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COMPARATIVE TOTAL AND PROPORTIONAL RATE OF GERMINATION OF BIPOLARIS SOROKINIANA AND CURVULARIA GENICULATA CONIDIA IS INFLUENCED BY CULTURE AGE AND TEMPERATURE

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Abstract

Comparative observations of *Bipolaris sorokiniana* and *Curvularia geniculata* conidia germination as influenced by culture age and temperature showed some distinct differences, but generally established the ability of these organisms to function under similar conditions. Total germination of *B. sorokiniana* conidia was favored by increasing culture age from 20 to 60 days and temperature to 25 °C; total conidia germination of *C. geniculata* was favored by increasing temperature to 25 °C, but increasing culture age decreased germination. These reactions seem associated with conidia age. Maximum proportional intrapopulation germination of conidia of each organism also varied with culture age and temperature. At temperatures of 5 °C and 15 °C, amplitude of maximum proportional germination of both organisms increased as culture age was increased from 20 to 40 days and then decreased among 60-day-old cultures. At 25 °C and above, amplitude of maximum proportional germination of conidia of both organisms decreased from each older culture. Progressively increasing temperature at a given culture age increased the amplitude of maximum proportional germination up to 25 °C for conidia of *B. sorokiniana*, but generally decreased it for conidia of *C. geniculata* (except 20-day-old cultures). Frequency (specific 2 h interval) of maximum proportional intrapopulation germination of *B. sorokiniana* shifted from 6 h to 2 h in response to increasing temperature and culture age; conidia from youngest cultures of *C. geniculata* shifted to intervals of 4 h and 2 h in response to increasing temperature to 25 °C, but among conidia from 60-day-old cultures, frequency shifted to 6 h intervals at all temperatures. Above 25 °C, maximum proportional germination of *C. geniculata* conidia from cultures of all ages occurred at 6 h. It was concluded that the germination response of *B. sorokiniana* and *C. geniculata* conidia to temperature and culture age (and, subsequently, conidia age) are enough similar that these organisms could function in a potential 'disease complex' on *Poa pratensis* and *Agrostis palustris*.

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Introduction

Species of *Curvularia* and *Bipolaris* have been implicated in what is termed a 'disease complex' on *Agrostis palustris* and *Poa pratensis* (4, 10, 12). Evidence in support of a complex of primary parasites has not been produced, and the relationship between the two organisms relative to a true 'disease complex' is unknown. It is of interest, however, that *C. geniculata* (Tr. & Earle) Boed. often is associated with *B. sorokiniana* Sacc. in Sorok. (=*Helminthosporium sorokinianum* Sacc. in Sorok. = *H. sativum* P.K.B.) on moldy grass seed (11) and that it commonly is isolated from *A. palustris*, displaying symptoms typical of *B. sorokiniana* infection (7). Such observations suggest that some type of relationship may exist between the organisms.

Most research with *C. geniculata* relative to infection of Gramineae indicates that it is a saprophyte (4, 6, 7, 11), but some studies indicate that it also may be primary parasite on *A. palustris*, *Festuca rubra*, and *P. pratensis* (1). Thus, the parasitic-saprophytic capabilities of *C. geniculata* are not clearly established.

Germination of *B. sorokiniana* and *C. geniculata* conidia show that culture age and temperature influence germination and growth and development of germ tubes (7, 8). These studies do not, however, include information on the relative proportions of conidia that germinate at specific intervals within the period that total germination occurs. Knowledge of the relative proportions of *B. sorokiniana*

and *C. geniculata* conidia that germinate at specific times during the time that total germination occurs might contribute additional knowledge about the relative aggressiveness of the respective organisms and subsequently about their possible relationship in a potential 'disease complex.' Therefore, the research reported herein was initiated to compare total germination and the intrapopulation proportional rate of germination of *B. sorokiniana* and *C. geniculata* conidia at specific intervals within the period that total germination was recorded.

