THE UNIVERSITY OF MANITOBA

AN INVESTIGATION OF POST-SEEDLING RESISTANCE IN FOUR CULTIVARS OF *TRITICUM AESTIVUM* TO *PUCCINIA GRAMINIS* F.SP. *TRITICI*

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PLANT SCIENCE

WINNIPEG, MANITOBA

October, 1973

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A dissertation submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

DOCTOR OF PHILOSOPHY

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. K.W. Buchannon for helpful direction and encouragement throughout the study. His constructive criticism and suggestions in the preparation of the manuscript are also acknowledged.

The author is deeply indebted to Dean L. H. Shebeski, the coordinator of a Canadian International Development Agency technical assistance project to the Kenyan government, for sending the author to the Plant Breeding Station, Njoro, Kenya where the majority of the research was conducted. Thanks are also due to Dr. R.C. McGinnis, the Head of the Department of Plant Science, for permitting the author to write up the research to satisfy the Ph.D. requirements. Gratitude is expressed to Drs. D.E. Harder, S.B. Helgason, J.W. Martens, W.C. McDonald, Messrs. G. Mathenge, I. Karanja, S. Kamau, and other staff members of the Plant Breeding Station, Njoro, Kenya, who contributed invaluably to the planning of the research and to the performing of the tests.

Gratitude is expressed to Dr. G.J. Green of Canada Department of Agriculture, Winnipeg, who arranged for testing facilities in the Canada Department of Agriculture, Research Station greenhouses and supplied cultures of Canadian stem rust races.

Thanks are also due to my colleagues, R. Morrison and N. Darvey, for helpful suggestions in the preparation of this manuscript.

Special thanks are due to my wife, Else Marie, for her infinite patience and loving encouragement given freely during the preparation of this manuscript.

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ABSTRACT

The seedling and post-seedling reactions of five cultivars of *T. aestivum* L. en. Thell. to eight races of *P. graminis* f. sp. *tritici* Eriks. and Henn. have been compared. The seedling and post-seedling reactions of Florence Aurore to the eight races of stem rust were similar. In both the seedling and post-seedling stages of development Florence Aurore expressed a susceptible reaction to races EA3(295), EA4(295), EA7(40), EA8(40), EA13(34), C10(15B-1), and C50(15B-5) and a moderately susceptible reaction to race C25(38). Seedlings of Hope, Africa Mayo, Kenya Page, and Conley were susceptible to some races of stem rust but post-seedlings of these cultivars expressed a degree of resistance to the same races. In the seedling stage Hope and Africa Mayo exhibited a susceptible reaction to all eight races of the pathogen. However, when Hope was inoculated at about the ear emergence stage, it expressed a moderately susceptible reaction to these same races. Similarly post-seedlings of Africa Mayo developed a moderately resistant reaction to races EA3(295), EA7(40), EA13(34) and C25(38) and a moderately susceptible reaction to races EA4(295), EA8(40), C10(15B-1) and C50(15B-5). In the seedling stage Kenya Page was moderately resistant to race EA3(295) but was susceptible to the other seven test-races. In the post-seedling stage Kenya Page expressed a moderately resistant reaction to all the test-races except C10(15B-1) and

C50(15B-5) to which it expressed a moderately susceptible reaction. Conley exhibited seedling resistance to races EA3(295), EA7(40), EA13(34) and C25(38). Races EA4(295), EA8(40), C10(15B-1) and C50 (15B-5) had virulence on seedlings of Conley. When Conley was inoculated at about the ear emergence stage, however, it expressed moderate resistance to these same races.

The stage of development at which resistance became effective was determined by inoculating each cultivar at various stages of growth. Hope and Africa Mayo exhibited moderate susceptibility between the 4th and 5th leaf stage to both races EA4(295) and EA8(40). Kenya Page and Conley exhibited moderate resistance to both races about the 3rd leaf-stage. There was no interaction between the race and the stage of development at which a degree of resistance was expressed. That is, the stage of development at which resistance was expressed was independent of the race used.

The number of genes controlling post-seedling resistance was determined by analyzing the pattern of segregation in the F_1, F_2, and F_3 generations of backcross populations that had been inoculated with stem rust races EA4(295) and EA8(40). One dominant gene determined the post-seedling reaction of Hope and Africa Mayo to each race of the pathogen. The same gene appeared to be effective against both races. The post-seedling resistance of Kenya Page and Conley was controlled by two duplicate dominant genes which were effective against both races

EA4 and EA8.

Inter-allelic relationships were established by analyzing the segregation ratios obtained from crossing in diallel the four sources of post-seedling resistance. No susceptible segregates were detected in the crosses Hope/Conley and Kenya Page/Conley. The population of the latter cross may not have been large enough to detect a four gene difference. The other four crosses of the diallel, Hope/Africa Mayo, Hope/Kenya Page, Africa Mayo/Kenya Page, and Africa Mayo/Conley segregated for post-seedling resistance to stem rust races EA4 and EA8. Therefore, these cultivars have among them at least four different genes controlling post-seedling reaction. Hope has one gene which is also carried by Conley. The other gene carried by Conley may be similar to one of the genes of Kenya Page. The second gene carried by Kenya Page and the one gene carried by Africa Mayo are different from each other and from the genes in Hope and Conley.

CHAPTER 1

INTRODUCTION

About one-sixth of Kenya's 225,000 square miles is suitable for cultivated crops. The 11,000,000 inhabitants and 3% population growth rate exert considerable pressure upon the available land resources. The staple foods consist of maize, bananas, potatoes, beans, millet, and sorghum. Wheat has become an important food crop. In 1970 the total internal consumption of wheat flour was 92,886 metric tons. At that time the Kenyan Wheat Board estimated an annual 5% increase in domestic wheat usage. Due to a maize shortage the following year internal consumption soared by an unexpected rate of 25.3% (Report of the Kenya Wheat Board 1971-72).

In 1968 the area planted to wheat in Kenya reached a peak of approximately 167,000 hectares. Since then the planted area has been declining and in 1972 it was about 110,000 hectares. This decline in wheat production has been influenced by a lower price paid to the wheat producer and by a higher price paid for maize, milk, and barley.

In Kenya *Puccinia graminis* f.sp. *tritici* Eriks. and Henn., *P. striiformis* Westend., and *P. recondita* Rob. ex. Desm. pose a threat to wheat production. Either stem rust or stripe rust can destroy a wheat crop (Pinto and Hurd 1970). During the wheat growing season climatic conditions favor stem rust development. There may be 10 - 20 days per month during which there is some precipitation. At Njoro, Kenya (0^{o}

20'S, 35° 56'E) the daily minimum temperature may range from 9 - 14°C and the daily maximum rarely exceeds 30°C.

Within the geographical wheat growing region of Kenya there are different growing seasons. Wheat is grown commercially somewhere within this geographical region all year round. Initial stem rust inoculum comes from infections on commercial plantings in an adjacent area and from infected volunteer wheat. Barberry spp. do not appear to function in the stem rust epidemiology of Kenya (Guthrie 1966). Changes in the virulence of the pathogen population may arise from an exchange of inoculum between the wheat growing regions of eastern Africa (Green _et_ _al_. 1970).

Genetic resistance provides an economical means by which to control cereal rusts. Since 1908 wheat research in Kenya has tried to match pace with the changes in virulence of the stem rust pathogen. From 1908 to 1973, 132 wheat cultivars have been released in Kenya. Because of the changes in prevalence of older races and the occurrence of new races, the average commercial life of a wheat cultivar during the period 1960 - 1970 was only 4.4 years. The two cultivars Africa Mayo and Kenya Page are a striking exception to this pattern. Africa Mayo and Kenya Page were released in 1960 and 1963 respectively. Each year they have expressed adequate resistance in field nurseries and commercial plantings to be recommended for production the following year.

The East African races of stem rust EA4(295), EA5(34), EA7(40), and EA8(40) have virulence on seedlings of Africa Mayo and Kenya Page (Evans et al. 1969). In 1966 race EA4 became prevalent. During 1968 - 70 these four races comprised over 70% of the isolates identified (Green et al. 1970; Harder et al. 1972). Consequently, the resistance of Africa Mayo and Kenya Page, which is ineffective in the seedling stage but becomes operative sometime later, has aroused interest as a means of controlling the stem rust pathogen.

The cultivars, Hope and Conley, have exhibited a type of resistance similar to that of Africa Mayo and Kenya Page. Hope and Conley have not been grown commercially in Kenya but they have been included in a stem rust parental collection which has been organized by the Plant Breeding Station, Njoro, Kenya. This parental collection was grown annually at two locations. They have expressed a satisfactory level of resistance in the field in spite of being susceptible in the seedling stage to a number of the prevalent races (Green et al. 1970; Harder et al. 1972).

The resistance of these four cultivars could be used in combination with seedling resistance genes in order to develop cultivars with durable resistance. To utilize this resistance on an efficient and systematic basis in the breeding program, however, it was necessary to determine:

(a) the stage of development at which this type of resistance becomes effective,

(b) the spectrum of resistance,

(c) the mode of inheritance,

(d) the number of genes controlling resistance,

(e) the inter-allelic relationships among the resistance genes.

Throughout this thesis the term "post-seedling resistance" is used in the sense that the first seedling leaf of a wheat cultivar is susceptible to one or more races of the pathogen but, at a later stage of development the plant expresses resistances to the same race or races (Anderson 1966). The term "seedling resistance" refers to that type of resistance which is effective at the first leaf stage, and generally is effective throughout the life of the plant.

CHAPTER 2

LITERATURE REVIEW

2.1 Resistance and Susceptibility

When a pathogen causes disease in the host, the host is said to be susceptible to the pathogen. Hence, susceptibility means "the sum total of qualities which make a plant a fit host for a pathogen" (Wood et al. 1940). The converse of susceptibility is resistance. A resistant host has the ability to prevent a pathogen from causing suffering (Horsfall and Dimond 1960). The ability to withstand the effects of the pathogen is a property of the host. Some hosts may offer no hindrance to the growth and spread of the pathogen whereas other hosts may cause the death of the pathogen. These two types of hosts can be placed at opposite ends of a continuum with varying degrees of resistance and susceptibility in between.

2.2 Classification of Disease Resistance Mechanisms

Disease resistance mechanisms are generally classified as escape, exclusion, host-parasite interactions after infection, and tolerance (Stakman and Harrar 1957; Fuchs 1971; Schafer 1971). Escape and exclusion are not true forms of resistance. In either case the pathogen fails to make contact with the host. That is, a host crop may pass through that developmental phase when it is potentially susceptible without the pathogen contacting it. Hence, the host crop appears resistant. Escape as a mechanism of resistance depends upon the ontogeny of the host in relation to the epidemiology of the pathogen.

Exclusion is another way of preventing the pathogen from making contact with potentially susceptible host tissue. Cleistogamous barley genotypes are resistant to Ustilago nuda since basidiospores are excluded from the florets (Macer 1960).

Other morphological features may hinder the pathogen from contacting susceptible tissue. Such features may include leaf pubescence, waxy bloom, and leaf angle (Fuchs 1971). Neatby and Goulden (1930) studied the segregation of leaf pubescence and stem rust resistance in the cross Marquis x H-44-24. They found no correlation between resistance and leaf pubescence.

Hayden (1956) noted differences in infectibility among spring wheat cultivars that were exposed to spore showers of race 15B of Puccinia graminis tritici. Susceptible type pustules developed on the stems of both Marquis and Sentry. However, a greater number of pustules per culm developed consistently on Marquis than on Sentry. Hayden concluded that the differences in infectibility were due to a mechanism other than a physiological one. Hart (1929) detected a correlation between the time of stomatal opening in the morning and the resistance of the wheat cultivars Hope, Webster, Acme, and Velvet Don. These cultivars were characterized by stomata that were slow to open in the morning. This contrasted with the susceptible cultivars, like Marquis and Little Club, whose stomata were open during the morning "dew-period". Peterson (1931), however, was unable to relate stomatal behaviour of H-44-24, Hope, and Pentad wheats with stem rust infection.

Tolerance has generally been defined as the capacity of the host to endure attack by a pathogen without sustaining a proportionate loss in yield or quality (Caldwell et al. 1958; Simons 1966; Schafer 1971; Leakey 1973). It is difficult to select cultivars with tolerance. Simons (1966, 1969) attempted to identify oat lines with tolerance to crown rust. All of the oat lines were heavily infected. Lines with tolerance exhibited a minimum reduction in yield and kernel weight. These criteria, however, do not distinguish between true tolerance and "slow-rusting" or "late-rusting" (Luke et al. 1971). A host with true tolerance has the capacity to carry the extra burden put on it by the energy demands of the parasite without suffering any substantial loss in vitality. Cultivars that are slow rusting exhibit reduced infectibility. Slow rusting cultivars usually exhibit a lower percentage of infection than normally susceptible cultivars throughout the growing season; whereas late rusting cultivars exhibit disease symptoms later than susceptible cultivars (Luke et al. 1971). Therefore, slow rusting and late rusting cultivars might exhibit the same percentage of plant tissue attacked at the end of the growing season as tolerant and susceptible cultivars. The slow rusting, late rusting, and tolerant cultivars might also show the same relatively small reduction in yield, kernel weight, or quality as compared to the susceptible cultivar. The resistance mechanism, however, would be different in each case.

Host-parasite interactions following infection are thought to be due to physiological processes (Stakman and Harrar 1957). This type of resistance mechanism is characterized by the hypersensitive reaction wherein the host and parasite are mutually incompatible, resulting in localized necrosis of host tissue and in death or limited growth of the parasite.

Physiologic resistance is generally operative throughout the entire life of a plant (Goulden _et al_. 1930; Green and Knott 1962). However, there are some reports of seedling resistance and post-seedling susceptibility (Campos _et al_. 1953; Duff 1954; Evans _et al_. 1969). Conversely, some cultivars may be susceptible in the seedling stage but at a later stage of development they become resistant. This maturescent type of resistance was first reported by Stakman and Piemeisel (1917) and Mains and Jackson (1921). The phenomenon of seedling susceptibility and post-seedling resistance has not been adequately investigated as to whether the resistance is physiological, morphological, or functional.

When physiologic specialization of the pathogen is considered, resistance to the rust fungus can be either specific or non-specific (Hooker 1967a; Caldwell 1968). Nonspecific resistance functions more or less effectively against all races of the pathogen. Specific resistance functions against certain races or biotypes but not against others. Specific resistance may be effective against a few or many races (Green

1971; Koo and Ausemus 1951). The spectrum of resistance obtained by combining a number of specific genes may appear to be similar to nonspecific resistance. Rajaram and Luig (1972) identified several wheat cultivars with a broad spectrum of resistance as indicated by a low coefficient of infection. This broad spectrum of resistance appeared to be similar to nonspecific resistance. A genetic analysis of this resistance revealed that it was due to the cumulative effect of a few genes for specific resistance.

Specific resistance in Triticum spp. to Puccinia spp. is not an "all or nothing" phenomenon. Loegering (1966) has proposed terminology to describe the host:pathogen interaction. Reaction type is a character of the host and may have a phenotype of either resistance or susceptibility. Likewise, the pathogen may have a character, pathogenicity, which may have a phenotype of either virulence or avirulence. Loegering (1966) coined the term aegricorpus to describe "the single living manifestation of specific genetic interactions in and between host and pathogen". Infection type is a character of the aegricorpus which may be either low or high.

2.3 The Inheritance of Disease Resistance Mechanisms

The inheritance of disease resistance has been reviewed recently by Hooker (1967a), Oggema (1972), Quinones (1972), and Walker (1965). These reviews point out that a disease resistance mechanism can be inherited by means of either a few major genes (oligogenically), or a number of minor genes each with a small effect (polygenically). Except perhaps for specific physiological resistance, the type of resistance mechanism does not necessarily determine the mode of inheritance.

Inheritance studies of host-parasite interactions have provided evidence that specific resistance is controlled by major genes. Flor (1953, 1956, 1959) proposed the gene-for-gene concept to explain the relation of pathogenicity genes in *Melampsora lini* to reaction genes in *Linum usitatissimum*. Evidence has been provided for a number of other host-parasite systems to show that they conform to this concept. For example, a gene-for-gene relationship has been demonstrated in the *Erysiphe graminis hordei*:*Hordeum* system (Moseman 1959), *Erysiphe graminis tritici*:*Triticum* system (Powers and Sandos 1960), *Puccinia graminis tritici*:*Triticum* (Green 1964; Luig and Watson 1961), *Puccinia recondita*:*Triticum* system (Dyck *et al*. 1966), and the *Puccinia graminis avenae*:*Avena* system (Martens *et al*. 1970).

Major genes segregate into discrete classes that are rather easy to determine. Minor genes segregate into continuous classes that are ill-defined, and therefore, estimation of the number of genes controlling a trait becomes difficult.

Toxopeus (1959) intercrossed clones of *Solanum tuberosum*, with and without non-specific resistance to *Phytophthora infestans*. The non-specific resistance appeared to be controlled by a series of minor genes. Similarly, the non-specific resistance of the strawberry clone

Cambridge Favourite to Phytophthora fragariae exhibited polygenic inheritance (Goodings 1973).

Hooker (1967b) investigated the inheritance of post-seedling resistance of maize to Puccinia sorghi using data from 65 crosses in the F_1, F_2, and F_3 generations. Disease scores of the F_2 plants resembled a normal distribution. The mean score of the F_1 was usually between that of the F_2 and the most resistant parent. The calculated heritability value was quite high. In general, the heritability values for polygenically inherited resistance to other maize diseases have been moderate to high (Kappelman and Thompson 1966; Hughes and Hooker 1971).

