Survival of *Puccinia recondita* and *P. graminis* Urediniospores Exposed to Temperatures from Subfreezing to 35 C

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ABSTRACT

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Viable urediniospores and dormant mycelial infections are the principal inoculum sources that contribute to the establishment and development of destructive wheat rust epidemics in the central Great Plains. Viability of urediniospores of *Puccinia recondita* and *P. graminis* was measured by exposure in environmental chambers set at constant temperatures ranging from −6 to 35 C. Exposure of *P. recondita* and *P. graminis* urediniospores for up to 120 h to constant temperatures of 5–35 C did not significantly ($P \leq 0.05$) affect spore viability among isolates. However, *P. recondita* and *P. graminis* urediniospore viability as measured by germination observed after exposure to constant temperatures of −6, −4, −2, 0, 5, 10, 20, 25, 30, or 35 C for 24, 48, 72, 96, or 120 h indicated significant differences in viability among temperature treatment means ($P \leq 0.05$). Urediniospores remained viable up to 864 h at constant temperatures between 10 and 30 C and up to 504 h at constant temperatures of 5 and 35 C. Viability of urediniospores exposed at temperatures ranging from −2 to 0 C decreased rapidly after the first 4 h of exposure. At below-freezing temperatures, viability of spores declined rapidly after 1–2 h of exposure. Viability of urediniospores exposed to freezing or below-freezing temperatures was not restored by heat shocking at temperatures typically encountered in the field during an epidemic. Infectivity of *P. recondita* and *P. graminis* urediniospores exposed at 5–35 C for 120 h was not significantly different from infectivity of unexposed urediniospores; an average of 58% of the viable urediniospores produced appressoria.

Additional keywords: dispersal, models, prediction, *Triticum aestivum*.

Viable urediniospores and dormant mycelial infections of *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* and *P. graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. are the principal sources of inocula that contribute to the establishment and development of destructive wheat leaf and stem rust epidemics in the wheat-growing areas of the central Great Plains (1–3,6,7). Survival of inoculum from harvest to fall stand establishment and/or subsequent survival through winter dormancy to spring green-up of wheat plants normally results in epidemics that reduce crop production by at least 2% (2,11). During years in which inoculum does not survive the oversummering or overwintering periods, the primary sources of inoculum are urediniospores transported into the field from other overwintering areas. Rapid development of wheat leaf rust epidemics on a susceptible host cultivar depends on the effectiveness of urediniospore production (16,17), dispersal (10), and survival (2,8,9,12). Each of those events, which have been extensively used in model development (1,4,6,7), can be affected by several different environmental variables that limit the rate or final level of epidemic progression.

Urediniospore viability in the field during the overwintering or spring initiation stage of leaf and stem rust epidemics was significantly reduced during periods of near-freezing or subfreezing temperatures (9). The objective of the present study was to measure the influence of temperatures from subfreezing to 35 C on the viability of *P. recondita* and *P. graminis* urediniospores. Such information could be useful in improving wheat leaf and stem rust development models by explaining why certain temperature variables in the oversummering or overwintering phase of an epidemic are important indicators in the determination of final severity levels.

MATERIALS AND METHODS

Urediniospores of four isolates each of *P. recondita* (PRTUS3, PRTUS6, PRTUS19, and PRTUS25) and *P. graminis* (TNM, QFB, RKQ, and MBC) were exposed to various temperatures in environmental chambers (Percival, Boone, IA) with measured relative humidities of 30–50% and without lights to test survival. Isolates of *P. recondita* were all increased from collections made in the wheat-producing areas of the central Great Plains. *P. graminis* isolates TNM, QFB, and RKQ were increased from collections made in the same area and identified to the avirulence pattern at the Cereal Rust Laboratory, St. Paul, Minnesota, whereas isolate MBC, also found in the area, was obtained from their collection. Urediniospores of *P. recondita* and *P. graminis* were increased on susceptible wheat cultivars Trison and McNair 701, respectively, grown at 20 C with a 14-h light (135 μmol $s^{-1}m^{-1}$) and 10-h dark cycle in environmental chambers. Urediniospores used for viability tests were collected by gently tapping infected leaves and allowing dislodged spores to settle on aluminum foil. Ten milligrams of urediniospores was dispersed over and entrapped in nylon hosiery fabric with a 50- to 75-μm mesh stretched over 8- × 8-cm^2 samplers constructed with 5-mm wire and placed in environmental chambers within 2 h after urediniospore collection. Germination of urediniospores was 95–100% when they were initially placed in the nylon samplers. The intent of this procedure was to simulate the exposure of spores suspended freely in the atmosphere (9).

