Sporulation in Helminthosporium maydis: Inhibition by Thiamine

M. O. Garraway

Associate Professor, Department of Plant Pathology, The Ohio State University, Columbus 43210, and Ohio Agricultural Research and Development Center, Wooster 44691.

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ABSTRACT

Thiamine (1 ppm) inhibits sporulation of *Helminthosporium maydis* by over 70 percent after 6 days incubation in the dark at 28 C on a basal agar medium containing glucose plus either L-asparagine or ammonium citrate. Linear growth (mm per day) of mycelia is relatively insensitive to this thiamine concentration. The differential response of sporulation and mycelial growth to thiamine provides a tool for physiological investigations of sporogenesis.

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The promotion of growth, reproduction, and metabolite production in fungi by appropriate concentrations of one or more vitamins (5, 8, 13, 14) usually provides the impetus for investigations of their biosynthesis and mode(s) of action (2, 6, 7). Fungi which are autotrophic, or whose growth is inhibited by a vitamin, usually are not used as test organisms in such biochemical investigations (5). However, fungi inhibited by thiamine appear to be an exception (1, 9, 10, 12). Thus, Rabinowitz & Snell (12) found that growth of *Saccharomyces carlsbergensis* 4228 was inhibited over 90 percent by thiamine (1 ppm) if vitamin B_6 was omitted from the growth medium. Their observations have been confirmed and extended by others (1, 9, 10).

Apparently, critical investigations of thiamine inhibition of fungal growth have been confined to yeasts (1, 9, 10, 12). Information on thiamine inhibition of growth of filamentous fungi is scant (3, 4). Moreover, there appears to be a complete lack of information on thiamine inhibition of sporulation. From the plant pathologist's point of view such information is vital. Thiamine inhibition of mycelial growth and sporulation of fungal pathogens might provide a physiological basis for investigations related to control of their survival and spread.

A study of the effects of nutritional factors on sporulation of *Helminthosporium maydis* Nisik. & Miyake, the southern corn leaf blight pathogen, revealed that thiamine (1 ppm) inhibits sporulation. A brief report follows.

MATERIALS AND METHODS.—The experiments were carried out with a single-spored isolate of *H. maydis* race T. The fungus was isolated from a corn ear obtained from a field in Franklin County, Ohio, in the summer of 1970. Its cultural and pathogenic characteristics appeared unchanged after 2 years in culture.

The fungus was grown on a basal medium containing either L-asparagine or ammonium citrate with various concentrations of glucose. The L-asparagine medium contained 4.0 g L-asparagine, 1.5 g KH_2PO_4, 0.75 g $MgSO_4 \cdot 7H_2O$, 1.0 mg each of $CuSO_4$, $FeSO_4$, $MnSO_4$, and $ZnSO_4$, and 20 g Difco agar/liter of distilled water. The ammonium citrate medium contained 4.0 g ammonium citrate, 1.8 g K_2HPO_4, 0.75 g $MgSO_4 \cdot 7H_2O$, 1.0 mg each of $CuSO_4$, $FeSO_4$, $MnSO_4$, and $ZnSO_4$, and 20 g Difco agar/liter of distilled water. The pH of each medium was adjusted to 6.0 with 3 N NaOH, prior to sterilization by autoclaving. Then 18 ml of melted (60 C) basal media were added to petri dishes (100 × 15 mm) containing 2.0 ml of sterile distilled water or sterile dilutions of glucose, to give 20 ml of media containing 0, 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, or 10.0 g glucose/liter. Media with each nitrogen source and glucose concentration were tested, with and without a supplement of thiamine, for their effects on mycelial growth and sporulation. For this purpose, 0.2 ml of a sterile dilution (0.1 mg/ml) of thiamine, prepared by autoclaving or filtration, was added to petri dishes with 2.0 ml of the glucose dilutions mentioned. The resulting concentration of thiamine (1.0 mg/liter, or 1 ppm) was in excess of that needed for severe inhibition of growth of *S. carlsbergensis* (1, 12) and *Fusarium roseum* (4). To compensate for the extra liquid, 0.2 ml sterile distilled water was added to the controls without thiamine. While the agar medium was still melted, petri dishes were gently shaken to ensure complete mixing of all added ingredients.