2.4 Epidemiological Effect of Disease Resistance Mechanism

Van der Plank (1963, 1968) defined two categories of resistance based on their effect on an epidemic. These two categories have been termed horizontal resistance and vertical resistance. The epidemiological characteristic of horizontal resistance is a slowing down of the epidemic or a more flattened out disease development curve. Vertical resistance results in an apparent reduction of initial inoculum so that the epidemic is delayed. Van der Plank (1963, 1968) defined these terms mathematically. Resistance is vertical if there is a differential interaction among races of the pathogen and cultivars. When there is no differential interaction among races and cultivars the resistance is horizontal. A differential interaction can be detected through an analysis of variance. A non-significant race x cultivar interaction would indicate horizontal resistance. Alternatively, a simpler, more crude test to detect horizontal resistance is available: if any host cultivar can rank in order the pathogenicity of the races or if any race can rank in order the resistance of the cultivars, then the resistance is horizontal (Van der Plank 1968).

Recently various resistance terms have been divided into two groups (Caldwell 1968; Abdulla and Hermsen 1971). The first group treats the following terms as synonyms: physiologic resistance, specific resistance, major gene resistance, oligogenic resistance, differential resistance, and vertical resistance. The second group includes the following names: non-specific resistance, generalized resistance, polygenic resistance, tolerance, uniform resistance, and horizontal resistance. This type of equating does not recognize the existence of different mechanisms of resistance, the way in which they are inherited, and how they affect an epidemic. There is no necessary relation between the resistance mechanism, the manner in which it is inherited, and how it affects an epidemic. For example, vertical resistance is not necessarily always inherited oligogenically, nor does horizontal resistance have to be inherited polygenically. Red-skin onion varieties are resistant to Colletotrichum circinans which causes onion smudge. Rieman (1931) obtained evidence that pigmentation in the outer scale of the onion developed under the influence of 3 genes, each having a specific, defined function. The inheritance of color in the outer scales appeared to be identical with the inheritance of resistance to disease. The genes controlling pigmentation had a pleiotropic effect on resistance to *Colletotrichum circinans*.

Cleistogamous flowering barley varieties are resistant to all races of *Ustilago nuda* (Macer 1960). Pedersen and Jorgensen (1965) reported that lodicule size affected cleistogamy in barley. Lodicule size was inherited monogenically. Differences in reciprocal crosses indicated that extra nuclear factors might also have affected the size of the lodicules and especially the tendency for open flowering. Fehr *et al*. (1964) doubted the effectiveness of the lodicule to determine cleistogamy in barley.

2.5 Inheritance of Post-Seedling Resistance

As early as 1815, Thomas Knight suggested that disease resistant wheats might be developed (Biffen 1905). Biffen crossed the variety Red King which was susceptible to *Puccinia striiformis* to Burt which was resistant. He noted that the F_1 plants were all susceptible. Segregation occurred in the F_2 according to the ratio 1 resistant to 3 susceptible. He concluded that resistance to *P. striiformis* was inherited in a Mendelian manner. Biffen developed the cultivar 'Little Joss'. It is now known to be susceptible to some races in the seedling stage. To this day, however, it has maintained a high level of post-seedling resistance to most races (Loegering *et al*. 1967).

The phenomenon that young wheat plants were sometimes more susceptible to P. graminis tritici than older plants was first reported by Stakman and Piemeisel (1917). A few years later the same phenomenon was noted with regard to Puccinia recondita (Mains and Jackson 1921). Mains and Jackson (1921) noted that Kanred wheat and two other lines had a high level of resistance in the field. When seedlings of these lines were inoculated under greenhouse conditions, they were fully susceptible. Seedling susceptibility and post-seedling resistance of Kanred to leaf rust was explained as being the result of a type of resistance that becomes expressed at later stages of maturity. Hayes and Aamodt (1923), Hayes et al. (1925), and Johnston and Melchers (1929) corroborated these reports that a maturescent type of resistance was the most logical explanation of the phenomenon of seedling susceptibility and post-seedling resistance.

About the same time Stakman and Levine (1918) detected an alarming plasticity of the stem rust organism based on the different physiological capabilities of stem rust isolates. Wheat breeders and pathologists quickly realized that in order for a wheat variety to have adequate stem rust resistance to be suitable for commercial production it must have resistance to all known races of the pathogen. The problem of developing a stem rust resistant variety focused on the identification of genetic sources of resistance.

Some of the resistant cultivars identified included the tetraploid wheats Acme, Velvet Don, Pentad, Iumillo, and Yaroslav emmer. When they were grown in field nurseries, they exhibited a good level of resistance to stem rust (Hayes and Aamodt 1923; Hart 1929; Goulden _et_ _al_. 1930). Whereas when they were inoculated in the seedling stage with pure race cultures under greenhouse conditions, they were susceptible to a number of races (Goulden _et_ _al_. 1930).

In an attempt to transfer the resistance from a tetraploid to a hexaploid wheat, McFadden (1930) crossed Yaroslav emmer to Marquis. He selected two lines from this cross which were resistant to all races in the field. These two lines were later named Hope and H-44-24.

Hope and H-44-24 appeared to have a broad spectrum of resistance. Genetic studies revealed that they possessed genes for both seedling resistance and post-seedling resistance, and that the seedling resistance genes were inherited independently from the post-seedling resistance genes (Goulden _et_ _al_. 1928; Goulden 1929; Clark and Humphrey 1933). Broad spectrum resistance, therefore, appeared to be simply inherited.

Neatby (1931) detected two genes controlling stem rust resistance in the cross Marquis by H-44-24. He classified 15 races of stem rust into 3 groups based on the correlated reaction of hybrid lines to these races. One of the two genes in H-44-24 determined the reaction to groups I and II, while the other gene conditioned the reaction to groups I and III. He concluded that a host gene conditioned resistance to a group of races. The production of resistant varieties appeared to be greatly simplified. He thought that it would not be necessary to test the reaction of the host to all races but only to key races within each group. Since then the experience of wheat breeders and pathologists has proved this optimism to be unjustified (Stakman and Christensen 1960).

Recent work has confirmed the earlier reports that Hope and H-44-24 possess both seedling resistance and post-seedling resistance genes. Seedling resistance genes Sr7b, Sr9d, and sr17 have been located on chromosomes 4B, 2B, and 7B respectively (Sears _et al_. 1957; Loegering and Sears 1966; Knott 1971). Identifying the chromosomes that carry genes for post-seedling resistance has been more difficult.

Sears _et al_. (1957) developed all 21 substitution lines of Hope by crossing Hope to the Chinese Spring nullisomics and then backcrossing five times to the appropriate Chinese Spring nullisomic. The substitution lines and parents were inoculated in the post-seedling stage with a culture of stem rust race 29. None of the substitution lines were as resistant as Hope. They concluded that the post-seedling resistance of Hope was the result of interaction between genes on different chromosomes. Knott (1971), however, reported that Hope carries a post-seedling resistance gene on chromosome 3B.

2.5.1 Single Gene

Post-seedling resistance of cereals to _Puccinia_ species has frequently been reported to be inherited as a Mendelian character. Goulden _et al_. (1928), Goulden (1929), Neatby and Goulden (1930), Hayes _et al_. (1934), Neatby (1936), Knott (1968, 1971), and Rajaram and Luig (1972) reported that the post-seedling resistance of some wheat cultivars to stem rust was controlled by a single dominant gene. Post-seedling resistance to *P. recondita* has also been attributed to a single major gene (Dyck *et al.* 1966; McIntosh and Baker 1966; Bartos *et al.* 1969; Dyck and Kerber 1970). Allen *et al.* (1966) concluded that post-seedling resistance to *P. striiformis* in some wheat cultivars was inherited monogenically. McKenzie and Martens (1968) identified the recessive gene pg-11 in the *Avena sativa* line CI3034, which conferred post-seedling resistance to *P. graminis* f.sp. *avenae*.

2.5.2 Two or More Genes

There are reports that the inheritance of resistance as expressed in the field is controlled by two or more genes. Both Neatby and Goulden (1930) and Clark and Humphrey (1933) studied the inheritance of stem rust resistance in the cross Hope x Marquis. The resistance of Hope was controlled by two independently inherited genes. The adult plant resistance of Thatcher to stem rust was conferred by two recessive complementary genes (Hayes and Ausemus 1936; Koo and Ausemus 1951; and Sears *et al.* 1957). Some inheritance studies of post-seedling resistance have merely concluded that the number of genes involved were more than one (Hayes *et al.* 1923; Hayes *et al.* 1934; Peterson and Love 1940; Pal *et al.* 1956; Allen *et al.* 1963).

2.5.3 Gene Interactions

Early geneticists thought that genes acted as autonomous units. Genetic research, however, has revealed that the integration of gene action is complicated. Gene interaction is the interaction between allelic or non-allelic genes of the same genotype in the production of a particular phenotypic character. Interaction between alleles may result in a gene being described as dominant or recessive. Dominance and recessiveness, however, are not properties of the gene *per se*. Rather they are the result of the action of the genetic locus in question within the total reaction system of the particular genotype (Murray 1972). Non-allelic interactions involve more than one locus to produce an effect. The expression of an allele can be modified by the external environment and by other genes of the genotype. Strickberger (1968) noted that the "mutual interdependence of all the developmental stages in an organisms growth makes it likely that most genes, if not all, affect more than one phenotypic character."

A character may be transmitted from parent to progeny in a dominant or a recessive manner. Post-seedling resistance of wheat cultivars to *Puccinia* species has been reported frequently to be inherited as a dominant trait (Goulden *et al*. 1928; Neatby and Goulden 1930; Dyck *et al*. 1966; McIntosh and Baker 1966; Knott 1968; Bartos *et al*. 1969; Dyck and Kerber 1970; Rajaram and Luig 1972). It has been well documented that the post-seedling resistance of Thatcher wheat to stem rust exhibits recessive inheritance (Hayes *et al*. 1936; Koo and Ausemus 1951; Sears et al. 1957).

Thatcher and Marquillo are Iumillo durum derivatives. Neither of the derivatives have as broad a spectrum of resistance as Iumillo durum (Peterson and Love 1940). The failure to recover the same level of resistance in a progeny line as was expressed in the parent could be the consequence of selecting within an F_2 population that was too small. The desired recombinant type that combined the resistance of the Iumillo parent with the T. aestivum characteristics of the other parent would be rather rare. Linkage between stem rust resistance and undesirable "durum-type" genes would further reduce the frequency of the desired recombinant type. Alternatively, the failure to identify progeny with the same spectrum of resistance as the Iumillo parent could have resulted from screening the segregating population with a pathogen population which failed to detect some of the resistance genes. Finally it is possible that cytoplasmic factors may have played some role in determining the resistance of Iumillo.

Cytoplasmic factors affect host:parasite interactions as evidenced by the outbreak of leaf blight of corn in the American corn belt (Hooker 1972). Sanchez-Monge et al. (1973) developed alloplasmic forms of tetra- and hexaploid wheats. They backcrossed the tetra- and hexaploid wheats a minimum of five times in order to transfer the nuclear material into a foreign cytoplasm. The donor cytoplasms were from Ae. ovata, Ae caudata, and T. timopheevi. The normal and alloplasmic forms were inoculated with races 21 and 133 of stem rust. A few of the alloplasmic forms, which had been susceptible to these races in their own cytoplasm, were resistant. Some alloplasmic forms, that had been resistant to these races in their own cytoplasm, were susceptible. In the development of these alloplasmic forms, however, they did not indicate whether they checked the stem rust reaction after each generation of backcrossing. Reaction genes can be gained or lost by meiotic crossing over between homologous chromosomes.

Because a given trait may be controlled by a major gene and one or more modifier genes, the trait may be difficult to transfer into another genetic background. Modifier genes are any genes that by interaction affect the phenotypic expression of genes at other loci. Depending on the type of modifying action such genes may be classified as enhancers or reducers (Knott and Anderson 1956; Sears _et_ _al_. 1957; Sunderman and Ausemus 1963; Knott and Green 1965; Williams _et_ _al_. 1966).

Dyck _et_ _al_. (1966) identified two independently inherited genes, Lr12 and Lr13, for post-seedling reaction to _P_. _recondita_. These genes were transferred by backcrossing into a Thatcher background. The single gene lines had a higher infection response than the parents, Exchange and Frontana. The parents seemed to carry modifier genes that enhanced the resistance of the major gene. The mode of inheritance of Lr13 was also sensitive to the genetic background. That is, Lr13 was partially dominant in Frontana whereas in the cultivar Manitou it behaved as a recessive gene.

The range of resistance conferred by the combination of two or more genes is usually the sum of each gene's range (Knott 1957a, 1957b; Sunderman and Ausemus 1963). No gene interaction appears to be involved. Some resistance genes, however, may interact with other resistance genes to enhance the expression of resistance to a particular race of the pathogen. That is, the combination of genes produces a lower infection type to a particular race than any gene singly (Berg *et al*. 1963; Schafer *et al*. 1963; Knott 1968).

Modifier genes have not been isolated. Sears *et al*. (1957) pointed out that the modifier genes may not necessarily be in the host. Since the detection of gene interaction is based on the infection type produced, modifier genes may be present in the host or the pathogen. Alternatively, the modified effect (the infection type) may be produced by the interaction of two sets of complementary genes, those of the host and pathogen. In view of the complexity that genetic interactions can assume, it is not surprising that genetic analyses have concentrated on major genes.

2.5.4 Linkage

Linkage is the association between different genes by virtue of their close proximity on the same chromosome, and consequently, the association of the phenotypic characters that are controlled by these genes. Complete linkage rarely occurs. Linkage between genes is broken by crossing over between homologous chromosomes during meiosis.

McFadden (1930) noted an association between susceptibility to discoloration on glumes, rachis, peduncle, and the stem area and resistance to the stem rust pathogen. Other workers have noted a similar association between the discoloration of certain plant organs and resistance to stem rust (Goulden and Neatby 1929; Ausemus 1934; Hayes *et al.* 1934; Pan 1940). Because stem rust resistant non-pigmented types were occasionally recovered, the linkage was not complete, or the rust free non-pigmented plants were escapes or spontaneous mutants. Waldron (1929) thought an antagonistic relationship between the host and pathogen produced the pigmentation. Hart and Zaleski (1935) failed to find an antagonistic relationship between the host and the stem rust organism. Occasionally the bacterial blight organism, *Xanthomonas translucens*, was isolated from the pigmented areas. They concluded that the pigmentation was produced by the interaction of host and stem rust pathogen as affected by the environment. Hagborg (1936) attributed the discoloration of plant organs of Hope, H-44-24, and their derivatives to three causes: one was the true black chaff organism, *Xanthomonas translucens*; the second was the fungus *Alternaria*; and the third was physiological in origin, that is pigmentation could also be produced under conditions of high humidity and high light intensity.

Recently Shuh-Ji *et al.* (1968) demonstrated that Conley, a Hope derivative, possessed a partially dominant gene that controlled physiological discoloration. This gene, located on chromosome 3B, had a linkage value of 21 - 24% with a gene for rust resistance. The physiologic origin of pigmentation is probably under genetic control, but the penetrance and expressivity of this gene appears to be incomplete as light and humidity seem to affect its expression.

The gene pg-11 in *Avena sativa* confers post-seedling resistance to *P. graminis* f.sp. *avenae* (McKenzie and Martens 1968). Plants which carry pg-11 resistance are green in the seedling stage and susceptible to oat stem rust. The plants change to a yellow green color and become resistant to the pathogen simultaneously. The gene pg-11 is associated with a decrease in total pigment content of the plant tissue (Harder *et al*. 1971). Attempts to separate these two characters by backcrossing and growing rather large populations has failed. They concluded that the gene pg-11 may be closely linked to genes affecting pigmentation levels or that pg-11 *per se* may not be a gene controlling rust reaction. It may be a gene which affects pigmentation and simultaneously affects rust reaction.

Reaction to two or more pathogens may be linked. The stem rust resistance of Hope, H-44-24, and their derivatives has been reported to be linked with reaction to *P. recondita*, *P. striiformis*, and *Erysiphe graminis* (Hayes *et al*. 1934; Neatby 1936; McIntosh *et al*. 1967).

The early attempts to determine the inheritance of post-seedling resistance were conducted in field nurseries and usually involved more than one year (Goulden *et al*. 1928; Neatby 1931; Clark and Humphrey 1933; Hayes *et al*. 1934). Both natural and artificial epidemics were used. When artificial epidemics were used, there was only limited control over the races present and the uniformity of their distribution. The segregating host populations exhibited maturity differences. Therefore, it was difficult to obtain uniform environmental conditions for infection and development of the pathogen throughout the phase which would have permitted differentiation between true resistance and the "resistance" due to senescence or escape. The observed segregation ratios might not have accurately reflected the actual genetic ratios. The interpretation of the data became difficult and confusing. Furthermore, when inheritance studies of disease reaction are conducted in the field it is difficult to distinguish between seedling resistance genes that are effective throughout the life of the plant and post-seedling resistance genes. The estimated gene number, therefore, may include both seedling and post-seedling resistance genes.

The detected number of genes in a segregating host population is determined by the number of genes in the pathogen population which fail to overcome the resistance genes of the host population. That is, when a segregating population is screened to a mixture of races, the number of genes detected will be those conferring resistance to the pathogen population. For example, assume that cultivar 'X' carried the independently inherited dominant genes SrA, SrB, and SrC which react as follows to races a, b, and c:

	SrA	SrB	SrC
race a	R	S	S
race b	R	R	S
race c	R	R	R

If cultivar 'X' is crossed to the universal suscept 'Y' and the F_2 progeny is screened to the mixture of races a, b, and c, monogenic segregation would be detected. The same result would be obtained if only race a is used as the test-race. If race b is used to screen the segregating material, then two genes would be detected. And if race c is the test-race, three genes would be detected.

