A STUDY OF RUST DEVELOPMENT AMONG FOUR CULTIVARS OF *TRITICUM AESTIVUM* L. AND FOUR RACES OF *PUCCINIA RECONDITA* ROB. EX. DESM.

By

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CHAPTER I

INTRODUCTION

Rust resistant cultivars of wheat, *Triticum aestivum* L., have been under development since before the turn of the century. However, specific resistance to wheat leaf rust, *Puccinia recondita* Rob. ex. Desm. f. sp. *tritici*, in the United States has only been emphasized since about 1940. No other control of the leaf rust fungus seems to be as economical as the use of resistant cultivars. Any cultivar or selection possessing a characteristic that reduces the probability of new infection occurring must be considered resistant (12). Specific resistance reduces a cultivar's infectability by means of genetic incompatibility between the host and certain races of the pathogen. On the other hand, cultivars that have a uniform resistance against all the races of a pathogen provide a non-specific type of resistance (21, 29, 33). This uniform resistance may function in many ways and may actually be a combination of genes for specific resistance (4, 33). Terms, such as "slow-rusting" are merely brief descriptions of the behavior of this non-specific resistance.

Thus, wheat cultivars exhibiting the "slow-rusting" type of resistance still may have a high infection type with certain or all known rust races but are infected to a lesser degree in the field than cultivars without this characteristic (18, 27). The nature of this characteristic of "slow-rusting" is not well established, but it may involve the physical exclusion of the parasite, its slow growth and development, or reduction of total spore production due to reduced intensity or duration of sporulation (14).

It is not unusual to observe slight differences in incubation time for plant cultivar-disease pathogen combinations that in other respects appear to be equally compatible in disease development (12, 15). The ability to delay infection of all races would seem to have the potential of outlasting race specific resistance. Similarly, the ability of certain pathogen types to survive in populations may be dependent upon their ability to germinate and penetrate more rapidly (10). This study was made to evaluate leaf rust disease development when four wheat cultivars and four leaf rust races were paired in all combinations.

CHAPTER II

LITERATURE REVIEW

Wheat leaf rust was distinguished from the other cereal rusts by DeCandolle over 150 years ago. Since that time, our knowledge of the disease has steadily increased. Vast amounts of facts and theories have accumulated, one of these being the idea of host resistance for disease control. The "slow-rusting" form of disease resistance has received much attention recently, but the idea is not necessarily new. Farrer (11) became aware of this slow rate of rust development as early as 1898. He stated, "...I look upon lateness in becoming affected by it [rust pathogen] as a measure of resistance to the parasite; ...when it has less time for injuring the grain." Marryat (20), in 1906, observed pustule development on two wheat cultivars. She found well-developed pustules on one cultivar 13 days after inoculation, and on another not until 18 days after inoculation. She concluded that fungus growth was retarded by either thicker cell walls or fewer stomata.

Brown and Shipton (5) reported that rust races will differ in their ability to penetrate depending on the host-pathogen combination. They believed that this interaction, not stomatal number or structure, controlled penetration.

Lapwood (18) noted that differences in host-parasite interactions caused changes in the amount of rust sporulation and proposed that the slower rate of infection should be attributed to reduced production of spores, not host resistance.

Variance in the amount of rust infection produced by different cultivars may make a major difference in the level of rust infection during the growing season. Bolley and Pritchard (2), on the other hand, observed that urediospores produced on various host cultivars had different colors and "strength of growth". They recommended carefully conducted infection experiments dealing only with spores from an individual pustule when studying this type of interaction of host and parasite.

