Moisture Effects on the Discharge and Survival of Conidia of *Septoria tritici*

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ABSTRACT

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The effect of relative humidity (RH) on the discharge of conidia of *Septoria tritici* and their longevity in cirri was tested by suspending pycnidia in leaf lesions above water and solutions of NaCl and NaOH in sealed test tubes held at 25 ±0.2 C in a water bath. Pycnidia held for 48 hr at about 100% RH discharged twice as many conidia as those held at 98%, and 11 to 23 times as many as those held at 86%. Conidia in cirri on top of pycnidia remained 100% viable for 15 days in atmospheres of 35, 45, 55, 65, 75, and 85% RH. But between the 15th and 20th day viability of conidia held at 65% and above dropped to 5–10%, then declined to less than 2% by the 30th day, and to 0 by the 50th day. At 55% RH, 60% of the conidia were viable, and at 45 and 35% RH, 80% were viable after 60 days. Conidia within pycnidia held at 35% RH were 100% viable after 132 days.

Additional key words: conidia, epidemiology, germination, speckled leaf blotch, *Triticum aestivum*, water potential.

Septoria tritici blotch of wheat caused by *Septoria tritici* Rob. ex Desm. (perfect state *Mycosphaerella graminicola* (Fuckel) Schroeter) is favored by prolonged periods of wet weather and moderate temperatures (1,5,11,13,15,21,23). The major source of primary inoculum derives from pycnidia (1,3,5,6,10,12,13,23,24) and perithecia (2,18,19) that survive the period between crops in plant debris. *M. graminicola* was recognized as the perfect stage of *S. tritici* in New Zealand in 1972 (17), and its importance as a source of primary inoculum in both New Zealand (18–20) and Australia (2) was soon reported. *M. graminicola* was reported on annual blue grass (*Poa annua*) and other grasses in the U.S. (14), but pathogenicity to wheat was not conclusively demonstrated. After initial infection by either conidia or ascospores, the disease spreads vertically and horizontally by the production and release of conidia (1,5,21,23).

Moisture requirements for liberation of conidia and their ability to survive the vicissitudes of ambient humidity are two of several factors that condition the development of speckled leaf blotch. Rain (12,23) or even high humidity (1,4,5) will induce the discharge of conidia, but data defining the lower limits of required moisture are lacking.

The purpose of this study was to determine the effects of relative humidity (RH) on the expulsion of conidia from pycnidia, and their survival in the expelled cirri. A preliminary report has been given (8).

MATERIALS AND METHODS

Source of pycnidia. Pycnidia were obtained from leaves of winter wheat (*Triticum aestivum* 'Triumph 64') inoculated with a mixture of two cultures of *S. tritici*. One culture, designated MT-5, was obtained from A. L. Scharen (ARS-USDA, Department of Plant Pathology, Montana State University, Bozeman 59715). The second culture, designated S-22, was isolated from a single pycnidium in a lesion from wheat grown near Stillwater, OK. The cultures were grown singly in 25 ml of a liquid medium that contained 2 g of malt extract (Difco Laboratories, Detroit, MI 48232) and 0.5 g of yeast extract (U.S. Biochemical Corp., Cleveland, OH 44128) in 1 L of distilled water. The cultures were incubated at about 22 C for 8 days. Equal volumes of medium containing the cultures were combined and strained through a double layer of cheesecloth to remove most of the mycelium. About 8×10^6 spores per milliliter were left suspended in the combined medium. One-half gram of unflavored gelatin dissolved in 20 ml of warm water was added to each 100 ml of the spore suspension as a sticker. The suspension was sprayed on the plants with an atomizer attached to a pressure pump (34.5 kPa). The plants then were placed inside a polyethylene chamber on a greenhouse bench and kept moist for 96 hr by a timeclock-controlled mist blower.

Twenty days after inoculation, green leaves bearing pycnidia in noncoalesing lesions were harvested and stored between paper towels at 4 C until used.

Expulsion of conidia. Eight days after harvest, individual lesions were cut from the stored leaves and suspended above deionized distilled water and molal solutions of NaCl in rubber-stoppered test tubes. The water and NaCl solutions were prepared with water activity (a_w) values (16) of 1.00, 0.98, 0.94, 0.90, 0.86 when maintained at 25 C (RH = $a_w \times 100$). To minimize weighing errors, the water and NaCl solutions were prepared in 500-g lots, then dispensed in 15-ml aliquants into the test tubes. To suspend the lesions above the water and NaCl solutions, paper clips (Gem pattern, size 2) were inserted to about one-third their length in slits cut in the small end of the test tube stoppers. Spring-loaded pin-curl clips (alligator type), each clasping a single lesion, were then placed between the exposed loops of the paper clips. The stoppers holding the clips and lesions were pushed firmly into the tops of the test tubes to create an airtight seal. Each a_w value was designated a treatment and was represented by one tube assigned to each of four replications in a randomized complete block design in a test tube rack. The rack and tubes were immersed to a depth of 1 cm from the top of the tubes in a water bath at 25 C ±0.2 C.

