Effects of Free Moisture, Head Development, and Embryo Accessibility on Infection of Wheat by *Ustilago tritici*

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ABSTRACT

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Varying the concentration of teliospores in a spore-talc mixture from 0.02 to 20% did not alter infection of intact florets by *Ustilago tritici*. Florets inoculated during anthesis were 3.1–3.8 times more susceptible than those inoculated before or after anthesis. Infection of florets was 18.5 and 22.9% for free-moisture periods of 8 and 32 hr, respectively; but was also relatively high (12.3%) with no wetting period. When glumes were clipped to improve floret accessibility, mean infection was 13.6–31.4% compared to 1.1–11.9% for intact florets. Teliospore germination inside and outside florets increased with ambient relative humidity, but germination was higher inside than outside florets at the two highest relative humidities. Embryo accessibility appears to be a limiting factor in infection of wheat by *U. tritici*.

Additional key words: epidemiology, loose smut of wheat, *Triticum aestivum*.

Loose smut, which is caused by *Ustilago tritici* (Pers.) Rostr., occurs sporadically in wheat (*Triticum aestivum* L.) in Michigan. Maximally infected fields may have up to 5% loose smutted heads, but seed from such fields may yield little or no infection the following year. Since wheat seed certification for loose smut in Michigan is based on the quantity of inoculum in the seed field, the lack of association between smutted heads and subsequent infection sometimes renders the regulation meaningless. The lack of disease buildup when ample inoculum is present suggests that other factors limit infection under field conditions. Factors sometimes associated with increased natural infection include extended periods of free moisture (1), high relative humidity (1,7), and the degree of floret opening (8). However, these factors have not been previously investigated under controlled conditions and the association between floret opening and susceptibility has not been consistent (6).

The primary objective of this study was to examine the effect of duration of wetness, floret development, and embryo accessibility on infection of wheat by *U. tritici*.

MATERIALS AND METHODS

Seeds of *T. aestivum* 'Genessee' (widely grown in Michigan and susceptible to *U. tritici*) were sown in flats of soil, held in the greenhouse until seedling emergence was complete (usually in 7 days), and transferred to a cold room at 3 C with 9 hr of incandescent light per day for 8 wk. Vernalized seedlings were transplanted to 10-cm-diameter pots and grown to heading in the greenhouse at 20–25 C. Tillers were secured to stakes to prevent lodging, and powdery mildew and insects were controlled with sulfur vapors and with occasional sprays of acephate, respectively.

Teliospores of *U. tritici* were collected from greenhouse or field-grown wheat plants by pulverizing air-dried smutted heads with a mortar and pestle, and sifting the spores through cheesecloth. Spores were stored at 4 C in small vials until used, and mixed with powdered talc before inoculation. Spore germinability was assayed by dusting the spore-talc mixtures onto one-third strength potato-dextrose agar and incubating the seeded plates for 24 hr at 22 C. Spores from lots that germinated ≥80% were dusted onto wheat heads in a 1-L plastic bag. Wheat heads in horizontal position were uniformly coated with inoculum by briefly shaking the plastic bag. The Romig numerical scale (RNS) was used to assess wheat growth (3). Plants within the range RNS 12 (heads at least 25% emerged from the uppermost leaf) to RNS 22 (kernels near the middle of the head less than three-quarters formed) were chosen for inoculation.

The effect of inoculum concentration on infection was investigated by dusting wheat heads of known growth stage with spore-talc mixtures containing 0, 0.02, 0.2, 2.0, or 20% teliospores (w/w), using 20 randomly selected heads per treatment. After inoculation, plants were placed in a mist chamber at 22 C for 48 hr, then removed to a greenhouse and allowed to mature. Mature seeds were harvested, allowed to air-dry at 22 C for 2 wk, reseeded, vernalized, grown to heading, and evaluated for production of loose-smutted heads.

The effect of duration of wetting on infection was investigated by incubating inoculated plants in a mist chamber for 6, 12, 24, or 36 hr at 22 C under continuous fluorescent light. Wheat heads were stroked before inoculation to stimulate floret opening and increase the accessibility of the embryos to inoculum. A talc-spore mixture containing 20% teliospores (w/w) was used to inoculate 90 heads per treatment. After misting, the plants were placed in the greenhouse and grown to maturity. Seeds were dried, reseeded, vernalized, grown to heading, and evaluated for infection.

The influence of the stage of head development and of accessibility on infection were investigated simultaneously. Growth stages (RNS 12 to 22) of individual heads were noted, then the heads were stroked to stimulate floret opening. The tips of the glumes were clipped to one-half their original length on one side of each head to expose the embryos, while the florets on the other side were left intact. Heads were dusted with a talc-spore mixture containing 20% teliospores (w/w), and the plants were incubated in a mist chamber at 22 C for 24 hr and placed in the greenhouse to mature. Seeds from the clipped and unclipped portions of individual heads were evaluated separately for infection. Seventeen to 36 heads were used for each treatment combination.