Hayden (13) discovered infectibility differences among cultivars early in the growing season that tended to become obscurred at maturity. He thought this factor might be responsible for notable differences in grain yield of supposedly rust-susceptible wheat cultivars. Mont and Rowell (21) determined that cultivars they studied did have variable "receptivity" to rust infection which was dependent on both age of the plant and type of tissue inoculated. They believe that low receptivity should be considered when developing resistant cultivars. Mont and Rowell listed many factors influencing rust development, one of which was the time needed between infection and onset of sporulation. Gallegly and Niederhauser (12) found variation between cultivars in this respect and referred to the phenomenon as "incubation resistance". In 1967, Katsuya and Green (16) examined the incubation period of two P. graminis races and found that one race began sporulation two days before the other. Johnston (15) found similar results with P. recondita as early as 1930. They both noted that the race with the shorter incubation period would have the advantage of earlier spore dispersal and, possibly, more uredinial cycles. Consequently, cultivars with the ability to delay this period can reduce the amount of inoculum produced. Shaner (24) has observed wheat cultivars with a slow-mildewing chacteristic. He suggested combining this character with other mechanisms of resistance to attain more stable resistance. Vowinckel (30) also discovered that potato varieties with general resistance to Phytophthora infestans caused the pathogen to produce smaller lesions and extended its incubation period. Stakman and Rodenhiser (26) found that it requires a greater amount of inoculum, a longer period of time, and more suitable environmental conditions for stem rust to damage wheat cultivars with generalized resistance.

Rowell and McVey (23) established that the slow-rusting character can be distinguished in the field and therefore would be useful in breeding programs. Luke, Chapman, and Barnett (19), however, cautioned that slow rusting may be linked to late maturity and should be considered when this type of resistance is used.

Browder (3, 4) pointed out that a single specific gene for resistance probably will provide only efemeral control of rust if used extensively. However, a combination of these genes may actually be the basis for certain types of "slow-rusting" resistance. He describes certain slow-rusting characters as being determined by "a gene or genes for low reaction which is easily measurable through infection types produced by different parasite cultures and which can be overcome by genes for high pathogenicity". If the gene or genes for low reaction in the host are overcome, the host may become "faster rusting".

CHAPTER III

MATERIALS AND METHODS

The wheat cultivars used in this study were the hard red winter wheats; Danne CI 13876, Wichita CI 11952, Triumph 64 CI 13679, and Scout 66 CI 13996. All of them are currently used for commercial production in Oklahoma. Danne, Wichita, and Triumph 64 do not possess any known genes for resistance to *P. recondita* and are susceptible in the seedling stage to the races used in this study. Scout 66 has one or more genes for resistance to the leaf rust fungus, but is susceptible to the races used here.

The races of *Puccinia recondita* f. sp. *tritici* used were UN 1, UN 2AAG, UN 6B, and UN 13A. With the exception of UN 1, these races were isolated from wheat fields in Oklahoma. Race UN 1 was obtained from Dr. L. E. Browder, USDA, ARS, Kansas State University. So far as is known at this time, races UN 6B and UN 13A have the most genes for pathogenicity while race UN 1 has the least. Race UN 2AAG is a relatively new race and has genes for pathogenicity on LR 3 and LR 24 at least. Identification of these races was made using differential cultivars and a diagnostic key published by Basile (1), and amended as follows: (1) "A" = virulence on the cultivar Westar C. I. 12110 (LR 10);

(2) "AG" = virulence on the cultivar Agent C. I. 13523 (LR 24); and (3) "B" = virulence on both Westar and Wesel C. I. 13090.

Urediospores required for the various inoculations in this study were produced on the wheat cultivar, Cheyenne C. I. 8885. Approximately 15 - 20 seeds of this cultivar were planted in each of five pots. After seven to eight days, the seedlings were inoculated by the brushing technique described by Browder (3) except when race UN 1 was used. In that case, the dipping method was used since the spores had been stored in liquid nitrogen. After inoculation, the plants were sprayed again with water plus a surfactant (Tween-20, polyoxyethelene 20 sorbitan monolaurate), placed in a moist chamber and covered with a sheet of glass. The inoculated seedlings were incubated for 15 - 18 hours at 15 - 20 C and then placed in a 60 cm. x 60 cm. x 45 cm. muslin cage in a greenhouse at 20 $\pm$ 4 C. The top of the cage was covered with clear plastic (mylar). After 10 - 12 days, fresh urediospores were available for inoculation of the test cultivars.

All cultivars to be tested were grown in 9 cm. x 11 cm. plastic cups. Ten 50% Thiram (Arasan) treated seeds of each cultivar were planted in each of eight cups filled with a clay loam soil mixture. Each cup was coded according to the cultivar and race used so that they could be placed at random in a growth chamber (Sherer-Gillet Model CEL 25 7HL) at 20 $\pm$ 2 C, with a light intensity of 21,500 lux and a 12 hour photoperiod. Just prior to inoculation, the eight-day old seedlings were thinned to four plants per cup. Inoculation of the test cultivar was accomplished with the brush technique, as before. When the seedlings were transferred from the moist chamber to the growth chamber, the length of each primary leaf was measured.