Materials and methods

Bipolaris sorokiniana Sacc. in Sorok. (= *Helminthosporium sorokinianum* Sacc. in Sorok. = *H. sativum* P.K.B.) and *Curvularia geniculata* (Tr. & Earle) Boed. were isolated from blighted leaves of *Agrostis palustris* Hud. and *Poa pratensis* L. on 4% Bacto-agar in double-distilled water and transferred to V-8 Juice agar (20% (Vol/Vol) 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, tissue-culture flasks.

Conidia of each organism were collected from 20-, 40-, and 60-day-old cultures and germinated at 5, 15, 25, 30, and 35 °C (±0.2 °C). Conidia were collected by placing 15 ml of sterile, distilled water in each culture flask and shaking the flask to suspend conidia. Mycelial fragments were removed from conidia by passing the suspension through a 43–47 μ sieve into a Syracuse dish. Five transfers of the conidia suspension were made with a 3 mm loop to a Sykes-Moore tissue-culture chamber (Bellco Glass, Inc.) containing ca. 1.0 ml of sterile, distilled water. The chambers were placed on a Cambion heating-cooling stage (Cambridge Thermionic Corp.) at the appropriate temperature (9).

Total germination of 300 conidia of each organism was recorded from cultures of each age at each temperature in samples of 100 for an 8 h observation period. The influence was recorded of culture age and temperature on the proportion of conidia germinating at a given 2 h interval (within 8 h) within each population of 300 conidia of each organism. The maximum proportion of conidia germinating at a specific 2 h interval (referred to as frequency) and the quantity of conidia constituting that maximum (referred to as amplitude) were recorded as a percentage of the total number of conidia that germinated within the population of 300 conidia from each culture age at each temperature.

Results

Total Conidial Germination

Culture age had opposite effects on total germination of *B. sorokiniana* and *C. geniculata* conidia at all temperatures. Among populations of *B. sorokiniana*, total germination of conidia generally increased at all temperatures with each older culture (table 1). Total germination of *C. geniculata* conidia decreased from each older culture at nearly all temperatures.

The effect of temperature on germination of *B. sorokiniana* and *C. geniculata* conidia from different age cultures was nearly the same between 5 and 30 °C. Number of germinating conidia of both organisms, from all culture ages, increased progressively as temperature was increased from 5 to 25 °C, with the exception of *C. geniculata* conidia from 60-day-old cultures (table 1). At 30 °C, total germination of all conidia decreased. At 35 °C, all conidia of *B. sorokiniana* and conidia from 60-day-old cultures of *C. geniculata* did not germinate (table 1).

Maximum Proportional Germination Within Conidial Populations

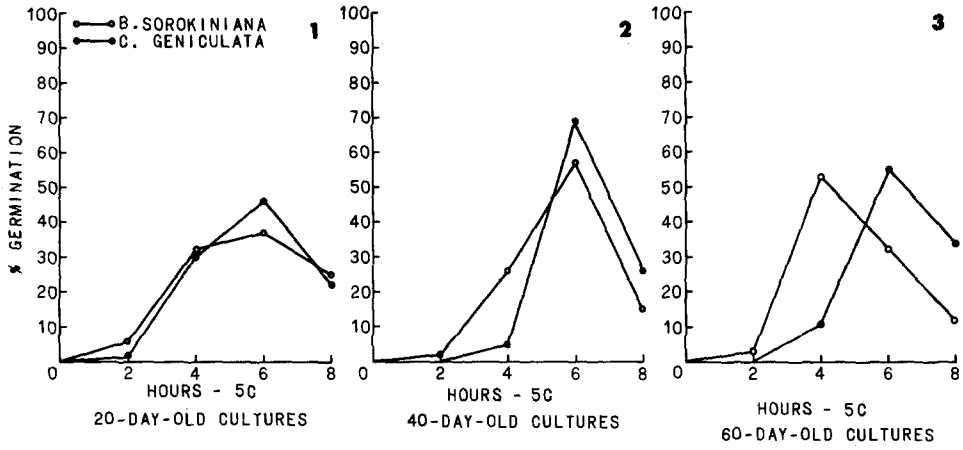
Age of the cultures from which conidia of *B. sorokiniana* and *C. geniculata* were collected directly influenced the amplitude and frequency (specific 2 h interval) of maximum proportional germination of conidia at a given temperature. At 5 and 15 °C, amplitude of maximum proportional germination of conidia of both organisms increased as culture age increased from 20 to 40 days; at 60 days, amplitude decreased slightly for both organisms (fig. 1–6).