Conidia expelled from pycnidia in each treatment were harvested and counted 48–50 hr after immersion in the water bath. The conidia were harvested by agitating each leaf lesion for 1 min in 200

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μl of water deposited in spot glass depressions with an Eppendorf syringe. A wire loop was used to stir the resulting conidial suspension and to transfer drops to a hemacytometer slide for counting. At the same time, four lesion-bearing leaf segments were removed from refrigerator storage and similarly treated. Conidia counts derived from these lesions provided an estimate of the numbers of conidia that may have been expelled from the pycnidia prior to collecting the lesions and during the 1 min wash period.

To facilitate counting pycnidia, immediately after the conidia were harvested the leaf segments were bleached in 5% KOH for 20 hr, briefly washed in distilled water, and placed in 70% alcohol for 1 hr. The leaf segments then were placed between microscope slides held together with adhesive cellophane tape. Counts of pycnidia per lesion per treatment were made by using a dissecting microscope. An estimate of the number of conidia released per pycnidium was calculated.

Longevity of expelled conidia. The longevity of discharged conidia in atmospheres having different RH values was tested in two similar experiments. Pycnidia in lesions stored at 4 C were held (in pin-curl clips supported with glass rods) above wet filter paper in petri dishes for 48 hr at room temperature. After 48 hr, exuded conidia had formed short cirri on the tops of the pycnidia. The lesions were then suspended (as described for the conidia discharge test) above molal solutions of NaOH that gave RH values 85, 75, 65, 55, 45, and 35% at 25 C (16) for periods of 5, 10, 15, 25, 30 and 45 days. In addition, one set of dry lesions containing pycnidia with unexpelled conidia was suspended above the 35% solution.

At the designated times, a lesion was removed from each humidity level, and five to ten cirri of expelled conidia from each lesion were transferred to drops of malt extract-yeast extract medium on cover glasses. The cover glasses were then inverted over wells of depression slides and their edges were sealed with petroleum jelly. After 24 hr at room temperature (about 22 C), the percentage of germinating conidia was estimated by observation through a microscope. The viability of conidia maintained in pycnidia in dry lesions at 35% RH was tested similarly after expulsion was induced by placing the lesions on moistened filter paper in petri dishes.

Statistical analyses. Data were subjected to an analysis of variance and Duncan's multiple range test.

RESULTS

Humidity and the liberation of conidia. Conidia were expelled from pycnidia at all humidity levels tested (Table 1). In each of two trials, pycnidia held for 48–50 hr in an atmosphere with a RH value of 100% expelled more than twice as many conidia as pycnidia held at 98% RH, and from 11 to 23 times as many as pycnidia held at 86% RH.

Humidity and conidial longevity. The longevity of conidia maintained at the different RH levels was very similar in two tests.

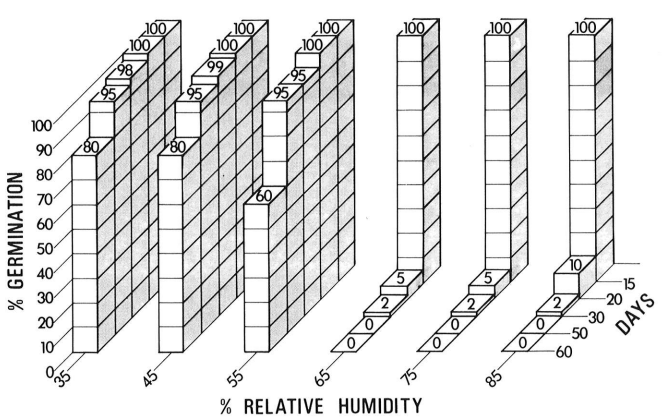

Fig. 1. The longevity of conidia of *Septoria tritici* in cirri held for 15–60 days in atmospheres having constant relative humidities over NaOH solutions at 25 C.

An analysis of variance, with each test representing a replication in a randomized block design, indicated that no differences existed between replications at the 10% level of significance (coefficient of variation = 21%). Consequently, results of only one test are presented (Fig. 1) and discussed. Viability of the expelled conidia in cirri was estimated to be 100% in all humidity levels after 15 days. Between the 15th and 20th day in atmospheres with RH values of 85, 75, and 65, viability dropped abruptly to about 5% and then gradually to 0 by the 50th day. By contrast, conidia in atmospheres of 55, 45, and 35% RH were, respectively, 60, 80, and 80% viable after 60 days. Viability of conidia in pycnidia maintained at 35% RH remained near 100% when the test ended after 132 days due to lack of available pycnidia.