Six replications of a Quasi-latin square were used. Two replications were run simultaneously in each of three trials. Each replication contained three variables: race, cultivar, and position.

Plants were checked for visible signs of infection beginning 26 hours after inoculation and continued at six hour intervals for about 200 hours. Cups of plants were removed from the growth chamber individually, examined and replaced immediately. The number of hours after inoculation required for the first visible fleck to appear, was recorded for each race - cultivar combination. Observations continued until the first pustule had erupted, at which time the number of pustules on each individual primary leaf were counted with the aid of a hand lens (10x). Only the adaxial side of the leaf was inspected.

CHAPTER IV

RESULTS

There were no significant differences between either races or cultivars in the time required for the first sign of rust infection (flecking) to appear. These data are given in Tables I and II. Therefore, the mean number of hours from inoculation to first visible sign of infection (fleck) for all race-cultivar combinations used in this study can be designated as a single value (46.6 hours).

The number of pustules at the time of first sporulation was totaled for each race-cultivar combination (six replications, four leaves each) since analysis indicated no significant difference between trials. These data are presented in Table III (all races on each cultivar), and Table IV (each race on all cultivars). No significant differences were found between either cultivars or races.

When the time from inoculation to the appearance of the first pustule was considered, a significant difference appeared. These data are presented in Tables V and VI. Triumph 64 differed significantly from the three other cultivars tested. The mean time for the appearance of the first spores for Triumph 64 (172 hours) is considerably longer than for any of the other cultivars, regardless of

TABLE I

THE NUMBER OF HOURS BETWEEN INOCULATION AND FIRST SIGN OF RUST INFECTION (FLECK) FOR EACH OF FOUR WHEAT CULTIVARS INOCULATED WITH FOUR RACES OF PUCCINIA RECONDITA

	REPLICATION						
Cultivar	1	2	3	4	5	6	Mean 1/
Danne	41 2/	43	58	46	49	58	49
Scout 66	44	44	44	46	46	49	45
Triumph 64	44	41	55	50	43	53	48
Wichita	49	50	41	44	47	38	45

1/ Mean number of hours for 4 primary leaves of 6 replications of 4 races (96 leaves). Differences are not statistically significant.

2/ Mean number of hours for 4 primary leaves of 4 races (16 leaves).

TABLE II

THE NUMBER OF HOURS BETWEEN INOCULATION AND FIRST SIGN OF RUST INFECTION (FLECK) FOR EACH OF FOUR RACES OF *PUCCINIA RECONDITA* ON FOUR WHEAT CULTIVARS

	REPLICATION						
Race	1	2	3	4	5	6	Mean[1/]
UN 1	44[2/]	47	55	35	44	56	47
UN 2AAG	44	49	44	52	44	46	46
UN 6B	44	43	38	47	49	47	45
UN 13A	46	40	61	52	47	49	49

1/ Mean number of hours for 4 primary leaves of 6 replications of 4 cultivars (96 leaves). Differences are not statistically significant.

2/ Mean number of hours for 4 primary leaves of 4 cultivars (16 leaves).

TABLE III

THE NUMBER OF PUSTULES ON THE PRIMARY LEAF AT THE TIME OF FIRST SPORULATION ON EACH OF FOUR WHEAT CULTIVARS INOCULATED WITH FOUR RACES OF *PUCCINIA RECONDITA*

	REPLICATION						
Cultivar	1	2	3	4	5	6	Mean 1/
Danne	53 2/	67	29	16	35	31	38
Scout 66	57	66	7	27	54	40	42
Triumph 64	68	47	19	18	36	43	38
Wichita	74	58	18	19	23	39	39

1/ Mean number of pustules on 4 primary leaves of 6 replications of 4 races (96 leaves). Differences are not statistically significant.

2/ Mean number of pustules on 4 primary leaves of 4 races (16 leaves).

