THE RELATION OF WHEAT CULTIVAR AND DISTANCE FROM AN INOCULUM SOURCE TO THE AMOUNT OF UREDO-SPORES RETAINED ON LEAF SURFACES AND RESULTING SEVERITY OF INFECTION e •

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CHAPTER I

INTRODUCTION

Leaf rust of wheat is a chronic and costly disease of wheat almost every year in the state of Oklahoma. This disease is spread by means of airborne uredospores which are blown by the wind from one region to another and from one area to another from within the same field. The distance and amount of uredospores moved by the wind and their deposition upon wheat plants in relation to distance from an inoculum source could have a direct effect upon leaf rust severity and, ultimately, wheat production throughout the state.

Celik (10) studied the relation of cultivar and distance from an inoculum source to the severity of leaf rust infection. He found that leaf rust severity which ultimately developed was related to distance from an inoculum source, velocity of wind, and wheat cultivar. In his study, plants closest to and most distant from the inoculum source developed the least severity. He also found a significant difference between the amount of infection that developed on two cultivars of wheat both of which produced high infection types with the culture used in his experiments.

This study was made to determine the cause of the different levels of severity on the two cultivars studied by Celik. The objectives were: (1) to determine the relationship between distance from an inoculum source and the amount of spores retained on the leaf surfaces when

uredospores of the leaf rust fungus were blown past seedling wheat plants; (2) to determine the relationship between the number of spores deposited on the leaf surface and the number of infections developed; and (3) to determine the relationship between the number of stomata on the adaxial and abaxial leaf surfaces of the two wheat cultivars and the number of spores desposited, and the number of infections that developed.

CHAPTER II

REVIEW OF LITERATURE

No other class of crop equals cereals in contributing to man's welfare, and no type of plant disease causes a loss comparable to that resulting from the rusts of cereals. Waterhouse (34) in 1936 estimated total annual loss for the main cereal growing areas of the world at \$500,000,000. Wheat leaf rust occurs wherever wheat is grown and is the most common and widely distributed of all cereal rusts. Some times epidemics of this disease become severe. An example was the leaf rust epidemic of 1938 which caused an estimated \$12,000,000 loss in Oklahoma (11).

The spread of rust from one host to another by means of wind-borne uredospores has had general recognition for a long time. A. P. DeCandolle in France and Sir Joseph Banks in England writing at the beginning of the 19th century took this method of spore distribution for granted. It was not until 1882 that Ward first demonstrated the action of the wind in his studies of the coffee disease (<u>Hemileia vastatrix</u>) in Ceylon, by catching uredospores on slides coated with glycerine (1).

The uredospores, which are almost exclusively responsible for the dissemination of wheat leaf rust, have no special mechanism for their expulsion from the uredia. On maturity, however, they are easily dislodged by the slightest air currents or vibrations.

Chester (12) in 1938 reported that leaf rust does not cover summer in Oklahoma but passes the summer months in other parts of North America

and returns via northerly winds and is deposited on fall sown wheat. This completes a cycle from crop to crop by remaining only in the uredial stage of the fungus.

Each uredium produces an average of some 2,000 spores each day for a period of about two weeks and heavily infected leaves may have 1,000 pustules. Assuming two infected leaves per tiller, an acre of heavily infected wheat would produce, in two weeks, about 100,000,000,000,000 spores. Upon liberation, the great majority of spores will not fall on susceptible wheat leaves. Some of those which do will have lost their viability. Others may be deposited on wheat leaves but lack environmental requirements for germination and infection. The enormous production of spores is counteracted by the failure of the vast majority of them to accomplish infection. If this were not so, wheat might long ago have become extinct (11).

Fungi with this type of spore production and distribution are economically destructive and have a good chance for biological success. The rapid increase of disease development in epidemics of cereal rusts is probably due at least in part to this highly efficient method of spore production and distribution. The uredospores are very effective as dispersal units and may remain viable for a long time (11). Although a delicate balance exists between the host-pathogen-environment interaction in the ensuing epidemic development, nevertheless, the most important factor in rust epidemic development is uredospore dispersal over wide areas (14).

Autogenous and exogenous inocula may be found within any given field (2). The former being from spores disseminated within the field from established infections and the latter from spores carried to the

field from outside sources of infection. Autogenous inoculum generally exceeds exogenous inoculum because of the dilution by distance from the source, after the initial stage of an epidemic. As a result, the rate of rust development within a field of susceptible wheat is governed ultimately by the dispersal of autogenous inoculum.