DISCUSSION

The expulsion of conidia from pycnidia during periods of high humidity and their tolerance to desiccation while in cirri indicated adaptation of *S. tritici* to an environment of cyclic humidity. Consequently, we examined weather data for March, April, and May of 1980, a year in which the measured loss from foliar diseases at one location in north central Oklahoma was 19.2% (*unpublished*). The humidity data, collected by L. S. Morrison, from a field near Stillwater, OK, revealed that during 85 monitored days the RH reached or exceeded 95% during 62 days and dropped to 55% or lower during 69 days. Except during times of rain, the periods of high humidity (≥95%) typically began at about 2400 hours, persisted for 1–10 hr, dropped to 55% between 1000 and 1200 hours, and continued downward to a low (mean of 26.9 ± 13.4%) at about 1400 hours. In conjunction with discriminatory temperature effects on conidial production (22), tolerance of expelled conidia to desiccation may partially explain why *S. tritici* is more common than *Leptosphaeria nodorum* in Oklahoma and other low-rainfall regions. Conidia of *S. nodorum* produced in culture (9) and liberated from pycnidia (23) are easily killed by drying and by direct sunlight. The effect of sunlight on spores of *S. tritici* has not been determined.

Wheat harvest in Oklahoma is often nearly complete by mid-June. Thus, infections occurring after 1 June are probably of no

TABLE 1. Number of conidia released per pycnidium of *Septoria tritici* in lesions suspended above deionized water and solutions of NaCl[w] of differing molal concentrations at 25 C

Relative humidities (%), dry checks, and statistical data	Pycnidia sampled (no.)	Conidia released per pycnidium[x] (no.)
Trial 1		
100.00	855	3,980 a
98.0	404	1,716 b
94.0	400	608 c
90.0	286	353 c
86.0	244	167 c
Dry check[y]	374	20
CV = 46.7%		
Trial 2		
100.00	609	2,952 a
98.0	357	1,407 b
94.0	201	983 b
90.0	300	587 b
86.0	171	258 b
Dry check	142	60
CV = 45.7%		

[w] Water activity (a_w) values for molal solutions published by Robinson and Stokes (16) were used to calculate relative humidites.

[x] All values represent the means of four replications. Numbers within trials followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test. Conidia obtained from the dry check were not included in the statistical analyses.

[y] Dry checks consisted of pycnidia bearing lesions in leaves that had been stored in a refrigerator at 4 C. They were washed in water at the same time and in the same manner as lesions suspended over the NaCl solutions.

[z] CV = coefficient of variation.

economic importance under normal weather conditions and harvest dates. Because *S. tritici* oversummers in plant debris, the RH during July, August, and September, or later, may affect inoculum available for fall infection. Morrison's (L. S. Morrison, *unpublished*) data indicated that during these months in 1980 the RH was below 55% and above 95%, respectively, 70% and 14% of the time. Djerbi (3) observed that conidia in pycnidia do not survive the summer in Tunisia. However, the sporophores remain viable and regenerate new spores in favorable conditions. It is not clear from Djerbi's report whether the failure of the conidia to germinate was a consequence of humidity, temperature, or both. Our work indicates that conidia in cirri of *S. tritici* are very tolerant to desiccation and should not be adversely affected by RH during the summer in Oklahoma unless fluctuations in RH are extreme.

Fournet (7) reported that in a laboratory atmosphere conidia of *S. nodorum* preserved in cirri were protected from dessication. Conidia in cirri were 67% viable after 28 days; whereas, germinability of conidia dispersed on a sterile slide with a droplet of sterile water was reduced 50% after 24 hr, and was practically nil after 5 days.

Luthra et al (12) reported that conidia of *S. tritici* smeared on seed and left exposed to dry conditions in the summer lost vitality in about 2 wk. Similarly, Arsenijević (1) noted that liberated conidia survived for 15 days in the laboratory and for 2 days in the open. These observations agree closely with our own for conidia kept in atmospheres with RH values above 55% at 25 C. Since we used RH values from 35 to 85% in 10% increments, the critical point between 55 and 65% conditioned viability or death was not determined.

The reason for the rapid decline in viability of the conidia between 15 and 20 days at RH values above 55% is unknown. We suggest, however, that above 55–65% RH the basal metabolism of the spores proceeds at a more or less constant rate which exhausts endogenous energy sources in about 2 wk.

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