A series of experiments, which were replicated four times and repeated five times, exposed urediniospores to constant temperatures in darkness by suspending the nylon samplers containing spores of each isolate in environmental chambers set at 5, 10, 20, 25, 30, or 35 C. Temperatures were assigned randomly to environmental chambers in each test.

Samples of exposed urediniospores were collected for viability tests at 24-h intervals, except as noted in each series of experiments,

by tapping the nylon to dislodge spores onto a 5-cm-diameter petri plate of 2% water agar. The nylon samplers were replaced in the environmental chamber within 1 min each time spores were removed for a viability test. The water agar plates were incubated for 16 h in an environmental chamber set at 20 C without lights. Viability was assessed by counting a total of 100 urediniospores per treatment of each isolate to determine the percentage of germinated spores. Viability was assumed if the germ tube was longer than the diameter of the spore. Sampling continued until either no urediniospores remained in the nylon sampler or no germinating urediniospores were observed from two consecutive collections on the water agar plates.

The same procedures were followed, except as noted, in conducting a second set of experiments replicated four times and repeated twice to measure infectivity of spores after exposure to temperature treatments. Eight 10-cm-diameter pots each with 10–15 10-day-old TAM 107 wheat seedlings were substituted for the water agar plates and then placed in a moist chamber without lights at 20 C for 16 h. Immediately after removal from the moist chamber, plants in four of the pots were air dried, and a thin layer of contact cement (Devcon, Wood Dale, IL) was applied to eight randomly selected oldest leaves in each pot. After drying for 15 min, the film of contact cement bearing the impression of the leaf surface, spores, and attached appressoria was removed. One hundred spores were observed for germination and appressoria formation by mounting the leaf impression in cotton blue plus lactophenol and examining it under a microscope. The other four pots were returned to a chamber set at 20 C with a 14-h light cycle for completion of the infection process. The numbers of uredinia that developed per square centimeter of leaf area on eight randomly selected oldest leaves in each pot were recorded.

