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Germination of Phyllachora maydis ascospores and conidia of Monographella maydis

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SUMMARY

The germination of ascospores and the formation of appressoria of the obligate parasite *Phyllachora maydis* increased from 10° to 20 °C, then decreased at 25 °C. A high percentage of conidia of the facultative parasite *Monographella maydis* germinated on the seven agar media substrates under test conditions. The conidia of *M. maydis* germinated between 5° and 35 °C and pH values of 3 to 8. The highest germination of conidia was obtained on mung bean agar at 25 °C. Dark conditions resulted in higher germination. Conidia of *M. maydis* survived for 109 days on detached leaves under ambient conditions, although their viability decreased rapidly after 81 days. Ascospores of *P. maydis* are released within 2 to 3 wk after formation of the stromata.

Introduction

Phyllachora maydis Maubl. and *Monographella maydis* Müller & Samuels with its anamorph *Microdochium maydis* Müller & Samuels, are the most important pathogens of the tarspot disease complex (2, 3) of maize (*Zea mays* L.). *P. maydis*, an obligate parasite, was described by Maublanc (7) on maize in Mexico as the cause of tarspot. About 80 years later *M. maydis* was described as the second component of this disease complex. The involvement of *M. maydis* leads to necrotic tissue that surrounds the tarspot stromata and is 7 to 10 times larger in size. This symptom is commonly called "fisheye".

There is little published information on the tarspot complex, particularly on the phases of the disease, its causal pathogens, and the factors that influence germination of the two causal fungi (CIMMYT, 1987).

Therefore, experiments were conducted at the International Maize and Wheat Improvement Center (CIMMYT), 20° N lat., 2249 masl, Mexico, to elucidate various factors affecting spore germination of these two fungi.

Materials and Methods

Spores were obtained from fresh lesions of maize leaves of Pool 15 (susceptible) and Population 22 TSR (resistant) collected at CIMMYT's Poza Rica station (21. 5° N lat., 60 masl) during the winter season of 1986–87. For *P. maydis* a spore suspension was prepared from 50 *Phyllachora* lesions. The ascostroma (tarspots) were scraped from the leaves, crushed on glass slides and mixed with 4 ml of distilled water al volume 10 (VORTEX genie TM). Conidial solutions of *M. maydis* were prepared by mixing excised "fisheye" lesions with distilled water at volume 10 (VORTEX genie TM). After the conidial solution was decanted through a 30 μ filter, the concentration ranged from 3.8×10^5 to 4×10^5 conidia/ml.

Ascospore suspensions were incubated in duplicate hanging drops in a moist chamber at temperatures of 5, 10, 15, 20 and 25 °C. Suspensions were also incubated on PDA media at the same temperatures. After 2, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 84 h, germination and appressorial formation were assessed by examining 150 spores for each sample.

Temperature in BOD incubators was regulated at 5, 10, 15, 20 and 25 °C at ± 0.5 °C for 12-h periods of light and darkness. Germination and appressorial formation were observed with a

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light compound microscope (400 ×) after staining with lactophenol. The mean values were derived and expressed as percentages.

The media used were water agar/Bioxon (WA), yeast agar/Bioxon (YA), potato dextrose agar/Merck (PDA), lima bean agar/Difco LBA), V8 agar, Sabouraud Nutrient Agar (SNA) (9), and mung bean agar (MBA, filtrate of 100 grs of boiled mungbeans in 1 l of water, 10 grs agar). To each medium 0.3 ml 90% lactic acid per litre was added. The pH factor was between 4 and 6. The effect of the media and temperature on conidia germination of *M. maydis* was studied in an replicated experiment. Using a pair of tweezers, non-disinfected "fisheye" lesions were passed lightly over WA. Agar sections with many conidia were transferred to the above mentioned media. Two petri dishes per sample were incubated at 5, 10, 15, 20 and 25 °C. The germination of two sets of 60 conidia was examined 2, 4, 6, 8, 24, 48, 72 and 96 h later by microscope at 400 × magnification.

To determine the effect of light on germination of *M. maydis*, conidial suspensions were transferred onto PDA in petri dishes. Two dishes in black plastic bags and two controls per temperature were kept at 5, 10, 15, 20, 25, 30 and 35 °C. After 4, 6 and 8 h, 200 spores per sample were examined and the number of germinated conidia recorded.

