and Methods

Leaves infected with Cp were obtained from Alabama, Florida, North Carolina, and Texas peanut fields in 1985. Conidia of Cp were harvested from several sporulating lesions with a cyclone spore collector (ERI Machine Shop, 124 ERI Building, Ames, Iowa 50011) and maintained as a common population representing each geographic source. Thai Cp populations were collected from local peanut cultivars at two sites each in North, Northeast, and South Thailand in a similar manner. Dry spores collected from diseased leaves in the spring of 1985 were shipped to the USA in plastic vials and refrigerated at 2-4 C until used to establish new cultures in July 1985. Isolates of Cp were cultured by inoculating leaves of Cp susceptible NC 3033 peanut. Ten milliliters of a suspension of Cp conidia (containing one drop Tween 80/100 mL H_2O) were atomized onto leaves and inoculated plants were placed individually in isolation chambers at 22-28 C in the greenhouse. Freshly produced conidia from Cp lesions produced on NC 3033 were used for all studies.

Effects of temperature on germination: Ten to 15 sporulating lesions of each isolate were collected by removing leaflets from NC 3033 culture plants. Leaflets were immersed in deionized H_2O containing Tween 80 (2 drop/100 mL) and conidia were removed from lesions by gentle stroking with a camel-hair brush. Microscope cover-glass slides (18 x 18mm) were coated with a thin film of 2% water agar and held in moist chambers until application of conidial suspensions. Conidial suspensions were uniformly atomized onto two slides per incubation temperature and incubation time. Slides were placed in small moist chambers to maintain high humidity and chambers were placed in incubators at 16, 20, 24, 28, 30, 32, 34 and 36 C. After 12, 24 and 48 h incubation, all cover-glass slides were removed from moist chambers, inverted, and placed on 76 x 25 mm glass slides with a drop of cotton blue in lactophenol. Approximately 100 conidia in five randomly chosen microscopic fields were observed for germination.

Morphological comparisons: Conidia were produced, collected, and sprayed onto agar-coated cover-glass slides as described for germination studies. Slides with conidia were placed in moist chambers and incubated for 12 h at 16 C. Cotton blue in lactophenol was used to stain conidia and germ tubes. At least 100 conidia were observed in five microscope fields of two slides per isolate. Conidia were measured and number of septa per conidium was determined. Number of germ tubes produced per conidium and location of conidial cells producing germ tubes were recorded.

Results

Effect of temperature on germination: Four USA isolates did not differ ($p = 0.05$) in germination percentages at a given temperature. Similarly, Thai isolates did not differ among themselves but all isolates had higher percent germination ($p = 0.05$) than USA isolates after 24 and 48 h incubation. After 12 h incubation, maximum percent germination for all conidia occurred at 20 C and was 58 and 54%, for Thai and USA isolates, respectively. After 24 h incubation, maximum percent germination of USA isolates occurred at 20 C (60%). Percent germination of Thai isolates at 20 C was 84%. After 48 h incubation, maximum percent germination for Thai and USA isolates occurred at 16 C (Fig. 1). Percent germination of Thai and USA isolates at 16 C was 87 ± 1.6 and 76% ± 2.2, respectively. Although percent germination decreased for all isolates as temperatures increased above 20 C, Thai isolates continued to germinate at a higher percentage at all temperatures. At 30 and 32 C, approximately 58 ± 3.0 and 22% ± 2.9 of conidia germinated for Thai isolates whereas only 33 ± 5.4 and 6% ± 1.0 of conidia for USA isolates germinated, respectively. At 34 and 36 C, approximately 2.2 ± 0.4 and 0.2% ± 0.1 of conidia germinated for Thai isolates while 0.6 ± 0.3 and 0.0% of conidia for USA isolates germinated, respectively. When percent germination was averaged across all temperatures, 50.7 ± 2.4 and 39.4 ± 2.7 percent germination occurred for Thai and USA isolates, respectively.

