Interaction of genes and environment conditioning inheritance of stripe rust resistance of wheat
by Robert Thomas Lewellen

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Genetics
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Abstract:
Seedling infection types of four wheat varieties and their F1, F2, F3, testcross, and testcross-F2 distributions were infected with a single unidentified race of Puccinia striiformis and used to study the inheritance of stripe rust resistance genes, their interaction with each other, and with temperature. Two rigidly controlled environment chambers were used, temperature being the important variable. Diurnal temperature profiles with gradual inclines and declines were used which simulated spring and fall conditions, 2 C night/18 C day, and early to mid-summer conditions, 15 C night/24 C day.

Wheat varieties P. I. 178383 (ovo) and Chinese 166 (ovo) had dominant genes for resistance which conditioned a ovo infection type. The heterozygote of these factors conditioned a oo to 0 infection type. The double heterozygote conditioned a ovo infection type. In addition, these varieties had minor genes which conditioned 0, 1, 2, and 3 infection types in certain additive combinations.

Rego (0 at 2/18 and 3 at 15/24) had a pair of dominant complementary genes which conditioned a 3 infection type at both profiles. However, at 2/18 Rego had temperature sensitive minor genes which conditioned a O infection type in combination with the two complementary genes.

Lemhi (4) was considered to be completely void of resistance genes.

Distributions of segregating progenies showed a significant interaction with the temperature profile. The minor factors from either P. I. 178383 or Chinese 166 conditioned greater resistance at 15/24 than at 2/18 and had just the opposite response of Rego's temperature sensitive factors. Combinations of factors from these parents were additive at both temperature profiles. These minor factors were not entirely in common and transgressive segregation was observed for increased resistance.

The minor factors were also effective in causing the major factors to condition greater resistance, particularly for the heterozygote of either major gene.

The infection types used, ovo, oo, 0-, 0, 1, 2, 3, and 4 were found to have a genetic basis and were good indications of the genotype at the controlled conditions. The infection type distribution for each cross at each temperature was unique as to proportion of different types and range of expression of various factors or combinations of factors.

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in

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ABSTRACT

Seedling infection types of four wheat varieties and their F_1, F_2, F_3, testcross, and testcross-F_2 distributions were infected with a single unidentified race of Puccinia striiformis and used to study the inheritance of stripe rust resistance genes, their interaction with each other, and with temperature. Two rigidly controlled environment chambers were used, temperature being the important variable. Diurnal temperature profiles with gradual inclines and declines were used which simulated spring and fall conditions, 2 C night/18 C day, and early to mid-summer conditions, 15 C night/24 C day.

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INTRODUCTION

Since the beginning of agriculture, man has selected and reseeded the most acceptable forms of a particular crop, including types resistant to disease. As a result of planting disease resistant types, genes which condition resistance have been accumulated into the present gene pool. The pathologist and plant breeder uses this gene pool to incorporate the desired disease resistance into agronomically desirable crop varieties.

In order for these factors to be efficiently and effectively employed in a breeding program, it would be ideal to have the resistance genes cataloged; that is, to know where these genes are located, how many there are, how they are inherited, what relationships and interactions they have with each other and possibly with other factors, and how the environment influences their potential expression of resistance.

Various aspects of disease resistance were studied in this investigation with the emphasis placed upon the influence of controlled temperature regimes upon the reaction of segregating wheat populations to stripe rust (Puccinia striiformis West). Two diurnal temperature profiles, 2 C night/18 C day and 15 C night/24 C day, were used to study the inheritance of stripe rust resistance. These two profiles simulated the two important environmental patterns present in areas where stripe rust is an economically important disease. The first profile, 2/18, is similar to the spring and fall conditions in which primary and early secondary infections occur. The second profile, 15/24, is similar to conditions in early or mid-summer in which economic losses caused by this disease would be greatest.

The influence of environment on the reaction of cereal varieties to rust fungi is well known. However, most disease resistance studies have been carried out in the field or greenhouse with little or no control of environmental factors, except possibly a constant temperature regime. When constant temperatures are used, an unnatural situation is created for both the host and pathogen. By using controlled environment chambers with simulated diurnal profiles for temperature, light, and relative humidity, the infection type conditioned in response to a specific host-pathogen interaction should be accurately expressed. Sharp (1965) has determined that the response of a host-pathogen complex at a constant temperature was different from the response at a diurnal temperature profile for stripe rust. He observed that the simulated diurnal profile matched the response of certain varieties to field conditions, whereas constant temperatures did not necessarily do so.

The use of broad response classifications for a host-parasite interaction has been a common practice in disease resistance inheritance studies. For example, in the rust diseases of cereals infection types 1 through 2 are generally considered resistant and infection types 3 and 4 are considered susceptible, regardless of the cross or situation. In the present study the infection types conditioned by each cross were investigated at both temperature profiles in an attempt to determine the association between the same genotypes at the two profiles.

REVIEW OF LITERATURE

Stripe rust is most commonly found in areas of the world that have a northwest marine or similar climate. This is the most destructive wheat disease in Europe (Lupton and Macer, 1962) and in the highlands of Northern India (Bahl and Kohli, 1960). It is found in cool intermountain areas (Orjuela, 1956) and the cool southern plains (Vallega, 1955) of South America, in the mountainous areas of Japan and China (Kajiwara, Ueda, and Iwata, 1964), and in the coastal and intermountain valleys of northwestern North America. A general discussion of the distribution of this disease and the favorable climatic conditions was given by Dickson (1956).