The dispersal of spores is a function of both vertical and horizontal forces of gravity and air movement (8). The first stage in dispersal is the liberation of spores from the structures in which they were formed. Uredospores of the leaf rust fungus are produced above the leaf surface where minute air currents can dislodge the mature spore.

Chamberlin (13), Gregory (18), and Schrodter (29) have all developed theories on spore dispersal. These theories were derived from: (1) the movement of spores and particles in relatively short wind tunnels; (2) the dissemination of spores released from point sources in the field; (3) the study of plant disease gradients; and (4) consideration of the physical laws of particle movement. Although these theories have been used to describe gradients from natural sources, empirical evaluations have been lacking. For example, wind turbulence, a most important factor under field conditions is minimal in wind tunnels; spore dispersal gradients from point sources are known to be steeper than those from area sources (18); plant disease gradients of the cereal rusts are dependent not only on spore dispersal but also on resistance or susceptibility, germination and growth of the pathogen and favorable environmental conditions each of which may often be a greater limitation than spore dispersal gradients.

Ukkleberg (33) in 1933 was the first to make a precise study of the rate of free fall of leaf rust uredospores in calm air. He found much

variation in the rate of fall of individual spores, averaging 12.62 mm/ sec. At this rate of fall, it would take a leaf rust uredospores six hours forty-three minutes to fall 1,000 feet.

McCubbin (21) related the dispersal of uredospores of <u>Cronartium</u> <u>ribicola</u> to their rate of fall in still air. Bilham (4), in England, reported from 3,625 observations at two locations that wind speeds over 96 percent of the time were above 0.3 m/sec. He found the highest speeds were from 3.5 to 7.9 m/sec. If such observations are generally applicable, the wind speeds are usually 100 times, and commonly 300 times as great as the rate of fall of spores under gravity. Knowing the altitude of spores, their rate of fall, and the wind velocity, one can calculate the theoretical distance to which the spores could be carried before coming to rest on the earth's surface.

Christensen (14) calculated the theoretical dispersal distance of spores falling under gravity from a height of one mile in a 20 m.p.h. wind to be 740 miles for uredospores of about 28 x 17 u. Ukkelberg (33) in 1933 has shown that theoretically a leaf rust uredospore of wheat at 500 feet elevation in a 30 m.p.h. wind would travel 1,000 miles of horizontal distance before reaching the ground. Numarov (23) in 1939 considered the theoretical limit of dissemination of <u>Puccinia triticina</u> uredospores to be 1,000 - 12,000 km. Stephanov (30), in 1935 has calculated the average limit of dissemination of the rust as 641 km. and the absolute limit of dissemination support these calculations. Undoubtedly wind is a major factor in controlling spores dispersal and may also play a part in the number of spores liberated into the air.

The degree and extent of local downwind uredospore travel depends

upon wind velocity, time of day, number of spores produced by the infected plant, and the location of pustules on the plant (2).

Rusakow (27) in 1929 reported trapping an average of 127 spores in a 15 m/sec. wind and only 30 in a 8.5 m/sec. wind. Boevski (5) showed that the direction of wheat field relative to the source of infection is fully as important as its distance from the source of infection. He also illustrated the effectiveness of barriers in preventing local rust dissemination. He observed the following: at 14 feet from the source there was half as much rust as at the source; at 245 feet one fourth as much; and at 700 feet only one eighth as much (28).

Upon deposition, the uredospore of leaf rust germinates forming a germ tube which penetrates the susceptible host plant and establishes infection by way of the stomata (31). Hart (17) indicated that the resistance of certain wheat cultivars to stem rust under field conditions may be due to the behavior of their stomata since this rust can only penetrate open stomata. Peterson (25) found some difference in stomatal behavior, but not enough to account for the differences in rust reaction on cultivars used in his test. Todd and his associates (32) studying wind velocity in relation to physiological activities in wheat found that wind velocities of four to five m/sec. significantly affected stomatal opening and closing.

Caldwell and Stone (9) studied the relation of the stomatal aperature at the time of penetration to the entrance of leaf rust into its host. In their study, they found that closed stomata offered no impediment to penetration of wheat seedlings by the leaf rust pathogen.