Some recent investigations of the genetics of post-seedling resistance have been designed in accordance with the gene-for-gene concept. Under greenhouse conditions the host material is inoculated with a pure race culture. As a result of this experimental design, Dyck _et al_. (1966) and McIntosh and Baker (1966) suggested that the inheritance of post-seedling resistance to leaf rust also conformed to the gene-for-gene model.

2.6 Factors That May Contribute to Non-specific Resistance

2.6.1 Entry of the Pathogen

Penetration of the pathogen into the host is an early phase of the infection process. Pathogens can enter the host through wounds, natural openings, and by direct penetration of the cuticle (Stakman and Harrar 1957; Fuchs 1971). In general, the cereal rusts enter through the stomata. A noted exception is _P. recondita_ which can produce germ tubes that are capable of directly penetrating the leaf cuticle (Caldwell and Stone 1936).

Two cultivars exhibiting the same infection response may differ in the percentage area infected. The cultivar with a lower percentage infection would seem to be offering some resistance to the entry of the pathogen. Race 15B had virulence on Hope, Thatcher, and Marquis. The percentage area infected was consistently less on Hope than on Thatcher and Marquis (Johnson 1949). Hayden (1956) reported differences in infectibility among spring wheat cultivars exposed to spore showers of race 15B. Both light and heavy spore showers caused more pustules per culm on Marquis than on Sentry. This differential rate at which infections develop on a cultivar has been called "slow-rusting" by Caldwell (1968) and Hooker (1967a).

Caldwell _et al_. (1970) studied the spread of leaf rust in winter wheats that were separated by 38 ft. buffer zones. The plot centers were inoculated. Comparison of the number of pustules at predetermined dates and locations within the plots were used to determine the rate of spread of leaf rust. Purdue selection 45 wheat was fast-rusting compared to Bulgaria 88 which was considered slow-rusting.

Browder (1973) obtained infection type data on seedlings of Bulgaria 88 and Purdue selection 45 to 25 races of leaf rust. He concluded from the infection type data that Bulgaria 88 has specific rather than general resistance and that the reaction of Bulgaria 88 was controlled by 2 genes.

Hart (1929) attributed the lower percentage of stem rust infections on Hope, Webster, Velvet Don, and Acme to stomatal movement. The stomata on these cultivars opened more slowly in the morning than the cultivars Little Club and Marquis which exhibited a higher percentage of infections.

Peterson (1931) was unable to relate stomatal behavior of Hope, H-44-24, and Pentad wheats to the percentage of stem rust infection. He artificially lengthened the dew period by spraying a fine mist of water onto the plants. The percentage of infections did not increase significantly. The role of stomata and their response to stimuli that might be received from the pathogen and host has not been adequately elucidated. A correlation between two traits does not imply a causal relationship but rather might reflect their mutual correlation to a third factor.

Various morphological features such as a waxy "bloom", leaf angle, and leaf pubescence have been attributed a role in preventing the entrance of the pathogen (Hooker 1967a). Neatby and Goulden (1930) reported that in the cross Marquis x H-44-24 leaf pubescence and stem rust resistance were inherited independently. Babayants (1969) detected a negative correlation between the lignin content of wheat stems and the percentage of the area infected with stem rust. There is only limited evidence that morphological features of cereal hosts do or do not inhibit penetration of the rust pathogens.

Cultivars that exhibit a reduced number of infections per unit of time have been identified in other host:parasite systems (Szeijnberg 1969; Luke _et al_. 1971; Gooding 1973). Ullstrup (1970) demonstrated that polygenic resistance of maize to _Helminthosporium turcicum_ conditions a reduction in the number of infections rather than any decrease in lesion size. Goodings (1972) attributed the resistance of some strawberry clones to _Phytophthora fragariae_ to a high capacity for root regeneration.

2.6.2 Resistance After Entrance

Stakman and Harrar (1957) described three general types of resistance after entrance. The first involves necrogenic effects of the pathogen and/or hypersensitivity of the host to infection. The second is associated with the relative ability of the pathogen to grow in the host. Thirdly, mechanical barriers that may inhibit the growth of the pathogen. Klement *et al.* (1967), Hooker (1967a), and Stakman and Harrar (1957) have reviewed the literature dealing with these aspects of resistance after entrance. A brief review of the mechanical barriers that limit the growth of the pathogen after entering the host is germane.

Peterson (1931) noted that larger stem rust pustules developed just above the nodes and on the peduncle of Hope wheat than on the other stem regions. Zadoks (1961) has reported similarly that the level of resistance to *P. striiformis* is not uniform in all plant organs. In some cultivars the primary leaves may be more susceptible than older leaves. The leaves may be resistant while the head is quite susceptible and vice versa.

Hursh (1924) detected stem rust mycelium growing only in collenchymous tissue. The ratio of collenchymous tissue to sclerenchymous tissue would be expected, therefore, to affect the size to which pustules can grow.

The wheat cultivar Webster is susceptible to several stem rust races in the seedling stage (Hart 1929). When it is grown in field nurseries it exhibits a fairly high percentage of infections but the pustules are always small. Hart (1931) attributed the resistance of Webster to the small collenchyma bundles and thick epidermis. Babayants (1969) also detected a positive correlation between the breadth of the chlorenchyma bands in wheat cultivars and the breadth of stem rust uredopustules.

The host may also affect spore production and release. Susceptible pustules of crown rust race 216 on Clinton oats yielded nearly twice the quantity of spores as did susceptible pustules on Cherokee (Torres and Browning 1968). Johnson and Taylor (1972) found some isolates of *P. striiformis* capable of producing twice the weight of spores as other isolates of the same race. This may be a feature of a specific race-cultivar combination or an attribute solely of the pathogen.

2.7 Factors That Influence the Expression of Non-specific Resistance

The expression of specific resistance can be modified by environmental factors such as light, temperature, and humidity and by genetic factors (Newton and Johnson 1941; Knott 1957; Anderson 1966; Dyck *et al.* 1966; Loegering 1966). The literature pertaining to the factors that influence the expression of specific resistance has been reviewed recently (Oggema 1972; Quinones 1972).

Environmental and genetic factors are similarly capable of modifying the expression of non-specific resistance. That there was an optimum light intensity for stem rust mycelial development and sporulation in Hope was demonstrated by Hart and Zaleski (1935). Hope was resistant to stem rust race 21 under normal intense sunlight. Under conditions of reduced sunlight, however, it was susceptible. McFadden (1939) claimed that H-44 wheat possessed two types of post-seedling resistance. He termed one type morphologic and the other type photologic because it was expressed only under conditions of high light intensity.

Slovencikova (1972) noted that post-seedlings of one wheat cultivar were resistant to P. striiformis when grown under long-day conditions in the greenhouse. But when the same cultivar was grown under short-day conditions, it was susceptible. Similarly the expression of non-specific resistance in Solanum tuberosum to Phytophthora infestans can be modified by day-length (Umaerus 1959). Clones adapted to long-day conditions are more susceptible when grown under short-day conditions in Mexico than when grown under long-day conditions in Minnesota. Environmental and/or genetic factors other than day-length, however, may be responsible for the observed difference.

Temperature is known to affect the expression of non-specific resistance. Hart and Zaleski (1935) determined that Hope was more resistant to stem rust at a temperature over 26°C than at a lower temperature. It may be that the temperature also affected the development and sporulation of the rust mycelium. Combinations of minor genes for resistance in wheat to P. striiformis gave a lower infection response at a higher temperature profile than at a lower temperature profile (Lewellen *et al*. 1967). The non-specific resistance of the cabbage cultivar Wisconsin Hollander to *Fusarium oxysporum* f.sp. *conglutinans* can be suppressed by a constant soil temperature over 26°C (Walker 1959).

Hayden (1956) provided evidence that the number of stem rust lesions produced per wheat culm was a function of the infectibility of the host and of the inoculum load. Light urediospore showers of 2,500,000 spores per 2 ft.^{2} caused 0.025 lesions per culm on Sentry and 5.25 lesions per culm on Marquis. A heavy spore shower of 57,450,000 spores per 2 ft.^{2} caused 1.25 lesions per culm on Sentry and 23.5 lesions per culm on Marquis. The late-rusting characteristic and small uredia reaction in some oat cultivars also seemed to be affected by inoculum load (Krull *et al*. 1965; Luke *et al*. 1971). To screen breeding material for differences in infectibility it is obvious that consideration must be given to provide for a uniform inoculum load throughout the critical test-phase.

The expression of non-specific resistance in potato to *Phytophthora infestans* can be influenced by nutrient supply. Lowings and Acha (1959) found a positive correlation between high nitrogen levels and increased blight resistance. They attributed the increased resistance to a delay in the onset of senescence. Main and Gallegly (1964) reported that when potato plants were grown in a balanced nutrient solution before inoculation those with non-specific resistance survived while those without non-specific resistance died. When the same clones were grown in a high concentration of nutrients before inoculation both those with and without non-specific resistance were killed.

Grainger (1956) detected two peaks during which potato clones are relatively more susceptible. These periods of increased susceptibility were related to times of slow growth, and to a high ratio of total carbohydrate in the whole plant to the residual dry weight of the shoot. The physiological condition of the plant at the time of inoculation or contact with the pathogen appeared to influence the expression of resistance.

Plant age has a pronounced effect on the expression of resistance. Seedlings may be quite susceptible. As they grow and develop, however, resistance becomes expressed. A maturescent type of resistance has been detected in wheat to the three species of *Puccinia*, in potato to *P. infestans*, in cabbage to *F. oxysporum* f.sp. *conglutinans*, in corn to *Puccinia polysora* and *P. sorghi* (Goulden et al.1930: Toxopeus 1958; Walker 1959; Zadoks 1961; Dyck *et al*. 1966; Hooker 1967b; Van der Plank 1968).

Another type of ontogenetic resistance is that type which is associated with the time required to reach maturity. The non-specific resistance of some potato and strawberry clones to *Phytophthora* spp., and the late- and slow-rusting characteristics of some oat cultivars is positively correlated with maturity (Toxopeus 1958; Luke 1971; Goodings 1972). The association is strong enough that these authors suggest that it may be difficult to combine early maturity with resistance.

Susceptibility and resistance are phenotypic characteristics of the host which result from the interaction of host, pathogen, and environment. These three elements make up what Van der Plank (1968) calls the disease triangle. The effects of each are equivalent. Modification of the expression of resistance, hence, is to be expected and not to be treated as an exception.

CHAPTER 3

EXPERIMENTAL MATERIALS AND METHODS

3.1 Selection of the Parental Cultivars

In order to study the inheritance of post-seedling resistance of a cultivar that cultivar must be susceptible to a race of stem rust in the seedling stage and become resistant to the same race in the post-seedling stage. In Kenya the stem rust reaction of all of the commercial cultivars and the lines being considered for release are assessed in field nurseries at two locations each year. The percentage of stem tissue infected on Africa Mayo and Kenya Page was consistently low relative to the susceptible lines in these nurseries. Because these two cultivars exhibited good resistance in field stem rust nurseries, they have been recommended for commercial production each year since their release in 1960 and 1963 respectively. These two cultivars were known to be susceptible to a number of races of stem rust in the seedling stage (Evans *et al*. 1969). Hence, Africa Mayo and Kenya Page were selected as sources of post-seedling resistance.

The cultivar Conley was selected because it expressed the highest level of resistance in field nurseries. It has also been used as a source of stem rust resistance in the wheat improvement program at Njoro.

The inheritance of stem rust resistance of Hope to North American strains of stem rust has been studied by Neatby and Goulden (1930), Clark and Humphrey (1933), Sears *et al*. (1957), and Knott (1968, 1971). Hope was included in this study to determine the inheritance of its post-seedling resistance to two East African races of stem rust, and secondly to determine if the other three cultivars carried the Hope gene(s) for post-seedling resistance.

The cultivar Florence Aurore was used as the susceptible parent in the genetic analysis. In the seedling stage and in all subsequent stages of growth it consistently gave a 3^+ to 4 infection response. It was also readily infected. The parentage and country of origin of each cultivar is shown in Table 1.

Table 1. The Parentage and Country of Origin of the Selected Cultivars.

Cultivar	Parentage	Country of Origin
Florence Aurore	Florence/Aurore[*]	Algeria
Hope	Marquis/Yaroslav emmer	U.S.A.
Africa Mayo	Africa/Mayo 48[+]	Mexico
Kenya Page	Mentana/Kenya 58/Bage /3/Kenya 184P[+]	Kenya
Conley	RL2563[+]/Lee[+]	U.S.A.

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[*] Pedigrees illustrated according to the method proposed by Purdy *et al.* (1968).

[+]

Mayo 48	Newthatch/Marroqui
Kenya 184P	Reliance/3/Nutcut/Florence/B256
RL2563	Thatcher/3/McMurachy/Exchange/2/2* Redman
Lee	Hope/Timstein

3.2 The Purity of Parental Cultivars

The genetic homogeneity of each cultivar was provided for by using single seed propagation. Two samples of 16 - 20 seeds per cultivar were inoculated at both the seedling and heading stage. One sample was inoculated with test-race EA4 and the other sample was inoculated with test-race EA8. One plant of each cultivar that had a typical reaction to the appropriate test-race was harvested and subsequently increased. All of the other plants were discarded. Thus two seed supplies of each cultivar were established. One seed supply was genetically homogeneous for its reaction to EA4 and the other was homogeneous for its reaction to EA8. The parental plants used in all cross-race combinations derived from the appropriate seed supply. For example, to produce a backcross population of Conley/2* Florence Aurore that was to be inoculated with EA4, the parental source of Conley and Florence Aurore derived from the original seed source that had a typical reaction to EA4.

By establishing two seed supplies of each cultivar rather than one, it was possible to reduce the amount of time required to carry out the study. The author had only a limited period of time in Kenya during which to conduct the study.

3.3 Growing the Parents and Progeny

In order to circumvent the problems associated with interpreting host-pathogen relationships based on data collected from field nurseries, the parents and progeny were grown and tested under greenhouse conditions.

Problems associated with field tests would be a lack of control over: (1) the distribution of stem rust races in space and time, (2) uniformity of inoculum in space and time, (3) maturity differences, and (4) micro-environment differences both within and between seasons.

The parents and progeny were grown in plastic pots 12.5 cm in diameter. Four or five seeds were planted per pot. The pots were placed on greenhouse benches 76 cm x 244 cm. Supplementary lighting for 18 hours per day was provided by a bank of 125 watt florescent tubes. The greenhouse consisted of four similar benches that were isolated from each other (Figure 1).

The seeds were dressed with 'Milstem' to control powdery mildew and 'Dieldrex M' to control soil borne fungi and insects. A small amount of 8-40-0 fertilizer with 1% copper was applied at the time of planting and again when the plants had headed.

Meterological data collected at the Plant Breeding Station, Njoro during 1971 is illustrated in Tables 2 - 4.

3.4 Size of Population Used

Because of the problems associated with inoculating and incubating adult plants and the greeenhouse space required to grow plants to maturity, minimum population sizes were used. In order to reduce the size of population required to establish a genetic ratio and to distinguish between more than one probable ratio, each source of post-seedling resistance was backcrossed once to the susceptible cultivar Florence Aurore.

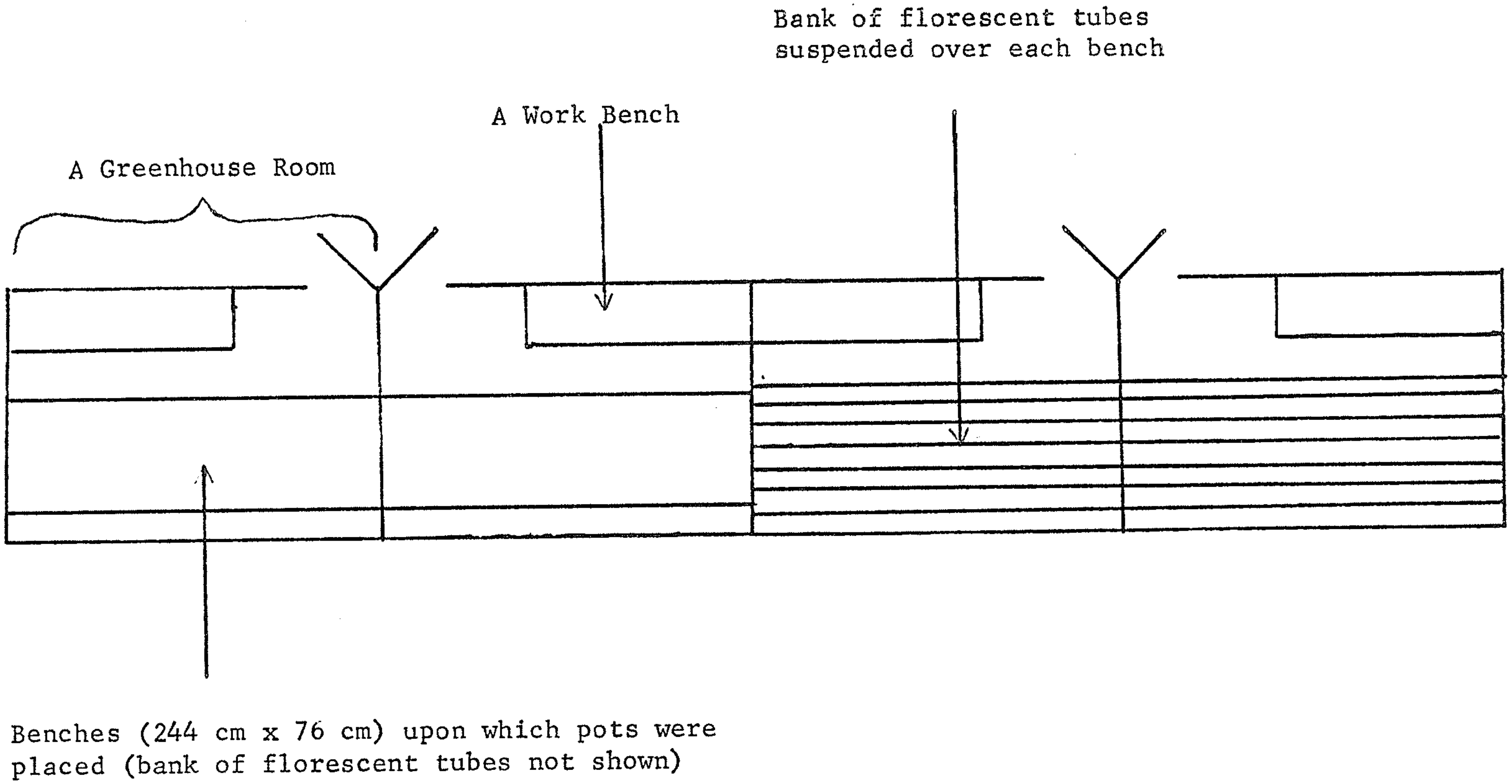


Figure 1. Top view of the greenhouse which consisted of four similar compartments.

