Influence of Culture Age and Temperature on Germination of Helminthosporium sorokinianum Conidia and on Pathogenicity to Poa pratensis

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Journal Paper No. J-7176 of the Iowa Agriculture and Home Economics Experiment Station, Ames; Project 1751. Supported in part by a research grant from the Golf Course Superintendents' Association of America.

I wish to thank E. S. Luttrell, Department of Plant Pathology and Plant Genetics, University of Georgia, College of Agriculture, Athens, for technical assistance in the classification of the organism used in this research.

Accepted for publication 10 April 1972.

ABSTRACT

Conidia of *Helminthosporium sorokinianum* germinated in larger numbers, at a faster rate, and produced more and longer germ tubes and germ tube branches as temperature was increased from 10 to 22 C; above 22 C, percentage germination and growth rates of germ tubes declined. Conidia from older cultures germinated faster and in larger numbers and produced more and longer germ tubes and germ tube branches at all temperatures. However, conidia from older cultures were less pathogenic to *Poa pratensis* than were conidia from younger cultures.

Phytopathology 62:1133-1137.

Additional key words: conidia maturity and pathogenicity, conidia growth, epiphytology.

Kentucky bluegrass (*Poa pratensis* L.) and creeping bentgrass (*Agrostis palustris* Hud.) are the primary perennial turfgrasses of the north central states. Both grasses are infected and damaged by *Helminthosporium sorokinianum* Sacc. ex Sorok. *H. sativum*). On Kentucky bluegrass, the pathogen infects leaves and roots (1, 2, 5, 15, 17); and on creeping bentgrass, the leaves, stems, and roots are infected (5, 7, 8, 12). The pathogen may produce leaf lesions on both hosts (1, 5, 12, 15, 17), or it may cause blighting of leaves (5, 12, 17).

Few studies have been done on conidia germination and pathogenicity of *H. sorokinianum.* Germination studies indicate that optimum temperature for germination occurs between 22 and 32 C and that conidia will germinate over a wide pH range (4). Guttation fluid from barley (*Hordeum vulgare*) and creeping bluegrass accelerates conidia germination, appressoria formation, and infection of creeping bentgrass seedlings (6, 7, 8). Guttation fluid from perennial ryegrass (*Lolium perenne*) is high in glutamine (3, 11), and infection of creeping bentgrass by *H. sorokinianum* is accelerated by L-glutamine (12). Some amino acids also cause attenuation of virulence in *H. sorokinianum* (13). Pathogenicity studies are complicated because isolates of *H. sorokinianum* are not equally pathogenic to *P. pratensis* and *A. palustris* (16), which may be the result of differences in their ability to produce toxins (9, 10, 14). The research reported herein was initiated to examine the interactions of culture age and temperature on conidia germination, germ tube development, and pathogenicity of *H. sorokinianum* to *P. pratensis.*

MATERIALS AND METHODS.—*Helminthosporium sorokinianum* Sacc. ex Sorok. (*H. sativum*) was isolated from leaf lesions on *P. pratensis* on 4% Bacto juice agar (20% [v/v] V-8 juice and 4% Bacto agar in double distilled water). All cultures were grown on 10 ml of V-8 juice agar in 30-ml disposable plastic culture flasks at 22 C under 200 ft-c of continuous light.

Conidia were collected from 20-, 40-, and 60-day-old cultures and germinated at 10, 16, 22, 28, and 34 C (±0.5 C). I collected conidia by placing 15 ml of sterile, double-distilled water in each culture flask and shaking the flask to suspend the conidia. I separated mycelial fragments from the conidia by passing the suspension through a 100-μ micro-sieve into a Syracuse dish. Five transfers of the conidial suspension were made with a 3-mm transfer loop to a Sykes-Moore tissue culture chamber (Bellco Glass, Inc.) containing ca. 1 ml of sterile, double-distilled water. The chambers were placed in the center of a Cambion heating-cooling microscope stage (Cambridge Thermionic Corp.) at the appropriate temperature, and conidia germination was observed and recorded at 1-hr intervals for a period of 8 hr. At the end of 8 hr, germ tubes were measured, and number of primary branches was recorded. A total of 300 conidia were observed for each treatment. Conidia were recorded as germinated when germ tubes reached 3 μ.