CHAPTER III

MATERIALS AND METHODS

A culture of race UN2 (3) of <u>Puccinia recondita</u> Rob. ex. Desm. f. sp. <u>tritici</u> Eriks. was used in this investigation. This culture was increased from a stock culture maintained in liquid nitrogen storage (19) at Oklahoma State University, Stillwater. This race has comprised approximately 50 to 70 percent of the leaf rust fungus population in the State of Oklahoma for many years. $\frac{1}{}$

The two cultivars of wheat, <u>Triticum aestivum</u> L. cm Thell., used in this investigation had a susceptible reaction to this culture in the seedling stage. One cultivar, Danne C. I. 13876 was released in 1970 by the Oklahoma Agricultural Experiment Station and the Crops Research Division, Agricultural Research Services, U. S. Department of Agriculture. It is a selection from the material bequeathed to Oklahoma State University in 1959 by a private plant breeder, the late Joseph E. Danne of El Reno, Oklahoma. Danne is one of the most widely grown cultivars in Oklahoma and has a high yield potential.

Triumph 64 CL 13679 (TMP64) (6) is also a selection produced by Mr. Danne and released by him with the name "Rust Resistant Triumph." In 1964, the seed source was purified and released by the Oklahoma Agricultural Experiment Station with the name Triumph 64.

H. C. Young, Jr., personal communication.

Air movement for the experiment was provided by a Dayton forward curved centrifugal fan Model 2C-944-9 modified at the outlet to provide a uniform air velocity of about 10 m/sec. (Figure 2).

Five distances from the inoculum source in increments of 25 cm., beginning with 25 cm. were used in this experiment. In this study, distance represented whole plots and cultivars were arranged in a complete randomized design. This design is shown in Figure 1.

Dista	ince	Replication		Randomization	of Cultivars
25	cm	1 <u>1</u> /		1	2
50	cm	2	·**	2	1
50	Cm	1	- 	2 2	1
75	cm	1 2	1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 -	1	2
100	cm	1		2	1 2
100	Cm	2		1	2
125	cm	1		2	1
		2		1	Z

Figure 1. A Diagram of the Experimental Design $\frac{1}{1}$ = cultivar Triumph 64 and 2 = cultivar Danne

Forty "Arasan" (50 percent Thiram) treated seeds of each cultivar were planted in each of ten 10 cm. pots. Each pot was filled to approximately 85 percent capabity with uniformly mixed soil composed of five parts clay loam, two parts sand, and one part Canadian sphagnum peat moss. The seeds were uniformly distributed on top of the soil surface and

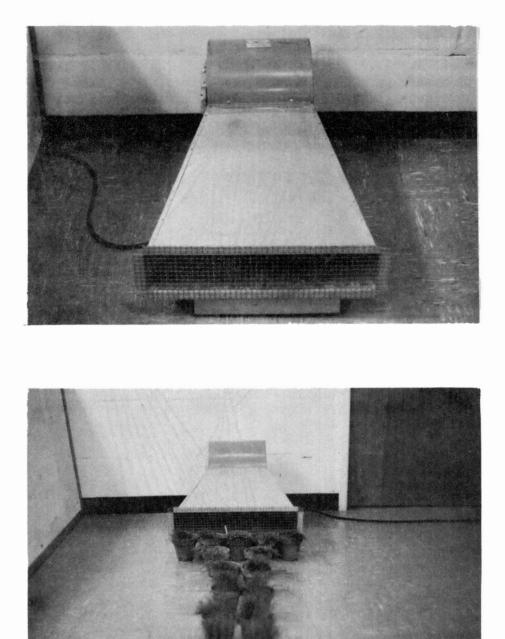


Figure 2. Dayton Forward Curved Centrifugal Fan Model 2C-944-9. Without (above) and With (below) Inoculum in Place. firmly covered with an additional 90 gm. of the soil mixture. Water was slowly added to each pot until a maximum holding capacity was attained.

The pots were placed in a greenhouse maintained at $21 \pm 3C$ for seven days at which time each pot was thinned to 30 plants. Beginning the second day after planting the pots were watered as needed. Soil moisture was maintained near optimum and the plants did not suffer from moisture stress during the course of this work.

The inoculum was obtained by increasing uredospores on the cultivar "Cheyenne" CI 8885. The first increase was made by dipping pots (7) of eight day old seedling plants in water on which uredospores of the culture were dispersed. Then, ten days after this inoculation, these rusted plants were used to brush inoculate other pots of seedlings which would be used as the inoculum source in the experiment.

