

Survival of Teliospores of *Sphacelotheca reiliana* in Soil

C. A. Matyac and T. Kommedahl

Department of Plant Pathology, University of Minnesota, St. Paul 55108. Present address of first author: Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

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ABSTRACT

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Teliospores of *Sphacelotheca reiliana* in soil of field microplots decreased to 42% of the original population in 6 mo and to 4% after 3 yr. In controlled environments, the greatest reduction in spore populations at all temperatures (0, 15, and 25 C) occurred at matric potentials of -0.5 bar, at which an average of 29% of the spores were recovered after 30 wk. At -11.1 and -44.0 bars, 52 and 89%, respectively, of the spores remained. Temperature and interactions of temperature \times matric potential also significantly affected decreases in spore number, with the greatest decreases

occurring at high temperatures and high matric potentials. Application of organic amendments with low C:N ratios significantly reduced inoculum density in microplots after 0.5, 1.0, 1.5, and 2.0 yr, but did not significantly affect the survival of teliospores either in microplots after 3.0 yr or in the controlled environments. In bioassays of incubated teliospores, incidence of infection also decreased with time, but soil type or organic amendments had no effect. Germination of spores on agar was reduced by 90% after 6 mo in microplots and by 63% in soils incubated at high moisture contents at 25 C.

Head smut is a systemic disease caused in corn (*Zea mays* L.) by *Sphacelotheca reiliana* (Kühn) Clinton (*Sporisorium reiliana* (Kühn) Langdon and Fullerton). Teliospores are the overwintering and survival structures of this fungus; however, the ability of teliospores to survive extended periods of time in soil or the effects of various environmental factors have not been investigated thoroughly. Brief reports from India, China, and the Soviet Union (4,8,13) have indicated that spores in soil remain viable for 2, 3, or 5 yr, respectively. Pia and Pan (10) indicated that the infectivity of teliospores decreased when stored at high moisture and temperature. However, changes in populations were not quantified in these studies. The purpose of this study was to investigate the survival, infectivity, and germination of teliospores incubated in various amended and unamended soils in the field and in a controlled environment at constant temperature and matric potential.

MATERIALS AND METHODS

Controlled environment. Teliospores of *S. reiliana* were incubated in natural and amended soil held at constant temperatures and matric potentials. At 6-wk intervals, the teliospores were counted and tested for germination and for infection of corn seedlings. Teliospores were collected from infected corn plants in field plots at the University of Minnesota Area Vocational Technical Institute (AVTI) at Staples, MN, and stored in an unheated building through the winter. Teliospores were removed from sori and put through a No. 16 sieve (openings 1.8 mm) and mixed with air-dried, sandy loam soil from the AVTI farm at Staples at the rate of 4.5 g of spores per liter of soil, which was equivalent to a spore-to-soil ratio of 1:75 (v/v), or approximately 3.5×10^6 propagules per gram soil. Some of the physical characteristics of this and other soils mentioned in this paper were described in a previous report (7). Dried alfalfa cubes were ground in a Wiley mill fitted with a 40-mesh screen and added to half of the soil at the rate of 1% of the oven-dry weight of the soil. A moisture release curve was determined using a 15-bar ceramic plate extractor. This curve was used as a guideline in the selection of three regimes of soil moisture. The percent moistures of the

amended and unamended soils were adjusted to 12.0, 5.0, and 1.2% by spraying water onto the soil while it was in a rotating soil mixer that had a 19-L capacity. Since water potentials during resorption differ from those during desorption, the final soil matric potential was checked with a soil psychrometer read with a dew point microvoltmeter (Wescor, Inc., Logan, UT). Moisture percentages of 12.0, 5.0, and 1.2% corresponded to -0.5, -11.1, and -44.0 bars, respectively. Approximately 150 g of soil was put into each 100-ml glass jar fitted with a metal screw cap. The jars were sealed with melted paraffin and incubated at 0, 15, or 25 C. Twenty replicates were used at each temperature. The soil matric potential, number of spores, and germination on agar were quantified after 6, 12, 18, 24, and 30 wk of incubation using assay procedures described below. Soils from three replicates were assayed at each sampling date. Infection of susceptible corn seedlings was determined after 6, 12, and 30 wk.