Poa pratensis 'Newport' was used for pathogenicity studies. Plants were vegetatively propagated from individual tillers of a single plant and grown in a steamed 2:1 loam-peat soil mixture in 3-inch-diam plastic pots. Plants were grown 30 days in the greenhouse (18-hr day-length, 20-35 C) and then placed in growth chambers at 22 C for 2 weeks prior to inoculation (18-hr day-length, 1,800 ft-c). At the time of inoculation, 10 plants were selected, and all but three tillers were removed from each plant; only the three youngest leaves were left on each tiller to reduce saprophytic development of *H. sorokinianum* on older leaves. Each leaf was cut to a height of 6 cm to simulate mowing injury. Leaves were rinsed with distilled water and inoculated with a conidial suspension of *H.*

TABLE 1. Germination of *Helminthosporium sorokinianum* conidia and the number and length of germ tubes and germ tube branches produced at different temperatures by conidia from cultures of different ages

Temp (C)	Culture age (days)	Characteristic observed: % Germination[a]	No. of germ tubes: One	No. of germ tubes: Two	No. of germ tubes: Mean length (μ)	Germ tube branches: No.	Germ tube branches: Mean length (μ)
10	20	58.0 a/a[b]	140	34	20.4 a/a	0	
	40	66.0 a/a	152	46	62.6 b/a	4	45.5 a/a
	60	74.3 a/a	137	86	76.2 c/a	15	18.4 b/a
16	20	58.7 a/a	155	21	102.7 a/b	22	54.1 a/a
	40	78.0 b/b	162	72	117.1 b/b	116	71.7 b/b
	60	92.0 c/b	140	136	122.8 b/b	177	58.7 a/b
22	20	97.3 a/b	124	168	187.7 a/c	416	72.5 a/b
	40	96.3 a/c	106	183	229.8 b/c	420	79.3 a/b
	60	90.0 a/b	103	191	250.3 c/c	421	98.3 b/c
28	20	89.0 a/b	154	113	116.6 a/b	62	24.9 a/c
	40	85.7 a/bc	118	139	135.7 b/d	105	48.3 b/a
	60	93.3 a/b	141	139	213.2 c/d	136	53.1 b/b

[a]Percentage germination based on 300 conidia from cultures of each age at each temperature.

[b]a/a = First letter indicates effect of culture age at the same temperature; figures followed by the same letter are not significantly different. Second letter indicates effect of different temperatures on cultures of the same age; figures followed by the same letter are not significantly different (> .5; Duncan's multiple range test).

sorokinianum by means of a compressed-freon atomizer. Fifty ml of a suspension of ca. 2×10^4 conidia were atomized onto the three tillers of each plant. Each plant was placed in a plastic bag and maintained in growth chambers at the appropriate temperature for 7 days, after which lesions were counted. Control plants were atomized with sterile distilled water.

RESULTS.—*Total conidia germination.*—Percentage germination of conidia from all treatments increased to 22 C, then decreased at 28 C; no conidia germinated at 34 C (Table 1). Percentage germination increased with increasing culture age at all temperatures.

Rate of conidia germination.—Except at 22 C, conidia from 60-day-old cultures germinated most rapidly at all temperatures, followed by conidia from 40- and 20-day-old cultures (Fig. 1-4). Only slight differences in rate of germination occurred at 10 C (Fig. 1); however, at 16 C, germination rate of conidia from 60-day-old cultures was faster than that of conidia from either 40- or 20-day-old cultures at and beyond 2 hr (Fig. 2). Between 2 and 8 hr at 16 C, germination of conidia from 20-day-old cultures differed very little from that of conidia from 20-day-old cultures at 10 C (Fig. 1, 2). At 22 C, conidia from 20-day-old cultures germinated most rapidly, followed by conidia from 40- and 60-day-old cultures (Fig. 3). Rate of germination of conidia from 60- and 40-day-old cultures at 28 C was greater than that of conidia from 20-day-old cultures at 2 through 5 hr (Fig. 4).

Germ tube production and growth.—Germ tube number and length increased on all conidia as temperature was increased from 10 to 22 C, and decreased above 22 C (Table 1). At 10 and 16 C, fewer conidia produced two germ tubes, but the proportion of conidia with two germ tubes was higher in older cultures. At 22 C, a greater proportion of conidia produced two germ tubes, whereas there was no consistent difference above 22 C.

Germ tube length increased on conidia from cultures of all ages as temperature was increased from 10 to 22 C; above 22 C, germ tube length declined (Table 1). Germ tube length also increased progressively at each temperature on conidia from each older culture. Maximum germ tube length occurred at 22 C on conidia from 60-day-old cultures.