Table 2. Average Weekly Maximum and Minimum Temperatures in the Greenhouse with Supplementary Lighting During 1971.

Date		Ave. Max. °C	Ave. Min. °C	Date		Ave. Max. °C	Ave. Min. °C
Jan.	1 - 7	30.5	9.4	July	2 - 8	29.5	10.0
	8 - 14	31.0	10.0		9 - 15	32.2	10.6
	15 - 21	30.0	11.7		16 - 22	30.5	10.6
	22 - 28	30.0	11.7		23 - 29	31.6	8.3
	29 - 4	30.0	11.7		30 - 5	32.8	8.9
Feb.	5 - 11	32.2	10.0	Aug.	6 - 12	30.5	10.0
	12 - 18	32.8	10.6		13 - 19	27.8	10.0
	19 - 25	35.6	10.0		20 - 26	27.8	11.7
	26 - 4	37.8	9.4		27 - 2	32.8	10.0
Mar.	5 - 11	35.0	11.1	Sept.	3 - 9	32.8	8.9
	12 - 18	35.0	11.1		10 - 16	35.0	8.9
	19 - 25	32.0	11.1		17 - 23	36.1	8.9
	26 - 1	31.0	11.7		24 - 30	29.5	10.6
April	2 - 8	28.9	11.7	Oct.	1 - 7	33.9	9.4
	9 - 15	32.2	13.9		8 - 14	32.2	8.9
	16 - 22	35.6	14.4		15 - 21	32.2	9.4
	23 - 29	28.9	12.8		22 - 28	33.9	10.0
	30 - 6	36.7	12.8		29 - 4	32.2	10.0
May	7 - 13	31.0	12.8	Nov.	5 - 11	32.2	8.9
	14 - 20	32.8	13.4		12 - 18	32.8	8.3
	21 - 27	32.8	11.7		19 - 25	28.3	8.9
	28 - 3	33.3	12.8		26 - 2	30.5	8.9
June	4 - 10	33.3	8.3	Dec.	3 - 9	31.6	8.3
	11 - 17	33.3	10.6		10 - 16	28.9	11.1
	18 - 24	30.5	10.6		17 - 23	30.0	10.6
	25 - 1	30.5	10.0		24 - 30	30.0	10.0

Table 3. Summary of Rainfall Observed in mm at the Plant Breeding Station, Njoro, Kenya During 1971.

Date	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1	-	-	-	-	7.0	-	-	-	0.1	2.4	-	-
2	-	-	-	-	-	2.1	0.1	-	33.1	1.9	-	-
3	-	-	-	6.4	1.5	8.3	-	-	1.2	0.1	-	-
4	-	-	-	8.4	17.1	-	0.2	0.1	5.0	-	0.3	-
5	-	-	-	-	36.3	7.4	7.2	1.6	1.9	-	6.2	-
6	-	-	-	3.8	-	-	2.0	-	-	-	0.6	-
7	-	-	-	0.7	-	14.8	Trace	2.1	-	-	-	5.3
8	-	-	-	1.9	-	32.6	6.7	0.6	-	0.4	6.0	-
9	-	-	0.5	0.3	4.2	1.4	3.2	7.0	-	3.2	-	-
10	Trace	-	8.5	-	0.2	-	0.7	5.2	3.0	-	-	-
11	-	-	8.4	-	0.5	34.0	-	8.2	-	-	-	-
12	-	-	-	Trace	0.6	-	-	19.2	Trace	-	0.6	-
13	-	-	-	2.4	17.3	-	-	1.3	2.2	-	-	-
14	-	-	-	4.3	Trace	-	-	-	0.4	-	-	-
15	0.8	-	-	-	-	-	8.7	5.2	-	-	-	0.4
16	25.4	-	Trace	50.0	-	1.2	1.8	13.1	-	-	-	2.0
17	16.8	-	-	17.3	11.6	-	8.7	7.0	-	-	0.5	14.0
18	Trace	-	-	1.2	8.4	-	0.1	-	-	-	0.2	1.0
19	-	-	Trace	3.2	-	-	-	2.1	-	-	-	1.9
20	-	-	-	7.2	Trace	-	-	8.5	-	-	2.4	0.4
21	-	-	Trace	11.9	10.5	-	-	18.2	-	-	-	5.4
22	-	-	-	-	Trace	9.3	-	-	8.2	Trace	1.3	4.6
23	-	-	-	-	-	-	-	23.8	-	-	8.4	2.6
24	-	-	-	-	-	-	-	5.9	-	-	2.5	16.3
25	0.9	-	-	0.6	-	3.2	2.5	4.5	-	0.1	6.0	4.2
26	-	-	1.2	13.7	-	2.4	-	-	-	0.4	0.3	1.2
27	-	-	-	-	0.4	0.2	1.8	5.5	9.4	4.7	13.6	0.5
28	-	-	0.7	0.5	12.5	4.1	0.2	8.5	1.4	-	5.7	0.7
29	-	X	0.6	7.1	6.2	18.7	Trace	18.1	9.3	1.0	0.4	Trace
30.	-	X	1.8	0.3	-	7.0	-	6.6	-	2.3	4.1	0.5
31	-	X	-	X	-	X	4.5	4.5	X	-	X	Trace
Monthly Total	43.9	Nil	21.7	134.2	134.2	146.7	55.4	173.8	75.3	16.4	51.1	61.3

Grand Total 914.1 mm.

Table 4. Average Number of Hours of Bright Sunshine Per Day at Plant Breeding Station, Njoro, Kenya During 1971.

Month	Hours	Month	Hours
Jan.	8.3	July	6.2
Feb.	9.3	Aug.	6.2
Mar.	7.3	Sept.	6.1
April	6.0	Oct.	7.8
May	6.4	Nov.	6.2
June	6.3	Dec.	7.0

The method proposed by Hansen (1959) was followed to calculate the minimum population size. More than 100 first generation backcross (BC_1F_1) seeds were produced for each cultivar-race combination. Each BC_1F_2 line derived from a single BC_1F_1 plant. The within BC_1F_2 line size was based on the probability of distinguishing between a line that was segregating from a line that was homozygous susceptible.

To determine the similarity or dissimilarity of the genes being carried by the sources of resistance a diallel analysis was conducted. Large numbers of F_3 lines were not screened because of the limitations imposed by the requirements to handle large numbers of adult plants in the greenhouse. The identification of susceptible F_2 plants that gave rise to homozygous susceptible F_3 lines was considered as evidence that the parental cultivars carried different genes for post-seedling resistance.

3.5 The Test-Races of *Puccinia graminis* f.sp. *tritici*

The spectrum of resistance of a cultivar was assessed by inoculating the cultivar in both the seedling stage and post-seedling stage with eight different races of stem rust. Races EA3, EA4, EA7, EA8 and EA13 were selected because of their virulence and prevalence in race surveys carried out in Kenya (Green *et al*. 1969; Harder *et al*. 1972). The races C10(15B-1), C25(38), and C50(15B-5) were chosen to evaluate the resistance of these cultivars to some Canadian strains. The East African and Canadian race numbers, virulence formulae, and "standard" race numbers of the eight races are listed in Table 5.

To keep the study within manageable limits only two East African races were selected to determine the inheritance of post-seedling resistance. Races EA4 and EA8 were selected because they were known to be virulent on seedlings of the five cultivars (Evans *et al*. 1969). These two races, therefore, would be able to detect those resistance genes that become effective after the seedling stage.

In addition, races EA4 and EA8 had virulence on a number of the commercial cultivars. They also had virulence on CI 12632, a *T. timopheevi* derivative, that had been used extensively as a sources of resistance in the wheat improvement program (Dixon 1960; Evans *et al*. 1969). New host genes with resistance to these two strains of the pathogen, were needed for the development of rust resistant cultivars.

3.6 The Purity and Increase of the Inocula

A differential set and 10 - 20 pots of seedlings of Florence Aurore were inoculated with a culture of each race. The inoculated material was kept in isolated compartments. If the race keyed out on the differential set without any trace of contamination, then the increase of the race-culture was deemed pure. The urediospores were collected and used immediately. This procedure was followed to check the purity and ensure the viability of the inocula used for every test.

Table 5. Stem Rust Race Numbers, Their Virulence Formulae, and "Standard" Race Equivalent.

	Virulence Formula	"Standard" Race
East African Race No.		
EA3	$\frac{2,4,5,8,15,22^{*}}{1,7,21,23}$	295
EA4	$\frac{2,4,8,15,22}{1,5,7,21,23}$	295
EA7	$\frac{5,15,22}{1,2,4,7,8,21,23}$	40
EA8	$\frac{15,22}{1,2,4,5,7,8,21,23}$	40
EA13	$\frac{4,5,7,8,15,23}{1,2,21,22}$	34
Canadian Race No.		
C10(15B-1)	$\frac{6,7,8,\ GB^{**}}{1,5,9a,9b,10,11,13,14,15,16,17}$	15B
C25	$\frac{}{5,6,7,10,11,15}$	38
C50(15B-5)	$\frac{7,8}{1,5,6,9a,9b,10,11,13,14,15,16,17}$	15B

* Differential cultivars: 1 = Reliance; 2 = Kota; 4 = Vernal; 5 = H-441; 7 = Marquis-Sr11; 8 = Giza 144; 15 = CI 8154-Frocor^2; 21 = Renown selection containing sr17; 22= Iumillo; 23 = Kenora.

** Differential cultivars: The differential number corresponds to the identified "single-gene" transferred into a Marquis background.

Adapted from Green (1971) and Harder *et al*. (1972).

3.7 Method of Inoculating Seedlings

When the first leaf was 7-10 cm long, the seedlings were inoculated by air-spraying urediospores of pure cultures suspended in Mobilsol-100 oil. The excess oil on the seedlings was allowed to dry off before they were placed in moist chambers. The seedlings were fogged with a fine spray of water and left in the moist chamber for 18 - 20 hrs. After incubation the seedlings were transferred to greenhouse benches. Infection types were recorded 13 - 15 days after inoculation.

3.8 Method of Inoculating Post-Seedlings

Pure fresh inoculum was collected from seedlings of Florence Aurore. A concentrated suspension of urediospores in Mobilsol-100 oil was sprayed on to plants anytime after the 5th leaf stage but before flowering. The excess oil on the plants was allowed to dry off before the plants were placed in moist chambers. The plants were fogged with a fine spray of water and left in the chamber at high humidity for 18 - 20 hours. After the incubation period the plants were transferred to greenhouse benches and the supplementary lighting switched on. In order to permit the pustules to develop to maximum size, the reaction to stem rust was recorded 19 - 22 days after inoculation.

3.9 Recording the Stem Rust Reactions

The classification of infection types on seedlings and post-seedlings was in accordance with the system proposed by Stakman _et al_. (1962). Infection types ranging from 0; to 1, 1^+ to 2, 2^+ to 3 and X, and 3^+ to 4 were considered to correspond to resistant, moderately resistant, moderately susceptible, and susceptible host reactions, respectively. Moderately resistant and moderately susceptible reactions were considered as degrees of host resistance. The percentage of the plant area infected was estimated in accordance with the modified Cobb scale. Whenever possible the infection type and level of infection were recorded for the flag leaf, flaf leaf sheath, and stem region below the flag leaf.

Post-seedlings were classified as susceptible if the pustules were large and coalesced, if the leaves, leaf-sheaths and stem were all equally susceptible to infection, and if there was more than 20% infection. Because the parents had only intermediate levels of resistance, the progeny were classified as moderately resistant, moderately susceptible, and susceptible.

3.10 Tests for Goodness of Fit

The Chi-square goodness of fit procedure was used to compute the probability that the observed segregation ratio fitted a hypothetical ratio. Yates correction factor (Snedecor and Cochran 1967) was used whenever the number of either of the expected classes was less than 30.

CHAPTER 4

EXPERIMENTS CONDUCTED

4.1 SEEDLING AND POST-SEEDLING REACTIONS OF FIVE WHEAT CULTIVARS TO EIGHT RACES OF STEM RUST

Introduction

In order to develop a cultivar of wheat resistant to the stem rust pathogen employing a program of hybridization and selection, the plant breeder requires parents which between them have resistance to all the races in the epidemiological area. A judicious selection of parents can be based on a knowledge of each cultivar's spectrum of resistance which can be determined by testing the host to a series of pure race cultures.

Wheat cultivars and lines have been screened in the greenhouse and field to some of the East African races of stem rust (Evans et al. 1969; Harder et al. 1972). From these Hope, Africa Mayo, Kenya Page, and Conley were chosen for further study because they exhibit a good level of resistance in the field.

The purpose of this experiment was: (1) to compare the seedling and post-seedling reaction of each cultivar to 5 East African races of stem rust and 3 Canadian races, and (2) to evaluate the spectrum of resistance of each cultivar.

Materials and Methods

The five cultivars Florence Aurore, Hope, Africa Mayo, Kenya Page, and Conley were grown in field stem rust nurseries together with numerous other entries. Their reaction to stem rust and percentage infection are listed in Table 6.

To compare the seedling and post-seedling reactions of the five cultivars to five East African stem rust races, five pots of each cultivar were planted. Five seeds were planted per pot. The seedlings were inoculated with pure race-cultures of EA3, EA4, EA7, EA8, and EA13 (cf. Chapter 3.6 and 3.7). The seedlings were incubated for 18 - 20 hrs. in a chamber at high humidity. Fourteen days after inoculation the infection types were recorded.

At about the heading stage the same plants were again inoculated (cf. Chapter 3.8) with pure race cultures. Plants that had been inoculated with a particular race in the seedling stage were inoculated with the same race. The infection type and percentage of infected area were recorded about 21 days after inoculation (cf. Chapter 3.9).

The same procedure was followed later in Canada to determine the seedling infection response of the cultivars to C10(15B-1), C25(38), and C50(15B-5). The technique of inoculating plants at about the heading stage was modified. The plants were sprayed with a mist of water containing Tween-20. Urediospores from sporulating pustules on Little Club were transferred by fingers onto the leaves and stems of the test-plants.

Results and Discussion

The field stem rust reactions and percentage infections for each cultivar for each of five years are recorded in Table 6. Each year the

Table 6. The Field Reactions of Five Wheat Cultivars to *P. graminis* f.sp. *tritici*.

	Cultivar				
Year	Florence Aurore	Hope	Africa Mayo	Kenya Page	Conley
1967	70S*	tR-MR	2S	5 MS	t MR
1968	80S	t MS	3R-S	R	10 MR
1969	60S	tR-tS	5S	10 MR - 10 MS	t MS
1970	60S	t MS	5 MR - MS	5 MR - t MS	t MR
1971	80S	10 MS	10 MS	5 MR	5 MR - ts

* Data adapted from stem rust reactions that were recorded on cultivars in the Stem Rust Parental Collection and Wheat Variety Trial I, Plant Breeding Station, Njoro, Kenya.

stems of Florence Aurore were covered with a large percentage of susceptible pustules. The reaction class and percentage infection of each of the other cultivars Hope, Africa Mayo, Kenya Page, and Conley varied considerably from year to year (Table 6). In some years a very low percentage of susceptible pustules occurred on the stems of Hope and Africa Mayo. It should be pointed out that these readings were not taken by the same person each year. Some workers might have scored the most susceptible reaction found in the plot as the typical reaction. Also the susceptible pustules recorded may have occurred in regions of greater susceptibility. Peterson (1931) noted that large stem rust pustules could be found around the node, on the stem near the auricle, and on the peduncle. In any case the level of resistance exhibited by these four cultivars would give them adequate protection under field conditions.

The seedling infection types and post-seedling reactions of the five cultivars to eight races of stem rust as obtained from greenhouse tests are presented in Table 7. Florence Aurore was susceptible in the seedling and post-seedling stage to all races except C25 to which it expressed moderate susceptibility in both stages of development. The seedling reaction of Florence Aurore, therefore, can be used as a reliable prediction of its post-seedling reaction.

In the seedling stage Hope was susceptible to all test-races except C25. Seedlings of Hope inoculated with race C25 produced a 2^+ infection response whereas it produced a 3^+ to 4 infection response when inoculated with the other test-races. In the post-seedling stage, however, Hope expressed moderate susceptibility to all races. Therefore, the seedling infection response failed to predict the post-seedling reaction. The post-seedlings of Hope expressed a degree of resistance to those races which were virulent on the seedlings.