Assays. Soil in the jar was mixed and two 10-g samples, corrected for moisture, from each replicate were placed in 50-ml test tubes containing 40 ml of water and one drop of Tween 20, a wetting agent. This soil slurry was allowed to set at 24 C for 1 hr, then was agitated by hand for 30 sec, poured through a No. 325 sieve (44- μ m openings), and rinsed with 10 ml of water. Spores were counted by using a hemacytometer, and 0.5 ml of the suspension was put into a petri plate containing water agar amended with 75 WP pentachloronitrobenzene at 5.0 mg/ml, streptomycin sulfate at 1.5 mg/ml, and aureomycin at 0.025 mg/ml. Plates were incubated at 25 C, and after 5 days an agar plug was placed on a glass slide and stained with acid fuchsin. One hundred spores were counted at $\times 400$ magnification and the percent germination was calculated. A control for the germination assay consisted of an air-dry mixture of spores and soil treated as already described and stored at 24 C.

The soils remaining from the three replicates were combined and a one-replicate bioassay for infectivity was made. This sample was mixed with 10 L of a greenhouse-mix soil previously described (6). Seeds of a susceptible hybrid, P 3978 (Pioneer Hi-Bred International, Inc., Des Moines, IA), were planted with the coleorhizal end down, four rows per flat, 15 seeds per row, and at a depth of 5 cm in galvanized steel flats (50 \times 35 \times 9 cm). Gypsum blocks calibrated with a soil psychrometer were installed. Soil was saturated at the time of planting and allowed to dry to approximately -1.5 bars as measured by a KS-1 moisture meter (Delmhorst Instrument Co., Towaco, NJ 07082) before being resaturated. Flats were placed in a greenhouse at 25 ± 5 C. Hygrothermographs were used to monitor air temperature and mercury thermometers were used to measure soil temperatures, which were recorded daily. Supplemental light consisted of 2.4 \times

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8 m banks, each holding six fluorescent lamps (30,000 lx). Seedlings were grown under these conditions for 4 wk and then were fertilized with 1 L of 20-20-20 (N-P-K) water-soluble fertilizer (Peters Fertilizer Products, Fogelsville, PA 18051) mixed at the rate of 25 g per 10 L of water and watered daily until the fourth or fifth leaf emerged. At this time, disease frequencies were determined by using the occurrence of chlorotic flecks on leaves as an indicator of infection (6). A control for the bioassays consisted of 1:75 (v/v) spore-soil mixtures prepared freshly at each planting date.

Microplots. Teliospores were incubated in natural and amended soil in microplots in the field. At selected intervals, the teliospores were counted and tested for germination on agar and ability to cause infection in corn seedlings. Teliospores were collected, stored, and screened as described previously and mixed with soils at a ratio of 1:125 (v/v), which resulted in approximately 2.5×10^6 propagules per gram of soil. Minnesota soils used in this experiment were: Webster typic haplaquolls clay loam from Waseca, Waukegan typic hapludolls silt loam from St. Paul, and Vernale udic agriboroll sandy loam from Staples. The sandy loam was either unamended or amended with dried corn stalks or alfalfa at a rate of 1% of the oven-dry weight of the soil. Both organic amendments were dried and ground as described previously. The C:N ratio of the alfalfa and the corn stalks was 18:1 and 52:1, respectively. C:N analysis was performed by the University of Minnesota Department of Soil Science Research Analytical Laboratory. Each soil mixture was placed in 15-cm-diameter clay or plastic pots and buried in a corn field on the AVTI farm at Staples or the University of Minnesota Farm at St. Paul with only the rim of the pot above the soil line. The number of spores, ability to germinate on agar, and ability to infect plants were quantified as described above after 0.5, 1.0, 1.5, 2.0, and 3.0 yr. Each treatment was replicated five times at each time interval.

