Heat Activation of Conidial Germination in Periconia circinata

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Research reported here was conducted under Project 21-20 and published as Journal Series Paper 4001, of the Nebraska Agricultural Experiment Station.

ABSTRACT

A brief exposure to elevated temperatures (45-48 C) substantially increases germination of conidia of *Periconia circinata*. After heat treatment, 50-75% of the conidia germinate within 18 hours on nutrient agar. Conidia of some isolates germinate without heat treatment and some isolates are more responsive than others, but the capacity for germination is not related to pathogenicity or virulence.

Phytopathology 65:1321-1322

Additional key words: milo disease, root and crown rot, *Sorghum bicolor*.

The fungus *Periconia circinata* (Mangin) Sacc. causes milo disease, a root and crown rot of susceptible milo cultivars of grain sorghum [*Sorghum bicolor* (L.) Moench] (3). Although the fungus sporulates abundantly on infected roots, germinated conidia have rarely been observed (3, 4, 5) and, hence, their role in the disease cycle is unknown. Our initial studies on the pathogenicity of *P. circinata* conidia necessitated development of methods for obtaining germination. This report summarizes results of experiments in which conidia of *P. circinata* were subjected to treatments and conditions known to activate spore germination in other fungal species.

MATERIALS AND METHODS.—Cultures of *P. circinata* were obtained by isolating single conidiophores from infected roots of sorghum plants. Mycelium growing from the conidiophore on water agar was transferred to potato dextrose agar (PDA) and cultured at 23 C. Pathogenic isolates, Pc1 and Pc9, were obtained from Texas and Kansas, respectively; nonpathogenic isolates, Pc3 and Pc49, were obtained from Nebraska. A culture of *Periconia macrospinosa* Lefebvre & A. G. Johnson, presumably a nonpathogen of sorghum (2), was isolated from sorghum roots also infected with *P. circinata*.

To obtain nearly synchronous production of conidia, 6 mm diameter disks of mycelium were cut with a cork borer from a 2-week-old culture and the plugs were transferred to water agar and incubated at 25 C, 30 C, or at room temperature (19-24 C). Conidia were harvested two, three, or four weeks later by shaking the plugs in sterile distilled water. In routine experiments in which spores from a single plug were harvested in 1 ml of water, it was not necessary to remove the few contaminating conidiophores. However, when large quantities of conidia were required, conidiophores and vegetative hyphae were completely removed by filtering the suspension repeatedly through six layers of cheesecloth.

RESULTS AND DISCUSSION.—Initial attempts to germinate conidia by incubating them on various media or solutions that stimulate spore germination in other fungal species (e.g., organic acids, aldehydes, alcohols, and amino acids) were unsuccessful. However, when conidia were suspended in 1 ml of sterile distilled water and heated in a water bath to 45 C for 15-45 minutes, nearly 25% of the conidia germinated on PDA at 25 C. Occasionally, the germ tubes branched profusely shortly after their emergence, or multiple germ tubes were produced from the same site in the spore wall; but no conspicuous germ pore was evident.

Heating to 48 C for 5-10 minutes followed by incubation on PDA at 30 C resulted in optimal germination (usually 50-70%). Lower temperatures (40-46 C) were also effective, but required longer treatments to stimulate comparable germination. Higher temperatures, below the thermal inactivation point (57-58 C), were slightly less effective, even for shorter durations. The optimal incubation temperature for germination was 30 C, but the vegetative growth rate was higher at 25 C (10 mm in 24 hours) than at 20 or 30 C (6-7 mm in 24 hours). Neither vegetative growth nor germination occurred at 40 C, but cultures resumed growth and heat-treated conidia germinated when they were incubated at 30 C after exposure to 40 C for 7 days or 12 hours, respectively.

Figure 1 shows the time course of conidial germination on PDA at 30 C following treatment at 48 C for 10 minutes. Germination began in about 9 hours and ended by 24 hours. Attempts to synchronize germination using different media and treatments were ineffective. Germination of nonheat-treated conidia was completed by 15 hours, indicating that the heat treatment influences the capacity for germination rather than the rate of germination.