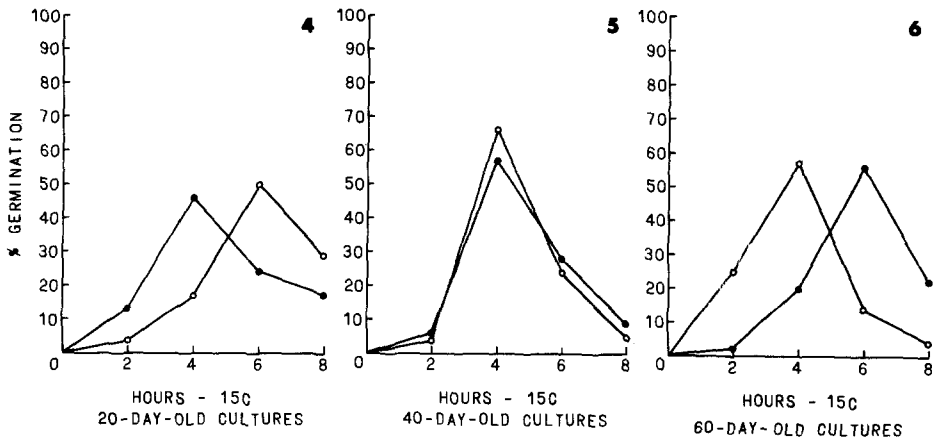
TABLE 1

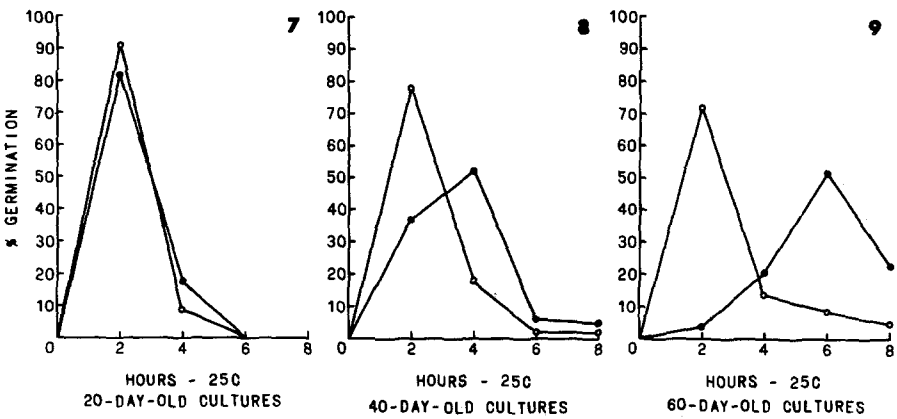
Number of germinating conidia of Bipolaris sorokiniana and Curvularia geniculata as influenced by culture age and temperature[a]

Organism	Culture age (days)	Temperature C 5	15	25	30	35
B. sorokiniana	20	88	180	294	228	0
	40	142	236	286	219	0
	60	174	280	289	241	0
C. geniculata	20	66	256	294	185	41
	40	53	268	274	86	8
	60	36	198	181	94	0

[a] 300 conidia of each organism were observed from each culture age at each temperature.


B. SOROKINIANA
C. GENICULATA
1
2
3
% GERMINATION
HOURS - 5C
20-DAY-OLD CULTURES
HOURS - 5C
40-DAY-OLD CULTURES
HOURS - 5C
60-DAY-OLD CULTURES