The causal organism of stripe rust is Puccinia striiformis West. The life cycle of this basidiomycete is similar to the organism that causes black stem rust of wheat (Puccinia graminis f. sp. tritici Eriks. & Henn.) except that the aecial stage is unknown. The life cycle of P. striiformis was studied in the United States by Hungerford (1923). He concluded that the fungus overwinters in the uredial stage, or may overwinter as mycelium in cereal or grass seedlings, especially when there is sufficient snow cover. He also determined that the hot, dry months of July and August were critical periods for the survival from one crop year to the next and that fall infection was important for heavy epiphytotics to occur the following year. Sharp and Hehn (1963) studied the manner in which this organism survived the winters in the Intermountain Valleys of Montana. They concluded that it overwintered as dormant mycelium in live leaves of winter wheat seedlings and that the native grasses were probably of little importance.

Just as the other rust fungi exhibit specialized forms and physiological races, so does P. striiformis. Hungerford and Owens (1923) found that there were probably two distinct forms, tritici and hordei, in the Northwest.

Within the form tritici many races have been defined. Hungerford and Owens (1923) found that the urediospores collected on one cereal crop, cereal variety, or grass would not always infect different cereal crops, cereal varieties, or grasses. Gassner and Straib (1932) and Straib (1937) found many races in Germany and Europe. Vallega (1955) reported that the races of Chile were different from those found in Argentina or Europe. Bever (1934) found that there were at least two races in the United States. Kajiwara et al (1964) showed that different races occurred on the several islands of Japan and that these races were generally different from those found on the China mainland.

Sharp (1965) reported that only a single race occurred in Montana when the standard differentials were used. However, Purdy and Allan (1963b) gave evidence for several races in Washington. Fuchs (1966) determined that the spore samples of Montana and California gave different infection patterns. Race 13 which is virulent on Chinese 166 was positively identified, but the predominant race appeared to be different and needed more experimentation before description.

Using two resistant and two susceptible varieties of both wheat and barley, Bever (1937) indicated that stripe rust reduced the yield of grain,

stunted root size, and influenced the water efficiency of both susceptible varieties of wheat and barley.

Allan and Vogel (1962) obtained a highly significant correlation of -0.494 between yield and stripe rust reaction when they used near isogenic lines of wheat. Allan, Vogel, and Purdy (1963) showed that yield and test weight losses caused by stripe rust were greater in susceptible short-strawed varieties than in standard-height varieties.

McNeal and Sharp (1963) used moderately resistant Thatcher spring wheat as a check and obtained a highly significant correlation coefficient of -0.839 between yield and the August coefficient of infection. Lemhi 53 sufferred a 34.4% loss, while other susceptible spring wheat varieties suffered losses in excess of 23%. They attributed no reduction in test weight to the epidemic of stripe rust.

However, Pope, Sharp, and Fenwick (1963) estimated yield losses as high as 60% and test weights as low as 48 pounds per bushel for Lemhi in Southern Idaho. In the mountain valleys of Montana, they estimated winter wheat losses from 25 to 60% on susceptible varieties.

Sunderman and Wise (1964) stated that stripe rust lowered the yield of susceptible plants by 37.5%, of which 19% was due to lower kernal weight and 17.6% was due to fewer seeds. The grain from these plants also had 1.7% lower protein content, and several test values used to measure the quality of flour were affected. They suggested that plant breeders should be cognizant of the effect of stripe rust on varieties in evaluation of nurseries, otherwise erroneous conclusions may be reached about

the milling and baking data.

Shortly after the rediscovery of Mendel's Laws in 1900, Biffen (1905) demonstrated that resistance to stripe rust was inherited in a Mendelian fashion. This was the first instance in which disease resistance was shown to segregate in a Mendelian ratio. In this classic experiment the very susceptible Red King (Triticum vulgare) was crossed with resistant Rivet (T. turgidum) to produce susceptible F_1 plants. In the F_2 there was clearly a one resistant to three susceptible segregation. In the F_3 the resistant plants remained resistant and the susceptible plants segregated for resistance and susceptibility. This clearly indicated that one recessive gene conditioned resistance. In a later experiment, Biffen (1907) showed that the immunity of American Club was also inherited as a single Mendelian recessive in a cross with susceptible Michigan Bronze.

Nilsson-Ehle (1911) was the next to show that resistance to stripe rust in cereal varieties was inherited in a Mendelian manner. Using parents that were different from those used by Biffen, he obtained data which indicated multigenic rather than monogenic inheritance for resistance.

Despite these three early demonstrations of genes for stripe rust resistance, the inheritance studies for this disease have lagged behind those for most of the other important cereal diseases. This may have been partially due to the sporadic occurance of stripe rust, or to the fact that several races have complicated the inheritance patterns, or to the difficulty in handling the inoculum.

Unlike stripe rust resistance studies, many cases have been reported for resistance to stem rust and leaf rust of wheat and barley and crown rust of oats. The early studies were summarized by Ausemus (1943). Active groups have continuously studied resistance to these diseases in most countries where they are prevalent.

Armstrong (1922) was next to report a factor for resistance to P. striiformis in wheat. In a cross of Wilhelmina x American Club, he also obtained a three to one segregation for susceptible to immune plants in the F_2 of adult plants.

Rudorf (1929) was the first to use seedling plants and a greenhouse to carry out resistance studies to stripe rust. He showed that resistance at the seedling stage was simply inherited and defined a number of resistant varieties. He showed that the hypersensitive reaction of Chinese 166 was unaffected by environmental conditions, and he was the first to demonstrate that this reaction was inherited as a dominant.

Straib (1934; 1937) also demonstrated that Chinese 166's immunity was inherited as a single dominant character to all 18 physiological races to which it was subjected. Other resistant varieties were also shown to be controlled by one or more dominant genes to various races.

In a study to determine if the factors that conditioned stem rust reaction were the same as those that conditioned stripe rust reaction in several crosses, Neatby (1936) found that the seedling reaction to P. striiformis was closely associated with the mature plant reaction to P.

graminis, and he concluded that the relationships were probably due to a plieotropic effect of the genes concerned rather than to genetic linkage.

