

Effects of Night Temperature and Mist Period on Infection of Sweet Corn by *Puccinia sorghi*

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ABSTRACT

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The effects of mist period and diurnal temperature fluctuation on the development of common rust (*Puccinia sorghi*) were evaluated on susceptible and partially resistant sweet corn hybrids in growth chambers. The optimal mist period for infection of sweet corn hybrids with *P. sorghi* was a 12-hr intermittent mist (30 min on/30 min off). A 6-hr constant mist period resulted in significantly fewer, yet abundant, uredinia. Urediniospore germination percentage was not significantly different for the 12-hr 30/30 or 6-hr constant mist periods. These results indicate that the 6-hr constant mist period was sufficient for urediniospore germination but may not have been adequate for complete infection structure formation. Infection was significantly reduced for plants exposed to 0, 3, or 6 hr of 30/30 mist or given a one-time mist with an atomizer. Night temperature appeared to be important in controlling uredinial formation, especially near the critical temperatures of 8 and 32 C. With day temperatures of 24 or 32 C, rust developed most rapidly at night temperatures of 24 and 16 C. Night temperature of 8 C resulted in an extension of the latent period by about 2 days over other treatments. On nights at 32 C, very few uredinia formed, although water-soaked lesions often developed and became necrotic without sporulating.

Additional key words: corn rust, disease forecasts, *Zea mays*

Common maize rust, caused by *Puccinia sorghi* Schw., occurs wherever corn (*Zea mays* L.) is grown. Rust epidemics on dent corn hybrids are rarely economically damaging in the corn belt region of the United States. This is

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(7), and *Phytophthora infestans* on potato (8) among others. Both temperature and moisture have been shown to greatly influence infection by *Puccinia sorghi* (11,13,18,19).

Previous investigations of environmental effects on *P. sorghi* generally focused on urediniospore germination (9,11,13,19). Generally, constant temperatures were used to define optimal conditions for rust development on dent corn genotypes (9,11,13,19). Weber (19) reported the minimum and maximum constant temperatures for urediniospore germination and infection to be 4 and 32 C and 8 and 32 C, respectively. Other studies (9,11,14) have supported these findings. Syamananda and Dickson (17,18) found that the rust reactions of inbred lines varied in response to diurnal temperature fluctuation and that the night temperature exerted a major influence on the reactions.

Kushalappa and Hedge (9) reported that urediniospore germination on water agar at 18–20 C was 85 and 90% within 2 and 8 hr, respectively. Mederick and Sackston (13) found that urediniospores on corn leaves in dew chambers at 9–18.5 C germinated within 3 hr. Longer dew periods did not significantly increase the percentage of germination. Mahindapala (11) reported maximum germination with a 6-hr dew period at 15 C.

The objectives of this investigation were to evaluate the effects of mist period length and diurnal temperature regimes on infection and uredinial formation on a susceptible and a partially resistant sweet corn hybrid.

MATERIALS AND METHODS

Mist period experiments. The first experiment was a 3 × 3 factorial

treatment design that included three replicates arranged in a split-plot experimental design. Urediniospore inoculum densities were main plots, and postinoculation mist periods were subplots. Each sampling unit consisted of an inoculated leaf plus the next emerged leaf of each of two plants per pot. Seedlings of the susceptible sweet corn hybrid Florida Staysweet were inoculated at the six-leaf stage with 1, 2, or 3 mg of dry urediniospores. The inoculum used in this and subsequent experiments was a composite of urediniospore isolates collected in Illinois and increased in the greenhouse on 11 sweet corn hybrids and two dent corn inbreds (4).

Plants were inoculated in a settling tower (1). Approximately equal-sized fourth or fifth leaves were taped flat to the settling tower tabletop. Microscope slides covered with a thin layer of petroleum jelly were placed next to each leaf to measure urediniospore deposition. Dry urediniospores were forcibly discharged into the upper cylinder of the tower by a 1-sec air blast through a glass tube bent at a right angle. After a 30-sec settling period, the settling tower shutter was opened and urediniospores settled for 2 min on leaves and greased slides on the tabletop. The shutter was then closed.