4
5
6
% GERMINATION
HOURS - 15C
20-DAY-OLD CULTURES
HOURS - 15C
40-DAY-OLD CULTURES
HOURS - 15C
60-DAY-OLD CULTURES


7
8
9
% GERMINATION
HOURS - 25C
20-DAY-OLD CULTURES
HOURS - 25C
40-DAY-OLD CULTURES
HOURS - 25C
60-DAY-OLD CULTURES

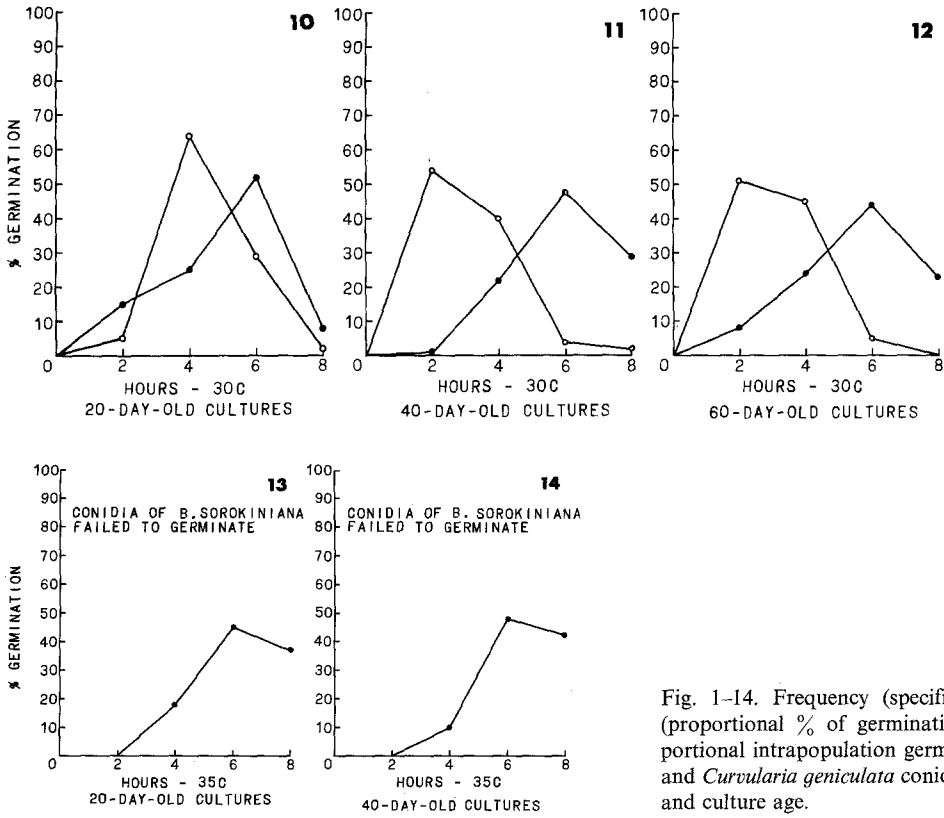


Fig. 1–14. Frequency (specific 2 h interval) and amplitude (proportional % of germinating conidia) of maximum proportional intrapopulation germination of *Bipolaris sorokiniana* and *Curvularia geniculata* conidia as influenced by temperature and culture age.

At 25 and 30 °C, amplitude of maximum proportional germination was greatest for conidia of both organisms from 20-day-old cultures and then progressively decreased as culture age was increased to 40 and 60 days (fig. 7–12). Conidia of *B. sorokiniana* from all cultures failed to germinate at 35 °C; conidia of *C. geniculata* from 20- and 40-day-old cultures germinating at 35 °C showed only slight differences in amplitude of maximum proportional germination (fig. 13 and 14).

Culture age also influenced the frequency at which maximum proportional germination of *B. sorokiniana* and *C. geniculata* conidia occurred at a given temperature. The frequency of maximum proportional germination of *B. sorokiniana* conidia shifted from 6 h to 4 h at temperatures of 5 and 15 °C as culture age was increased from 20 to 60 days (fig. 1–6); at 25 and 30 °C, *B. sorokiniana* conidia from cultures of all ages showed maximum proportional germination at 2 h, except for those from 20-day-old cultures at 30 °C (fig. 7–12). Maximum proportional germination of *C. geniculata* conidia occurred at 6 h from cultures of all ages at 5, 30, and 35 °C (fig. 1–3 and 10–14). At 15 and 25 °C, frequency of maximum proportional germination of *C. geniculata* conidia shifted from 4 h and 2 h to 6 h, respectively as culture age increased from 20 to 60 days (fig. 4–9).