Teliospore populations at each sample date for microplots and the controlled environment study were subjected to analysis of variance; means were compared using the least significant difference value at $P = 0.05$. Data were also transformed to percentage of the initial populations, converted to logarithms, and subjected to linear regression with time as the independent variable. Student's t tests and covariance analysis were used to compare heterogeneity of slopes and elevations, as suggested by Benson and Baker (2). Half-lives ($t_{0.5}$) were calculated by using the equation $t_{0.5} = [(T \log 2) / (\log P_0 - \log P_1)]$ in which P_0 = original population, P_1

= population after time T , T = the interval of the observation, and $t_{0.5}$ = time interval for half the individuals to be inactivated (14).

RESULTS

Survival in field microplots. After one winter in the field, spore populations in sandy soil amended with alfalfa decreased to 22% of the original populations and those in unamended sandy soil decreased to 35% (Fig. 1). The decrease in populations from fall to spring for all treatments averaged 42% of the original spore population. The least reduction in spore population occurred in silt loam soil, in which the population decreased only to 80% of the original population. Spore populations in clay soil and in alfalfa-amended sandy soils were significantly lower than populations in unamended and corn stalk-amended sandy soils after 0.5, 1.0, 1.5, and 2.0 yr. After 3.0 yr, only 4.2% of the original population of spores survived and there were no statistical differences among treatments (Fig. 1).

Regressions of transformed data from each treatment were highly significant ($P = 0.0001$). Slopes ranged from 0.38 for the unamended sandy soil to 0.52 for the silt soil. Elevations ranged from 1.8 for the clay soil to 2.0 for the silt soil. There were no significant differences in slope or elevation among treatments according to analysis of covariance. Therefore, these data were combined, resulting in one regression line (Fig. 1) that had F and R^2 values of 371.6 and 0.74, respectively, both significant at $P = 0.001$. The calculated half-life value for the combined data was 0.66 yr.

There were no significant differences among treatments in the number of infected plants in the bioassay at any sample date. The average percentage of infected plants for all treatments was 29.6, 34.9, 19.5, 9.9, and 2.8% for the 0.5-, 1.0-, 1.5-, 2.0-, and 3.0-yr sample dates, respectively. Disease incidence in the controls for this and later experiments averaged $34 \pm 7.9\%$. Germination of spores on agar was only 3% after 0.5 yr and was less than 1% thereafter. Germination of spores in the controls for this and later experiments was $30 \pm 8.5\%$.

Survival in a controlled environment. The enhancement of decreases in spore populations after 6 mo in the presence of alfalfa amendments in the field did not occur in the controlled-environment study. In general, there were no significant differences in spore survival between amended and unamended soils at any temperature or matric potential (Fig. 2A-C).

After 6 wk of incubation, spore populations in all treatments decreased to 79% of the original population. The greatest reduction occurred at high matric potentials and high temperatures. Teliospore populations at -0.5 and -11.1 bars at 25 C were reduced to an average of 51% of the original population during this 6-wk period. Analysis of variance indicated significant F values for temperature, matric potential, and interactions of temperature \times matric potential at this and every succeeding sample date. There were no significant differences in mean spore populations at 0 C among the three matric potential treatments, but at 15 and 25 C the matric potential treatments of -0.5 and -11.1 were not significantly different from each other. However, these populations were different from those in the -44.0 -bar treatment.

After 12 wk of incubation, spore populations decreased an additional 16–63% of the original population. This additional decrease resulted from changes in the -0.5 - and -11.1 -bar treatments, while populations in the -44 -bar treatment remained nearly constant at all temperatures. At 18 wk the population changed only 0.2% from that of the earlier sample date, and by 30 wk 57% of the spores still remained, suggesting some stability in population survival for a considerable time.