Africa Mayo had no seedling resistance to the test-strains of the pathogen (Table 7). Post-seedlings, however, developed a moderately resistant reaction to races EA3, EA7, EA13, and C25. To the other races EA4, EA8, C10, and C50 post-seedlings of Africa Mayo expressed moderate susceptibility.

When seedlings of Kenya Page were inoculated with race EA3, a ;(2) infection response was obtained (Table 7). Seedlings of Kenya Page were susceptible to all the other test-strains of the pathogen (Table 7). In

Table 7. The Seedling Infection Type and Post-Seedling Reactions of Five Wheat Cultivars to Eight Stem Rust Races.

		Cultivar				
Formula No.	"Standard" Race No.	Florence Aurore	Hope	Africa Mayo	Kenya Page	Conley
EA3	295					
Seedlings		3+	3+	3+	;2	;1
Post-Seedlings		S	MS	MR	MR	R
EA4	295					
Seedlings		4	3+	3+	3+	3
Post-Seedlings		S	MS	MS	MR	MR
EA7	40					
Seedlings		3+	3+	3+	3+	;2
Post-Seedlings		S	MS	MR	MR	R
EA8	40					
Seedlings		4	3+	3+	3+	3
Post-Seedlings		S	MS	MS	MR	MR
EA13	34					
Seedlings		4	3+	3+	3+	;1
Post-Seedlings		S	MS	MR	MR	R
C10	15B-1					
Seedlings		4	3+	3+	3+	23^{cn}
Post-Seedlings		S	MS	MS	MS	MR
C25	38					
Seedlings		2+	2+	3	3	2+
Post-Seedlings		MS	MS	MR	MR	MR
C50	15B-5					
Seedlings		4	4	4	4	3+
Post-Seedlings		S	MS	MS	MS	MR

the post-seedling stage Kenya Page was moderately resistant to all races except to C10(15B-1) and C50(15B-5) to which it was moderately susceptible.

Conley differed from the other cultivars in that it expressed seedling resistance to several races (Table 7). In the post-seedling stage Conley continued to be resistant to these races. On the other hand races EA4, EA8, C10, and C50 had virulence on seedlings of Conley. In the post-seedling stage Conley expressed a moderately resistant reaction to these same races.

Typical post-seedling reactions of the five cultivars to races EA8, C10(15B-1), and C50(15B-5) as expressed under greenhouse conditions are shown in Figures 2 and 3. The pustules that developed on the stems of Florence Aurore were large, confluent, and ruptured the epidermis. No chlorosis or necrosis were observed around the pustules. Florence Aurore offered no restriction to the development of the stem rust fungus.

The pustules that developed on the stems of the other cultivars remained distinct and did not coalesce (Figures 2 and 3). Chlorotic and necrotic areas surrounded the pustules. Occasionally the chlorotic areas developed only at either end of the pustules on the stems of Hope and Africa Mayo. The development of the stem rust pathogen was restricted as evidenced phenomenologically by the characteristics of the aegricorpus.

Generally, the seedling reaction of a cultivar can be considered a reliable indication of the level of resistance it will have at subsequent stages of development (Goulden et al. 1930; Green and Knott 1962). However, some cultivars may be resistant in the seedling stage and

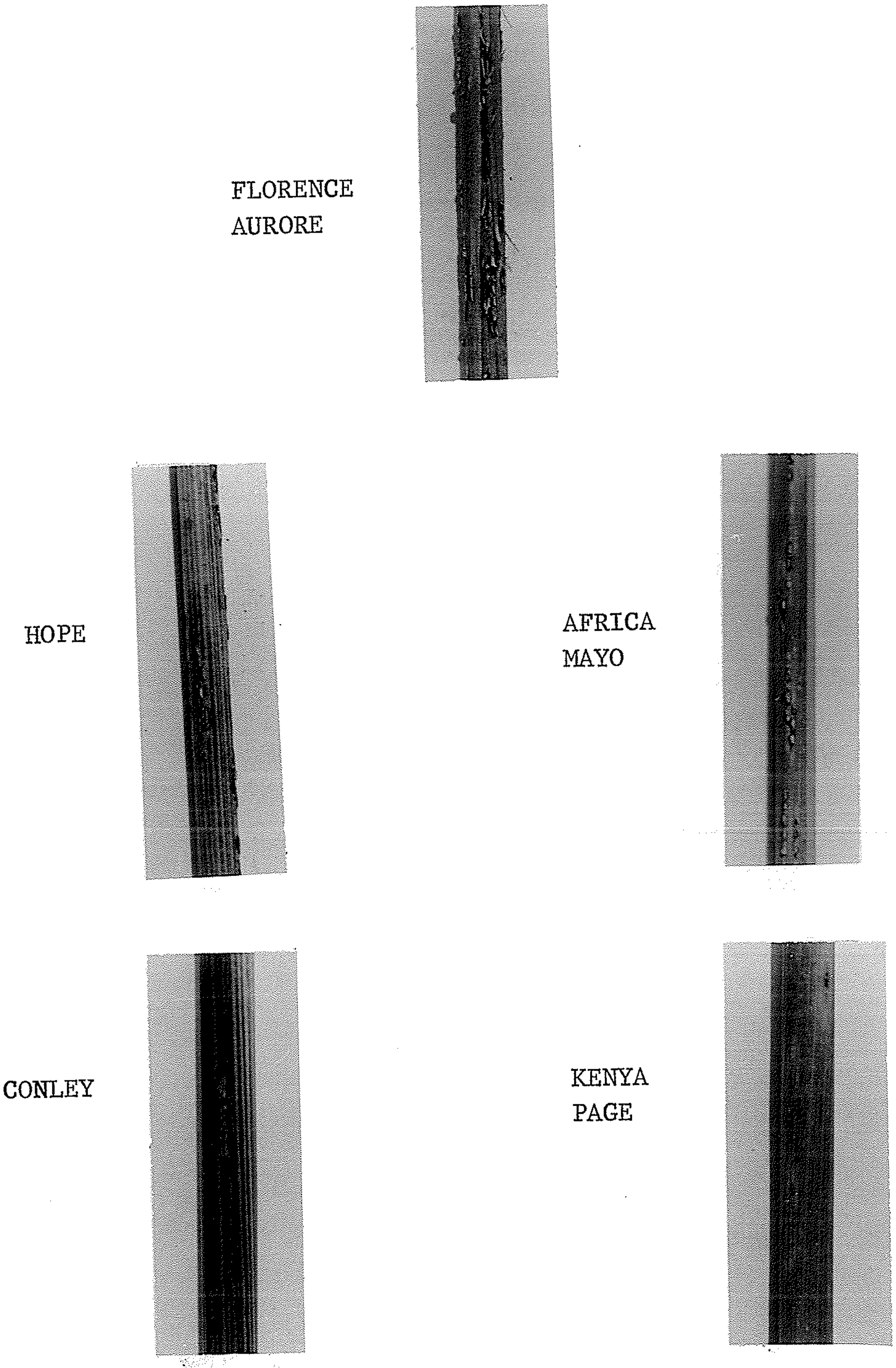
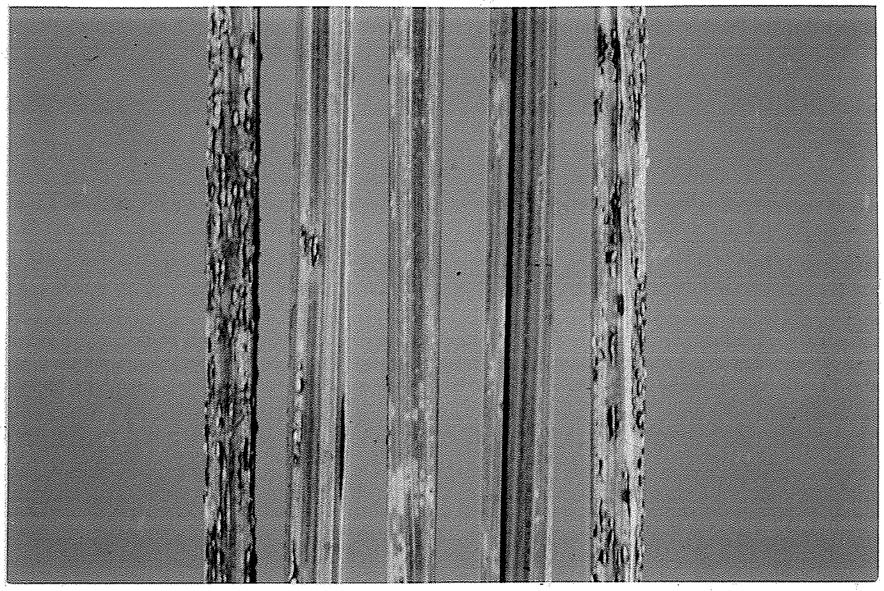


Figure 2. Typical stem rust reactions on stems of Florence Aurore, Hope, Africa Mayo, Conley, and Kenya Page inoculated with race EA8 (40).



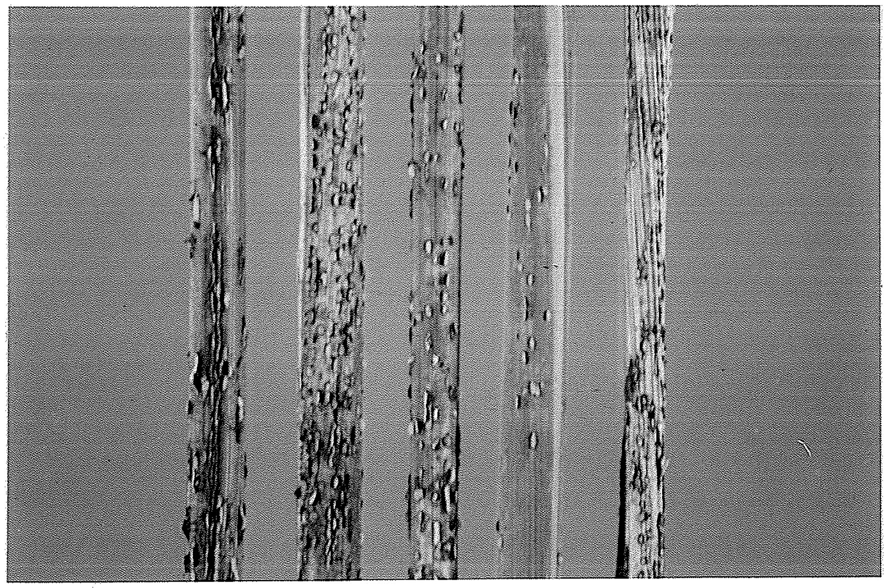


Figure 3. Typical stem rust reactions on stems of Florence Aurore, Hope, Africa Mayo, Conley, and Kenya Page to races C10 (15B-1) (upper photograph) and C50(15B-5) (lower photograph).

subsequently lose their resistance (Duff 1954; Evans et al. 1969). Other cultivars may be susceptible in the seedling stage but become resistant sometime after the seedling stage (Stakman and Piemeisel 1917). The resistance of Hope, Africa Mayo, Kenya Page, and Conley fall into the latter category.

A cultivar may possess more than one type of resistance. Conley has seedling resistance to EA3, EA7, EA13, and C25 and post-seedling resistance to the other four races. In general, the seedling resistance of Conley conditions a more resistant reaction than does its post-seedling resistance. The seedling resistance is effective throughout the life of the plant. Therefore, seedling resistance is epistatic to post-seedling resistance.

A cultivar in the post-seedling stage did not necessarily express a uniform reaction to all test-races. In the post-seedling stage Hope was moderately susceptible to all races whereas Africa Mayo and Kenya Page were moderately resistant to some races and moderately susceptible to other races.

It is noteworthy that none of the eight test-races produced a 3^+ or 4 infection type on post-seedlings of Hope, Africa Mayo, Kenya Page, or Conley. This may be an indication of horizontal resistance as defined by Van der Plank (1968). The eight test-races, however, constitute a very small sample of the virulence within the total stem rust pathogen population. At some future date an isolate of stem rust may be identified that has virulence on Conley but not on Hope, Africa Mayo, and

Kenya Page. This would result in a differential interaction and thereby prove that post-seedling resistance is not equivalent to horizontal resistance. Adult plant resistance to *P. recondita* apparently conforms to the gene-to-gene model (Dyck *et al*. 1966). Bartos *et al*. (1969) demonstrated that the adult plant resistance of Thatcher to leaf rust was race specific. This precedent should prevent one from assuming *a priori* that post-seedling resistance to wheat stem rust is identical with horizontal resistance.

4.2 THE STAGE OF DEVELOPMENT DURING WHICH RESISTANCE TO STEM RUST BECOMES EFFECTIVE

Introduction

In 1917 Stakman and Piemeisel reported the phenomenon that young plants of some wheat cultivars were more susceptible to *Puccinia graminis* than older plants. Cultivars such as Yaroslav emmer, Iumillo, Acme, Pentad, and Kota became resistant as they approached flowering to races of stem stem rust to which they had been susceptible in the seedling stage (Hursh 1924; Goulden *et al*. 1930). Craigie (1957) noted that Regent and Redman wheat developed resistance about the fifth leaf stage to a number of races to which they had been susceptible in the first leaf stage. Since then very little work has been done to determine the stage of development during which the post-seedling resistance of a cultivar becomes effective.

The object of this experiment was: (1) to determine the stage of development during which the cultivars, Hope, Africa Mayo, Kenya Page, and Conley developed a degree of resistance to stem rust races EA4 and EA8, and (2) to test for a race by stage of development interaction.

Materials and Methods

Each of the five cultivars Florence Aurore, Hope, Africa Mayo, Kenya Page, and Conley was planted on a series of seven dates. There were seven days between planting dates. The cultivars were planted and grown as described in Chapter 3.3. Duplicate sets of each planting date were grown.

When the plants from the first planting date of the fastest growing cultivar, Florence Aurore, were in the pre-milk stage, the stage of development for all of the cultivars in each planting date was assessed and recorded. The stage of development was determined by utilizing the modified Feekes scale as published by Large (1954). The number of leaves that had completely emerged was considered the "leaf-stage" of the plant. One set of all seven planting dates of all the cultivars was inoculated with a pure culture of race EA4 (Chapter 3.7 and 3.8). The other set was inoculated with stem rust race EA8. The plants were incubated for 18 - 20 hours in a moist chamber. Fourteen days later the infection types on the plants of the last three planting dates were recorded. Twenty days after inoculation the stem rust reaction and percentage infection on the plants of the first four planting dates were assessed (Chapter 3.9). The above procedure was repeated about five months later.

Results and Discussion

The stages of development for each planting date that the cultivars had attained at the time of inoculation are listed in Table 8. The cultivars did not grow at the same rate. For the first planting date the fastest growing cultivar, Florence Aurore, was in the pre-milk stage while the slowest growing cultivar, Conley, was in the boot stage. All of the cultivars in the last planting date were in the first leaf stage.

Table 8. The Stage of Development* for Five Cultivars in Each of Seven Planting Dates at the Time of Inoculation.

Planting Date	Florence Aurore	Hope	Africa Mayo	Kenya Page	Conley
I	Pre-milk	Boot	Flowering	Flowering	Boot
II	Flowering	Flag Leaf	Boot	Boot	Flag Leaf
III	Boot	5th Leaf	Flag Leaf	6th Leaf	6th Leaf
IV	Flag Leaf	4th Leaf	5th Leaf	5th Leaf	5th Leaf
V	5th Leaf	4th Leaf	4th Leaf	4th Leaf	4th Leaf
VI	3rd Leaf	2nd Leaf	2nd Leaf	2nd Leaf	2nd Leaf
VII	1st Leaf	1st Leaf	1st Leaf	1st Leaf	1st Leaf

* As described by Large (1954).

The reaction of the five cultivars to race EA4 at different stages of development is presented in Table 9. Florence Aurore was susceptible at all stages of development. The reaction class of Hope changed from S to an X and then to an MS. Thereafter the Hope reaction to stem rust race EA4 remained unchanged. The critical stage of development during which Hope became resistant would have been the 4th leaf stage.

The reaction class of Africa Mayo changed from S to MS between the 4th and 5th leaf stage. Kenya Page became progressively more resistant to race EA4, reaching a maximum by the 5th leaf stage. The reaction class of Kenya Page changed from S to X, to MS, and finally to MR. Similarly the resistance of Conley increased and became MR by the fifth leaf stage. The critical stage of development during which the resistance of

Kenya Page and Conley first became expressed would have been the late 2nd leaf and 3rd leaf stage.

Table 9. The Reaction of Five Wheat Cultivars at Different Stages of Development to *P. graminis* f.sp. *tritici* Race EA4.

Planting Date	Cultivar Florence Aurore	Hope	Africa Mayo	Kenya Page	Conley
I	S	MS	MS	MR	MR
II	S	MS	MS	MR	MR
III	S	MS	MS	MR	MR
IV	S	MS	MS	MR	MR
V	S	X	S	MS	MS
VI	S	S	S	X	MS
VII	S	S	S	S	S

The reaction class of the 5 cultivars inoculated with race EA8 at different stages of development are recorded in Table 10. Florence Aurore was susceptible at all stages of development. The other four cultivars began to express resistance during the same stages of development to race EA8 as they did to race EA4. That is, the resistance of Hope developed during the 4th leaf stage. Similarly the critical stage of development during which the resistance of Africa Mayo became expressed was the 4th to 5th leaf stage. Kenya Page and Conley first began to express resistance during the late 2nd leaf stage and the expression of resistance intensified up to the 5th leaf stage and thereafter remained constant.

Table 10. The Reaction of Five Wheat Cultivars at Different Stages of Development to P. *graminis* f.sp. *tritici* Race EA8.