Varying the temperature at which conidia of *B. sorokiniana* and *C. geniculata* were germinated, from a given culture age, also had the effect of varying the amplitude and frequency of maximum proportional germination. Amplitude of maximum proportional germination increased from 5 to 25 °C and decreased at 30 and 35 °C for conidia of both organisms from 20-day-old cultures (fig. 1, 4, 7, 10, & 13). The same pattern of germination occurred among conidia of *B. sorokiniana* from 40- and 60-day-old cultures; maximum proportional germination of conidia from 40- and 60-day-old cultures of *C. geniculata*, however, progressively decreased as temperature was increased from 5 to 35 °C (fig. 2, 5, 8, 11, 14, and 3, 6, 9, 12).

Temperature variation also affected the frequency at which maximum proportional germination occurred for conidia of *B. sorokiniana* and *C. geniculata* from a given culture age. As temperature was increased from 5 to 25 °C, frequency of maximum proportional germination of conidia of both organisms from 20-day-old cultures shifted

from 6 h to 2 h (fig. 1, 4, & 7); above 25 °C, frequency shifted to 4 h and 6 h, respectively, for *B. sorokiniana* and *C. geniculata* conidia (fig. 10 & 13). Among 40-day-old cultures, as temperature was increased from 5 to 25 °C, frequency of maximum proportional germination shifted from 6 h to 2 h for *B. sorokiniana* and from 6 h to 4 h for *C. geniculata* (fig. 2, 5, & 8); above 25 °C, frequency remained at 2 h for *B. sorokiniana*, but shifted back to 6 h for *C. geniculata* (fig. 11 & 14). As temperature was increased from 5 to 25 °C, frequency of maximum proportional germination of *B. sorokiniana* conidia from 60-day-old cultures shifted from 4 h to 2 h and remained at 2 h above 25 °C; for conidia of *C. geniculata*, however, frequency remained at 6 h over the entire temperature range (fig. 3, 6, 9, & 12).

Discussion

The results of the research contained herein indicate that culture age and temperature have independent and interacting effects on total and maximum proportional intrapopulation germination of *B. sorokiniana* and *C. geniculata* conidia. As culture age increased and, subsequently, the average age of the conidia collected, *B. sorokiniana* conidia germination increased, and *C. geniculata* conidia germination decreased (table 1). This reaction strongly suggests that *B. sorokiniana* conidia mature in their ability to germinate more slowly than those of *C. geniculata*, which evidently are most viable shortly after production and then rapidly lose their viability with age. Although the conidia of each organism react differently with age, their reaction to temperature is similar; total germination of conidia of both organisms increased with increasing temperatures (table 1). Increasing temperature to 25 °C and progressively increasing culture age seems to interact to increase total germination of *B. sorokiniana* conidia; among conidia of *C. geniculata*, increasing temperature to 15 and 25 °C seems to compensate for decreasing germination of conidia associated with progressively older cultures.

Optimum conditions for maximum proportional intrapopulation germination of conidia from both organisms occur among conidia from 20-day-old cultures at 25 °C (fig. 7). Amplitude of maximum proportional germination of *B. sorokiniana* conidia from cultures of all ages increased as temperature was increased from 5 to 25 °C; except for conidia from 20-old-day cultures, the opposite reaction was observed for *C. geniculata*. In general, increasing culture age at a specific temperature ultimately decreases amplitude of maximum proportional germination of conidia of both organisms. These reactions probably reflect the decreasing viability of *C. geniculata* conidia; however, in the case of *B. sorokiniana* where total germination increased with age between 5 and 25 °C, the slight decrease in amplitude among 60-day-old cultures at these temperatures suggests that, as culture age increases, the conidia germinate more evenly over the observation period. Decreases in total germination and amplitude of maximum proportional germination of both organisms above 25 °C are indicative of supraoptimal temperature.