Bever (1938) studied the varietal reaction to stripe rust of 317 American wheat varieties including common, club, durum, emmer, poulard, and polonicum wheats and 1284 foreign introductions. He found that the common white group was more susceptible than either the soft red winter, hard red winter, or durum groups. The durum group was the most resistant. The club wheats, as a whole, were the most susceptible, there being only one resistant variety in the entire group.

Pal (1951) and Pal, Sikka, and Rao (1956) made a number of crosses between varieties resistant to the Indian races and found that single dominant genes were responsible for the resistance of Chinese 166, Spalding's Prolific and Carsten's V, and they found that there was a trifactorial inheritance with the resistant Frondoso with susceptible NP. 789.

Favret and Vallega (1953) reported that Chinese 166 had one dominant factor for resistance to the Argentina races. Later Vallega (1955) indicated that the races of Chile were able to overcome the resistance of this variety. Fuchs (1966) reported that race 13 of the United States was also virulent on Chinese 166. Kajiwara et al (1964) also showed that the races of Japan were virulent to Chinese 166.

Konzak et al (1956) irradiated the susceptible Gabo variety of wheat and induced a resistant true-breeding line. The mutation was inherited as a recessive. The Gabo parent had a 4 infection type while the mutant

possessed a 1 infection type.

Allan and Vogel (1961) showed that one dominant gene controlled adult resistance of Suwon 92 to the prevalent race that occurred in Eastern Washington at that time.

Singh and Dhillon (1963) found that the resistance of Rio Negro, Centaria, and La Prevision was controlled by a single dominant gene and that resistance in Frontana was controlled by two dominant genes.

Gosh, Sikka, and Rao (1958) found a single dominant gene governing resistance of Cometa Klein to the most prevalent Indian races.

Bahl and Kohli (1960) reported the results of two crosses using two resistant parents, Cometa Klein and Rio Negro, and two races of P. striiformis, 13 and H. The resistance of Cometa Klein against both races was controlled by two pair of complementary genes, both of which are contributed by Cometa Klein. A nine to seven ratio was obtained with seedling F_2 plants. The genetic constitution of Cometa Klein was proposed to be $R_1R_1R_2R_2$ and that of the susceptible parent as $r_1r_1r_2r_2$. R_1 and R_2 were independent, and both were required to condition resistance. Although this cross fit nine to seven ratios for both races, the genes contributing to resistance appeared to be different on the basis of a chi-square test of independence on 97 F_3 families.

In the second cross reported by Bahl and Kohli, the inheritance of resistance of Rio Negro to race 13 in a cross with Pb. C. 518 revealed that interaction of a gene for resistance and an inhibitory gene was

involved. Rio Negro contributed the dominant gene R for resistance while Pb. C. 518 contributed the dominant inhibitory gene, I. The presence of R and the absence of I were necessary for resistance. The resistant parent then had the genotype RRii and the susceptible parent the genotype rrII. There was an F_2 ratio of 3 resistant to 13 susceptible. Infection types were read according to the scheme of Gassner and Straib (1932). Infection types 0, 1, and 2 were considered resistant, and types 3 and 4 were considered susceptible. The actual infection types of the parents, F_1, or F_2 seedling plants were not given.

In a later paper by Nambisan and Kohli (1961), Cometa Klein was again reported to have two dominant complementary genes for seedling resistance to races 13 and H. They also showed that the resistant variety La Prevision had a dominant gene which was inhibited by a dominant gene of Selection NP. 770.

The earlier results of Singh and Swaminathan (1959) were not entirely in agreement with the later findings of Bahl and Kohli (1960) and Nambisan and Kohli (1961). Using monosomic analysis, they identified three recessive genes on three chromosomes of Cometa Klein that conditioned resistance to P. striiformis. They reported that recessive factors which controlled resistance to race H were located on chromosomes IV and VI and one recessive gene which controlled resistance to race 13 was located on chromosome IX. In this monosomic study Cometa Klein was crossed with the 21 monosomes of Chinese Spring. All 21 F_1 lines were susceptible. The

F_2 deviations from the expected were used to locate the chromosomes carrying resistant factors. With race H, recessive complementary genes were expected to condition resistance, or there should have been a seven resistant to nine susceptible ratio. With race 13, a single homozygous recessive gene was expected to condition resistance, or the F_2 data were fit to a one resistant to three susceptible ratio. These results were nearly opposite those obtained by Bahl and Kohli (1960).

These deviations are unexplained unless the infection types that were considered resistant or susceptible varied. For this study, susceptible infection types were considered to be 3 and 4 types while resistant types were considered to be 0, 1, and 2 types. Plants that were between types 2 and 3 were grouped separately. The manner in which these separate studies were classified into response groups may then account for the differences in the proposed genetic systems involved. Since it is also known that some varieties respond differently at different temperatures (Sharp, 1965), there may have been enough temperature variation to significantly change the apparent results between the two studies.

Up to the time of Lupton and Macer's (1962) report, there had not been a published account of the relationship between the various factors that condition resistance to P. striiformis. No deliberate effort had been made to find the commonness of factors between the resistant varieties or the allelism that may occur for resistance to different races. Also, except for the account of Singh and Swaminathan (1959), the linkage

relationships were not known.

To determine the relationship between genes for resistance in some of the important wheat varieties grown in England, Lupton and Macer made a diallel set of crosses between seven varieties. In the F_1, F_2, and F_3 of these crosses, the rust reaction to four physiological races was used to identify seven genes conferring resistance at four loci. In general, they found resistance to be inherited as a dominant character, although cases of recessive resistance to very aggressive races of the rust organism occurred.