Experimental inoculations were made when the two cultivars to be inoculated were seven days old and when the inoculum was ten days old. There were two replications in this experiment, and they were both inoculated in the same manner. The source of inoculum was placed adjacent to the outlet opening of the fan. The spores from the inoculum source were then dispersed by a 30 sec. air blast from the fan outlet (Figure 3).

An attempt was made to count the number of spores retained on the leaf surface after inoculation without removal of the leaves for placement on microscope slides. However, undulations on the leaf surface and twisting of the leaf blade prevented adequate microscopic examination; therefore, at the end of the air blast, five leaves from these inoculated plants were then selected at random from the 30 plants in each pot. The leaves were carefully removed with forceps and surgical scissors for a minimum disturbance of spores retained on the leaf surface. These leaves

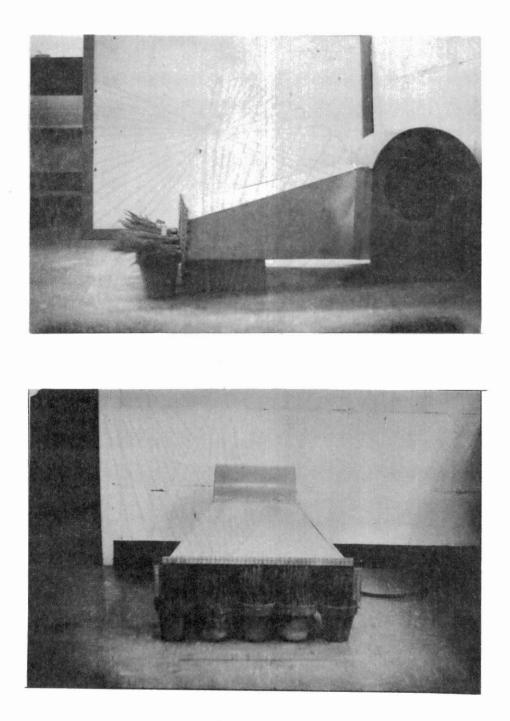


Figure 3. Inoculation Process in Operation.

were then placed on slides and a spore count was made. The remainder of the inoculated plants were then placed in glass covered moist chambers for a period of 12 hours after which they were moved to growth chambers (Percival model E-30) for ten days. At the end of that time five leaves were again randomly selected from each pot and the number of pustules were counted.

A microscopic examination of leaves of the two cultivars was also undertaken to determine the number of stomates per unit area. Seven leaves from ten day old seedlings of each cultivar were removed, placed in a clear saran wrap covering and run through a stationary LiCar portable area meter (Model LI-3000) for a measurement of leaf size. The leaves were then painted with a mixture of half clear nail polish and acetone (22). Upon drying, the film was removed and a count of the positive images of stomata was made.

CHAPTER IV

RESULTS

Distance from the inoculum source did effect the amount of spores retained on the leaf surface of both cultivars. Highest spore concentration was found closest to the inoculum source (25 cm.) while the lightest spore load was found farthest from the inoculum source (125 cm., Table I, Figure 4).

The severity of leaf rust pustule development followed the same trend. The largest number of pustules developed on plants closest to the inoculum source and decreased with distance from the source to a minimum at the longest distance (Table II, Figure 5). There was no significant interaction between distance and cultivar in the test as far as spore retention on the leaves or subsequent pustule development was concerned.

There was no significant difference between cultivars in spore retention (Table IV). However, there was a significant difference between cultivars in relation to ensuing leaf rust development. The largest number of leaf rust pustules was found on the cultivar Danne and the differences between Danne and Triumph 64 at all distances from the inoculum source were significant statistically (Table III).

There was no significant difference between the number of stomata on the adaxial and abaxial leaf surfaces of the cultivars, Danne and Triumph 64 (Table V).