To investigate spore germination inside wheat florets, heads that were 95% emerged from the upper leaf (RNS 15) were detached

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from greenhouse-grown plants. Culms were trimmed to 15 cm and placed in split sponges inside wax-coated cups with distilled water. Millipore filters (0.45 μm, Millipore Corp., Bedford, MA 01730) were cut into 1.5 × 1.5 mm pieces and placed in a vial containing teliospores (1.9 mg), and the vial was shaken to distribute the spores on the squares. Florets were inoculated by gently pulling back the lemma, inserting a spore-covered filter square, and pushing the lemma back into position. Glass chimneys lined with filter paper wicks were used to increase the relative humidity around the wheat heads. Relative humidity inside the chimneys was controlled by adjusting the amount of filter paper and the quantity of ambient air allowed through the top of each chimney. Spore-covered squares also were suspended in filter paper (Whatman No. 1) packets inside the glass chimneys to determine the level of spore germination outside the florets. The ambient vapor pressure was monitored with a dew point hygrometer (YSI Model 91, Scientific Divisions, Yellow Springs Instrument Co., Yellow Springs, OH 45387). Ambient air temperature was measured inside the chimneys with thermocouples attached to a dew point microvoltmeter (Model HR-33T, Wescor, Inc., Logan, UT 84321). Ambient relative humidity was calculated from temperature and vapor pressure. All treatments were incubated for 48 hr at 22 C in diffuse light. Filter squares were stained with cotton blue in lactophenol and examined microscopically for germination. Two hundred spores per square were counted with four replicates per treatment.

RESULTS AND DISCUSSION

Although inoculum concentration was varied by a factor of 10^3, no statistically significant ($P = 0.05$) differences in infection resulted. The ratios of smutted to total wheat heads were 14/834 (1.7%), 10/616 (1.6%), 2/320 (0.63%), and 10/624 (1.6%) for inoculum concentrations of 0.02, 0.2, 2.0, and 20.0%, respectively. This is consistent with the lack of association between inoculum levels and subsequent infection that has been observed in the field (9). When the data were examined according to the stage of flowering at inoculation, significant differences in infection were detected. The ratios of smutted heads to total wheat heads were 7/870 (0.80%), 25/996 (2.5%), and 4/609 (0.66%) for the preanthesis (RNS 12 to 15), anthesis (RNS 16 to 18), and postanthesis (RNS 19 to 22) growth stages, respectively. Thus, heads inoculated at anthesis had 3.1 and 3.8 times more infection than those inoculated before and after anthesis, respectively. Because florets open during anthesis, while those at preanthesis and postanthesis do not, accessibility to the interior of the floret, rather than inoculum concentration, may have limited infection in this experiment. This suggests that infection rates might be increased by clipping the glumes or stroking the wheat heads before inoculation to increase accessibility. In a previous study, 1 g of spores per liter of water produced maximum infection when the spore suspension was forced into wheat florets in vacuo, and infection decreased with increasing dilutions of the spore suspension (5).

In an experiment in which heads were stroked to stimulate floret opening prior to inoculation, florets were observed to open in response to stroking, and infection was increased dramatically relative to the previous experiment using the same inoculum level. The incidence of smutted heads was higher with longer wetting periods, but differences between treatments were not statistically significant. In this case, the ratios of smutted to total heads examined were 68/554 (12.3%), 164/1,106 (14.8%), 120/1,012 (11.9%), 162/877 (18.5%), 122/726 (16.8%), and 131/573 (22.9%) for wet periods of 0, 2, 4, 8, 16, and 32 hr, respectively. Significant infection in the absence of misting indicates that wetting is not necessary for infection by spores contained inside the floret.

High rates of infection also were obtained by clipping the glumes to expose the embryos to inoculum. The rate in clipped florets was increased over the rate in unclipped florets at all growth stages except those heads in midflower (RNS 17) (Fig. 1). Mean infection levels ranged from 13.6 to 31.4% for clipped florets and from 1.1 to 11.9% for unclipped florets. When data were grouped according to the stage of anthesis at the time of inoculation (preanthesis, anthesis, and postanthesis), infection of clipped florets was significantly greater ($P = 0.05$) than for unclipped florets at all three stages (Table 1). Clipping florets reduces the effect of growth stage on infection, suggesting that the embryo is susceptible at all growth stages studied and that the period of susceptibility to natural infection is determined by floret opening. This is consistent with studies which show embryos susceptible in most stages of development when inoculum is introduced directly into florets. However, the stage of maximum susceptibility has varied from preanthesis to postanthesis, depending on the inoculation technique used (2,4,5).