Inoculum for seeding the media, consisted of 6-mm agar disks taken from 3-week-old cultures grown in the dark at 24 C on a L-asparagine medium with 10 g glucose/liter. Seeded media were incubated for 6 days in the dark at 28 C. Linear mycelial growth was measured daily after the third day. At termination of the experiment on the sixth day, six 12-mm disks with mycelia and conidia (spores) were selected randomly from each petri dish, transferred to screw-cap vials (15-ml capacity), then inactivated by suspending in 6.0 ml of a 0.6 N NaOH solution containing 20% ethanol and 5% Clorox. Conidia per ml were determined with a hemacytometer, then the number per cm^2 of medium surface was estimated. Sporulation data for each medium represents the mean of three replications with 10 hemacytometer grids (1 × 1 mm) counted per replication. Data presented are representative of several similar experiments.

RESULTS.—On either nitrogen source, sporulation increased with increasing glucose concentrations (Fig. 1).

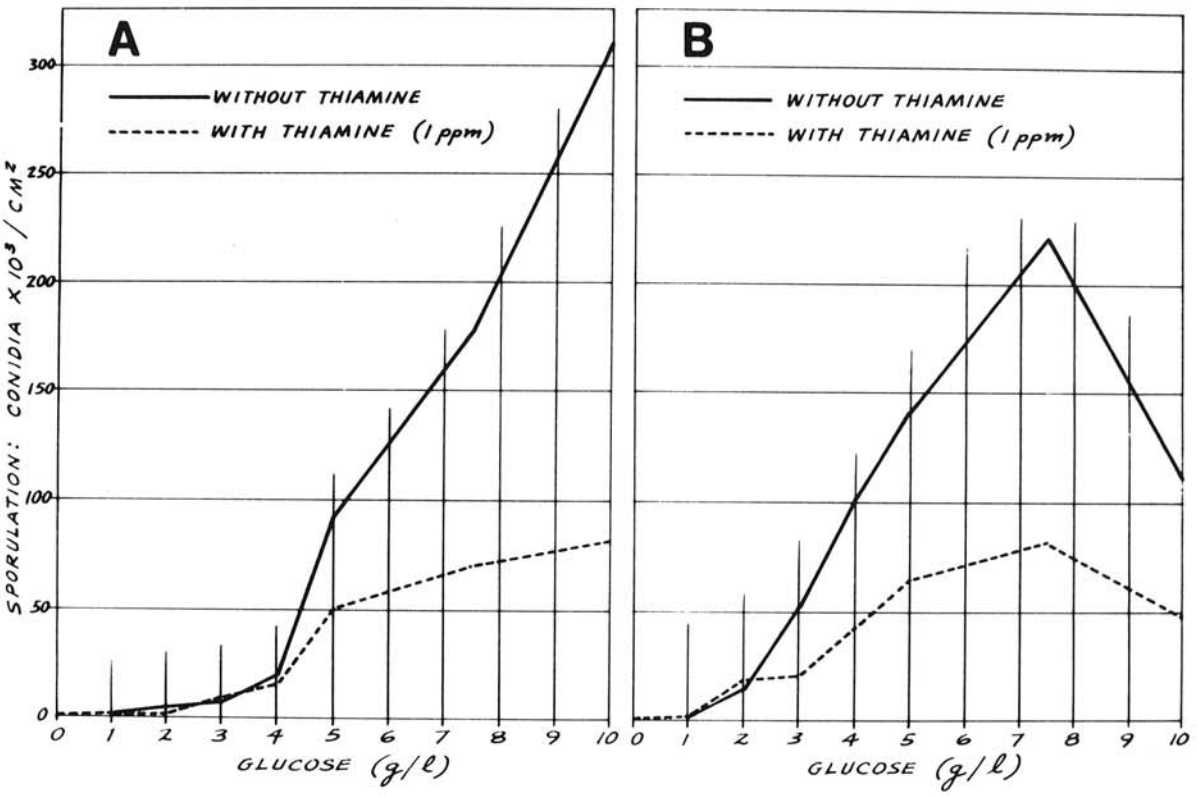

Fig. 1. Sporulation response of *Helminthosporium maydis* race T to various glucose concentrations with (-----) and without (——) thiamine (1 ppm). **A)** Comparison of sporulation response on L-asparagine media with and without thiamine. Note the steeper increase in sporulation in response to glucose concentrations above 4.0 g/liter without thiamine. **B)** Comparison of sporulation response on ammonium citrate media with and without thiamine. Note the steeper increase in sporulation in response to glucose concentrations between 2.0 and 7.5 g/liter without thiamine. Sporulation decreases above the latter glucose concentration.

The presence of thiamine (1 ppm) reduced this increase at glucose concentrations above 4 g/liter on L-asparagine media (Fig. 1-A), and above 2 g/liter on ammonium citrate media (Fig. 1-B).