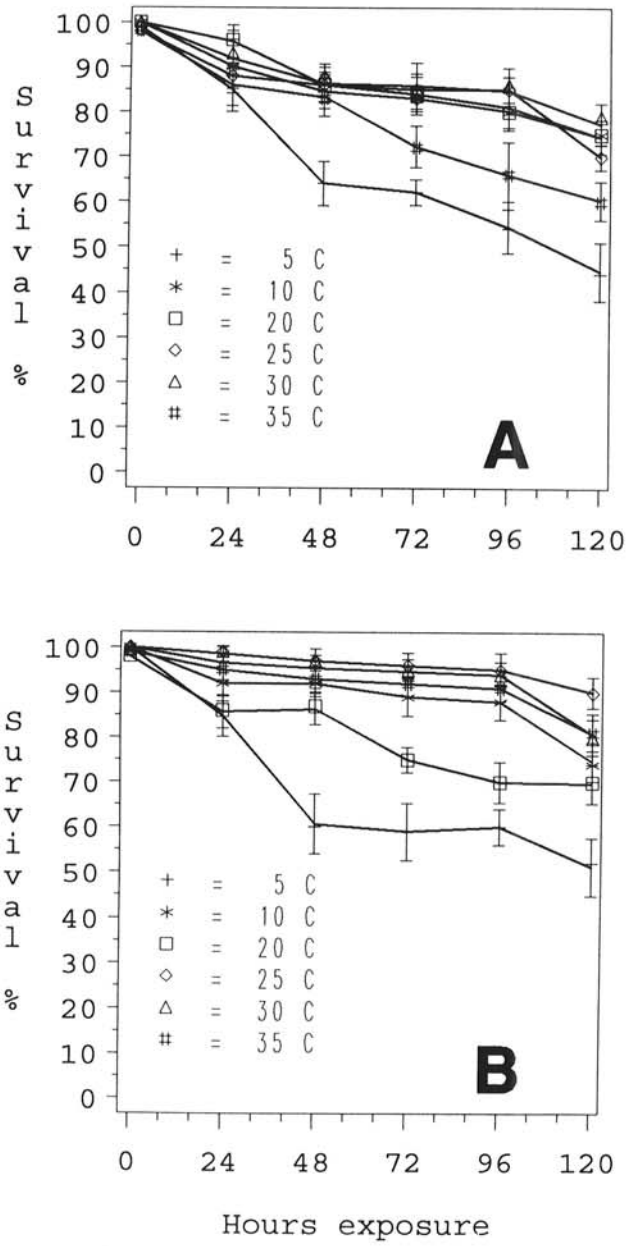

Fig. 1. Survival of **A,** *Puccinia recondita* and **B,** *P. graminis* urediniospores exposed to constant temperatures of 5, 10, 20, 25, 30, and 35 C for 24, 48, 72, 96, and 120 h. Data points for each temperature represent means of four isolates replicated four times and repeated five times. Length of line drawn through each point is twice the standard error of the mean.

A third set of experiments replicated six times and repeated three times was conducted in environmental chambers set at −6, −4, −2, 0, 2, and 5 C to determine effects of subfreezing, freezing, and near-freezing temperatures on spore viability. The 2% water agar procedure was used to assess urediniospore viability, and sampling was conducted at 1-, 2-, 3-, 4-, 5-, 6-, 7-, and 24-h intervals. A second set of samples was taken from each temperature treatment and heat shocked in environmental chambers set at 10, 20, 30, or 40 C for 5 min prior to incubation for 16 h at 20 C to determine whether loss of viability after exposure to freezing or subfreezing temperatures could be reversed by exposure to temperatures found in the Great Plains.

All statistical analyses were performed by procedures from the Statistical Analysis System (SAS Institute, Cary, NC) for analysis of variance. Arcsine square root transformations of viability data were performed prior to statistical analysis, but all data are reported as untransformed values. Univariate analyses of variance were performed for repeated measures of viability data. Means were separated by t tests at the $P < 0.05$ level.

RESULTS

Exposure of *P. recondita* urediniospores for up to 120 h to constant temperatures of 5, 10, 20, 25, 30, and 35 C did not significantly ($P < 0.05$) affect viability among isolates with temperature treatment. Therefore, mean viability of all isolates in a temperature treatment are shown in Figures 1 and 2. Exposure for periods longer than 120 h at 5, 30, or 35 C resulted in 5–10% reductions in viability among means of isolates PRTUS3 and PRTUS6. Viability of urediniospores exposed at 10, 20, and 25 C for 24-, 48-, 72-, 96-, and 120-h intervals did not result in significant differences among the temperature treatment means at each successive exposure time when separated by t tests. Urediniospore viability was reduced by 10–25% after exposure at 5 C compared with viability after exposure at 10–30 C. Similarly, exposure to 35 C for 48 h reduced spore viability by 10% compared with temperature treatments at 10–30 C. Viability remained above 70% at the end of 120 h of exposure, except for urediniospores exposed to 5 and 35 C, for which viability had decreased to 45 and 60%, respectively (Fig. 1A).