In this study, seedling plants were divided into susceptible and re-resistant groups. Plants having infection types 0 or 1 were classified resistant, and those having infection types 2, 3, or 4 were classified susceptible for all crosses. Gassner and Straib's (1932) scheme was used. The plants were grown in greenhouses and, except for one year, were subjected to wide temperature variations. The greenhouse was maintained at a constant 60 $\pm$ 5 F during the last year of the experiment. The results of chi-square tests of goodness of fit to many of the F_2 ratios were very poor.

They found that Chinese 166 possessed a single gene, Yr_1, for resistance to all races. Heine VII and Soissonais possessed the same gene, Yr_2, for resistance to three of the four races. Holdfast was the susceptible variety and possessed no genes for resistance. Cappelle had a third dominant gene Yr_{3a}, for resistance to two of the races, and two recessive

genes yr_{3a} and yr_{4a}, for resistance to a third race. Hybrid 46 carried two dominant factors for resistance, Yr_{3b} and Yr_{4b}, one being allelic to the Cappelle factor, Yr_{3a}. Hybrid 46 may also have had an allele of the second Cappelle locus, Yr_{4b}.

Lupton and Macer stated, "Resistance is thus determined by a relatively simple major gene system, similar to that shown by Knott and Anderson (1956) to determine the reactions of wheat to stem rust."

Even after this account, the interactions between resistant loci in the same plant were very meagerly known. The majority of papers reported one, two, or three dominant or recessive factors for resistance. Simple cases of interallelic action were reported by Bahl and Kohli (1960), Nambison and Kohli (1961), and Singh and Swaminathan (1959). Lupton and Macer (1962) also noted gene dosage effects between two dominant genes to give greater resistance. However, all of these reports supposedly dealt with major genes, and resistance or susceptibility fell into qualitative classes. The influence of minor or modifying genes that more accurately fall into a quantitative system were not discussed.

The first possible mention of quantitative inheritance of stripe rust resistance was by Allan et al (1963). Studying the influence of stripe rust on yield of closely related lines, they noted that some fourth-generation backcross lines of (Norin 10 - Brevor 14) x Burt were more resistant than the susceptible parents in 1962. This moderate level of field resistance indicated that complementary gene action was involved in

the expression of resistance between susceptible or moderately susceptible varieties and that, in effect, transgressive segregation for greater adult field resistance had occurred.

Purdy and Allan (1963a) found that seedling reactions were generally indicative of the mature plant reaction, although the adult reaction of certain semi-dwarf selections could not be reliably predicted at the seedling stage.

In a later study, Allan et al (1966) determined the relationship between seedling and adult resistance. They found that an association existed between adult and seedling reactions for every cross studied. There were two crosses, however, Itana x Spinkcota and Dickson 114 x Itana, that had major discrepancies between field and greenhouse reactions. Generally, more lines were resistant as adult plants than as seedlings. They also encountered transgressive segregation for adult resistance to stripe rust in the Itana x Burt cross. Simple monogenic inheritance occurred in crosses between C. I. 13431 x Golden, C. I. 13431 x Itana, and Spinkcota x Itana. Two complementary loci, or epistasis between loci in the greenhouse seedling stage, were found for Norin 10 - Brevor 14 x Nord Desprez, Spinkcota x Golden, and Dickson 114 x Itana. In all of the Dickson 114 crosses there were two or three loci involved in the seedling stage.

Using field observations on pure-line wheat varieties and their segregating progeny, Pope and Henriksen (1964; 1965), Henriksen and Pope (1965), and Pope (in press) found that in all wheat varieties observed, only Lemhi had no genes for resistance. All varieties with near total

susceptibility contributed a heritable resistance to stripe rust by transgressive segregation in appropriate crosses. Varieties that were intermediate for stripe rust reaction showed either no segregation (genes in common) or transgressive segregation, suggesting that all gene combinations were more or less additive. Reactions with the tested wheat varieties suggested at least 20 different resistance genes to P. striiformis. They stated that these genes were apparently recessive.

Using a controlled diurnal temperature profile of 15/24 C, Lewellen, Sharp, and Hehn (1965) showed that P. I. 178383 possessed a major dominant gene and several recessive genes for stripe rust resistance in seedling plants. By selecting F_2 plants that had an infection type 4 and growing out F_3 lines, they demonstrated that the minor factors in P. I. 178383 conditioned various levels of resistance and were probably additive.

Metzger and Silbaugh (1966), using monosomic analysis, indicated that P. I. 178383 carried a single dominant locus for resistance and that this gene is linked with the gene for brown glume color in P. I. 178383 with two crossover units distance. McCallum, Welsh, and Sharp (Personal communication, 1965) located this gene on chromosome 2B.

All of the investigations with stripe rust have been carried out under field conditions or in greenhouses where temperatures were either not controlled or were controlled at a single level. However, various investigations have shown that the resistance of some wheat varieties to P. striiformis is quite variable under changing environmental conditions. Gassner and Straib (1932) indicated that varieties resistant to P. glumarum

(P. striiformis) at 20 C were severely rusted at slightly lower temperatures. Newton et al (1933) found that all the wheat varieties tested were resistant to stripe rust at 78 F, but that many varieties were susceptible at 54 F.

Generally it was found and observed that increasing temperatures increased resistance, but Manners (1950) showed that high temperatures increased the susceptibility of some differential hosts to certain stripe rust races but decreased that of others. Sharp (1962a,b) indicated that the amount of infection or severity varied with different host-parasite combinations, but was usually greater when plants were grown at lower temperatures prior to inoculation. He suggested that physiological changes that influence the reaction of a particular race with a particular variety must take place before as well as after inoculation.

The influence of temperature upon the expression of resistance of wheat varieties to P. striiformis has been most accurately studied by Sharp (1965). The host plants were grown in walk-in environment chambers rigidly controlled and programmed for temperature, relative humidity, and light. Both constant and diurnal temperature profiles were investigated. In the latter, declines and inclines from extreme temperatures were gradual. A single unidentified race of P. striiformis was used for all inoculations.