Immediately after inoculation, one of three mist treatments was applied. Plants were placed in a mist chamber for periods of 3 or 6 hr or were not misted. Non-misted plants were moved to a growth chamber (EGC, Environmental Growth Chambers, Chagrin Falls, OH, or ISCO, Instrument Specialty Co., Lincoln, NE) at 20 C and 12 hr light/12 hr dark immediately after inoculation. The remaining plants were moved to the growth chambers after the appropriate mist period. Humidifiers (Kaz DynaMist, Kaz, Inc., New York, NY) were operated for 48 hr after plants were placed in each chamber to ensure high relative humidities without resulting in condensation on leaf surfaces.

Disease was measured by counting the total uredinia on the inoculated leaf and on the next emerged leaf for two plants per replicate. Counts were made 9, 10, 13, 15, 17, 20, and 23 days after inoculation.

Half of the greased microscope slides (one from beside each leaf) were placed in the mist chamber for 6 hr. The other half were not misted. Both sets were stored in a refrigerator at 2 C for a maximum of 2 wk, then examined under the microscope at 100X. Total urediniospores and germinated urediniospores were counted.

The second experiment was identical to the first, with three exceptions. The sweet corn hybrid Gold Cup, which is less susceptible to rust than Florida Staysweet, was grown. A one-time misting with a Model 15 DeVilbiss atomizer (DeVilbiss Co., Somerset, PA) replaced the 3-hr mist treatment. Urediniospore deposition was not measured.

The third experiment was a 2 × 3 factorial with four replicates of preinoculation leaf treatments as main plots and postinoculation dew periods as subplots. Each sampling unit consisted of an inoculated leaf plus the next emerged leaf of a single plant per pot. Fourth or fifth leaves of Florida Staysweet were either lightly sprayed with polyoxyethylene sorbitan monooleate surfactant (Tween 80, ICN Nutritional Biochemicals, Cleveland, OH) or were untreated before inoculation. Inoculation and settling tower techniques were the same as those in the first two experiments, with 2 mg of dry urediniospores used for each inoculation. After inoculation, plants were placed in a mist chamber for 6 hr of constant mist or for 6 or 12 hr of intermittent mist (30 min on/30 min off). The leaves exposed to the intermittent mists did not dry during the 30 min between mists, although the moisture on the leaves was less than on leaves of plants exposed to a constant mist. After misting, plants were moved to a growth chamber at 20 C with a 12/12 day/night cycle. Total uredinia on the inoculated leaf plus the next emerged leaf were counted every day from 7 to 15 days after inoculation in each replicate. Greased microscope slides also were placed in the settling tower during inoculation and were exposed to each of the three mist chamber treatments. Data for all three experiments were log₁₀-transformed to stabilize variance before analysis of covariance. Treatments were analyzed over time, with time as a quantitative independent variable.

Temperature experiments. Two temperature experiments were repeated four times. Day temperature was 32 or 24 C for either experiment. Night temperatures were 8, 16, 24, or 32 C in both experiments. Both day experiments (32 and 24 C) were replicated four times (four trials), and night temperatures were randomized among four growth chambers. There were five to eight subsamples per experimental unit (growth chamber) per replicate. Each subsample consisted of a single plant each of the sweet corn hybrids Florida Staysweet (susceptible) and Sugar Loaf (partially resistant). Total uredinia on the inoculated leaf plus the next emerged leaf were counted. Six-leaved seedlings were inoculated with 2 mg of dry urediniospores by the settling tower procedure. Seedlings were placed in a mist chamber for 6 hr immediately after inoculation. Plants were then moved to growth chambers at one of the four day/night temperature regimes. Humidifiers were operated for 48 hr after placing plants in growth chambers to ensure high relative humidities but not to provide free moisture. Uredinia were counted 6, 7, 8, 10, 12, and 14 days after inoculation. Data were log₁₀-transformed to stabilize variance before analysis of covariance. Treatments were analyzed

over time, with time as a quantitative independent variable.