Primary branching of germ tubes.—The number of germ tube branches increased as temperature was increased from 10 to 22 C and decreased above 22 C (Table 1). The number of germ tube branches was also higher on conidia from older cultures at all temperatures.

The length of germ tube branches increased as temperature was increased from 10 to 22 C, and decreased above 22 C (Table 1). The age of cultures had no consistent effect on branch length. At 10 and 16 C, maximum branch length occurred on conidia from 40-day-old cultures and was shorter on conidia from 60-day-old cultures. At 22 and 28 C, germ tube branch length was longer on conidia from 60-day-old cultures; the maximum length occurred at 22 C.

Pathogenicity.—Disease severity increased with conidia from cultures of all ages as temperature was increased to 22 C; above 22 C, disease severity decreased (Fig. 5). Except at 10 and 28 C where no differences occurred, conidia from 20-day-old cultures were more virulent to *P. pratensis* than were

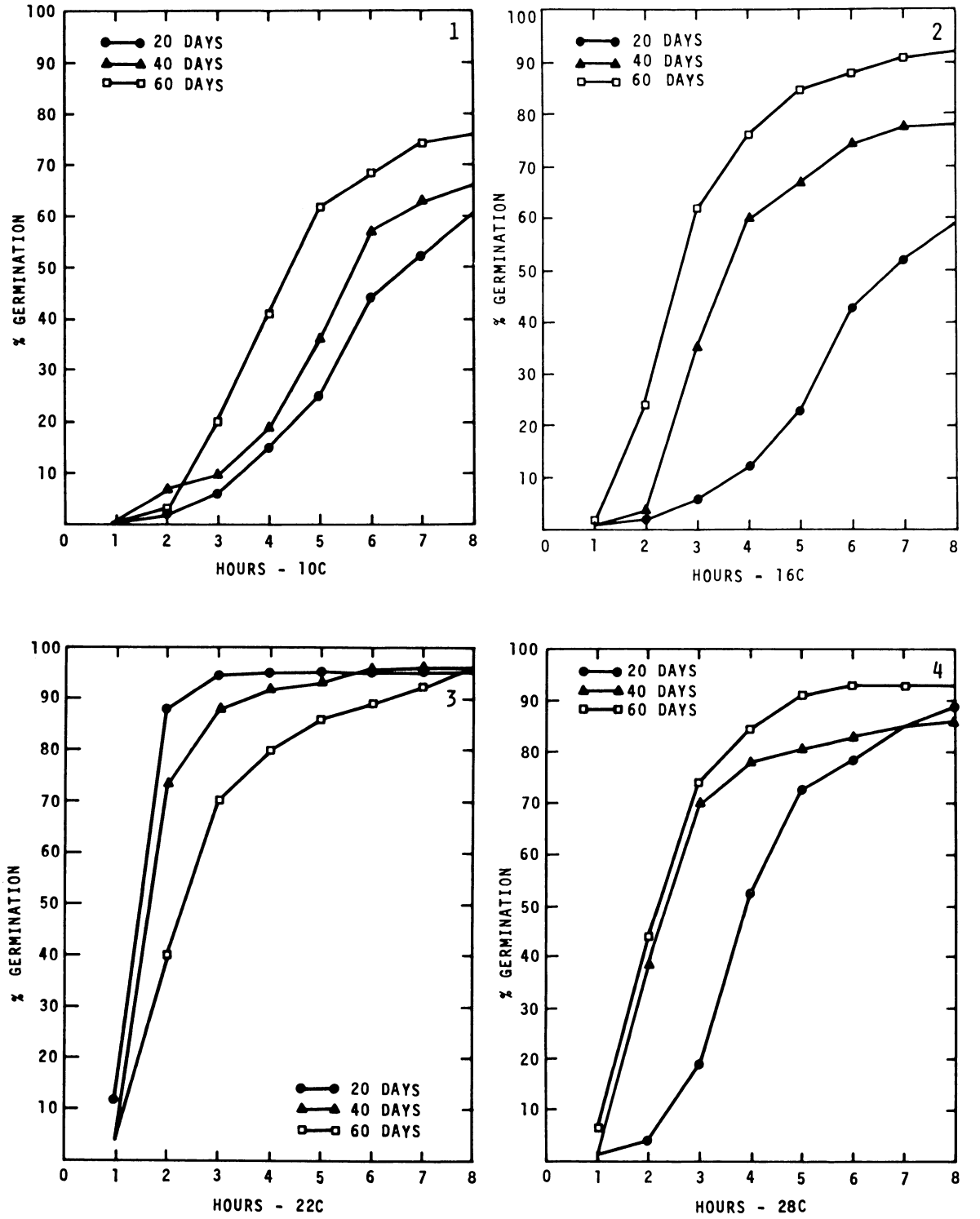

Fig. 1-4. Rate of germination of *Helminthosporium sorokinianum* conidia from cultures of different ages at different temperatures.