Ambient relative humidities inside the glass chimneys were 13.5, 71.8, 96.4, and 98.9%. Germination of spores both inside and outside florets increased with increasing ambient relative humidity and ranged from 0 to 49.7% and 0 to 10.7%, respectively (Table 2). Germination at the two highest relative humidities was significantly ($P = 0.05$) greater inside florets than in ambient air. This suggests

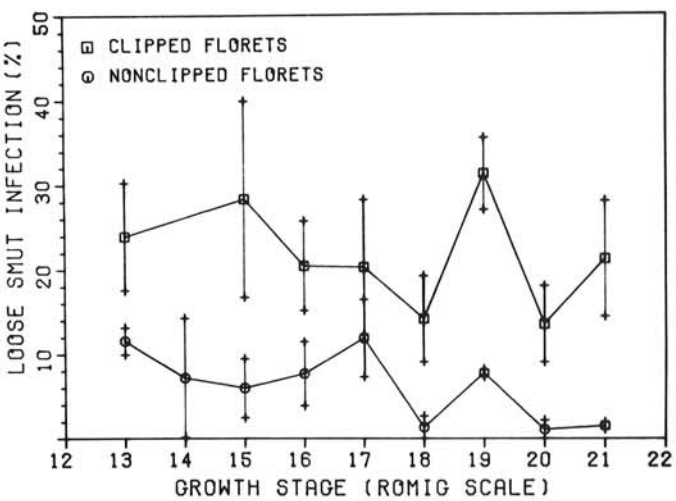

Fig. 1. Infection of clipped and unclipped florets of Genessee wheat heads by *Ustilago tritici* in relation to the stage of head development. Vertical lines indicate standard deviations of the mean.

TABLE 1. Effects on infection of the wheat cultivar Genessee by *Ustilago tritici* caused by clipping the glumes to expose the embryos to inoculum at different stages of development

	Growth stage range[x]		
Infection	12–15 (preanthesis)	16–18 (anthesis)	19–22 (postanthesis)
Unclipped			
Ratio[y]	19/292	30/355	24/585
Percent[z]	6.5 a	8.5 a	4.1 a
Clipped			
Ratio	38/193	56/302	107/533
Percent	19.7 b	18.5 b	20.1 b

[x] Romig numerical scale for assessment of wheat growth.
[y] Number of smutted heads over total number of wheat heads.
[z] Means in the same column followed by the same letter are not different by Duncan's multiple range test, $P = 0.05$.

TABLE 2. Germination of teliospores of *Ustilago tritici* on Millipore filter squares inside and outside of Genessee wheat florets in response to ambient vapor pressure

	Teliospore germination at indicated relative humidity			
Location	13.5%	71.8%	96.4%	98.9%
Outside floret	0.0 a[z]	0.0 a	4.1 a	10.7 a
Inside floret	0.0 a	8.4 a	42.3 b	49.7 b

[z] Means in the same column followed by the same letter are not different by Duncan's multiple range test, $P = 0.05$.

that conditions suitable for spore germination may be present inside florets even though ambient conditions may be inadequate or suboptimal.

This study confirms that floret opening is an important factor in the epidemiology of loose smut of wheat and suggests that accessibility of the embryo is more critical to infection than inoculum concentration or duration of wetness. The need to coordinate floret opening and spore dispersal for good infection may explain the sporadic occurrence of loose smut in Michigan fields.

Future investigations of loose smut infection should consider the importance of floret opening. Breeding programs that screen wheat cultivars for loose smut resistance by placing spores directly into florets may overlook the mechanical resistance of restricted floret opening. The development of wheat cultivars with florets that remain open longer for the production of hybrid wheat seed may increase susceptibility to loose smut.

LITERATURE CITED

1. Atkins, I. M., Merke, O. G., Porter, K. B., Lahr, K. A., and Weibel, D. E. 1963. The influence of environment on loose smut percentages, reinfection, and grain yields of winter wheat at four locations in Texas. Plant Dis. Rep. 47:192-196.
2. Batts, C. C. V. 1955. Loose smut (*Ustilago tritici*) [Pers.] Rostr.) of wheat: Physiological specialization and reaction of varieties in England. Ann. Appl. Biol. 43:533-537.
3. Calpouzos, L., Roelfs, A. P., Madson, M. E., Martin, F. B., Welsh, J. R., and Wilcoxson, R. D. 1976. A new model to measure yield losses caused by stem rust in spring wheat. Minn. Agric. Exp. Stn. Tech. Bull. 307. 22 pp.
4. Gothwal, B. D. 1972. Proper stage for the inoculation of wheat plants with loose smut pathogen. Indian J. Mycol. Plant Pathol. 2:89.
5. Oort A. J. P. 1939. Inoculation experiments with loose smuts of wheat and barley (*Ustilago tritici* and *U. nuda*). Phytopathology 29:717-728.
6. Tapke, V. F. 1929. The influence of varietal resistance, sap acidity, and certain environmental factors on the occurrence of loose smut in wheat. J. Agric. Res. 39:313-339.
7. Tapke, V. F. 1931. Influence of humidity on floral infection of wheat and barley by loose smut. J. Agric. Res. 43:503-516.
8. Tapke, V. F. 1948. Environment and the cereal smuts. Bot. Rev. 14:359-412.
9. Tyler, L. J. 1965. Failure of loose smut to build up in winter wheats exposed to abundant inoculum naturally disseminated. Plant Dis. Rep. 49:239-241.