RESULTS

Mist period experiments. In the first experiment, the number of urediniospores deposited on the greased microscope slides was directly proportional to inoculum density ($r = 0.99$). Concentrations were 51.6, 83.1, and 133.3 urediniospores per square centimeter for 1, 2, and 3 mg of spores discharged. Dew period had a significant effect on urediniospore germination on the slides. Germination averaged 55.9% on slides exposed to a 6-hr period in a mist chamber and 0.7% on slides not misted.

The number of uredinia formed was greater as inoculum density increased, but the effect was not significant because of substantial variation among replicates. Mist period significantly affected uredinial formation at all evaluation times (Fig. 1). The number of uredinia on plants exposed to the 6-hr dew period was significantly greater than on plants exposed to the 0- or 3-hr dew periods, which were not significantly different from each other.

In the second experiment, the number of uredinia formed also increased with inoculum density, but the differences were not significant. As in the first experiment, mist period significantly affected uredinial formation over time. Uredinia developed rapidly on plants exposed to a 6-hr mist period, whereas very few uredinia developed on plants that were misted with the atomizer or on plants that were not misted. Twenty days after inoculation, the log₁₀ number of uredinia averaged 0.28, 0.05, and 1.84 for no mist, atomized mist, and 6-hr mist chamber treatments, respectively.

In the third experiment, mist period had a significant effect on germination of urediniospores on greased slides (Table 1). Percentage of germination was 42.3, 24.1, and 47.9 for the 6-hr, 6-hr 30/30, and 12-hr 30/30 treatments, respectively. The difference between the 6-hr constant and 12-hr 30/30 dew periods was not significant; however, both treatments were significantly greater than the 6-hr 30/30 dew period.

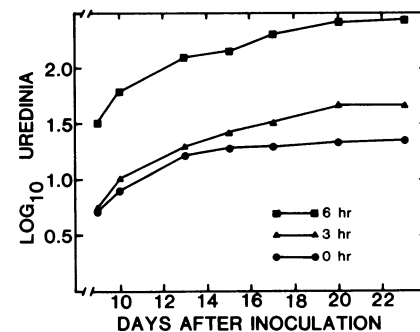


Fig. 1. Log₁₀ number of *Puccinia sorghi* uredinia on two inoculated leaves per plant of the sweet corn hybrid Florida Staysweet after exposure to 0, 3, and 6 hr of mist.

Preinoculation treatment with Tween 80 inhibited uredinial formation because of phytotoxicity. Therefore, mist period effects were compared on untreated plants. The greatest number of uredinia resulted from exposure to a 12-hr intermittent mist period with 30 min on/30 min off. Mist treatments were significantly different from each other (Table 1, Fig. 2).

Temperature experiments. Microscopic examination of greased slides placed in the settling tower during inoculation showed an average of 55 urediniospores per square centimeter per milligram of urediniospore inoculum. On the basis of mean leaf area and number of uredinia produced on the susceptible hybrid Florida Staysweet at a constant 24 C after exposure to a 6-hr mist period, the infection frequency was about 1.2%, or about 80 urediniospores were required for every uredinium produced.

For days at 32 or 24 C, night temperatures had a significant effect on uredinial formation (Fig. 3). For both day temperatures, 24 C at night was the most conducive for infection. The rate of uredinial formation at night temperatures of 16 and 24 C was rapid from 6 to 10 days after inoculation and decreased from 12 to 14 days (Fig. 3). For nights at 8

C, the latent period was extended by about 2 days, although the number of uredinia nearly equaled that of the treatment at 16 C by 14 days after inoculation. Very few uredinia developed at night at 32 C for both day temperatures, even though latent period was unaffected. Water-soaked lesions that became chlorotic and necrotic without sporulating were common on both hybrids at the night temperature of 32 C.

DISCUSSION

Night temperature appears to greatly influence common rust development on sweet corn given a minimum period of moisture.

For days at 24 and 32 C, very few uredinia developed when night temperature was 32 C. However, when night temperatures ranged from 8 to 24 C, uredinial formation was comparable to that seen in the field under favorable environmental conditions. The similar effect of night temperatures on uredinial formation at both day temperatures emphasizes the importance of night temperature in the rust reaction.