TABLE I

THE NUMBER OF SPORES RETAINED PER SQUARE CENTIMETER OF LEAF SURFACE OF TWO CULTIVARS LOCATED AT FIVE DISTANCES FROM AN INOCULUM SOURCE WHICH WAS BLOWN OVER THE LEAVES BY A 10 M/SEC. WIND

		Distance from I	noculum Se	urce in Cm.	
Cultivar	25	50	75	100	125
Danne	515	192	116	166	86
Triumph 64	562	138	129	59	44

TABLE II

THE NUMBER OF PUSTULES PER SQUARE CENTIMETER OF LEAF SURFACE OF TWO CULTIVARS LOCATED AT FIVE DISTANCES FROM AN INOCULUM SOURCE WHICH WAS BLOWN OVER THE LEAVES BY A 10 M/SEC. WIND

		Distance fro	m Inøculum S	ource in Cm.	• • • • • • • •
Cultivar	25	50	75	100	125
Danne	95	75	59	52	51
Triumph 64	59	51	51	33	34

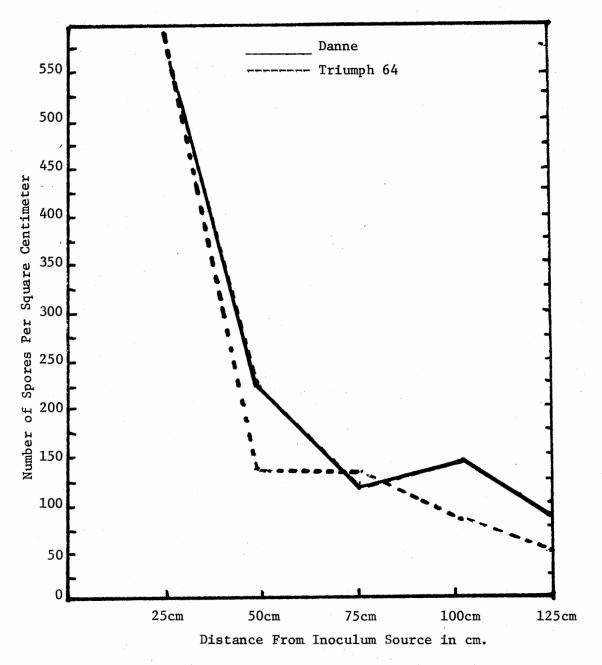


Figure 4. Number of Uredospores Per Square Centimeter Deposited on Leaves of Two Cultivars of Wheat at Five Distances From an Inoculum Source by a 10 m/sec. Wind.

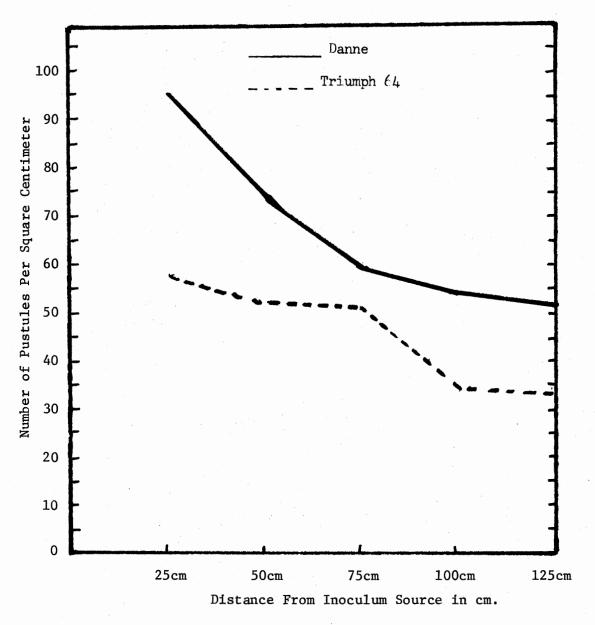


Figure 5. Number of Pustules Per Square Centimeter of Leaf Surface of Two Cultivars of Wheat at Five Distances From an Inoculum Source Dispersed by a 10 m/sec. Wind.

TABLE III

THE NUMBER OF PUSTULES DEVELOPED PER SQUARE CENTIMETER OF LEAF SURFACE FOR TWO CULTIVARS LOCATED AT FIVE DISTANCES FROM AN INOCULUM SOURCE BLOWN OVER THE PLANTS BY A 10 M/SEC. WIND

	Distar						
Cultivar	25	50	75	100	125	x _	<u>1</u>
Danne	95	75	59	52	51		67
Triumph 64	59	51	51	33	34		46
<u>x</u> <u>1</u>	77	63	55	43	43		· · · · · · · · · · · · ·

¹LSD ().05) for comparing cultivar means = 12.