TABLE IV

THE NUMBER OF PUSTULES ON THE PRIMARY LEAF AT THE TIME OF FIRST SPORULATION PRODUCED BY EACH OF FOUR RACES OF *PUCCINIA RECONDITA* ON FOUR WHEAT CULTIVARS

	REPLICATION						
Race	1	2	3	4	5	6	Mean[1/]
UN 1	53[2/]	35	16	24	33	37	33
UN 2AAG	46	73	22	18	36	31	38
UN 6B	95	65	21	17	49	37	47
UN 13A	57	64	15	21	32	49	39

1/ Mean number of pustules on 4 primary leaves of 6 replications of 4 cultivars (96 leaves). Differences are not statistically significant.

2/ Mean number of pustules on 4 primary leaves of 4 cultivars (16 leaves).

TABLE V

THE NUMBER OF HOURS REQUIRED FOR THE FORMATION OF THE FIRST PUSTULE ON EACH OF FOUR WHEAT CULTIVARS INOCULATED WITH FOUR RACES OF PUCCINIA RECONDITA

	REPLICATION						
Cultivar	1	2	3	4	5	6	Mean1/
Danne	146 2/	154	168	160	178	178	163
Scout 66	158	166	157	152	178	170	163
Triumph 64	172	166	163	161	185	185	172
Wichita	161	167	158	157	173	179	165

LSD (0.05) for comparing cultivar means = 4.7

1/ Mean number of hours for 4 primary leaves of 6 replications of 4 races (96 leaves).

2/ Mean number of hours for 4 primary leaves of 4 races (16 leaves).

TABLE VI

THE NUMBER OF HOURS REQUIRED FOR THE FORMATION OF THE FIRST PUSTULE BY EACH OF FOUR RACES OF *PUCCINIA RECONDITA* ON FOUR WHEAT CULTIVARS

	REPLICATION						
Race	1	2	3	4	5	6	Mean[1/]
UN 1	155[2/]	163	160	155	185	182	167
UN 2AAG	160	167	160	158	185	182	169
UN 6B	161	154	158	154	172	170	161
UN 13A	161	169	163	163	172	178	167

LSD (0.05) for comparing race means = 4.7

1/ Mean number of hours for 4 primary leaves of 6 replications of 4 cultivars (96 leaves).

2/ Mean number of hours for 4 primary leaves of 4 cultivars (16 leaves).

the race of the pathogen used. This is shown diagrammatically in Figure 1. This incubation period was always the longest on Triumph 64 and the shortest on Danne although the latter cultivar was not significantly different from either Wichita or Scout 66.

Races UN 1, UN 2AAG, and UN 13A were found to be statistically equal in their incubation periods (inoculation to first spore production) on these four wheat cultivars. Race UN 6B, however, proved significantly more virulent on all cultivars having a significantly shorter incubation period than any of the other races (Figure 2).