Sharp found that the wheat varieties Westmont, Omar, Lemhi, and Idaed were all highly susceptible at a constant 15 C. As temperatures were

increased to 24 C, all varieties became resistant with Lemhi remaining the most susceptible at all temperatures. Using the diurnal temperature profiles, Sharp found that P. I. 178383 and Westmont showed no shift, and this tended to be the case for all varieties which were either highly resistant (P. I. 178383) or highly susceptible (Westmont). Turkey-1 and Idaed showed decreasing compatibility with increasing temperature profiles, whereas Rego reacted in just the opposite manner. He showed that the temperature during the dark period was most important.

Investigations to determine the inheritance of the temperature sensitive factors have not been reported in the literature. However, an active project is underway in Dr. Sharp's laboratory at Montana State University to determine the same as well as to more accurately determine the inheritance of the recessive minor factors which appear to be additive in conditioning stripe rust resistance.

Investigations to determine the quantitative nature of disease resistance and the influence of environment have been carried out for several other plant diseases. However, the general procedure has been to account for those resistances caused by major genes which are not influenced by the environment. The gene-for-gene systems first described by Flor (1956) for flax rust and later found to occur in late blight of potatoes (Toxopeus, 1956), powdery mildew of barley (Moseman, 1959), stem rust of wheat (Loegering and Powers, 1962; Williams, Gough, and Rondon, 1966), and theoretically for any disease (Person, 1959) have been based entirely upon these major dominant factors for resistance. As an example, in the recent studies

by Berg, Gough, and Williams (1963), Knott and Anderson (1956), Knott (1962; 1956), Rondon, Gough, and Williams (1966), and Sunderman and Ausemus (1963) with stem rust resistance only the major, dominant genes were described.

Green et al (1960) and Green and Knott (1962) have shown that minor or modifying genes were involved in the expression of stem rust resistance in both seedling and adult plants. In these studies, resistance genes were transferred into a completely susceptible background using substitution lines. They stated:

> "The reaction of the substitued lines and the Marquis lines carrying the same genes were similar, although the substituted lines were more susceptible to some races. With the Sr_{10} gene for resistance, resistance was lost with each backcross and the gene became increasingly difficult to detect. This gene was variable with temperature change and resistance was diminished at high temperatures."

Knott (1957a; 1957b) encountered the "modifier effect" in earlier work and explained:

> "The 'modifier effect' probably explains many of the difficulties encountered in maintaining full resistance while backcrossing to produce rust resistant varieties. While the genes reported determine whether a variety is or is not resistant to races of 15B and 56, the degree of resistance they condition is variable in different crosses, and it is not known whether the difference is due to specific modifiers or to the genetic environment in general. For example, Thatcher's resistance to race 56 is probably due to modifiers that this variety apparently possesses. These modifiers are necessary in connection with other major genes in order to get good resistance to race 15B."

Schafer et al (1963) used varieties with less than immune reactions to leaf rust of wheat (which, they stated, has generally not been done for

any rust disease) and showed that they could get better resistance from true breeding F_3, F_4, or F_5 lines than was apparent in either parent. All combinations of the moderately resistant parents yielded some lines with better resistance than either parent, even though the actual genetic constitutions of the parents were not known. They suggested that these factors may provide a better source of long term resistance than single major gene resistances that can be overcome by a single mutation in the pathogen.

A similar interaction of resistance factors was reported earlier by Finkner (1954) for crown rust of oats.

Diseases caused by rust fungi are not the only ones that respond in this manner. For example, Wasuwat and Walker (1961) reported that the degree of resistance to cucumber mosaic virus controlled by a major gene may be increased or reinforced by an undetermined number of modifying genes.

In the review paper by Wingard (1941) and the book by Walker (1957), the effects of environment upon the level of resistance in various host-pathogen interactions were discussed. However, few of these related to the rust diseases of cereals and most related to the diseases in which the pathogens were soil borne. There have been only a few accounts of the influence of environment on the cereal rusts since.

In a study to determine at which constant temperature a temperature sensitive variety changes response to several races of P. graminis, Bromfield (1961a) found that the breakdown period for seven sensitive

varieties was between 70 F and 77 F. In general, the varieties were resistant at 70 F, mixed in reaction at 72 F to 74 F, and susceptible at 76 F. In a later study Bromfield (1961b) determined that the temperature sensitive wheat varieties Kenya N. B. 263 and Lerna 52 were resistant to P. graminis at 70 F and when transferred to either a constant 77 F or 85 F did not retain the resistance. Conversely, varieties that were grown at constant 77 F or 85 F and moved to 70 F did not retain the susceptibility. He concluded that the effect of temperature was most likely on the host-parasite complex and not on either of the components independently.

The implication of results from Green and Johnson (1955) would be that specific temperature sensitive genes occur in the host, the parasite or both. In this study the reaction of adult plants of ten wheat varieties to ten races of stem rust at temperatures of about 60 F and 80 F was investigated. The temperature at which resistance or susceptibility for certain combinations of host variety and rust race broke down was specific. If a one to one genic relationship is valid for host-parasite interactions, then the differences observed by different combinations would be due to specific temperature sensitive factors present in one or both of the component parts.

The classic example of the influence of temperature upon disease expression is the reaction of cabbage to Fusarium oxysporium f. sp. conglutinans (Wr.) Snyder & Hansen (Walker and Smith, 1930; Blank, 1937; Walker, 1963). Two types of resistance were shown to exist in cabbage. Type A

resistance was shown to be monogenic, dominant, and stable to temperature changes. The type B resistance was shown to be multigenic, only partially dominant, and varied with the temperature. At a low soil temperature the type B conditioned resistance equal to that of type A, however, as soil temperatures increased the plants with type B resistance became more and more susceptible. It was shown that the more type B genes possessed by a variety of cabbage, the higher the soil temperature needed to be to break down the resistance.