Investigations on the effects of constant temperatures on common rust (17,19) defined the maximum temperature for infection as 32 C. Day temperatures of 32 C can be common during the sweet corn growing season in areas where common rust occurs; however, a range of rust severity is often observed over time. Thus, rust epidemic development may be regulated by cool night temperatures. Previous investigators (18) also have

observed night temperatures to regulate the reaction of corn to *P. sorghi*, especially near the critical temperatures.

Day temperatures may influence the rust reaction when night temperatures approach the minimum for infection just as night temperatures influence the rust reaction when day temperatures approach the maximum for infection. In other words, the minimum and maximum temperatures for infection under diurnally fluctuating conditions appear to represent a wider range than those under constant conditions. Thus, within a prescribed range, the rust reaction may be explained on the basis of accumulated heat units. The exception to this would be night temperature of 32 C, which appeared to inhibit uredinial formation even when day temperatures were moderate. Additional experimentation is needed to confirm this. Other studies (14,19) have reported that little to no rust develops at a constant 8 C. In the present investigation, the number of uredinia formed on plants exposed to an 8 C night was only slightly less than the number formed at night temperatures of 16 and 24 C, although the latent period was extended by about 2 days (Fig. 3). Thus, infection may not occur at a constant 8 C, but increasing the day temperature to the range of 16–32 C may result in disease. Although the cool temperature (8 C) is below the range expected during the main sweet corn growing season, such temperatures may be important in late-planted fields during the fall, when inoculum levels are generally high.

For day temperature of 32 C, there were significantly more uredinia on the susceptible hybrid Florida Staysweet than on the partially resistant Sugar Loaf. However, for days at 24 C, there was no significant difference between hybrids (Fig. 3). Florida Staysweet has been more susceptible to rust than Sugar Loaf in previous studies (2,4). When compared with days at 32 C, perhaps the near optimal conditions for rust development for days at 24 C may have masked the differences in hybrid susceptibility in the seedling stage, where these differences can be subtle. Also, in these experiments, there was insufficient time for secondary spread of disease, which would amplify differences in partial resistance under field conditions. The number of uredinia resulting from a single inoculation increased up to 20 days after inoculation in these experiments. Pataky (16) also observed the maximum number of uredinia on seedlings of several sweet corn hybrids to occur at 19–20 days after inoculation.

The optimal mist period for infection of sweet corn hybrids with *P. sorghi* in these experiments was a 12-hr intermittent mist. A 6-hr constant mist resulted in significantly less yet abundant uredinia. Urediniospore germination percentage was not significantly different for the 12-hr-30/30 or 6-hr constant mist. These

Table 1. Percent urediniospore germination and log₁₀ number of uredinia of *Puccinia sorghi* that developed after exposure to three mist periods

Mist period ^x (hr)	Germination ^y (%)	Log ₁₀ no. uredinia ^z
6	42.3 b	1.72 b
6 (30/30)	24.1 a	1.45 a
12 (30/30)	47.9 b	2.19 c

^x Constant mist of 30/30 = intermittent mist (30 min on/30 min off).

^y Measured on greased microscope slides. Means followed by the same letter are not significantly different (FLSD_{0.05} = 6.2).

^z Log₁₀ number of uredinia on two inoculated leaves plus next emerged leaves 18 days after inoculation (FLSD_{0.05} = 0.24).

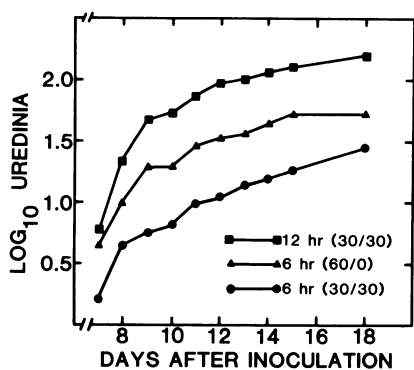


Fig. 2. Log₁₀ number of *Puccinia sorghi* uredinia on two inoculated leaves per plant of the sweet corn hybrid Florida Staysweet after exposure to mist periods of 6 hr of constant mist, 6 hr of 30 min on/30 min off, and 12 hr of 30 min on/30 min off.