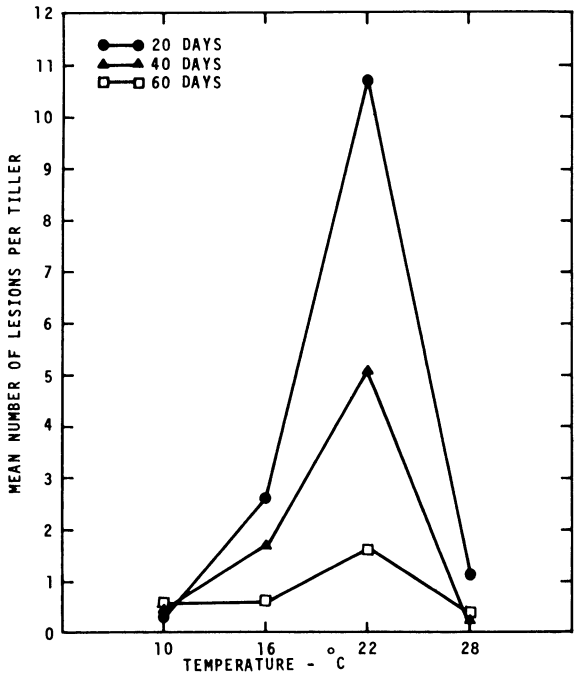

Fig. 5. The number of *Helminthosporium sorokinianum* lesions on *Poa pratensis* from cultures of different ages and incubated at different temperatures.

conidia from 40- and 60-day-old cultures. The number of lesions produced by conidia from 20-, 40-, and 60-day-old cultures at 22 C were 10.7, 5.1, and 1.6, respectively.

DISCUSSION.—The effects of age and temperature on germination of conidia suggest that morphological and physiological characteristics of *H. sorokinianum* conidia function independently. Conidia from 20-, 40-, and 60-day-old cultures are morphologically mature; i.e., conidia from all cultures were fully developed with septa and could not be separated morphologically on the basis of the age of the culture from which they were collected. Conidia from 60-day-old cultures, however, with few exceptions, germinated faster and in larger numbers, and produced more and longer germ tubes and germ tube branches than did conidia from younger cultures. Thus, conidia from 60-day-old cultures are more physiologically mature than are conidia from 20-day-old cultures; i.e., they germinate and grow more rapidly than conidia from younger cultures at most temperatures. Factors other than age may be implicated in physiological maturity, but they are not discernible without additional research.

The relationship between morphological and physiological maturity has significance relative to pathogenicity. Conidia from 20-day-old cultures were generally more virulent than conidia from 40- and 60-day-old cultures (Fig. 5). It is interesting that only at 22 C did conidia from 20-day-old cultures germinate faster and in larger quantities than those from 60- and 40-day-old cultures (Fig. 3). This characteristic of conidia from 20-day-old cultures, in combination with their greater virulence at 22 C (Fig. 5), suggests that younger conidia are more sensitive to temperature and that the combination of conidia age and temperature may directly influence virulence. On the basis of germination characteristics and number of lesions produced, it seems that physiologically immature conidia are most virulent and that, as conidia mature, they become less virulent. The rapid loss of virulence by maturing conidia may also provide a partial explanation for the mutual survival of the pathogen and *P. pratensis* in a turf monoculture. If all conidia were equally pathogenic, the continued survival of a *P. pratensis* monoculture would be improbable. This study suggests, however, that only a small portion of the conidia of a given population may be pathogenic at any one time.

Research concerned with virulence of *H. sorokinianum* conidia grown in vitro must be conducted under very precise conditions if the findings are to be meaningful. The rapid loss of virulence by conidia makes even relatively young cultures unacceptable for such studies. Therefore, it is apparent that in vitro studies must be conducted with fresh isolates of the pathogen from living plants, that the pathogen should be grown in vitro only long enough to produce morphologically mature conidia, and that, culture age of conidia to be used for inoculations should not exceed 20 days.

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