The effect of pH values was studied on WA, MBA, PDA and SNA. After autoclaving either lactic acid or potassium hydroxide was added to adjust the pH to 3, 4, 5, 6, 7 and 8, as determined by pH indicator strips (Merck). After the agar had set, a pH-meter with a flat surface electrode (CAT 13-639-83) was used and there was a deviation of ± 0.3. The conidial seeded media were incubated at 10, 15, 25 and 30 °C. After 2, 4, 6 and 8 h, 200 spores were examined for germination.

The viability of *M. maydis* is maize leaf tissue was determined by recording the percent germination of conidia from lesions of different ages. Infected leaves from Pool 15 and Population 22 were harvested and stored in the laboratory at about 20 °C and 50% r.h., wrapped in paper. Tests of viability were made at weekly intervals from 60 to 109 days by preparing spore suspensions on PDA plates from 30 lesions from the cultivars. The resulting suspensions had the following concentrations (Pool 15/Population 22): 21.2/4.1, 9.1/4.5, 9.0/4.6, 9.1/4.8, 11.3/7.1 and 5.9/1.5 × 10^5 conidial/ml. Two PDA dishes per sample were seeded and incubated at 25 °C for 6 h, and 200 conidia were observed in duplicate.

Table 1: Mean maximum germination (%) and formation of appressoria of *Phyllachora maydis* as conditioned by temperature.

Medium	Maximum germination			Maximum appressoria formation		
	%	at °C	after h	%	at °C	after h
lesions[1)]	18	25	36	18	20	36
PDA	40	10–25[2)]	12	26	25[3)]	48
hanging drops	32	10–20	8	40	10–20	24

[1)] 100 surface sterilized lesions were incubated in humid chambers. For counting spores were removed and stained; 200 spores in 2 replicates were counted per temperature.

[2)] At 25 °C the germination continued on PDA and reached more than 90% after 72 hours.

[3)] At 5 °C after 60 hours 18% of the ascospores had formed appressoria, at 15 and 25 °C after 48 hours 22 and 25%.

Results

Mean maximum germination of *P. maydis* ascospores and formation of appressoria on lesions, PDA and in hanging drops are shown in Table 1. Appressoria formation, the necessary first step to infection, is highest and fastest in the hanging drop. Germination in hanging drops began within 2 h at 15 to 25 °C and reached a maximum by 8 h at 10 to 20 °C (Fig. 1). A few appressoria formed in 8 h, with the peak occurring between 12 and 24 h at 10 to 20 °C (Fig. 2). The appressoria are dark brown and have

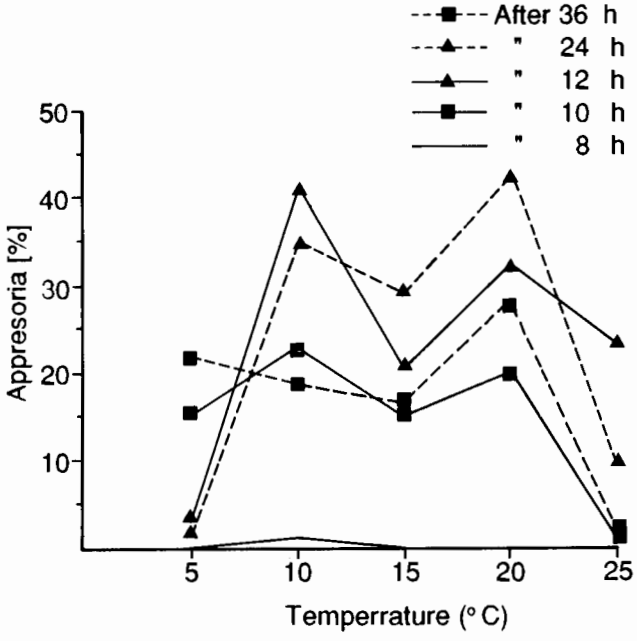

Figure 1: Effect of temperature (°C) on percent germination of *Phyllachora maydis* ascospores in hanging drops of distilled water.

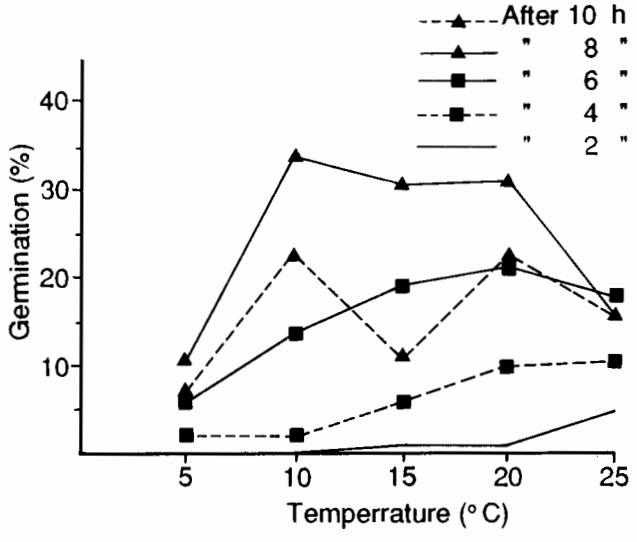

Figure 2: Effect of temperature (°C) on percent of ascospores of *Phyllachora maydis* that formed appressoria in hanging drops of distilled water.