Planting Date	Cultivar				
	Florence Aurore	Hope	Africa Mayo	Kenya Page	Conley
I	S	MS	MS	MR	MR
II	S	MS	MS	MR	MR
III	S	MS	MS	MR	MR
IV	S	MS	MS	MR	MR
V	S	S	S	MS	MS
VI	S	S	S	X	MS
VII	S	S	S	S	S

There was no race by stage of development interaction. The cultivars became resistant at different stages of development, but the stage of development at which a cultivar became resistant was a characteristic of the cultivar and was not influenced by the race.

It should be emphasized that the determination of "leaf-stage" was based on the number of leaves that had completely emerged. This criterion does not take into account the extent to which the next leaf had protruded up through the previous leaf's sheath. The choice of this criterion, even if arbitrary, permitted an easily recognizable "leaf-stage".

When seedlings of cultivars known to possess post-seedling resistance are inoculated with a race culture, the infection type on the 1st leaf should be taken as characteristic of the seedling only. Clipping back the young plant and re-inoculating it or inoculating successive leaves may not necessarily provide an accurate assessment of its seedling reaction.

Studies of the post-seedling resistance of some wheat cultivars to *Puccinia recondita* also show that all cultivars do not become resistant at the same stage of development (Samborski and Ostapyk 1959; Anderson 1966). Furthermore, the expression of leaf rust resistance may intensify and reach a maximum at about the flag leaf stage. Samborski and Ostapyk (1959) concluded that the distinction between seedling resistance and post-seedling resistance was not very clear.

Green and Knott (1962) hypothesized that in the seedling stage a seedling resistance gene may be ineffective against some races but it may become effective against these same races at some subsequent ontological stage. In such cases the resistant reaction may be due to a metabolite whose production is controlled by this gene. In the early growth phases the concentration of this metabolite may be too low for resistance to be expressed. As production of this metabolite continues the concentration of it passes a critical threshold and resistance becomes expressed. Alternatively the resistance may not be controlled by a physiological mechanism but rather by a morphological mechanism. In any event the plant must reach a definable ontogenic stage before resistance is expressed. If the pathogen determines the stage of host development at which resistance becomes expressed, then a race by stage of development interaction would be expected. No such interaction was detected in this experiment.

4.3 THE INHERITANCE OF POST-SEEDLING RESISTANCE IN FOUR WHEAT CULTIVARS TO TWO RACES OF STEM RUST

Introduction

Biffen (1905) reported that the resistance of some wheats to *Puccinia striiformis* was inherited as a Mendelian character. His report met scepticism. Yet by 1946 the list of wheat genetic studies compiled by Ausemus *et al.* (1946) contained a substantial number of works that dealt with the inheritance of disease resistance. Most of these inheritance studies of disease resistance were performed in field nurseries with little control over the variability of virulence within the test-pathogen in space and time, and no control over environmental conditions within and between seasons. Consequently the results were often difficult to interpret.

Flor (1956) proposed the gene-for-gene concept to explain host-pathogen interactions. Since then the experimental design used to study host-parasite interactions has been based on this concept. The progeny from a cross between a resistant and a susceptible host is tested to a pure race-culture of the pathogen. The ratio of resistant to susceptible progeny provides an estimate of the number of genes controlling resistance in the host.

An experiment was designed to investigate the manner in which post-seedling resistance was inherited and the number of genes controlling its expression in four wheat cultivars to two races of stem rust.

Materials and Methods

The four sources of post-seedling resistance, Hope, Africa Mayo, Kenya Page, and Conley, were each crossed and backcrossed to Florence Aurore which is susceptible in all stages of growth. About 250 - 300 backcross seeds (BC_1F_1) were produced for each donor parent. The parents and each BC_1F_1 population were planted in plastic pots in the greenhouse (Chapter 3.3). At about the ear emergence stage, the parents and progeny were divided into two groups of equal size. One group was inoculated with a pure culture of stem rust race EA4(295) and the other group was inoculated with a pure culture of race EA8(40) (Chapter 3.8). Rust reactions were recorded three weeks after inoculation (Chapter 3.9)

The progeny of a random sample of 25 BC_1F_1 plants from each race-cross combination were also tested to races EA4 and EA8. The parents and about 16 - 25 seeds per BC_1F_2 backcross line were planted. At about the ear emergence stage the backcross lines and parents were inoculated with the appropriate test-race. If the BC_1F_1 plant had been inoculated with race EA4, then the progeny of this plant was also inoculated with EA4. Similarly the BC_1F_2 lines that were inoculated with race EA8 derived from BC_1F_1 plants that had been inoculated with EA8. Because there was limited greenhouse space, only two race-cross combinations were grown and inoculated at a time.

The term "breeding behavior of a line" is used in this thesis to indicate that a line is either homozygous for reaction or segregating for reaction. After the BC_1F_2 lines of each cross had been inoculated with race EA4 and their breeding behavior determined, they were grown to maturity. The plants within each BC_1F_2 line were bulked together at harvest to produce BC_1F_3 bulk seed. A sample of 5 - 7 BC_1F_3 bulks per cross and the parents were planted. At about the ear emergence stage the material was inoculated with a pure race culture of EA8. Rust reactions were recorded 21 days after inoculation. The breeding behavior of each BC_1F_3 bulk inoculated with EA8 was compared to the breeding behavior of its BC_1F_2 progenitor that had been inoculated with EA4.

Control plants of Florence Aurore were included each time that segregating material was tested to a race. If the average level of infection on Florence Aurore was less than 20%, the inoculation and/or incubation conditions for that particular test were considered to have been less than optimum. In such a case the rust reactions were not recorded. The material was grown to maturity and subsequently progeny tested.

The Chi-square goodness of fit test was used to compute the probability that the observed segregation ratio fitted a hypothesized ratio.

Results and Discussion

If resistance is inherited in a dominant manner, then it will be expressed by a proportion of the BC_1F_1 plants. On the other hand, if resistance is inherited in a recessive manner, all of the BC_1F_1 plants will be susceptible.

The number of genes controlling resistance can be estimated by the probability of an observed segregation ratio fitting a hypothesized segregation ratio. If resistance is controlled by a single dominant gene, then the ratio of resistant to susceptible BC_1F_1 plants would be 1:1. The progeny of the resistant plants would segregate whereas the progeny of the susceptible plants would breed true for susceptibility. Therefore, the expected ratio of segregating BC_1F_2 lines to homozygous susceptible lines would also be 1:1. The segregation ratio within the segregating BC_1F_2 lines would be 3:1.

On the other hand if resistance is controlled by two independent dominant genes, the expected ratio of resistant to susceptible BC_1F_1 plants would be 3:1. The AaBb, Aabb, and aaBb genotypes would be resistant and the aabb genotype would be susceptible. The progeny of the first three genotypes would segregate whereas the progeny of the aabb genotype would be homozygous susceptible. Hence, the expected ratio of segregating BC_1F_2 lines to homozygous susceptible lines would be 3:1. The segregation ratio within the lines derived from AaBb progenitors would be 15:1, while the within line segregation ratio derived from Aabb and aaBb progenitors would be 3:1.

Hope

The post-seedling reactions of Hope, Florence Aurore, and the backcross progeny to races EA4 and EA8 are listed in Tables 11 and 12. Resistance to both races was expressed in about one half of the BC_1F_1 plants. Hence, the resistance of Hope was inherited as a dominant character. The observed segregation ratio of both the BC_1F_1 plants and the

BC_1F_2 lines for reaction to race EA4 fitted a single gene segregation ratio ($0.7 < P < 0.9$, $0.95 < P$, Table 11).

Table 11. The Stem Rust Reactions of Parents and Progeny of Cross Hope/ 2* Florence Aurore+ to Race EA4(295).

Parent or Generation	Stem Rust Reaction			Expected	P-value
Hope	MS				
Florence Aurore (FA)			S		
Hope/2* FA					
BC_1F_1 Single Plants	32 MS		35 S	1:1	0.9 - 0.7
BC_1F_2 Lines		10Seg	9 S	1:1	0.95

+ The pedigree has been written according to the system suggested by Purdy *et al*. (1968).

The segregation of BC_1F_1 plants to race EA8 conformed to a hypothesized single gene ratio ($0.3 < P < 0.5$, Table 12). The observed 17 segregating BC_1F_2 lines to 6 homozygous susceptible lines did not conform to the hypothesized single gene ratio ($P < 0.05$). The within line segregation, however, confirmed that Hope possessed a single gene for resistance to race EA8(148MS:54S, P = 0.7 - 0.5). The excess number of segregating lines may have been fortuitous.

Table 12. The Stem Rust Reactions of Parents and Progeny of Cross Hope/ 2* Florence Aurore to Race EA8(40).

Parents or Generation	Stem Rust Reaction			Expected	P-value
Hope	MS				
Florence Aurore (FA)			S		
Hope/2* FA					
BC_1F_1 Single Plants	62 MS		51 S	1:1	0.5 - 0.3
BC_1F_2 Lines		17Seg	6 S	1:1	0.05 - 0.025

Africa Mayo

The post-seedling reactions of parents and backcross progeny of the cross between Africa Mayo and Florence Aurore to races EA4 and EA8 are recorded in Tables 13 and 14. Resistance was expressed by about one half of the BC_1F_1 plants. Therefore,resistance was inherited as a dominant trait. The observed segregation of BC_1F_1 plants and BC_1F_2 lines for reaction to race EA4 exhibited single gene inheritance ($0.1 < P < 0.2$, $0.7 < P < 0.9$, Table 13). The within line segregation did not conform to this hypothesis (76 MS:40 S), $0.02 < P < 0.05$). There were a few too many plants in the susceptible category which may have been a chance occurrence.

Table 13. The Stem Rust Reactions of Parents and Progeny of Cross Africa Mayo/2* Florence Aurore to Race EA4.

Parent or Generation	Stem Rust Reaction			Expected	P-value
Africa Mayo	MS				
Florence Aurore (FA)			S		
Africa Mayo/2* FA					
BC_1F_1 Single Plants	43 MS		56 S	1:1	0.2 - 0.1
BC_1F_2 Lines		9Seg	11 S	1:1	0.9 - 0.7

Similarly, the segregation of the BC_1F_1 plants and BC_1F_2 lines for reaction to race EA8 fitted an assumed single gene ratio ($0.9 < P < 0.95$, $0.5 < P < 0.7$, Table 14). The within line segregation (81 MS:40 S, $0.05 < P < 0.1$) also conformed to the single gene model. Therefore, the post-seedling resistance of Africa Mayo to race EA8 was inherited monogenically.

Table 14. The Stem Rust Reactions of Parents and Progeny of Cross Africa Mayo/2* Florence Aurore to Race EA8.

Parent or Generation	Stem Rust Reaction			Expected	P-value
Africa Mayo	MS				
Florence Aurore			S		
Africa Mayo/2* FA					
BC_1F_1 Single Plants	49 MS		50 S	1:1	0.95 - 0.90
BC_1F_2 Lines		9Seg	13 S	1:1	0.7 - 0.5

Kenya Page

The post-seedling reactions of parents and backcross progeny from the cross between Kenya Page and Florence Aurore to races EA4 and EA8 are listed in Table 15 and 16. Because resistance to either race was expressed by a large proportion of the BC_1F_1 plants the resistance of

Table 15. The Stem Rust Reactions of Parents and Progeny of cross Kenya Page/2* Florence Aurore to Race EA4.

Parent or Generation	Stem Rust Reaction			Expected	P-value
Kenya Page	MR				
Florence Aurore (FA)			S		
Kenya Page/2* FA					
BC_1F_1 Single Plants	75 MR		29S	3:1	0.7 - 0.5
BC_1F_2 Lines		14Seg	7S	3:1	0.7 - 0.5

Table 16. The Stem Rust Reactions of Parents and Progeny of Cross Kenya Page/2* Florence Aurore to Race EA8.

Parent or Generation	Stem Rust Reaction			Expected	P-value
Kenya Page	MR				
Florence Aurore (FA)			S		
Kenya Page/2* FA					
BC_1F_1 Single Plants	55 MR		22 S	3:1	0.7 - 0.5
BC_1F_3 Bulks		15Seg	4 S	3:1	0.9 - 0.7

Kenya Page was inherited as a dominant trait. The observed segregation of both the BC_1F_1 plants and BC_1F_2 lines to race EA4 fitted a two gene model ($0.5 < P < 0.7$, $0.5 < P < 0.7$, Table 15). Resistant plants fell into only one category. Those progeny with one gene were able to express the same level of resistance as those progeny with two genes. Therefore, the post-seedling resistance of Kenya Page to race EA4 is controlled by two duplicate dominant genes.

The segregation ratio of the BC_1F_1 plants indicated that two independent genes were segregating for reaction to race EA8 ($0.5 < P < 0.7$, Table 16). The rust reactions of the BC_1F_2 lines were not recorded because the level of infection on the check plants of Florence Aurore was less than 20%. The BC_1F_2 lines were progeny tested as BC_1F_3 bulks. The ratio of 15 segregating to 4 homozygous susceptible BC_1F_3 bulks fitted a 3:1 ratio ($0.7 < P < 0.9$, Table 16). There was only one level of resistance (MR) expressed by the progeny. Hence, the two resistance genes of Kenya Page were acting as duplicates.

Conley

The post-seedling reactions of the parents and backcross progeny from the cross between Conley and Florence Aurore to races EA4 and EA8 are recorded in Tables 17 and 18. Resistance to either race was expressed by about three-quarters of the BC_1F_1 plants. Hence, the post-seedling resistance of Conley to both races was inherited as a dominant character. The observed segregation of both the BC_1F_1 plants and BC_1F_2

Table 17. The Stem Rust Reactions of Parents and Progeny of Cross Conley/2* Florence Aurore to Race EA4.

Parent or Generation	Stem Rust Reaction			Expected	P-value
Conley	MR				
Florence Aurore (FA)			S		
Conley/2* FA					
BC_1F_1 Single Plants	54 MR		12 S	3:1	0.3 - 0.2
BC_1F_2 Lines		17Seg	5 S	3:1	0.95

Table 18. The Stem Rust Reactions of Parents and Progeny of Cross Conley/2* Florence Aurore to Race EA8.

Parent or Generation	Stem Rust Reaction			Expected	P-value
Conley	MR				
Florence Aurore (FA)			S		
Conley/2* FA					
BC_1F_1 Single Plants	48 MR		17 S	3:1	0.95 - 0.9
BC_1F_3 Bulks		19Seg	2 S	3:1	0.2 - 0.1

lines for reaction to race EA4 conformed to a hypothesized 3:1 ratio ($0.2 < P < 0.3$, $0.95 < P$, Table 17). The two genes appeared to be acting as duplicates.

The observed segregation of 48 moderately resistant BC_1F_1 plants to 17 susceptible BC_1F_1 plants to race EA8 indicated that resistance was controlled by two genes ($0.9 < P < 0.95$, Table 18). The rust reactions of the BC_1F_2 lines of this cross to race EA8 were not recorded because the level of infection on the check plants of Florence Aurore was less than 20%. The BC_1F_2 lines were progeny tested as BC_1F_3 bulks. The ratio of 19 segregating to 2 homozygous susceptible BC_1F_3 bulks confirmed that Conley has two genes for post-seedling reaction ($0.1 < P < 0.2$, Table 18).

Comparison of the breeding behavior of a BC_1F_3 bulk inoculated with race EA8 to the breeding behavior of its BC_1F_2 progenitor that had been inoculated with race EA4

If we assume that a given cultivar has one gene that confers post-seedling resistance to two races of stem rust, then any one hybrid line that derived from a cross between this cultivar and a susceptible one would exhibit the same breeding behavior to both races. That is, if the hybrid line carried the resistance gene, it would be homozygous resistance or segregating for reaction to both races, but if the hybrid line did not carry the gene, it would be homozygous susceptible to both races.

On the other hand, if a cultivar possesses a gene that conditions post-seedling resistance to one race and a different gene for reaction to a second race, then a proportion of the hybrid lines would exhibit a dissimilar breeding behavior to each race. That is, under such circumstances hybrid lines that are homozygous susceptible to one race may be either homozygous susceptible or segregating for reaction to the other race. The probability of failing to detect a line that exhibits dissimilar breeding behavior to the two races depends upon the number of genes that condition resistance to each race and the number of lines that are inoculated with both races (cf. Appendix I).

The breeding behavior of the BC_1F_3 bulks inoculated with race EA8 and the breeding behavior of their BC_1F_2 progenitors inoculated with race EA4 for each source of post-seedling resistance that was backcrossed to Florence Aurore are listed in Table 19. The three assumptions were made that Kenya Page had two genes controlling post-seedling reaction to race EA4, that it had two different genes for reaction to EA8, and that the genes were inherited independently. The four BC_1F_3 bulks that segregated for reaction to EA8 derived from BC_1F_2 lines that had also segregated to EA4 (Table 19). Similarly the two BC_1F_3 bulks that were homozygous susceptible to EA8 derived from two BC_1F_2 lines that were homozygous susceptible to EA4. If the three assumptions were true, then the probability of failing to detect one or more BC_1F_3 bulks that would have exhibited a dissimilar breeding behavior from its BC_1F_2 progenitor is $(3/4)^4 \times (1/4)^2 < 0.02$ (Table 19 and Appendix I). The assumptions,

Table 19. The Breeding Behavior of BC_1F_3 Bulks Inoculated With Stem Rust Race EA8 and the Breeding Behavior of Their BC_1F_2 Line Progenitors That Were Inoculated With Race EA4.