Walker (1963) stated the same type of A and B resistances occur for fusarium wilts of tomato varieties and for some potato varieties resistant to some races of Phtyphthora infestans (Mont.) DeBy.

This second type of resistance (type B) is probably what accounts for field resistance in many varieties of agronomic and horticultural crops.

MATERIALS AND METHODS

Parent varieties used were P. I. 178383, Chinese 166, Lemhi (C. I. 11415), and Rego (C. I. 13181).

P. I. 178383 was screened from the world collection as a source of resistance to several races of dwarf bunt, Tilletia controversa Kühn, and it was subsequently found to possess resistance to stripe rust. It has been used as a parent in several breeding programs specifically designed to obtain an agronomically acceptable wheat that is stripe rust and/or bunt resistant. It was used as a parent in the new stripe rust resistant variety 'Moro'. Except for resistance to smut and rust, P. I. 178383 has little agronomic value because of undesirable field characteristics.

Chinese 166 has been the most commonly used variety for stripe rust inheritance studies. It was included by Gassner and Straib (1932) as one of the standard differentials. Lupton and Macer (1962) give the history of this variety, which was collected in Western China early in the 20th century. Chinese 166 is a winter wheat with soft, red kernels and has been used both as a variety and parent in Western Europe.

Lemhi is the most susceptible variety known to prevalent Northwestern races of P. striiformis (Pope, 1965; Sharp, 1965) and, therefore, it made an ideal susceptible parent for inheritance studies. It is a soft, white, spring wheat most commonly grown in the irrigated valleys of Southeastern Oregon, Southern Idaho, Nevada, and Utah. Lemhi was selected from a cross between Federation and Dicklow and released in 1939 by the Idaho Agricultural Experiment Station.

Rego was selected for this study because of its unusual temperature response in regard to stripe rust infection. Sharp (1965) showed this variety to be more incompatible to P. striiformis at low temperatures than at high temperatures. This hard, red, winter variety was selected from a Yogo x Rescue cross as a solid-stemmed, sawfly resistant variety by the Montana Agricultural Experiment Station and released in 1956. Since 1962 it has been grown on some Montana acreage because of its moderate resistance to stripe rust.

Originally, a diallel set of crosses was to be made with these varieties, with each F_1 being backcrossed to the parents, except where one of the parents was immune to P. striiformis. The Rego x Chinese 166 cross was missed. The parents were grown in a crossing block in a greenhouse bench in the spring and summer of 1963. The parents were grown from selfed plants which had previously been checked for uniform rust reactions.

The parents, F_1, F_2, F_3, testcross, and testcross-F_2 seedlings were used to determine the inheritance of resistance. All seedling plants checked for their infection type to stripe rust were grown in one of two controlled environment chambers described earlier by Sharp (1965).

> "The chambers were rigidly controlled and programmed for temperature, relative humidity, and light. Two diurnal temperature profiles were used in which declines and inclines from extreme temperatures were gradual. The low temperature profile was maintained at cyclical temperatures of 2/18 (2 C at night to 18 C during the day), and the high temperature profile was maintained at 15/24 (15 C at night to 24 C during the day). Relative humidity was about 95% during the dark period and 65% during the light. Photoperiods were 12 hours. Light intensities

were increased stepwise from 300 to 1,800 to a maximum of 3,500 ft-c at the middle of the photoperiod and then decreased through a similar range."

The same collection of inoculum was used for the entire experiment. The inoculum was collected in the nursery plots west of Bozeman in August, 1963. After germination tests, the inoculum was stored by lyophilization in a manner previously discussed by Sharp (1965) and Sharp and Smith (1952). Samples of this inoculum and cultures developed from single pustules and single spores of the collection were tested on both the standard differentials of Gassner and Straib (1932) and on supplemental wheat varieties and gave no evidence for more than one physiologic race (Sharp, 1965). This race is believed to be the same race which Fuchs (1966) was unable to describe and is probably still the predominant race in Montana.

The infection types used are basically those of Gassner and Straib (1932) with some modifications and additions. Gassner and Straib defined six infection types: i = immune, no evidence of infection; 0 = chlorosis or necrosis without pustules; 1 = chlorosis or necrosis with few very small pustules; 2 = chlorosis or necrosis with small pustules; and 3 = chlorosis without necrosis; and 4 = normal pustulation without chlorosis.

Instead of the i infection type, the $\overset{v}{o}o$ infection type was substituted. When even the most resistant plants were inoculated and given a proper dew period, there appeared very minute chlorotic flecks as evidence that the pathogen had penetrated but had very quickly been destroyed by the host tissue. The next most resistant infection type used was designated oo. The characteristic of this infection type was a larger,

symetric fleck, chlorotic or necrotic, about one to two times as large as the period at the end of this sentence. The 0- infection type was the next most resistant averaging midway between the oo and 0 types. This infection type was less symetric, generally necrotic, and larger than the oo, but did not span across the entire leaf. The 0 infection type is much as described earlier, but encompassed more of the leaf than any of the other previously mentioned types. These types, $\overset{y}{o}o$, oo, 0-, and 0, represent varying degrees of host-parasite hypersensitivity, with the $\overset{y}{o}o$ being so hypersensitive that immediate death occurs to the pathogen and probably to the layer of cells surrounded by the penetration tube in the host. Hypersensitivity then decreases to the 0 type in which death to both the host and pathogen cells are delayed, and a very incompatible interaction is established, but death eventually occurs to the parasite without pustulation occuring.