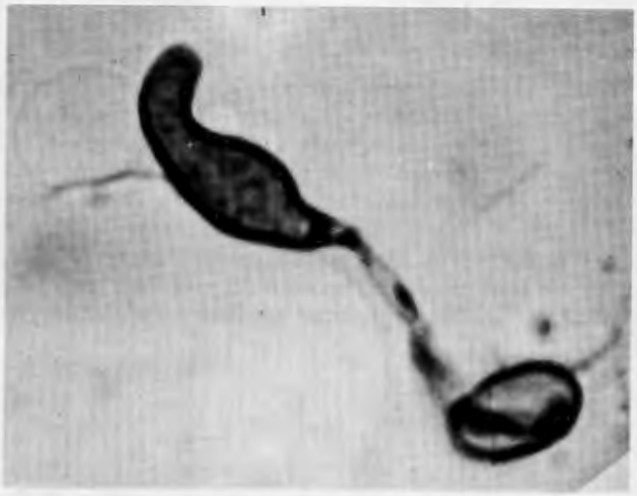

Figure 3: Appressorium of *Phyllachora maydis* developing from an ovoid ascospore.

an allantoid ellipsoid shape according to the classification of Parbery (11) for other *Phyllachora* spp. (Fig. 3).

Germination of *M. maydis* conidia was almost 100% on all the media used. Best results were obtained on MBA and PDA. On these media the colonies were initially white but subsequently changed to a light pink or salmon color. On V8A many septae of the conidia formed germtubes and the colony was very dense. On WA however mycelia were thin, fewer germtubes per conidia were formed and the mycelia grew very rapidly.

Conidia of *M. maydis* were found to germinate over a wide temperature range. Germination was slower and less at 5 °C than at 10 to 35 °C (Fig. 4). The optimum temperature was found to be near 25 °C, based on the rapidity of germination at the 4 and 6 hr intervals. However, about 85 to 95% of the conidia had germinated in 8 hr at 10 to 35 °C under dark conditions. Twenty four percent more conidia consistently germinated in dark than under light conditions during the test period (Fig. 4). The growth of hyphae and subsequent colony formation were found to have the same temperature requirements as that for conidial germination.

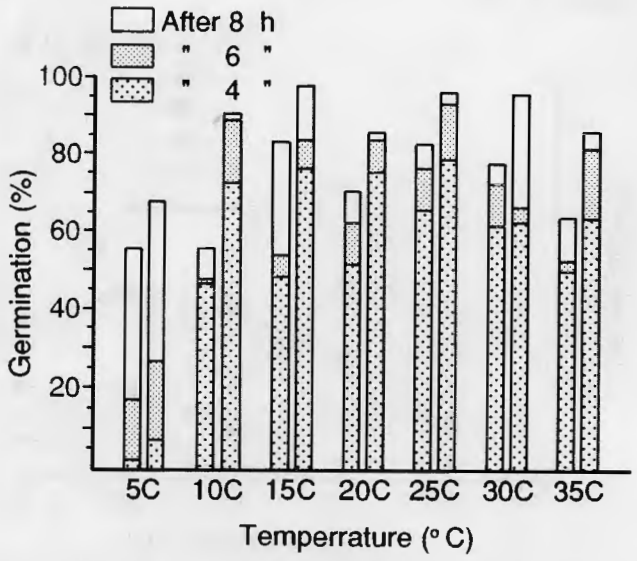

Figure 4: Influence of a dark period/temperature combination on germination of *Monographella maydis* conidia on PDA. The second in each pair of bars shows germination under darkness.

Almost 90% of the *M. maydis* conidia germinated within 6 h at all pH levels (3–8) at the near optimum temperature of 25 °C on PDA (Fig. 5). A pH of 3 or 4 appears to slow germination, particularly in the first 4 h. At a pH of 5 to 8 at 10 °C (Fig. 6) and also at all pH levels at 25 and 30 °C (Fig. 5), germination became rather uniform after 6 h.