Kenya Page/2* Florence Aurore

			EA8	
			Seg	Homo S
EA4	Seg	4	4	0
	Homo S	2	0	2

Conley/2* Florence Aurore

			EA8	
			Seg	Homo S
EA4	Seg	3	3	0
	Homo S	3	0	3

Hope/2* Florence Aurore

			EA8	
			Seg	Homo S
EA4	Seg	6	6	0
	Homo S	1	1	0

Africa Mayo/2* Florence Aurore

			EA8	
			Seg	Homo S
EA4	Seg	3	2	1
	Homo S	2	0	2

therefore, were rejected and it was concluded that Kenya Page has two genes for post-seedling resistance which are effective against both races EA4 and EA8.

The previous three assumptions were also made with regard to Conley. The three BC_1F_2 lines that segregated for reaction to EA4 each gave rise to a BC_1F_3 bulk that segregated for reaction to EA8 (Table 19). The three BC_1F_2 lines that were homozygous susceptible to EA4 each gave rise to a BC_1F_3 bulk that was also homozygous susceptible to EA8. If the assumptions were true, the probability that the six bulks would exhibit a similar breeding behavior as its progenitor to each race was $(^3/4)^3 \times (^1/4)^3 < 0.01$ (Appendix I). The very small probability of occurrence indicated that the assumptions were false. It was concluded that Conley has only two genes that determined post-seedling resistance and that these two genes are effective against both races EA4 and EA8.

The assumption was made that Hope had one gene for reaction to race EA4 and a second gene for reaction to EA8. The probability that the six BC_1F_2 lines that segregated for reaction to EA4 would also segregate to race EA8 was $(^1/2)^6 = 0.016$ (Table 19 and Appendix I). The one BC_1F_2 line that was homozygous susceptible for reaction to EA4 segregated for reaction to EA8. This was an expression of dissimilar breeding behavior. Only a few plants in this BC_1F_3 bulk exhibited a moderately susceptible reaction. It is possible that they arose from seed admixtures during threshing and/or planting. To confirm that this particular BC_1F_3 bulk exhibits a dissimilar breeding behavior to races EA4 and EA8, it was intended to inoculate a sample of it simultaneously to each race. Unfortunately the author's contract in Kenya expired before the test could be conducted.

It is probable that Hope has one gene for post-seedling reaction which is effective against both races. The results obtained corroborated Knott's (1968, 1971) study of the inheritance of stem rust resistance in Hope. Knott (1968, 1971), using pure race-cultures to inoculate BC_1F_2 families of Hope/2* Marquis, reported that Hope possessed three genes for reaction to stem rust. Two of these genes, Sr9d and sr17, conferred seedling resistance. The third gene, however, conditioned post-seedling resistance to race 56.

The comparison of the breeding behavior of the BC_1F_3 bulks from the cross Africa Mayo/2* Florence Aurore was more difficult to explain. It was assumed that Africa Mayo had one gene for reaction to EA4 and a second gene for reaction to EA8. One of the five BC_1F_3 bulks exhibited a dissimilar breeding behavior to its BC_1F_2 progenitor. The probability that x number of BC_1F_3 bulks would express a dissimilar breeding behavior was calculated by:

$$f(x) = \binom{n}{x} \; p^{x} \; q^{n-x}$$

where x is the number of BC_1F_3 bulks exhibiting dissimilar breeding behavior

n is the total number of BC_1F_3 bulks being tested p is the probability that dissimilar breeding behavior will be observed

q is the probability of failing to observe dissimilar breeding behavior.

The probability that one out of five BC_1F_3 bulks would exhibit dissimilar breeding behavior was 0.156. This is a rather low probability of occurrence. To confirm that this BC_1F_3 bulk did in fact have a dissimilar breeding behavior to its progenitor, a sample of the progeny from this bulk was to be inoculated with race EA4 and another sample of the progeny was to be inoculated with race EA8. Unfortunately a time limitation prevented the author from progeny testing this BC_1F_2 line as well as the one BC_1F_2 line from the cross Hope/2* Florence Aurore that showed anomolous breeding behavior. Therefore no definite conclusion was drawn as to whether Africa Mayo had one gene for reaction to EA4 and a second gene for reaction to EA8 or whether it had one gene which was effective against both races.

If the inoculated susceptible control plants for any test had less than 20% infection, then the inoculation and incubation conditions were considered to have been less than optimum. Under such conditions infections on both resistant and susceptible plants were more likely to occur in regions of greater susceptibility. Also some plants might not show any sign of infection. It was difficult to distinguish between resistant and susceptible plants. Consequently, the rust reactions were not recorded and the material was progeny tested. Under near optimum conditions for inoculation and incubation infections developed on all plants. However, susceptible type pustules developed on all plant parts of only Florence Aurore and homozygous susceptible genotypes. Therefore, it was possible to distinguish between plants with and without resistance.

Usually the characteristics of the pustule can be used to assess the reaction of the host. Occasionally 3^- and/or 3 infection type pustules occurred close enough so as to coalesce and to form a larger pustule. Such plants might erroneously have been classified as susceptible. Since the inoculation and incubation procedure was designed to obtain a high level of infections, this might explain why there was generally more plants scored as susceptible than expected.

Because the donor parents, Hope, Africa Mayo, Kenya Page and Conley, had only intermediate levels of resistance, the progeny would be expected to have intermediate resistance or no resistance. Hence, the progeny were classified as resembling either the resistant parent or the susceptible one. Transgressive segregation for resistance would be unlikely. A progeny plant showing no infection was considered an escape rather than a transgressive segregant. Such plants were not included in the analysis.

The infection type on a plant with resistance may vary from a 1^+ to a 3^+. Consequently, the "resistance" category was kept broad. A 3^+ infection type may occur in the region of greater susceptibility. The 1^+ and 2 infection types would occur on the lower stem regions.

When the BC_1F_2 lines of the crosses Kenya Page/2* Florence Aurore and Conley/2* Florence Aurore were inoculated with race EA8, less than

20% infection developed on Florence Aurore. Instead of scoring the rust reactions of the progeny, they were grown to maturity and subsequently tested as BC_1F_3 bulks. The expected ratio of segregating to homozygous susceptible BC_1F_3 bulks would be the same as the expected ratio of segregating to homozygous susceptible BC_1F_2 lines.

A small number of plants constituted each line (cf. Chapter 3.4). The size of each line was determined by the number of plants required to be able to distinguish between segregating lines and homozygous susceptible lines. The number of plants within a line was inadequate to permit distinguishing between a 3:1 ratio and a 15:1 ratio. The minimum number of plants required to distinguish between these two ratios at the 5% probability level would be about 38 (Hansen 1959). Therefore, the within line segregation was computed only in those crosses which had indicated single gene segregation.

In summary, post-seedling reaction was inherited as a dominant character. It was controlled by one gene in Hope and probably by one gene in Africa Mayo. Kenya Page and Conley both possessed two duplicate dominant genes for post-seedling reaction that were effective against both races EA4 and EA8. The post-seedling resistance of these cultivars to East African races of stem rust would be easy to manipulate in a breeding program.

4.4 DIALLEL ANALYSIS OF FOUR WHEAT CULTIVARS FOR POST-SEEDLING RESISTANCE TO STEM RUST

Introduction

Since Biffen (1905) demonstrated that cultivars within *T. aestivum* had genetic resistance to *P. striiformis*, wheat breeders have been using resistance genes to control diseases caused by *Puccinia* species. The number of resistance genes that can be incorporated into one cultivar depends upon the number of loci for resistance. The number of alleles for resistance that can be combined within a cultivar depends upon the number of loci that condition resistance, whether there is an allelic series at each locus, and whether the cultivar being developed is to be used as a homozygous line or an F_1 hybrid.

A diallel analysis can be used to determine whether several resistant cultivars have a locus in common. And if they do have a locus in common, it can be determined whether they possess the same or different alleles. If they have different resistance genes, the degree of recombination between them can be determined.

A diallel analysis was carried out to determine the genetic relationship of the genes for post-seedling resistance carried by Hope, Africa Mayo, Kenya Page, and Conley.

Materials and Methods

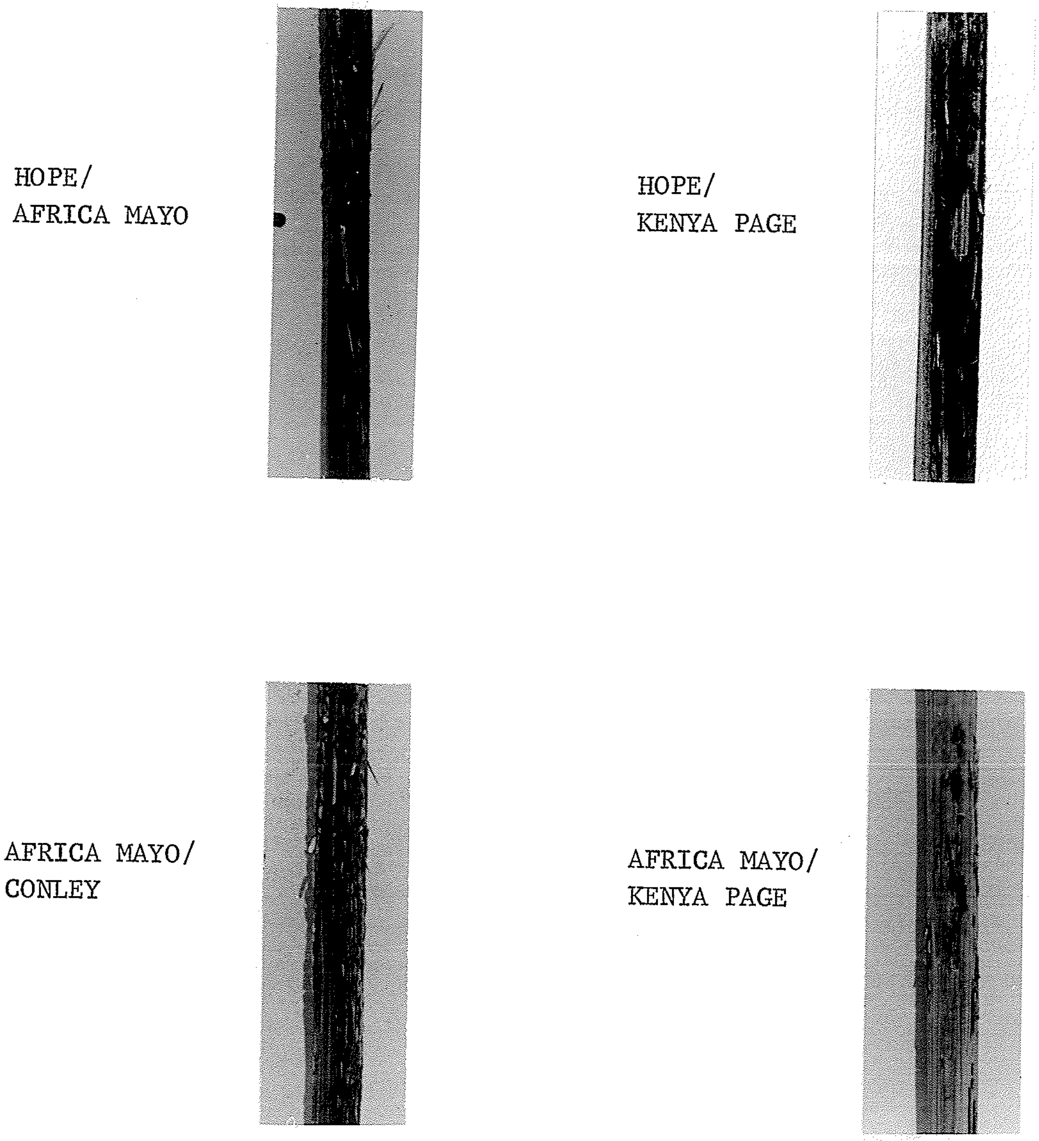
The cultivars, Hope, Africa Mayo, Kenya Page and Conley, were crossed in diallel. The parents and 400 - 600 F_2 seeds of each cross were planted in 12.7 cm diameter plastic pots (cf. Chapter 3.3). At about the flag leaf stage one half of each population was inoculated with a pure culture of race EA4(295) and the other half was inoculated with a pure culture of race EA8(40) (cf. Chapter 3.8). Rust reactions were recorded 18 - 21 days after inoculation (cf. Chapter 3.9). The F_2 plants that appeared to be susceptible to EA8 were progeny tested (cf. Chapter 3.4). The Chi-square test was used to determine the goodness of fit of the observed segregation pattern to the hypothesized ratio (cf. Chapter 3.10).

Results and Discussion

The stem rust reactions of the progeny from the diallel cross to race EA8 are recorded in Table 20. Some F_2 plants had susceptible type pustules on all plant parts. Some F_2 plants had a fairly high percentage ($>$20%) of 2^+ and/or 3 infection response. Both types of F_2 plants were classified as susceptible and subsequently progeny tested. If an F_2 plant was in fact genotypically susceptible, then it would be expected to breed true for susceptibility since it was shown that each cultivar possessed dominant genes for reaction. Homozygous susceptible F_3 lines were identified in the following crosses: Hope/Africa Mayo, Hope/Kenya Page, Africa Mayo/Kenya Page, and Africa Mayo/Conley. Pustules on stems from F_3 lines that bred true for susceptibility to race EA8 are shown in Figure 4.

Table 20. The Stem Rust Reactions of F_2 Plants and F_3 Lines of Four Sources of Post-Seedling Resistance, That Had Been Crossed in Diallel, to Race EA8.

Cross or Generation	Stem Rust Reaction			Expected	P-value
Hope/Africa Mayo					
F_2 plants	94 MS		6S		
F_3 lines		3Seg	3S		
Adjusted F_2	91 MS		3S	15:1	0.5 - 0.3
Hope/Kenya Page					
F_2 plants	167 MS		7S		
F_3 lines		3Seg	4S		
Adjusted F_2	170 MS		4S	63:1	0.7 - 0.5
Hope/Conley					
F_2 plants	157 MS		3S		
F_3 lines		3Seg	0		
Adjusted F_2	160 MS		0	63:1	-
Africa Mayo/Kenya Page					
F_2 plants	184 MS		3S		
F_3 lines		1Seg	2S		
Adjusted F_2	185 MS		2S	63:1	0.9 - 0.7
Africa Mayo/Conley					
F_2 plants	118 MS		4S		
F_3 lines		3Seg	1S		
Adjusted F_2	121 MS		1S	63:1	0.9 - 0.7
Kenya Page/Conley					
F_2 plants	214 MS		1S		
F_3 lines		1Seg	0S		
Adjusted F_2	215 MS		0S	255:1	-



HOPE/
AFRICA MAYO

HOPE/
KENYA PAGE

AFRICA MAYO/
CONLEY

AFRICA MAYO/
KENYA PAGE

Figure 4. Stem rust reactions on stems taken from homozygous susceptible F_3 lines derived from the crosses Hope/Africa Mayo, Hope/Kenya Page, Africa Mayo/Conley, and Africa Mayo/Kenya Page that were inoculated with race EA8 (40).

Some of the F_3 lines segregated. Therefore the F_2 progenitors of such lines were not genotypically susceptible. The numbers of homozygous susceptible and segregation F_3 lines were used to adjust the F_2 segregation ratio (Table 20).

Hypothesized segregation ratios were based on the number of genes for post-seedling resistance known to be carried by each cultivar (Table 21). In those populations which segregated for resistance the observed segregation pattern fitted the hypothesized ratio (Table 20). The segregation ratios to race EA8 indicated that Hope and Africa Mayo differed by two genes, and that Hope and Kenya Page, Africa Mayo and Kenya Page, and Africa Mayo and Conley each differed by three genes.

Table 21. The Number of Genes for Post-Seedling Reaction Carried by Each Cultivar to Races EA4 and EA8.

Cultivar	Race EA4	Race EA8
Hope	1	1
Africa Mayo	1	1
Kenya Page	2	2
Conley	2	2

No homozygous susceptible F_3 lines were identified from the crosses Hope/Conley and Kenya Page/Conley. Hope is involved in both parents of Conley (Table 1). Therefore, it is quite possible that Conley carries the Hope gene for post-seedling resistance.

It is more difficult to justify the lack of segregation in the cross Kenya Page/Conley. They do not have any immediate common ancestors (Table 1). Linkage between the resistance genes carried by either parent would result in an increased frequency of susceptible genotypes. Linkage between the genes carried by either parent was not detected (cf. Chapter 4.3). On the other hand, if the resistance genes of Kenya Page were linked in repulsion with the resistance genes of Conley, there would be a decreased frequency of the susceptible recombinant. If in fact the genes carried by either parent were different and independently inherited or linked in repulsion, it is quite possible that the population size was too small to detect segregation. Of the original 457 F_2 seeds of the cross Kenya Page/Conley rust reactions were obtained on only 215 plants. Not all of the seeds germinated, and some plants died during growth. The systemic insecticide 'Roguer-40' was sprayed on the plants to control aphids and this resulted in damage to the stem tissue of some plants. The stem rust reaction was not assessed on these damaged plants. Furthermore, some adult plants failed to show any indication of infection. These plants were considered escapes and were excluded from the analysis. The possibility of transgressive segregation for resistance, however, cannot be entirely ruled out.

F_2 plants susceptible to race EA4 were identified in the following crosses: Hope/Africa Mayo, Hope/Kenya Page, Africa Mayo/Kenya Page, and Africa Mayo/Conley (Table 22). The observed segregation fitted the hypothesized ratio which was based on the number of genes that each cultivar possessed (Tables 21 and 22). The segregation ratios indicated that Hope and Africa Mayo differed by two genes, and that Hope and Kenya Page, Africa Mayo and Kenya Page, and Africa Mayo and Conley, each differed by three genes. No susceptible plants were detected in the cross Hope/Conley. This provided further evidence that Conley possessed the Hope gene for post-seedling resistance.

All of the F_2 seed of the cross Kenya Page/Conley had been tested to EA8. Therefore no conclusion could be drawn about the genetic relationship of the genes for reaction carried by these two cultivars to race EA4.