After 30 wk of incubation, spore populations at each matric potential were significantly different from each other at 0 and 15 C. Populations in soils at 25 C and -0.5 and -11.1 bars were significantly lower than populations at -44 bars, but the former two treatments were not different from each other.

Survival curves using the transformed data, which did not have significantly different slopes or elevations, were combined into one of four groups (Fig. 3). The first group consisted of all -44 -bar treatments. These dry soils allowed spore populations to remain

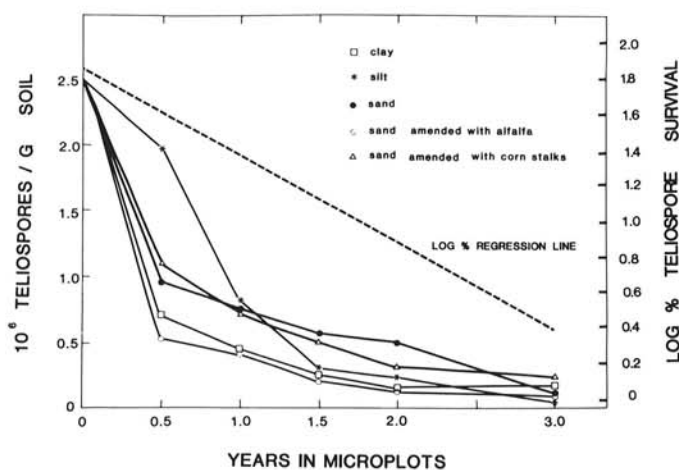


Fig. 1. Recovery curves of raw and transformed data of teliospores of *Sphacelotheca reiliana* from various soils in microplots under ambient environmental conditions at various time intervals. Soils were: Webster clay loam, Waukegan silt loam, and Vernale sandy loam. Each point represents the mean of five replicates, two analyses per replicate. LSD values at 0.5, 1.0, 1.5, 2.0, and 3.0 yr were 0.28, 0.20, 0.13, 0.14, and 0.8, respectively. Slopes and elevations of individual treatment regression lines of transformed data were not significantly different; therefore, all data were combined. The regression equation of the combined and transformed data was $y = -0.469x + 1.87$.