Conidia of *M. maydis* had a viability of 90% after leaves had been stored for 60 days at a room temperature of 20 °C and approximately 50% r. h. However, the viability decreased rapidly to 20% during the following 7 wk of storage (Fig. 7). Ascospores of *P. maydis* are released within 2 to 3 wk after the formation of the stromata (2).

Discussion

The germination of ascospores of *P. maydis* followed a pattern similar to that described for other *Phyllachora* species (5, 10, 11). Liu (5) observed germination in distilled water after 2 h at 24 °C but none at 36 °C. Parbery (11) found that the best temperature for germination and appressorial formation for other Phyllachoraceae is 14 °C. Our observation that at 25 °C germination and appressoria formation are lower coincides with the sharp decline of spore germination at 22 to 26 °C described by Parbery (11). The optimum temperature range for germination and appressorial formation of 10 to 20 °C for *P. maydis* confirms that the fungus prefers cool, humid regions in a subtropical climate (4, 6).

Studying *P. maydis* ascospores in hanging drops better simulates the natural process and is more convenient and precise than other methods (2). Ascospore germination in lesions was low (8 to 18% at 10 to 20 °C after 36 to 60 h), but on PDA the percentage was higher (45 to 95% at 10 to 25 °C after 12 to 72 h) than in the hanging drop. Appressoria formation on PDA, however, was very low (24 to 26% at 15 to 25 °C after 48 h). One disadvantage of the water drop is the lack of nutrients or other essential substances to prevent dissolution of appressoria and spores. On the lesions this phenomenon was observed to a lesser extent.

We found the optimum temperature for germination of *M. maydis* conidia to be 25 to 30 °C. This supplements the findings of Müller and Samuels (8), who found the growth of mycelia had a minimum temperature of 5 °C, an optimum of 24 to 27 °C and a maximum of 30 °C.

Differences in optimal temperatures for the two pathogens could help to explain why disease severity of the tarspot complex appears to be less in regions where high temperatures prevail during the growth period. The laboratory results support the findings of Hock et al. (4) that

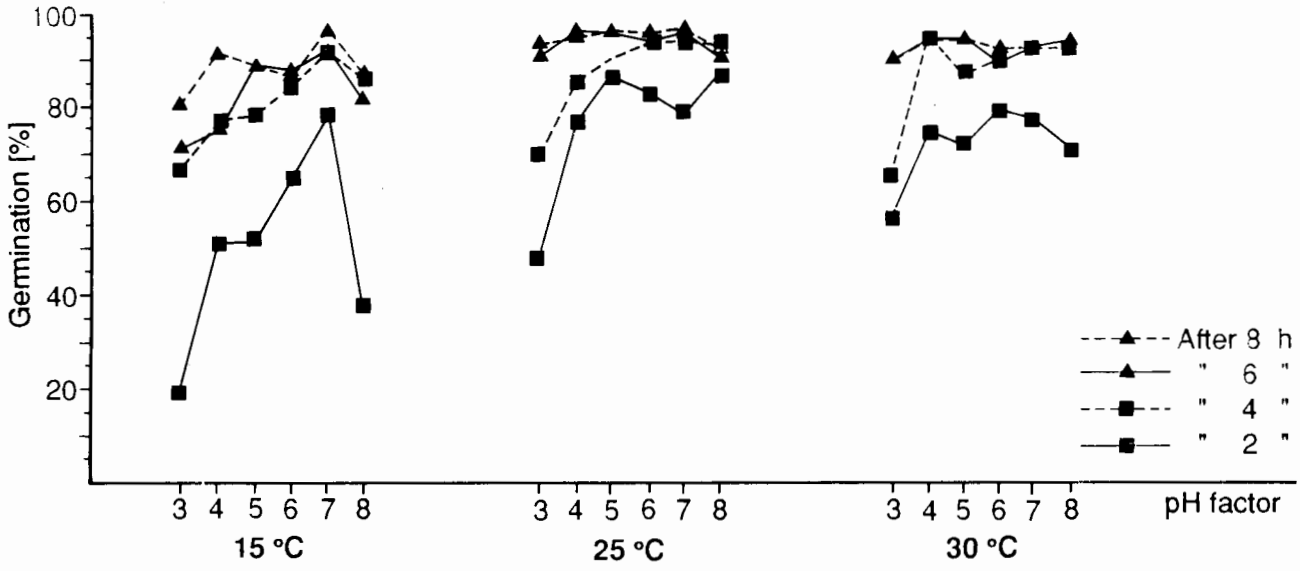

Figure 5: Conidial germination of *Monographella maydis* on PDA as influenced by 6 pH levels at 3 temperatures.

the complex is more prevalent in regions with a monthly average temperature of 16 to 18 °C and a daily oscillation of 5 to 7 °C, since a preceding infection by *P. maydis* seems to be required for *M. maydis* development (2). Several media proved to be adequate for germination of *M. maydis*. Difficulties arise if sporulation is needed, since after two months of growth, no sporulation was observed on any of the seven media (2).