In contrast to *P. recondita* (Fig 1A), viability of *P. graminis* (Fig 1B) isolates remained above 50% for 120 h for all temperature treatments. No significant differences were observed in spore viability among isolates exposed to constant temperatures of 5–35 C for up to 120 h. Exposure of *P. graminis* urediniospores for more than 120 h resulted in mean viability rates among of isolates QFB and MBC that were 15–30% lower than those of isolates TNM and RKQ. Viability of *P. graminis* urediniospores exposed at 5 C was 10–30% lower than viability of spores exposed to temperatures above 5 C. Viability of *P. graminis* urediniospores exposed to 10 C for more than 72 h was 10–15% lower than viability of spores exposed to temperatures above 10 C (Fig 1B). *P. recondita* and *P. graminis* urediniospores retained 1% viability for at least 934 h (end of experiment) at temperatures of 10–30 C and for at least 504 h at 5 or 35 C.

Maximum viability of urediniospores of each isolate of *P. recondita* combined across temperatures after exposure for 120 h was observed at 10–30 C (Fig. 3A). Viability decreased rapidly

from the maximum of 80–90% to 55–65% at temperatures below 10 C and to 70–75% at temperatures above 30 C. In contrast to *P. recondita,* for which viability was 80–90% over the wide range of temperatures (10–30 C), maximum viability (85–95%) of urediniospores of each isolate of *P. graminis* was measured only at 25–30 C. Viability was reduced to 75–90% at 35 C and to 60–80% at 5–20 C (Fig. 3B). The same general profile (Fig. 3A and B) was observed for viability of both *P. recondita* and *P. graminis* urediniospores after 48, 96, 144, 168, or 240 h of exposure to the temperature treatments (data not shown). The profiles were moved only up or down on the survival axis.

Viability of *P. recondita* and *P. graminis* urediniospores decreased rapidly from 96–100% to 1–15% in the first 7 h after exposure to subfreezing temperatures (Fig. 2A and B). Viability after exposure to −4 and −6 C for 1–2 h was 4–15% and after 24 h was 1–5%. Heat shocking urediniospores exposed to 0, −2, −4, or −6 C for 1, 2, 3, 4, 5, 6, 7, or 24 h by exposure of urediniospores to 10, 20, 30, or 40 C for 5 min did not increase viability of any of the treated urediniospores.

Infectivity of *P. recondita* and *P. graminis* urediniospores was not significantly altered by exposure for 120 h to temperatures of 5, 10, 20, 25, 30, or 35 C (Table 1). Of those urediniospores that germinated, an average of 58% produced appressoria. Although the present data did not allow for determining the percentage of viable urediniospores with appressoria that formed uredinia, comparative numbers of uredinia per unit area were recorded (Table 1).

DISCUSSION

Exposure of urediniospores to constant temperatures of 5–35 C in environment chambers with no photoperiod and relative humidities of 30–50% indicated that viability of inoculum during various phases of an epidemic could be affected by temperature.