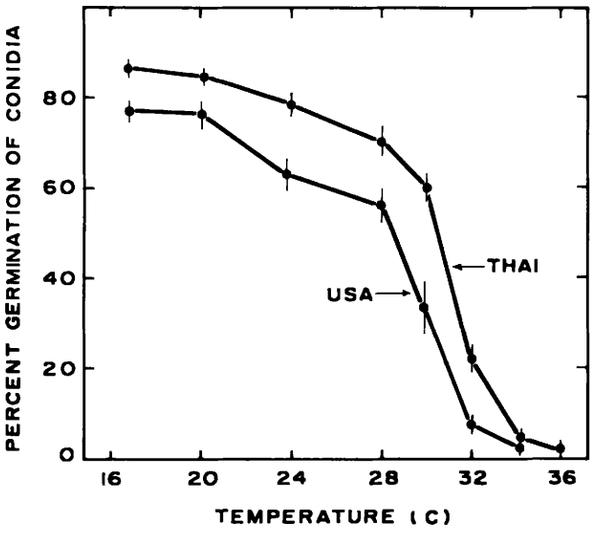

Fig. 1. Effect of temperature on % germination of conidia for Thai and USA isolates of *Cercosporidium personatum* after 48 h. Percent germination of conidia did not differ (P = 0.05) among individual Thai or USA isolate groups. However, at each temperature tested, germination of Thai isolates was higher than USA isolates according to t-test analysis.

Morphological comparisons: Conidia from USA isolates ranged in length from 19.8 to 89.1 μm (Table 1). Conidia from Thai isolates were slightly shorter, ranging in length from 16.5 to 85.5 μm. Width of all conidia ranged from

Table 1. Comparison of conidium size and septum characteristics for *Cercosporidium personatum* as reported from 1933 to 1986.

Length X width per conidia (um)	Number of septa	Reference cited (year reported)
20-70 x 4-9	1-9 (3-4)[a]	Woodroof (1933)
18-60 x 5-11	1-8	Jenkins (1938)
20-70 x 4-9	1-9 (3-4)[a]	Mulder (1974)
17-70 x 5-11	—	Paningatang (1981)
20-70 x 4-9	1-9 (3-4)[a]	Porter (1984)
26-100 x 7-10	2-8	Sommartya (1983)
20-90 x 5-10	1-11 (3-6)[a]	USA isolates [b]
16-86 x 5-10	1-11 (3-6)[a]	Thai isolates [b]

[a] Most commonly occurring number of septa per conidium.

[b] Conidium size and number of septa observed in this study.

5-10 μm. Most (77%) conidia observed in this study ranged in length from 33-60 μm. Most (78%) conidia had 3-6 septa; however, number of septa per conidium for all isolates ranged from 1-11. Furcated conidia were observed from both USA and Thai isolates (Fig. 2). Forked conidia germinated normally, producing both intercalary or terminal germ tubes. A single forked conidium generally produced germ tubes from all three terminal cells.

Fig. 2. Furcate conidium of *Cercosporidium personatum* occurring within both USA and Thai isolates.

Discussion

Conidial germination was greater ($p = 0.05$) at all temperatures for Thai isolates than for USA isolates (Fig. 1). Although isolates could easily be grouped for percent germination on the basis of geographic origin (Thai vs USA), no difference in germination was observed within isolate groups originating from either Thailand or USA. Magnitude of differences in conidial percent germination between Thai and USA isolates remained fairly constant from 16-28 C (Fig. 1). However, almost twice (58 vs. 33%) as many Thai conidia germinated at 30 C and a three-fold difference (22 vs. 6%) between Thai and USA isolates was noted at 32 C. Incremental increases in germination of conidia of Thai isolates may have a major epidemiological impact within semi-tropical environments characteristic of Thailand. If the proportion of conidia germinating at each temperature reflect (potential) subsequent infections, differences in inoculum density for local Cp populations could be magnified greatly because of the high "apparent infection rate" ($r = 0.25$-0.60) reported for Cp on susceptible genotypes (10).

The optimum temperature for germination of conidia of all isolates, was 16-20 C. This temperature range (especially 16 C) is lower than reported by Das (1) or Miller (7). Greater germination percentages at lower temperatures may explain why numerous researchers (3, 4, 12) have concluded that epidemics of late leafspot (Cp) occur more frequently during cool and wet periods, such as late in the USA growing season. However, Cp is a major disease problem in tropical and semi-tropical areas of the world (3, 9, 12) and serious epidemics of late leafspot regularly occur because local Cp populations have adapted to warmer environments.

There was no distinct morphological differences between Thai and USA isolates of Cp; however, conidia of all isolates were longer by 28-50% than previously reported (6,8,9,11,13) (Table 1). Sommartya, *et al.* (12) also observed that conidia of both *C. personatum* and *C. arachidicola* exceeded published lengths when Cp was cultured on local Thai peanut cultivars. Distinguishing between Cercospora leafspot pathogens based on conidial measurements should be done with extreme care if field (leaflet) produced conidia are used in diagnosis. Although conidia observed in this study generally had more septa (1-11) than previously reported in literature, number of septa per conidia may be a reflection of longer conidia produced on peanut leaflets. This is believed to be the first report on the occurrence of furcated conidia for *C. personatum*.

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