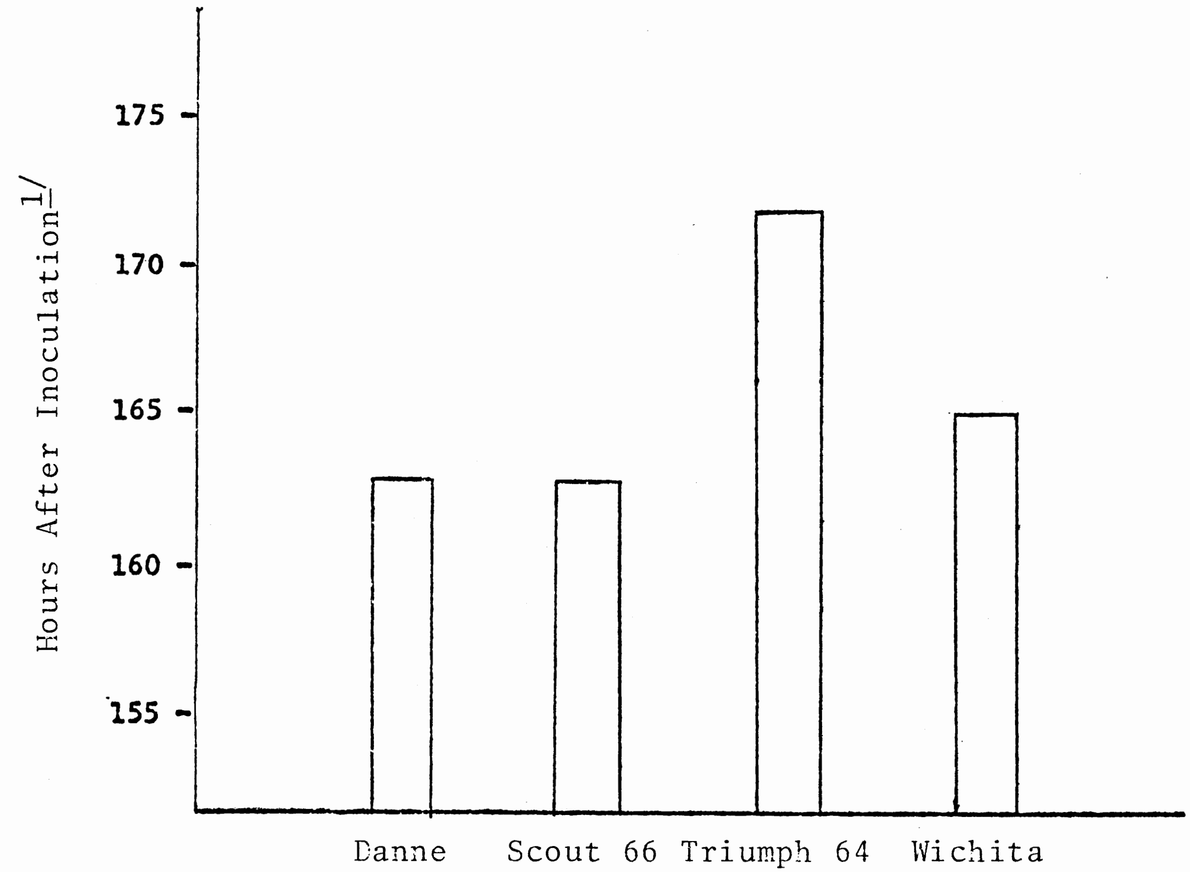

Figure 1. The Number of Hours Required for the Formation of the First Pustule on Each of Four Wheat Cultivars Inoculated with Four Races of *Puccinia recondita*.

1/ Mean Number of Hours for 4 Primary Leaves of 6 Replications of 4 Races (96 Leaves).