Since conidial germination of *M. maydis* is most rapid under dark conditions, they probably germinate more readily on cloudy or foggy days, at night or on lower leaves, where disease development begins (2, 3). Samuels and Hallett (1983) observed faster mycelia growth for *Monographella stoveri* under darkness.

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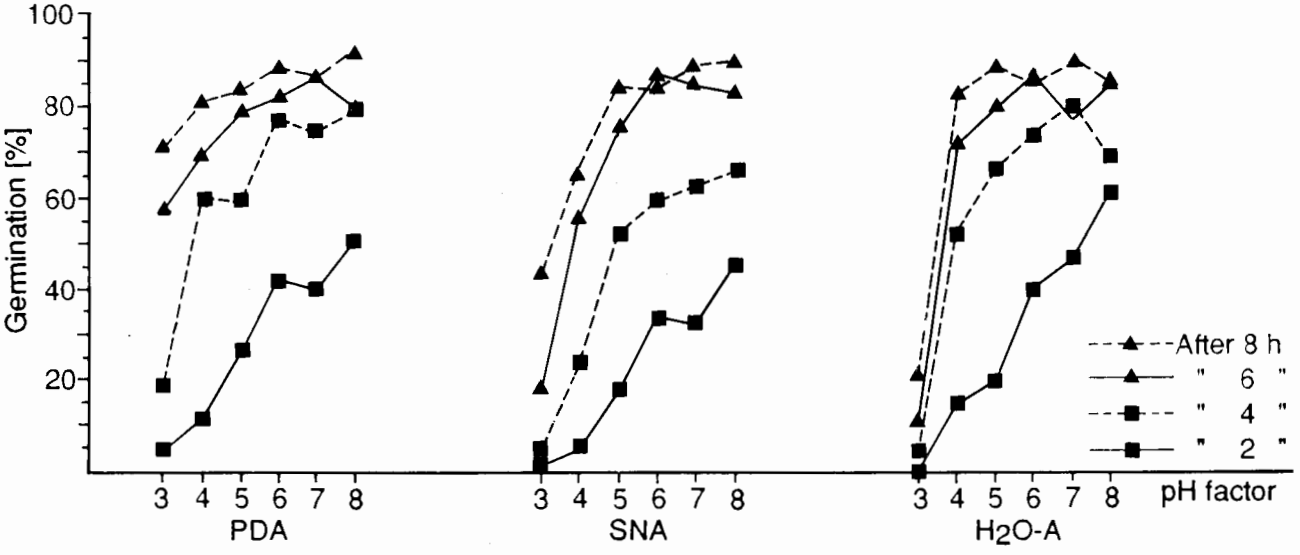

Figure 6: Conidial germination of *Monographella maydis* as influenced by 6 pH levels on three media at 10 °C.

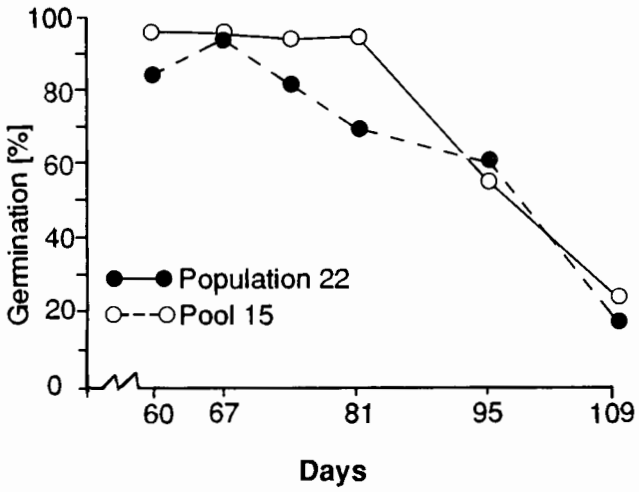

Figure 7: Viability over time of conidia of *Monographella maydis* on leaves of two maize materials stored in the laboratory at about 20 °C, 50% r. h. and darkness.

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