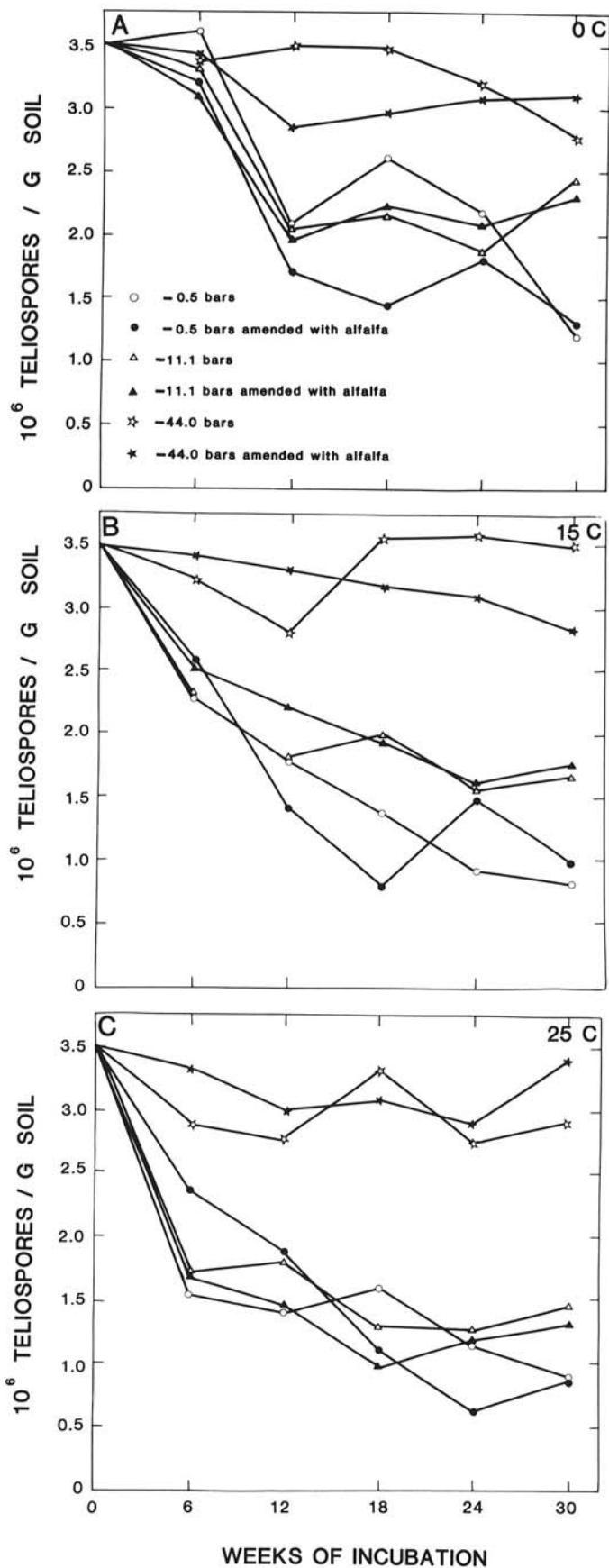


Fig. 2. Recovery of teliospores of *Sphacelotheca reiliana* from an amended or unamended, nonautoclaved, sandy loam soil at: A, 0 C, B, 15 C, and C, 25 C at three soil matric potentials at various time intervals. Each point represents the mean of three replicates, two analyses per replicate. LSD values for 0 C at 6, 12, 18, 24, and 30 wk were 1.0, 0.58, 0.70, 0.79, and 0.50; for 15 C, 0.65, 0.70, 1.0, 0.5, and 0.36; and for 25 C, 0.93, 0.55, 0.56, 0.66, and 0.73, respectively.

nearly constant. The $t_{0.5}$ value for this group was 137.9 wk. The second group consisted of soils held at -0.5 and -11 bars at 0 C and -11 bars at 15 C; $t_{0.5} = 26.0$ wk. The slope of this line, but not its elevation, differed from that of group 1. The third group consisted of soils at -11 bars at 25 C. This survival curve had the same slope as group 2, but the elevation was significantly lower and the $t_{0.5}$ value was 18.1 wk. The fourth group consisted of soils at -0.5 bar and 15 and 25 C. The slope and the elevation of this line were significantly different from the values of the other lines and the $t_{0.5}$ value was 13.3 wk. Survival curves of groups 1-4 had F values of 10.4, 136.5, 35.5, and 300.7, respectively, and all were significant at $P = 0.01$.

The combined average of disease incidence in bioassays at all sample dates reflected the effects of matric potential and temperature on spore populations. Disease incidence was 33, 25, and 15% for the spores incubated at 0, 15, and 25 C, respectively. Spores incubated at -0.5 , -11.1 , and -44 bars produced 16, 18, and 38% incidence of diseased plants, respectively.

Similarly, the factors that affected spore survival also affected spore germination. Spores incubated at 0, 15, and 25 C germinated 26, 16, and 10%, respectively. Spores incubated at -44 , -11.1 , and -0.5 bars germinated 30, 14, and 7%, respectively. Since only one bioassay and germination test was done for each treatment at each sample date, the data were not analyzed statistically.

DISCUSSION

Head smut was discovered affecting 1,250 ha in four counties of Minnesota in 1980, but its occurrence within those counties the following year was diminished markedly (12). Several factors may be responsible for such a rapid decrease in the incidence of head smut. Environmental conditions favorable for infection may not have occurred. The inoculum potential may have diminished rapidly due to the germination of teliospores in the absence of hosts and to hyperparasitism that may have decreased spore populations. Nutrient deprivation may also have decreased the germinability of the remaining spores.