Heat treatment stimulated germination of conidia from several *P. circinata* isolates and was effective with conidia produced on infected roots as well as those produced in culture. However, heat shock was not equally effective with all isolates; some (Pc9 and Pc49) germinated more efficiently even in absence of heat treatment (Table 1). The response to heat treatment and the capacity for germination were not correlated with pathogenicity or virulence of the isolates. Conidia of the non-pathogen *P. macrospinosa* germinated approximately 90% whether heat-treated or not.

In general, conidia produced at 30 C germinated better than those produced at 25 C or at room temperature (Table 1). Furthermore, conidia produced at 30 C apparently matured more rapidly, since they responded to heat shock earlier than those at the lower temperatures.

No consistent effect of harvesting the conidia in water was observed (Table 1). Although a higher percentage of conidia of isolate Pc9 germinated after water harvest, neither the addition of concentrated spore leachates nor increasing the spore concentration influenced the extent of germination. Apparently, spore germination is not regulated by endogenous inhibitors that are released from the spores into water. The inability of conidia to germinate in liquid media precluded more definitive experiments.

Temperature and duration of heat treatment effective in stimulating germination of *P. circinata* conidia are strikingly similar to those conditions that activate sporangiospores of *Phycomyces blakesleeanus* (48-53 C for 3 minutes) (1). In the latter species, the spores are also

TABLE 1. Effect of heat treatment of 48 C for 10 minutes on germination of conidia from four isolates of *Periconia circinata* produced for 3 weeks at room temperature (RT) or at 30 C

Treatment	Production temperature	Germination (%)[a] of isolate:			
		Pc1	Pc3	Pc9	Pc49
Dry conidia-no heat shock	RT	8	3	7	26
	30 C	12	11	35	42
Water harvested-no heat shock	RT	4	2	53	20
	30 C	8	16	54	22
Water harvest-heat shock	RT	40	40	53	71
	30 C	51	54	67	64

[a]Values determined after 18 hours on PDA at 30 C.

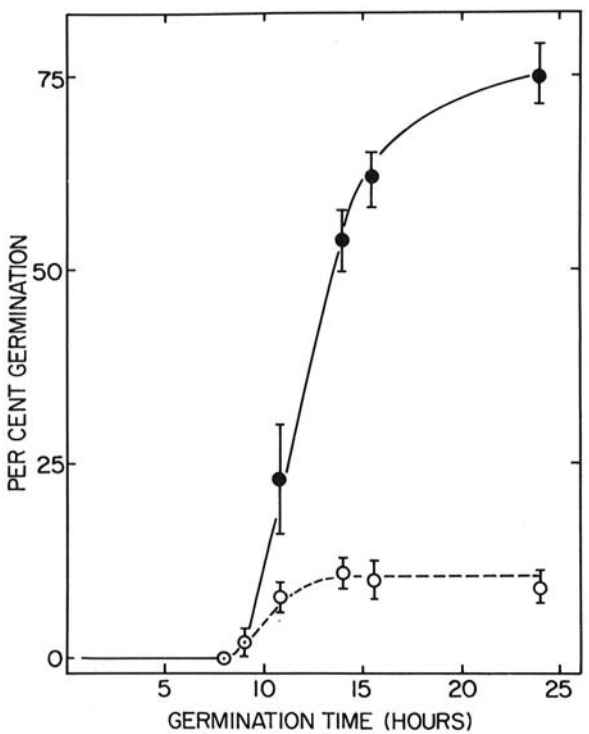

Fig. 1. Rate of germination of a population of *P. circinata* (Pc49) conidia on PDA at 30 C following a heat treatment of 48 C for 10 minutes (•) or no heat treatment (o). Germination was defined as production of a germ tube longer than the diameter of the spore. Percent germination was determined by counting 200 to 300 conidia on three separate plates and the range of values is indicated by the bars.

activated by several chemicals, including ammonium acetate. However, we were unable to obtain significant germination by treating the conidia with a number of chemicals over a range of concentrations.

The reasons for the failure of previous investigators (3, 4, 5) to observe low germination of nonheat-treated *P. circinata* conidia (Table 1) are not obvious. Perhaps differences in incubation and production methods are critical. We observed, for example, that nutrients, temperature, and humidity influenced sporulation and maturation of conidia.

That the conidia have the capacity to germinate suggests that they can function as inoculum in nature. Our preliminary studies (Dunkle et al., *unpublished*) indicate that germinating conidia of pathogenic *P. circinata* isolates can penetrate and colonize roots of susceptible seedlings of grain sorghum.

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