From the 0 type through the 4 type compatibility increases, until the most compatible relationship or interaction possible between a host and parasite is established in the 4 infection type. The infection types 1, 2, 3, and 4 were used much as defined by Gassner and Straib (1932). The 1 infection type was divided into 1- and 1 types. With some plants that first appear to be 0 types, a few pustules eventually form on the perimeters of the necrotic host tissue. These plants are described as having the 1- types. The 2 type was used as described. The 3 type was split into 3 and 3- types. With some plants, particularly those derived from Rego parentage, the decision to call the infection type 2 or 3 was

questionable, because of the nature of the chlorosis or necrosis. These plants were given the 3- infection type. Plants that were very susceptible were called 4 types. From observing the infection types 0 through 4, one begins to see reactions that no longer appear to be hypersensitive *per se* but begin to take on the appearance of a "nutritional basis" of incompatibility or compatibility, with a struggle being waged by the host, pathogen, or both to maintain themselves in a beneficial equilibrium.

Unlike most other rust fungi, *P. striiformis* grows vegetatively throughout a compatible host leaf by runner hyphae. Many infections are not necessary, because one infection will soon cover most of a leaf. The infection types were the only disease readings used. Severity (average per cent of leaf area infected) and prevalence (percentage of plants showing infection) were not used. Plants that were definite escapes were not counted.

Generally, twenty seeds were planted in a row across a four-inch clay pot containing Gallatin sandy loam with one-fourth part peat moss. The pots were subirrigated in galvanized steel trays. The seedlings were inoculated in the first leaf stage just as the secondary leaf began to appear. The leaves were bound in a horizontal position by the use of metal backboards and rubber bands. Inoculations were made in a modified settling tower (Sharp, 1965) in which a CO_2 pistol was used to disperse the urediospores and to allow an even distribution on the adaxil surface of the leaves. Two lyophilized tubes, each containing 40 mg of spores,

were used for each set of 15 to 20 pots. After allowing four minutes for the spores from the first tube to settle on the plants below, the pots were rotated 180° and the second tube was fired.

After the backboards were removed, the pots were placed in a dark dew chamber which is a small chamber within a larger, walk-in chamber. The air temperature of the walk-in chamber was maintained at a constant 2 C. In the small chamber, the water bath temperature was 10 C and the air temperature was 5 C, allowing ample dew to form on the leaves. After forty-eight hours the plants were returned to their original environment chamber. To check spore germination, either a collodion strip or a polyethylene membrane was used (Sharp, 1965).

The plants were scored for rust reaction when the pustules on the susceptible check were adequately developed. This required 14 to 16 days at 15/24 and 21 to 23 days at 2/18. Before reactions were read, the secondary and tertiary leaves were removed. The plants were scored twice to insure accurate readings. Plants that germinated late were generally removed and not counted.

Because the plants scored as seedlings were used to produce the following generation, specific seedling plants were marked with paper tags and saved. All unwanted plants were removed from the pot. The selected plants were moved to a vernalization chamber and maintained at a diurnal cycle of 2/10 C for six to eight weeks. The plants were then transplanted into eight-inch pots which were placed on greenhouse benches.

Because the top florets of the heads appeared to be male sterile, all heads were covered with glycine bags prior to anthesis to insure only selfed seed. Standard procedures were used to make the desired crosses. Morphological checks were made on all progenies to insure that the proper crosses had been made. The harvested seed from each crossed head or selfed plant was maintained separately. These lots of seed were considered a random sample for that particular experiment and were pooled for analysis.

Two chi-square tests were employed to analyze the data. Chi-square tests of goodness of fit were used to analyze data from segregating populations for one, two, or three factors. Chi-square tests of independence were used to test the hypothesis of the independence of two segregating distributions, either of the same cross at different temperature profiles or of different crosses at the same temperature profile. This test is also called a test of interaction and is described by Steel and Torrie (1960).

> "The hypothesis of independence implies that the ratio of numbers of plants emerging from treatment to treatment is the same, within random sampling errors. If there is no independence, there is said to be interaction. In the case of interaction, the ratio from treatment to treatment is dependent on the variable, i. e., the variables are not independent. No assumptions need be made about the true ratios for either variable, either within any category of the other variable or over all categories. If there is a significant chi-square, the table of contributions would supply information on possible causes."

An analysis of variance and Duncan's New Multiple Range Test were used to draw conclusions about mean infection types for the Rego x Lemhi cross.

EXPERIMENTAL RESULTS

I. Lemhi x P. I. 178383

Lemhi x P. I. 178383 at 2/18 The infection types of the parents, F_1, F_2, and Lemhi testcross distributions are presented in Table I. The oo type for the F_1 plants indicated that the gene or genes in P. I. 178383 were partially dominant.

The F_2 and the Lemhi testcross are shown giving the total number of plants observed for each infection type. The F_2 infection types were divided into three classes in which resistance was equal to P. I. 178383, $\overset{v}{o}o$, resistance was equal or slightly more susceptible than the F_1, oo, O-, and O, and susceptibility was nearly equal to the Lemhi parent, 3 and 4.

When the F_2 segregation was subjected to a chi-square test for either a 3:1 or 1:2:1 ratio, a good fit was obtained. This indicated either one completely dominant gene for resistance or one partially dominant gene for resistance depending upon which infection types were included in the various classifications.

In the testcross segregation the $\overset{v}{o}o$ infection type was absent. The absence of this type plus a good fit to a chi-square test of a 1:1 ratio further indicated that P. I. 178383 contained one major gene for resistance and that it expressed itself as being partially dominant.

Thus, the homozygous dominant and heterozygous condition of the major gene were distinguishable. The heterozygote apparently conditioned a oo to O infection type range at this temperature profile. Table II demonstrates that F_2 plants that were $\overset{v}{o}o$ types in the F_2 remain homozygous for approximately P. I. 178383's resistance, F_2 plants

Table I. The infection types of the parents, P. I. 178383 and Lemhi, the F_1, the F_2, and the testcross distributions at 2/18 and results of chi-square tests for goodness of fit.