Increasing temperature from 5 to 30 °C and/or culture age from 20 to 60 days shifted frequency of maximum proportional germination of *B. sorokiniana* conidia from 6 h to 2 h; germination of *C. geniculata* conidia from cultures of all ages at all temperatures occurred at 6 h (except conidia from 20- and 40-day-old cultures at 15 and 25 °C). Frequency seems to be most affected by temperature; under optimal conditions (fig. 7) conidia of both organisms germinate rapidly. Frequency also is influenced by differences in the rate of conidia maturity of the respective organisms. At 25 °C, the frequency of maximum proportional germination of *B. sorokiniana* conidia from cultures of all ages remains at 2 h; however, frequency shifts from 2 to 6 h among conidia of *C. geniculata* from progressively older cultures (fig. 7–9). This is probably associated with the loss of germinability of *C. geniculata* conidia from progressively older cultures (table 1).

It has been hypothesized that *C. geniculata* may be a secondary invader of lesions caused by *B. sorokiniana* (7). This hypothesis was arrived at on the basis that *C. geniculata* has not been established as a strong primary parasite (7), that *Curvularia* sp. are stronger saprophytic competitors than *B. sorokiniana* (2, 3, 5), and that *C. geniculata* is frequently isolated from lesions typical of *B. sorokiniana* during periods of hot westher (7). Both organisms have similar responses to moderate temperatures and the differences in germination relative to conidia age (table 1) would be negated under field condition where conidia would be of various ages. Also, the continued germination of *C. geniculata* conidia at 35 °C (fig. 13 & 14) further suggests that this organism remains active at temperatures that may slow or inhibit *B. sorokiniana*. Under such circumstances, it is possible that development of a lesion by *B. sorokiniana* could be slowed; and, saprophytic colonization of the necrotic tissue of the lesion by *C. geniculata* could follow. The ability of *C. geniculata* to remain active at high temperatures also

might account for the isolation of this organism from lesions typical of *B. sorokiniana* during periods of hot weather. To confirm or reject this hypothesis, studies are in progress to determine the ability of *C. geniculata* to infest lesions initiated by *B. sorokiniana* on *P. pratensis* and *A. palustris*.

1. Brown, G. E., H. Cole Jr. & R. R. Nelson. 1972. Pathogenicity of Curvularia sp. to Turfgrass. Plant Dis. Rep. 56: 59–63.
2. Butler, F. C. 1953a. Saprophytic Behavior of some Cereal Root-Rot Fungi. I. Saprophytic Colonization of Wheat Straw. Ann. Appl. Biol. 40: 284–297.
3. Butler, F. C. 1953b. Saprophytic Behavior of Some Cereal Root-Rot Fungi. II. Factors Influencing Saprophytic Colonization of Wheat Straw. Ann. Appl. Biol. 40: 298–304.
4. Couch, H. B. 1962. Diseases of Turfgrasses. Reinhold Publ. Corp., New York.
5. Garrett, S. D. 1970. Pathogenic Root-Infecting Fungi. Cambridge Univ. Press, New York.
6. Groves, F. W. & A. J. Skolko. 1945. Notes on Seed-Borne Fungi. III. Curvularia. Can. J. Res. C., 23: 94–104.
7. Hodges, C. F. 1972a. Interactions of Culture Age and Temperature on Germination and Growth of Curvularia geniculata and on Virulence. Can. J. Bot. 50: 2093–2096.
8. Hodges, C. F. 1972b. Influence of Culture Age and Temperature on Germination of Helminthosporium sorokinianum and on Pathogenicity to Poa pratensis. Phytopathology 62: 1133–1137.
9. Hodges, C. F. 1974. Use of the Sykes-Moore Tissue-Culture Chamber and the Cambion Heating-Cooling Stage for Spore Germination Research. Phytopathology 64: 903–905.
10. Howard, F. L. 1953. Helminthosporium-Curvularia Blights of Turf and Their Cure. Golf Course Rep. 21: 5–9.
11. Sprague, R. 1950. Diseases of Cereals and Grasses in North America. Ronald Press, New York.
12. Wernham, C. C. & R. S. Kirby. 1941. A New Turf Disease. Phytopathology 31: 24.