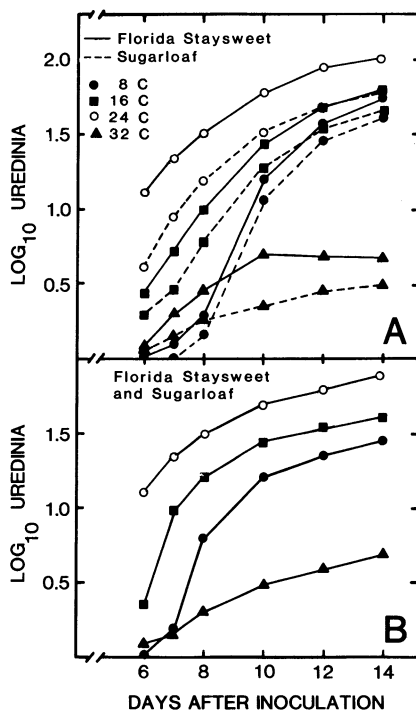


Fig. 3. Log₁₀ number of *Puccinia sorghi* uredinia on Florida Staysweet and Sugar Loaf at four night temperatures (8, 16, 24, and 32 C) with day temperatures of (A) 32 C and (B) 24 C.

results indicate that the 6-hr constant mist period was sufficient for urediniospore germination but may not have been adequate for complete infection structure formation. This could explain the relatively low (1.2%) infection frequency observed with the 6-hr mist period.

Similarly, in previous experiments with *P. sorghi* (9,11,13) and *P. polysora* (5), dew periods longer than 4 hr at optimal temperatures did not significantly increase urediniospore germination. However, dew periods longer than 6–12 hr did increase infection structure formation (11) and pustule density (5). Mederick and Sackston (13) observed near-maximum germination of *P. sorghi* urediniospores in vivo after a 3-hr dew period at 15.5–18.5 C. Kushalappa and Hedge (9) reported that urediniospores of *P. sorghi* began to germinate within 2 hr on both water agar and detached leaves and began to form appressoria on detached leaves within 4 hr at optimal temperatures. Mahindapala (11) found that a dew period of at least 3–4 hr was necessary for the initiation of infection structure formation in urediniospores of *P. sorghi*. In those experiments, only about 3% of the germinated urediniospores had formed appressoria within 3 hr and only 2% had substomatal vesicles within 4 hr. By 6 hr, 20% had formed appressoria and 10% had formed substomatal vesicles. Infection structure formation was not complete for 24 hr. In the present study, 6-hr 30/30 and 3-hr constant mist periods resulted in limited urediniospore germination and infection. The 0.7% urediniospore germination observed on greased slides not exposed to mist may have resulted from moisture present in the petroleum jelly and/or from condensation formed during storage in the refrigerator prior to examination.

The uredinia on nonmisted plants in the first experiment were apparently a result of unnoticed condensation on leaf

surfaces in the growth chamber. Although the humidifiers in the growth chambers may have affected uredinial formation, the number of uredinia on plants exposed to postinoculation mist periods of 0, 3, and 6 hr were significantly different. Mahindapala (11) demonstrated germination and germ tube growth of *P. sorghi* uredinia at relative humidities of 98.5–100% in the absence of free water but did not study infection under the same conditions. Such conditions may have existed in humidified growth chambers and contributed to infection in the present study.

Under field conditions in the absence of drought stress, moisture is often present in the whorls of actively growing corn plants for 6 hr or more. The regular presence of moisture in the whorl implies that temperature may be of greater importance in regulating the infection process because moisture is not the limiting factor. Cool night temperatures may also have the indirect effect of prolonging the moisture period in the whorl, which may improve infection efficiency.

Night temperature will be an important component in the development of a common rust forecasting model. A 6-hr moisture period may serve as a minimum value for infection, with longer moisture periods increasing the level of infection at a decreasing rate.

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