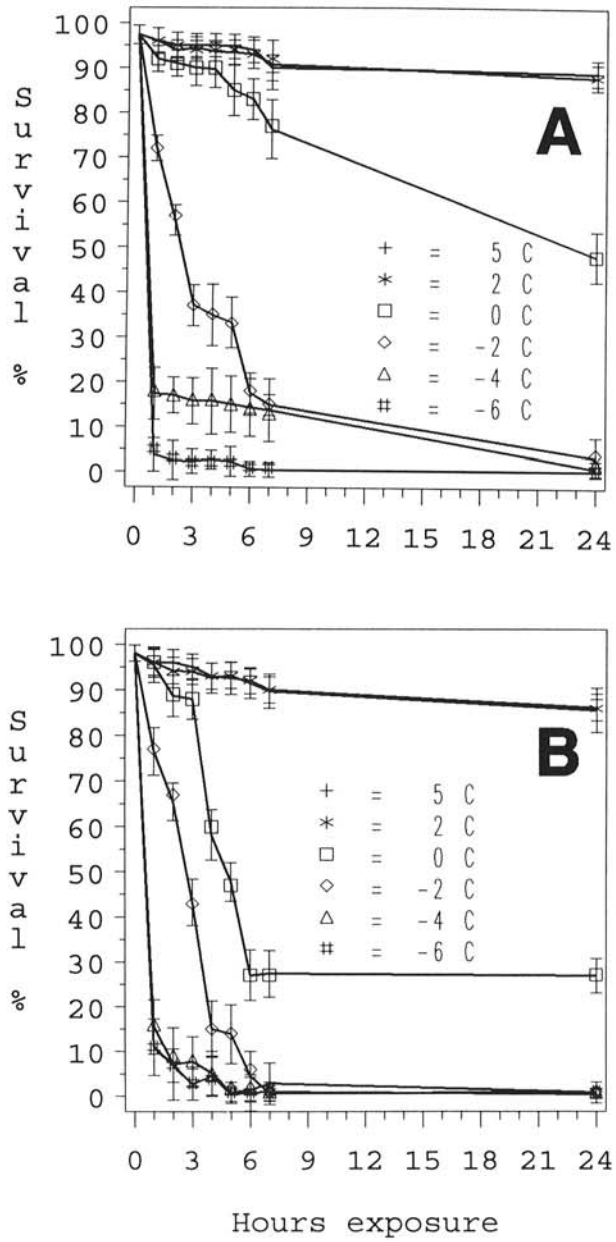

Fig. 2. Survival of **A,** *Puccinia recondita* and **B,** *P. graminis* urediniospores exposed to constant temperatures of 5, 2, 0, −2, −4, and −6 C for 1, 2, 3, 4, 5, 6, 7, or 24 h. Data points for each temperature represent means of four isolates replicated six times and repeated three times. Length of line drawn through each point is twice the standard error of the mean.

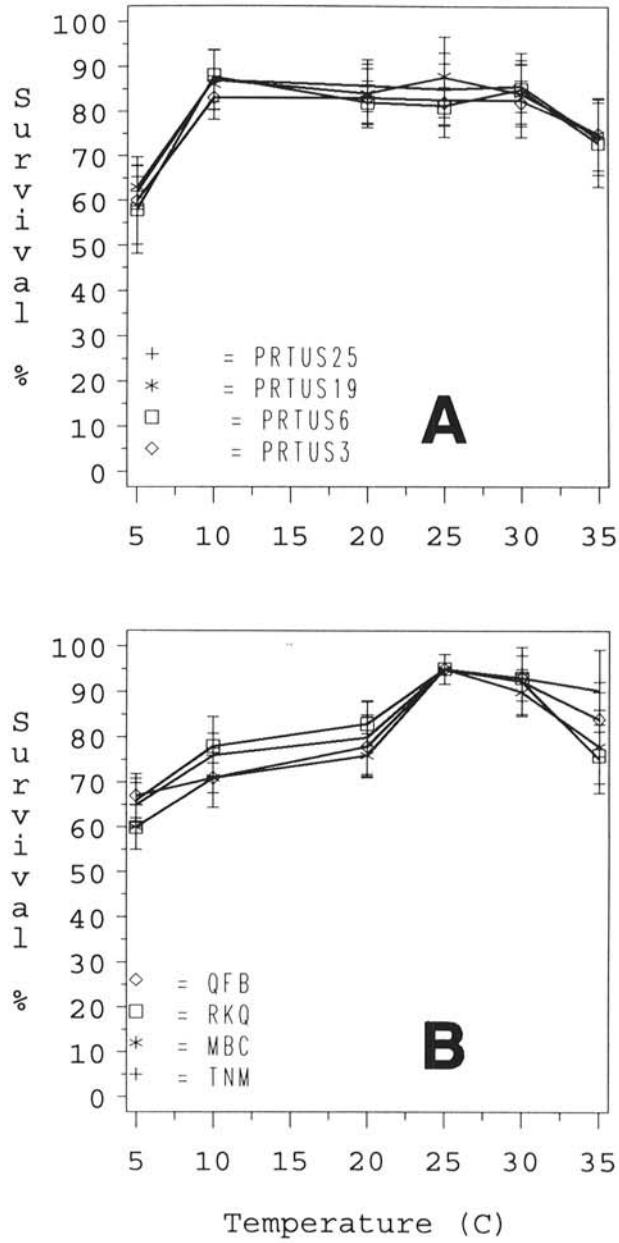

Fig. 3. Survival of urediniospores of **A,** four isolates of *Puccinia recondita* and **B,** four isolates of *P. graminis* exposed at constant temperatures between 5 and 35 C for 120 h. Data points for each isolate represent means of 20 samples. Length of line drawn through each point is twice the standard error of the mean.