Teliospore populations of *S. reiliana* diminished rapidly under natural conditions in Minnesota in all the soils tested; however, a low percentage of the spores remained viable (Fig. 1) and were able to infect corn seedlings after 3 yr in soil. The greatest decrease in spore populations occurred in the first 6 mo, and decreases were smaller with each succeeding time period. The percent decrease between each sample period was 58, 18, 10, 6, and 4%. These correspond to cumulative percent decreases in populations of 58,

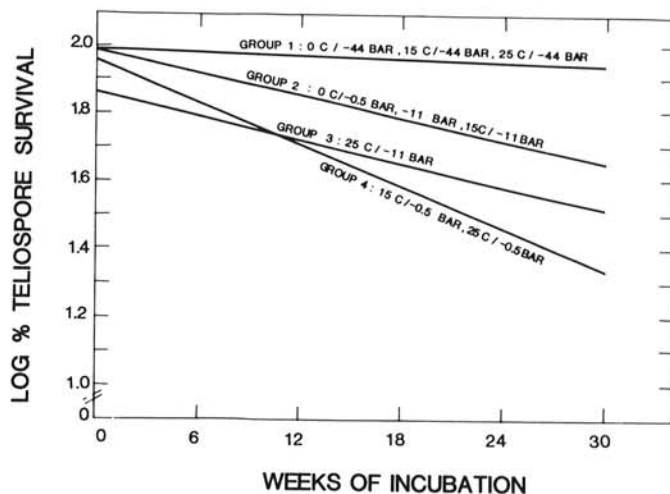


Fig. 3. Semilogarithmic transformations of the percentage of remaining teliospores at various time intervals. Curves with slopes and elevations that were not significantly different were combined. Regression equations were: for group 1, $y = -0.002x + 1.97$; for group 2, $y = -0.011x + 1.98$; for group 3, $y = -0.012x + 1.86$; and for group 4, $y = -0.021x + 1.95$.

75, 86, 89, and 96% for the 0.5-, 1.0-, 1.5-, 2.0-, and 3.0-yr sample dates, respectively. The decrease in number of spores recovered from soil was probably responsible for the decreased percentages of infected plants in bioassays at each of three time intervals. These changes resemble the idealized curve for survival of plant pathogen propagules introduced into the soil as described by Baker (1), excluding the slight initial increase in populations due to fragmentation of propagules or to saprophytic growth. That curve depicts a period of rapid death rate of propagules presumably not adapted to the soil environment followed by a period of lesser decline by more resistant propagules.

Under controlled conditions, teliospore populations decreased most rapidly at the high matric potentials and at temperatures of 15 and 25 C, whereas populations in dry soils (-44.0 bars) remained nearly constant at all temperatures. These data are similar to those reported by Papavizas and Lewis (9) and Lifshitz and Hancock (5) on the effect of high moisture and temperature on the survival of endoconidia of *Thielaviopsis basicola* Berk. and Br. and oospores of *Pythium ultimum* Trow. These authors suggest that hyperparasites may be involved because of the increased microbial activity under conditions of high moisture and temperature. In addition, teliospores may be stimulated to germinate in soil under these same conditions even in the absence of a host, since it has been shown that teliospores of *S. reiliana* on agar surfaces have increased rates of germination at high temperatures and matric potentials (7,11).

Teliospores incubated for 6-30 wk in soil showed a decrease in ability to germinate on agar that progressed with time, increase in temperature, and increase in matric potential. These findings are similar to reports by Filonow et al (3) on the decreased virulence of *Cochliobolus victoriae* Nelson due to nutrient deprivation during incubation in soil or sand. In the present study, however, the germination test was not an extremely sensitive measure of spore viability in long-term experiments, as indicated by the ability of smut spores from the 1.0-, 1.5-, 2.0-, and 3.0-yr sample dates to infect corn seedlings, whereas they only occasionally germinated on agar. Thus, teliospores of the head smut fungus would not likely survive in soil for longer than 3 yr, especially if soil temperatures were above 0 C and soil moisture was high.

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