Parents and progeny	Infection types and observed no. of plants									Total	Ratio*	X^2 value	P value
	v oo	oo	0-	0	1-	1	2	3	4				
P. I. 178383	all												
Lemhi									all				
F_1		all											
F_2	30	39	13	20				1	35	138			
		92					36				3:1	.09	.75-.90
	30		72				36				1:2:1	.78	.50-.75
(Lemhi x 178383) x Lemhi		4	13	5				5	28	55			
			22				33				1:1	2.20	.10-.25

* The 3:1 includes types v oo, oo, 0-, 0 to types 1-, 1, 2, 3, 4. The 1:2:1 includes type v oo to types oo, 0-, 0 to types 1-, 1, 2, 3, 4.

that were oo, O-, or O types in the F_2 segregated in a similar manner as did the $F_{2'}$ and F_2 plants that were 4 types segregated from 0 to 4 types (see Table I and Table II in the appendix).

Table II also presents the results of the F_3 segregation of forty-five randomly selected F_2 plants. If P. I. 178383 contains only a single major gene, then there should be a 1:2:1 ratio in the segregation patterns produced by these F_3 lines. A satisfactory fit to this ratio by a chi-square test confirmed this prediction.

To determine if the oo and O types were conditioned by variations of the same genotype or whether they were the result of a heterozygous major gene and differences in minor modifying genes, the F_3 lines that were selected from each of these infection types were pooled and compared. The individual F_3 lines of each infection type were tested for homogeneity and a non-significant value was obtained.

Because the oo and O lines are conditioned by a heterozygous major gene, these pooled F_3 lines should still segregate for either a 1:2:1 or 3:1 ratio. When the pooled oo lines were tested for a fit to these ratios, significance occurred (see Table III). The lack of fit to a 3:1 may have resulted from a number of escapes occurring and being called $\overset{v}{oo}$ types. These families were checked in January when Sharp and Pool (1965) showed that the germination of P. striiformis spores may be very low. When the pooled O lines were checked for goodnes of fit to a 1:2:1 or 3:1 ratio, a good fit was obtained only for the 3:1 ratio.

Although neither of the combined families segregated for a

Table II. Seedling infection types of F_3 lines from randomly selected F_2 plants from Lemhi x P. I. 178383 at 2/18.

F_2 infection type at 2/18	F_3 distribution of infection types from randomly selected F_2 plants								No. of F_3 lines	Obs. no.	Exp. no.	P value*	Probable F_2 genotype
v o o	v o o								8				AA
	v o o	oo							3				AA
	v o o	oo	0-						2	13	15		AA
oo, 0-, 0	v o o	oo	0-	0	1	2	3	4	1				Aa
	v o o	oo	0-	0		2	3	4	1				Aa
	v o o	oo	0-	0			3	4	6				Aa
	v o o	oo	0-	0				4	7				Aa
	v o o	oo	0-			2	3	4	3				Aa
	v o o	oo	0-				3	4	6				Aa
	v o o	oo				2	3	4	6				Aa
	v o o	oo					3	4	3	33	30		Aa
4				0	1	2	3	4	2				aa
					1	2	3	4	1				aa
						2	3	4	6				aa
							3	4	3				aa
	died								2	14	15	.50-.75	aa

* Chi-square test for goodness of fit to a 1:2:1

Table III. F_3 distributions of pooled $\overset{y}{oo}$, oo, 0, and 4 lines from Lemhi x P. I. 178383 and the goodness of fit of the pooled oo and 0 lines to ratios of 3:1 and 1:2:1 when tested at 2/18.

F_2 infection types at 2/18	Pooled F_3 infection types and observed no. of plants								Total	Ratio*	X^2 value	P value
	$\overset{y}{oo}$	oo	0-	0	1	2	3	4				
Pooled $\overset{y}{oo}$ lines	516	35	3	2					556			
Pooled oo lines	423	288	44	5	1	19	48	123	951			
		760				191				3:1	12.25	<.005
	423		337			191				1:2:1	192.14	<.005
Pooled 0 lines	59	82	81	53			14	66	355			
		275				80				3:1	1.15	.25-.50
	59		216			80				1:2:1	19.18	<.005
Pooled 4 lines				2	5	48	77	411	543			

* Ratios include same infection types as Table I.

1:2:1 ratio, they appeared to deviate from this ratio for different reasons. The oo lines had too many $\overset{v}{o}o$ types in relation to oo, O-, and O types while the O lines had too few $\overset{v}{o}o$ types in relation to oo, O-, and O types. The pooled $\overset{v}{o}o$ types also presented in Table III indicated that plants that contained the homozygous major gene still segregated for types more susceptible than a $\overset{v}{o}o$ type at this temperature profile. The pooled 4 type lines given in the same table also showed that a few O types were conditioned in plants wtih a homozygous recessive major gene.

A chi-square test for independence was run to compare the segregation of the pooled oo and O lines to determine if they were conditioned by different genotypes. A significant chi-square value indicated that the two F_3 distributions were different. The greatest deviations occurred in the excess numbers of $\overset{v}{o}o$ and oo types for the oo lines and the excess of O- and O types for the O lines (see Table IV). There were then different genotypes that conditioned the expression of the F_2 oo and O types, and it was possible to read these infection types as different responses to this disease. These factors would appear to be minor genes that modify the expression of the heterozygous major gene. The effect of these factors could then be responsible for the lack of fit to a 1:2:1 ratio.

A comparison was also made between the parts of the pooled oo and O lines that have a homozygous recessive major gene (i.e., types 1, 2, 3, and 4) to determine if these minor factors have any influence without the presence of the heterozygous major gene. Table V presents the