TABLE 1. Percent urediniospore germination, percentage of germinated spores that formed appressoria, and number of *Puccinia recondita* and *P. graminis* uredinia that developed per square centimeter of leaf surface on winter wheat cultivar TAM 107 after exposure of urediniospores to constant temperatures of 5, 10, 20, 30, or 35 C for 120 h

Temperature (C)	*P. recondita*[a]			*P. graminis*[a]		
	Germination (%)	Appressoria (%)	Uredinia per cm^2	Germination (%)	Appressoria (%)	Uredinia per cm^2
35	71	55	15	75	56	12
30	74	56	16	76	57	13
20	72	59	21	72	58	15
10	70	61	18	71	59	14
5	66	66	17	67	54	12
Standard error	4.71	4.92	3.17	5.60	5.23	2.98

[a]Data are means of four isolates replicated four times and repeated twice. Numbers within a column are not significantly different at $P \leq 0.05$ according to t tests.

Survival of urediniospores, as measured by percent germination, declined significantly with increasing hours of exposure to constant temperatures between −6 and 35 C in environmental chambers. There was a tendency for isolates of both species most prevalent in the Great Plains populations (PRTUS19, PRTUS25, TNM, and RKQ) to survive longer (>20 days) and in higher proportions (>1%) than those isolates less prevalent in the population. With few exceptions, more than 50% of the urediniospores of both *Puccinia* species survived for at least 120 h (5 days) when exposed at temperatures above 0 C.

Urediniospores of *P. recondita* and *P. graminis* are able to retain viability for a considerable length of time; however, the exact length of time spores remain viable may be influenced by a variety of exogenous as well as endogenous factors. A summary of early studies (3) indicates that temperature, humidity, and light may have pronounced effects on spore longevity. Protection provided by the uredium for urediniospores while they were subjected to high temperature (40 C) was shown to increase viability; however, when host tissues were killed or spores were separated from living tissue, a significant loss in germination occurred over a 24-h period (6,10). Burleigh et al (2) collected viable urediniospores from uredinia on dead leaf tissue or field debris that had been subjected to summer, fall, and winter environmental conditions in the Great Plains. Urediniospores placed on wheat leaves and subjected to temperatures of 5–8 C for 1,080 h were still viable and caused infection when placed in a dew chamber (5). The greatest reduction in viability was found (12) when urediniospores were separated from the uredinia and/or host tissue prior to exposure to the higher temperatures (35–40 C) normally found in the Great Plains during the oversummering phase of a cereal rust epidemic.

Assuming that most *P. recondita* and *P. graminis* urediniospores are transported in air masses below 3,000 m above sea level (10,15), we expect inoculum viability to be affected by the temperature of the air mass during transport. Most urediniospores from wheat fields in the Great Plains are released into the atmosphere after the canopy has dried and as wind velocity and temperature increase during the morning and early afternoon (13,14). During the exponential phase of a leaf rust epidemic, most of the dispersal of inoculum is within the local wheat canopy or surrounding fields until urediniospores are produced in the upper portions of the canopy and are easily released in large quantities into the air mass moving across that canopy. As indicated by the effect of low temperatures in these tests, the adiabatic cooling of the air with increasing height above ground level will have an adverse effect on spore viability. Winter or early spring air temperatures at 1–2,000 m above sea level are usually below 10 C during the daytime hours, when inoculum is most likely to be in the air masses above the Great Plains. We believe viability of inoculum during fall establishment and early spring initiation of an epidemic is of more importance than viability at other stages because inoculum concentrations are lowest during these phases of a cereal rust epidemic (1,6,7). These environmental chamber studies indicate that a few hours of freezing or below-freezing temperatures are extremely detrimental to survival of single, unprotected urediniospores of both *P. recondita* and *P. graminis*. However, exposure to a wide range of temperatures above 0 C does not significantly reduce viability or usefulness of exogenous airborne urediniospores for initiation of an epidemic.