On L-asparagine media without thiamine, sporulation at 4 and 10 g glucose/liter was 19.0×10^3 and 309.0×10^3 conidia/cm^2, respectively. In contrast, sporulation at these glucose concentrations in the presence of thiamine was 15.5×10^3 and 81.6×10^3 conidia/cm^2. Thus, with thiamine the sporulation increase of *H. maydis* in response to increasing glucose concentrations from 4 to 10 g/liter was 22.8% of that which occurred without thiamine.

On ammonium citrate media with thiamine, the sporulation increase in response to glucose concentrations from 2 to 7.5 g/liter was 30.8% of that which occurred without thiamine. At the latter glucose concentration, sporulation with and without thiamine was, respectively, 81.6×10^3 and 221.0×10^3 conidia/cm^2. At 10 g glucose/liter, sporulation decreased to 49.5×10^3 conidia/cm^2 with and 110.3×10^3 conidia/cm^2 without thiamine. Thus, glucose at a concentration above 7.5 g/liter suppressed sporulation in the presence and absence of thiamine.

Mycelial growth appeared to be less sensitive to the presence of thiamine than was sporulation. Linear rate of growth (mm/day) on L-asparagine media was slightly less in the presence of thiamine than in its absence, for each glucose concentration. The growth rate on the various concentrations of glucose ranged from 9.4-9.8 mm/day in the presence of thiamine and 9.8-11.2 mm/day in its absence. On ammonium citrate media, linear growth appeared to be insensitive to the presence of thiamine. The average growth rate, however, was about 21% less than on L-asparagine media.

Cultures of *H. maydis* grown on media without glucose had surface and subsurface mycelia with nonpigmented hyphae. In contrast, cultures on glucose-supplemented media had compact mycelia with heavily pigmented surface and subsurface hyphae and nonpigmented aerial hyphae. The intensity of pigmentation and the amount of aerial hyphae appeared to be greater on L-asparagine than on ammonium citrate, and at the higher glucose concentrations. These cultural features were unaffected by the presence of thiamine.

DISCUSSION.—Three conclusions about the physiology of *H. maydis* revealed by this study are: (i) the fungus is autotrophic for thiamine; (ii) sporulation is inhibited by thiamine; and (iii) mycelial growth, as measured by linear extension, is relatively insensitive to thiamine.

The finding that *H. maydis* is autotrophic for thiamine indicates that it has the capacity for biosynthesis of thiamine pyrophosphate, which is the biologically active

form for growth of organisms (2, 6, 7, 11). In this study, thiamine was added to the media as the biologically inactive thiamine hydrochloride. Accumulation of the latter compound in the fungus could induce a deficiency by inhibiting synthesis or biological activity of thiamine pyrophosphate (2, 6). Thus, thiamine may inhibit thiamine pyrophosphokinase activity and impede the synthesis of thiamine pyrophosphate (6). Alternatively, the antimetabolite activity of the pyrimidine moiety of thiamine or a derivative may inhibit the catalytic functions of thiamine pyrophosphate (2, 6, 15).

Since thiamine pyrophosphate is essential for several key biochemical reactions (11), one would expect a complete deficiency to cause almost complete impairment of mycelial growth and sporulation. However, mycelial growth appeared to be relatively insensitive to the concentrations of thiamine used. Therefore, thiamine acting by the mechanisms proposed above would produce only a partial thiamine pyrophosphate deficiency. In view of Hawker's observation (8) that sporulation is more readily altered by nutritional deficiencies than is vegetative growth, an induced partial deficiency of thiamine pyrophosphate could explain the observed effect of thiamine on sporulation of *H. maydis*.

An indirect effect of thiamine on sporulation may be the induction of a partial deficiency of vitamin B_6 or pyridoxal phosphate (1, 9, 10, 12, 15), since Rabinowitz & Snell (12) and others (1, 9, 10) found that thiamine inhibition of *S. carlsbergensis* growth was reversed non-competitively by vitamin B_6.

From the foregoing it is assumed that the mechanism of thiamine inhibition of *H. maydis* sporulation is comparable to that proposed for inhibition of *S. carlsbergensis* growth (1). The potential involvement of ethanol with this phenomenon is suggested by the work of Chiao & Peterson (1), who observed that thiamine inhibition of *S. carlsbergensis* growth was accompanied by a stimulation of ethanol production.

In addition to sporulation inhibition by thiamine, this study has revealed sporulation inhibition by a high glucose concentration (Fig. 1-B). The mechanism involved may or may not be the same as that proposed above for thiamine inhibition of sporulation in *H. maydis*.

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