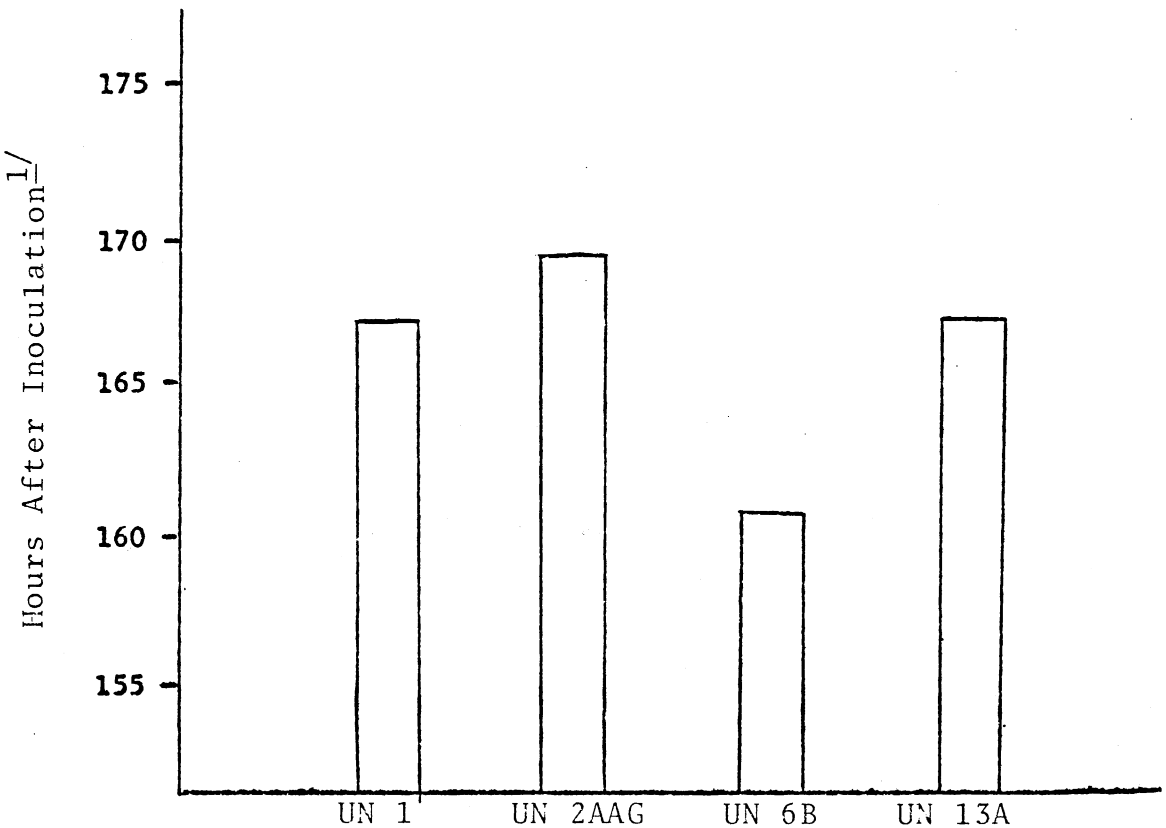

Figure 2. The Number of Hours Required for the Formation of the First Pustule by Each of Four Races of *Puccinia recondita* on Four Wheat Cultivars.

1/ Mean Number of Hours for 4 Primary Leaves of 6 Replications of 4 Cultivars (96 Leaves).

CHAPTER V

DISCUSSION

Extending the incubation period of the rust fungus slows the rate at which the fungus can reproduce and reach epidemic proportions. This ability to delay the time from infection to sporulation depended on the cultivar in this study irrespective of the pathogen race. Thus, delayed sporulation takes its place along with slower rust penetration, slower pustule development, and low receptivity as a form of "slow-rusting resistance" (21, 23, 28).

Although in this study there was only a few hours difference between the incubation period of Triumph 64 and the other cultivars used, it may be enough to give that cultivar an advantage over others under field conditions, and it has been noted in past years that Triumph 64 has consistently had lower leaf rust severity readings in field plots in Oklahoma than the cultivar Danne, for example (33).

It is interesting that there was also a difference in the incubation period (inoculation to first appearance of spores) among races, regardless of cultivar. Race UN 6B clearly had the shortest incubation period of any race used in the study. This is all the more significant since UN 6B only represents a small fraction of the rust population on host cultivars completely susceptible to it (33). It would normally be expected to be a major part of the population if this characteristic alone was considered. Perhaps some other factor limits the increased prevalence of this race.

It was found that all the races used in this study produced about the same number of pustules at the time of first sporulation on all of the cultivars tested. Excessive inoculum amounts used in these experiments could, however, mask the "slow-rusting" characteristic of these cultivars (29). This may be another expression of the same phenomenon reported by Celik (8) and Smith (25) who found that fewer pustules were produced on Triumph 64 than on Danne when equal inoculum was blown past wetted seedlings.

As a result of these studies, one type of "slow-rusting" resistance can be detected in wheat seedlings. Cultivars with the "slow-rusting" characteristic of shorter incubation period (inoculation to first spore production) may be identified in the seedling stage with only one race of the pathogen.

CHAPTER VI

SUMMARY

1. Four wheat cultivars (Danne, Scout 66, Triumph 64, and Wichita), and four leaf rust races (UN 1, UN 2AAG, UN 6B, and UN 13A) were used to study the effect of race - cultivar combinations on wheat leaf rust development.

2. All cultivars were equal in the number of hours between inoculation and first appearance of infection (flecks).

3. All races were equal in the number of hours required from inoculation to first appearance of infection (flecks).

4. The number of pustules developed by the time of the appearance of the first spores was the same for all cultivars and for all races.

5. Appearance of the first fruiting pustule was consistently later on the cultivar Triumph 64 than on the other three cultivars used.

6. Race UN 6B invariable produced fruiting pustules earlier than any other race.

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