Temperature conditions to which urediniospores are exposed prior to deposition in the field are similar to those evaluated in these tests. These conditions may have a pronounced effect on the development of cereal rust epidemics during those periods when exogenous inoculum is necessary for their initiation or continuation.

LITERATURE CITED

1. Burleigh, J. R., Eversmeyer, M. G., and Roelfs, A. P. 1972. Development of linear equations for predicting wheat leaf rust. Phytopathology 62:947-953.
2. Burleigh, J. R., Schulze, A. A., and Eversmeyer, M. G. 1969. Some aspects of the summer and winter ecology of wheat rust fungi. Plant Dis. Rep. 53:648-651.
3. Chester, K. S. 1946. The Nature and Prevention of the Cereal Rusts. Chronica Botanica, Waltham, MA.
4. Eversmeyer, M. G., Browder, L. E., and Young, H. C., Jr. 1975. Effect of leaf and stem rust on 1974 Kansas wheat yields. Plant Dis. Rep. 59:604-607.
5. Eversmeyer, M. G., and Burleigh, J. R. 1968. Effect of temperature on the longevity of *Puccinia recondita* f. sp. *tritici* urediniospores on dry wheat foliage. Plant Dis. Rep. 52:186-188.
6. Eversmeyer, M. G., and Burleigh, J. R. 1970. A method of predicting epidemic development of wheat leaf rust. Phytopathology 60:805-811.
7. Eversmeyer, M. G., Burleigh, J. R., and Roelfs, A. P. 1973. Equations for predicting wheat stem rust development. Phytopathology 63:348-351.
8. Eversmeyer, M. G., and Kramer, C. L. 1989. Survival and germination of urediniospores of *Puccinia recondita* and *P. graminis*. Trans. Kans. Acad. Sci. 92:159-175.
9. Eversmeyer, M. G., and Kramer, C. L. 1994. Survival of *Puccinia recondita* and *P. graminis* urediniospores as affected by exposure to weather conditions at one meter. Phytopathology 84:332-335.
10. Eversmeyer, M. G., Kramer, C. L., and Browder, L. E. 1984. Presence, viability, and movement of *Puccinia recondita* and *P. graminis* inoculum in the Great Plains. Plant Dis. 68:392-395.
11. Eversmeyer, M. G., Kramer, C. L., and Browder, L. E. 1988. Winter and early spring survival of *Puccinia recondita* on Kansas wheat during 1980–1986. Plant Dis. 72:1074-1076.
12. Hassan, Z. M., Kramer, C. L., and Eversmeyer, M. G. 1986. Summer and winter survival of *Puccinia recondita* and infection by soilborne urediniospores. Trans. Br. Mycol. Soc. 86:365-372.
13. Hirst, J. M., and Hurst, G. W. 1967. Long-distance spore transport. Pages 307-314 in: Airborne Microbes. Soc. Gen. Microbiol. Symp., 17th. P. H. Gregory and J. L. Monteith, eds. Cambridge University Press, London.
14. Kramer, C. L., and Pady, S. M. 1966. A new 24-hour spore sampler. Phytopathology 56:517-520.
15. Pady, S. M., Kramer, C. L., Pathak, V. K., Morgan, F. L., and Bhatti, M. A. 1965. Periodicity in airborne cereal rust urediniospores. Phytopathology 55:132-134.
16. Tomerlin, J. R., Eversmeyer, M. G., Kramer, C. L., and Browder, L. E. 1983. Temperature and host effects on latent and infectious periods and on urediniospore production of *Puccinia recondita* f. sp. *tritici*. Phytopathology 73:414-419.
17. Tomerlin, J. R., Eversmeyer, M. G., Kramer, C. L., and Browder, L. E. 1984. Environmental and host effects on colony development of *Puccinia recondita* f. sp. *tritici*. Phytopathology 74:225-229.