 2 LSD (0.05) for comparing distance means = 19.

TABLE IV

THE NUMBER OF SPORES DEPOSITED PER SQUARE CENTIMETER OF LEAF SURFACE FOR TWO CULTIVARS LOCATED AT FIVE DISTANCES FROM AN INOCULUM SOURCE BLOWN OVER THE PLANTS BY A 10 M/SEC, WIND

	Distan						
Cultivar	25	50	75	100	125	x	<u>1</u>
Danne	515	192	116	166	86		215
Triumph 64	562	138	129	59	44		186
<u>x</u> 2	539	165	123	113	65		

¹LSD (0.05) for comparing cultivar means = 39.

 2 LSD (0.05) for comparing distance means = 62.

TABLE V

THE NUMBER OF STOMATA PER SQUARE CENTIMETER ON THE ADAXIAL AND ABAXIAL SURFACE OF TWO WHEAT CULTIVARS

		Number of Ct	moto/8a Cm	
			omata/Sq. Cm. face	
	A	daxial		baxial
Leaf	Danne	Triumph 64	Danne	Triumph 64
1	4,000	3,120	2,346	2,040
2	3,488	4,053	2,152	1,962
3	4,080	4,069	2,386	2,106
4	4,200	4,280	2,229	2,057
5	3,881	4,667	2,346	2,040
6	4,606	4,183	2,152	1,962
7	4,157	5,000	2,386	2,106

CHAPTER V

DISCUSSION

Plants near to the inoculum source (at 25 cm.) had the greatest spore retention. In fact, plants near to the inoculum source retained a spore concentration approximately three times that of the plants at the next closest distance (at 50 cm.) and seven times that of the plants most distant from the inoculum (at 125 cm.). The deposition of spores, then, followed the pattern that one would anticipate with heavy deposition at the closest distance and a proportional reduction of spore deposition with distance from the source. This was true for both cultivars, Danne and Triumph 64. The manner in which this situation would relate to actual field distribution of autogenous inocula would warrant further study. However, the situation might be somewhat different since eddies and turbulence would be expected to have some amount of influence upon deposition.

Leaf rust pustule development followed the same trend as that established for spore deposition, with heavy pustule development at the closest distance (25 cm.) and a proportional reduction in pustule development with distance from the inoculum source.

This was in contrast to that of Celik (10) where he found that cultivars nearest to and most distant from the inoculum source developed the least leaf rust severity. Celik used five cultivars in his study resulting in more plants in the air flow and might more closely approximate an

actual open field situation than this study where only two cultivars were used. However, in Celik's tests a wall was adjacent to one side of the fan providing the wind blast for distribution of inocula, and this factor may have established air flow distortion. Certainly, the contrasting results of the two series of experiments would suggest further study of the effect of plant spacing and other factors affecting eddies and turbulence.

My study did confirm that more pustules developed on the cultivar Danne than on Triumph 64, as Celik had found. Since it appeared that the same number of spores was deposited on both cultivars it was thought that some morphological difference between the two cultivars might explain the difference in numbers of pustules that developed. Therefore, a count of stomata on both adaxial and abaxial leaf surfaces was undertaken. The result of this count was that there was no significant difference between the two cultivars on either leaf surface. However, it was found that both Danne and Triumph 64 had larger number of stomata on the upper surface than was anticipated (25).

Since the number of stomata did not appear to influence leaf rust severity, other factors such as pubescence or some other morphological difference in leaf structure must be responsible for the difference between cultivars and would warrant further study.

Both Danne and Triumph 64 have no known genes for specific resistance (15, 16, 24). However, it is possible that Triumph 64 might have a gene for resistance, unrecognized because of the lack of a pathogen culture to identify it. If this were true, then that gene might also influence the severity of infection, as has been shown by Martin (20).

CHAPTER IV

SUMMARY

1. Two wheat cultivars (Danne and Triumph 64) were used to study the relationship between distance from an inoculum source to the amount of spores retained on the leaf surface and the ensuing wheat leaf rust development. A count of the stomata per unit leaf area was made for both cultivars.

2. Spore retention for both cultivars followed the same model with the greatest spore numbers closest to the inoculum source. Spore retention was similar for both cultivars.

3. The largest number of pustules also was found on plants closest to the inoculum source. However, there were more pustules found on Danne than on Triumph 64 at each distance and the difference between the two cultivars was significant statistically.

4. There was no significant difference between the two cultivars in the amount of stomata present on either adaxial or abaxial leaf surface, although both cultivars had more stomata on the adaxial than on the abaxial leaf surface.

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