The progeny from each cross exhibited the same breeding behavior to both races. The progeny from the cross Hope/Conley did not segregate for reaction to either race EA4 or race EA8. Also the progeny from each of the crosses Hope/Africa Mayo, Hope/Kenya Page, Africa Mayo/Conley, and Africa Mayo/Kenya Page segregated for the same number of genes for reaction to race EA4 as segregated for reaction to race EA8. This pattern would be expected since the gene(s) in each cultivar that determine post-seedling reaction was effective against both races EA4 and EA8 (cf. Chapter 4.3). Therefore, Hope, Africa Mayo, Kenya Page and Conley have among them at least four different genes for post-seedling reaction. Hope has one gene which is also carried by Conley. Kenya Page and Conley

Table 22. The Stem Rust Reactions of F_2 Plants Derived From Four Sources of Post-Seedling Resistance, That Had Been Crossed in Diallel, Inoculated with Race EA4.

Cross or Generation		Stem Rust Reaction		Expected	P-value
Hope/Africa Mayo	(F_2 Plants)	100 MS	8S	15:1	0.9 - 0.7
Hope/Kenya Page	(F_2 Plants)	173 MS	4S	63:1	0.7 - 0.5
Hope/Conley	(F_2 Plants)	131 MS	0	-	-
Africa Mayo/ Kenya Page	(F_2 Plants)	134 MS	2S	63:1	0.9 - 0.7
Africa Mayo/ Conley	(F_2 Plants)	140 MS	5S	63:1	0.2 - 0.1

may have a gene in common which is different from the Hope gene. The other gene in Kenya Page and the gene for post-seedling reaction in Africa Mayo are different from each other and from the genes in Hope and Conley.

CHAPTER 5

GENERAL DISCUSSION

Some wheat cultivars are known to have a type of resistance to one or more of the Puccinia species that becomes effective sometime after the first leaf stage but before flowering. The expression of post-seedling resistance may intensify until it reaches a maximum at about the flag-leaf stage, as reported by Samborski and Ostapyk (1959).

The post-seedling resistance of Kenya Page and Conley developed about the 3rd leaf stage. Hope and Africa Mayo began to express resistance at the 4th to 5th leaf stage. Neither the infection response nor the percentage of infection decreased appreciably from that stage of development at which resistance was first expressed until the end of the flowering stage. It was observed, however, that the infection type and the percentage of infection decreased after the pre-milk stage. Even the susceptible cultivar, Florence Aurore, expressed some resistance after the pre-milk stage. This was not likely a true expression of resistance rather it was probably an expression of resistance that was associated with senescence. That is, as the plants approached maturity the collenchymous tissue began to senesce; and therefore, the stem rust mycelium was no longer capable of growing in this tissue. As the guard cells died the stomata remained permanently closed and penetration of the stem rust pathogen was thereby prevented.

The cultivars began to express a degree of resistance at different ontological stages. Each cultivar, however, began to express a degree of resistance at the same ontological stage regardless of the test-race used. The stage of development at which resistance became expressed was a unique characteristic of the cultivar, and was independent of the pathogen strain.

The post-seedling resistance of the four cultivars was effective against all test-races of the pathogen. It would seem, therefore, that the post-seedling resistance of these cultivars was "broad spectrum" resistance. Each cultivar did not have an identical degree of resistance as measured by the infection response to each stem rust race. Hope had the lowest level of resistance, Africa Mayo and Kenya Page had intermediate levels of resistance. Conley had the highest level of resistance.

Each cultivar did not have the same degree of resistance to all test-races. For example, Kenya Page was moderately resistant to stem rust races EA3, EA4, EA7, EA8, EA13, and C25, whereas it was moderately susceptible to races C10 and C50.

According to Van der Plank (1968) if any cultivar has horizontal resistance, then this cultivar would be able to rank in order the virulence of a series of pathogen races. A second cultivar which has horizontal resistance would rank these races of the pathogen in exactly the same order. And conversely any race of the pathogen would be able to rank in order the level of horizontal resistance of several cultivars. A second race of the pathogen would rank the cultivars in exactly the same order. The cultivars Kenya Page and Africa Mayo did not rank in order the virulence of the eight stem rust races. Post-seedling resistance to stem rust, therefore, would not seem to be equivalent to horizontal resistance, sensu Van der Plank (1968).

The assessment of the horizontal resistance of these cultivars was very crude because the categories of resistance were broad. The characteristics of the aegricorpus were used to determine the infection type. No attempt was made to evaluate differences in the infectibility of host cultivars. The coefficient of infection (Kilpatrick and Harmon 1972) which weights the infection type and the level of infection may be more sensitive to detect different levels of horizontal resistance.

It is noteworthy that 3^+ (4) infection types are not consistently produced on post-seedlings of Hope by any stem rust race as far as the author is aware. Johnson (1949), Stakman (1954), and Green and Johnson (1955) reported that isolates of stem rust race 15B produced a higher infection type on Hope than any other North American race. The percentage of infected area, however, was less than on Thatcher or Marquis (Stakman and Rodenhiser 1958).

The post-seedling resistance of corn to P. sorghi seems to be inherited polygenically (Hooker 1967b). P. sorghi has not been a disease problem in the U.S.A. in spite of conditions favorable for disease development. Some of the inbred lines used to produce the hybrid cultivars are quite susceptible. The environmental conditions are favourable for the fungus. Hooker (1967b) attributed race non-specificity to polygenically inherited resistance. This is only circumstantial evidence that post-seedling resistance is race non-specific.

On the other hand, Bartos et al. (1969) provided conclusive evidence that the post-seedling resistance of Thatcher and Marquis to leaf rust was race specific. Isolates of P. recondita with virulence on the post-seedling resistance gene Lr13 have been identified (Samborski 1972).

The rate of disease increase is called the infection rate (Van der Plank 1968). It is affected by the incubation period, the time from infection to sporulation, the length of time that sporulation continues, and size to which lesions enlarge. Pustules on post-seedlings of the susceptible cultivar Florence Aurore developed to their maximum size within 14 - 16 days after inoculation, whereas the pustules on the cultivars with post-seedling resistance reached a maximum size 19 - 21 days after inoculation. Pustules on the stems of Florence Aurore were large and coalesced whereas the pustules on stems of Hope, Africa Mayo, Kenya Page and Conley were smaller. Chlorotic and necrotic tissue developed around the pustules or at both ends and thereby limited the size to which they enlarged. Under favourable conditions, however, the pustules just above the node would coalesce. Some pustules were long and very narrow.

These characteristics of post-seedling resistance tend to reduce the "infection rate". The cultivars Hope, Africa Mayo, Kenya Page, and Conley would be expected to have a relatively lower infection rate than a cultivar like Florence Aurore. In this way post-seedling resistance may have the same effect on an epidemic as horizontal resistance.

Post-seedling resistance may be due to a major 'Sr' gene. This gene would be ineffective in the seedling stage to some races of stem rust, but it would become effective to the same race(s) sometime before flowering (Green and Knott 1962). Alternatively, post-seedling resistance may be due to another set of genes which become effective only in the post-seedling stage.

This study was not designed to establish which alternative was correct. Speculation about the nature of the genes controlling post-seedling resistance may prove to be helpful for future studies. The answer may depend in part on the nature of the disease resistance mechanism, that is, whether it is due to physiological incompatibility or whether it is due to a morphological feature. If post-seedling resistance is due to physiological incompatibility, then differential interactions among races and cultivars is to be expected. The identified 'Sr' genes control hypersensitive resistance. Post-seedling resistance does involve a degree of incompatibility because chlorotic and necrotic tissue is generally produced around the pustules. The host offers restriction to the growth of the fungus mycelium. A limited differential interaction among races and cultivars was evident (Table 7), but none of the cultivars with post-seedling resistance were susceptible to any of the test-races.

Green and Knott (1962) demonstrated that the "single-gene" lines Sr9a and Sr10 were more resistant in the adult plant stage than in the seedling stage to race 29-1 (Can.). They attributed this post-seedling resistance to the genes Sr9a and Sr10 since they considered it unlikely that other gene(s) could have been transferred together with the major gene during the production of the Sr lines.

Harder et al. (1972) reported that seedlings of the line Sr6 did not show the usual temperature sensitive reaction to stem rust race EA2(21). They thought that the stable resistance of the line Sr6 may be due to a second gene.

Crow and Kimura (1970) have developed a formula to determine how large a chromosome segment can be transferred intact together with a desired gene. After ten backcrosses the average segment remaining intact would be 10 map units on either side of the gene being transferred. Therefore, it is possible that the 'Sr' lines could carry other resistance genes linked to the gene being transferred.

It is interesting to note that all of the Sr lines when grown in field nurseries at Njoro, Kenya, consistently exhibited at least 90% susceptible type pustules. It is unlikely, therefore, that any of the identified 'Sr' genes are responsible for the post-seedling resistance of the four cultivars studied.

Alternatively post-seedling resistance may be produced by a set of genes other than the "Sr-genes". Abdulla and Hermsen (1971) hypothesized that there are specialized polygenes that confer non-specific resistance. These specialized polygenes are responsible for the production of the substrates necessary for a host to exhibit resistance. If this hypothesis is true, the major genes detected in the four cultivars may act as switching genes which "turn on" a set of polygenes that determine the expression of post-seedling resistance.

One possible procedure that could be used to resolve which alternative is true would be to establish isogenic lines of each cultivar. The seedling resistance genes of each cultivar would have to be bred out. Isogenic pairs could be developed by backcrossing. A universal suscept would be used to contribute the alleles for seedling susceptibility. One line of each pair would not have any seedling resistance genes. The other member of the pair would still have both seedling resistance genes and post-seedling resistance genes. The post-seedling reaction of the isogenic lines to the test-races would then be compared.

A discussion of the problems associated with developing cultivars with post-seedling resistance is appropriate. The level of post-seedling resistance that a cultivar has may be adequate in some areas to permit its successful commercial production. For example, Kenya Page and Africa Mayo have been grown commercially in Kenya for 10 and 13 years respectively. Similarly, the post-seedling resistance to the Hope derivatives was adequate in the spring wheat region of North America to protect them until the build-up of the race 15B complex. Although the yield of Hope and its derivatives was reduced by the stem rust epidemics of 1953 and 1954, the cultivars without the Hope post-seedling resistance suffered a greater yield loss (Stakman and Rodenhiser 1958).

Genes that condition seedling resistance can be incorporated into a cultivar to provide protection against those races that are virulent on the post-seedling resistance genes. The seedling resistance genes and the post-seedling resistance genes may act synergistically. The seedling resistance genes would provide specific protection against those races which are most virulent on the post-seedling resistance genes. Post-seedling resistance tends to reduce the infection rate, and therefore, would inhibit the build-up of a race with virulence on the seedling resistance gene.

Seedling resistance genes frequently confer a lower infection response than do genes for post-seedling resistance. That is, a seedling resistance gene is epistatic to a post-seedling resistance gene. Therefore, to combine seedling resistance with post-seedling resistance each type of resistance must be screened for separately. To screen for each type of resistance one requires at least two test-races, one for each type of resistance. The test-races would not be mixed; rather only one race at a time would be used to inoculate plants. Because the testing of adult plants in the greenhouse requires a lot of space and is difficult to handle, it would be preferable·to screen for seedling resistance first. Since post-seedling resistance is rather simply inherited, it would be expected that a reasonable number of plants that expressed seedling resistance would also have post-seedling resistance. Where conditions for stem rust epidemics are near ideal, early generation material could be screened in field nurseries for genes that condition resistance throughout the life of the plant at the same time as quantitative characters are being assessed. The selected material could subsequently be screened in the greenhouse for post-seedling resistance using a test-race which has virulence on the seedling resistance but not on the post-seedling resistance.

An alternative screening procedure would be to make use of virulence combinations in different epidemiological regions. For example, seedling resistance and post-seedling resistance genes could be incorporated into wheat cultivars for the rust area of Western Canada. The seedling resistance genes could be screened for in Winnipeg. The East African races of stem rust generally have virulence on the identified seedling resistance genes that are being used to develop resistant wheats for Western Canada (Green _et_ _al_. 1970; Harder _et_ _al_. 1972). Therefore, screening for post-seedling resistance could be conducted in field nurseries in Kenya.

The expressivity of post-seedling resistance is subject to environmental modification. The inoculum load can affect the expression of post-seedling resistance. Some 2^+ and/or 3 type pustules that occur close together may coalesce and form what looks like one large pustule that resulted from a single infection. When there is a heavy inoculum load, there will be more infections per unit culm area. The frequency of coalescing pustules would be expected to increase and may result in lines being scored phenotypically susceptible. The inoculum load, the uniformity of the inoculum load, variation in maturity dates of the segregating material, and the uniformity of the micro-environment in space and time will affect the efficiency of selecting for post-seedling resistance.

Linkage of the genes controlling post-seedling resistance with undesirable traits would also complicate a breeding program. The post-seedling resistance of Hope, H-44-24, and Conley is linked to a gene controlling pigment production which results in "brown-necrosis" (Goulden and Neatby 1929; Shuh-Ji et al. 1968). Africa Mayo and Kenya Page do not express this brown-necrosis and hence they would be more desirable sources of post-seedling resistance.

It may be possible that the different genes controlling post-seedling resistance would segregate transgressively for a higher level of resistance. The four sources of post-seedling resistance used in this study have been combined by double crossing. Promising material is in the F_4.

Post-seedling resistance can be used in a breeding program together with seedling resistance. Combining seedling resistance and post-seedling resistance may provide a means of producing wheat cultivars with more durable resistance than the cultivars which had been developed in Kenya.

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APPENDIX

APPENDIX I

A Test to Determine Whether a Cultivar Has One Gene for Reaction to Two Races or Wheather it Has One Gene for Reaction to Each Race.

A given wheat cultivar 'A' has resistance to stem rust races EA4 and EA8, while cultivar 'B' is susceptible to both races. Cultivar 'A' is crossed and backcrossed to cultivar 'B'. A sample of the backcross progeny is inoculated with race EA4 and another sample is inoculated with race EA8. Segregation ratios indicate that reaction to each race is controlled by one dominant gene. The question that arises is whether cultivar 'A' has one resistance gene which is effective against both races, or whether it has one gene for reaction to race EA4 and a second gene for reaction to race EA8.

One possible procedure to determine which alternative is true is to test a series of hybrid lines to both races. If every line exhibits the same breeding behavior to each race, then cultivar 'A' has one gene for reaction which is effective against both races. On the other hand, if some hybrid lines exhibit dissimilar breeding behavior to race EA4 and race EA8, then cultivar 'A' has one gene for reaction to each race. The probability of failing to detect a line which exhibits dissimilar breeding behavior when the second hypothesis is true can be derived from the expected segregation ratios and the number of lines being tested to both races.

Of those BC_1F_2 lines that segregate for reaction to race EA4, 1/2 would be homozygous susceptible to race EA8 (Table 23). The probability of failing to detect a BC_1F_2 line that would segregate for reaction to race EA4 and would also be homozygous susceptible to race EA8 would be $(^1/2)^n$, where n is the number of BC_1F_2 lines segregating for reaction to race EA4 that are also tested to EA8.

Table 23. The BC_1F_1 Genotypes, the Breeding Behavior of the BC_1F_2 Lines Inoculated with Race EA4 and the Breeding Behavior of Their Corresponding BC_1F_3 Bulks Inoculated with Race EA8.

Genotype of Resistant Parent		Genotype of Susceptible Parent
AABB[+]		aabb
BC_1F_1 Genotypes	Breeding Behavior of BC_1F_2 Lines Inoculated with EA4	Breeding Behavior of BC_1F_3 Bulks Inoculated with EA8
AaBb	Seg	Seg
Aabb	Seg	Homo. Susc.
aaBb	Homo. Susc.	Seg
aabb	Homo. Susc.	Homo. Susc.

[+] Assuming (1) that gene - A confers post-seedling resistance to race EA4
(2) that gene - B confers post-seedling resistance to race EA8
(3) that the two genes are independently inherited.

Similarly of those BC_1F_2 lines that are homozygous susceptible to race EA4, 1/2 would segregate for reaction to race EA8. The probability of failing to detect a BC_1F_2 line that would be homozygous susceptible to race EA4 and would also segregate for reaction to race EA8 would be $(^1/2)^m$, where m is the number of BC_1F_2 lines homozygous susceptible to race EA4 that are also tested to race EA8. When a series of BC_1F_2 lines, some of which segregate for reaction to race EA4 and some of which are homozygous susceptible to it, are also tested to race EA8, the probability of failing to detect a BC_1F_2 line which would exhibit a dissimilar breeding behavior would be $(^1/2)^n \times (^1/2)^m = (^1/2)^r$, where r is the sum of n and m.

The assumptions are made that a cultivar has four genes for reaction, that the genes A and B confer resistance to race EA4 and the genes C and D confer resistance to race EA8, and that the four genes are inherited independently (Table 24). Of the four BC_1F_2 lines that are homozygous susceptible to race EA4, 3/4 would segregate for reaction to race EA8 (Table 24). The probability of failing to detect a BC_1F_2 line that would be homozygous susceptible to race EA4 and would segregate for reaction to EA8 would be $(^1/4)^n$, where n is the number of BC_1F_2 lines that are homozygous susceptible to race EA4 and are also tested to race EA8. Similarly of the BC_1F_2 lines that segregate for reaction to race EA4, 1/4 would be homozygous susceptible to race EA8 (Table 24). The probability of failing to detect a BC_1F_2 line that would segregate for

Table 24. The BC_1F_1 Genotypes, the Breeding Behaviour of the BC_1F_2 Lines Inoculated with Race EA4, and the Breeding Behaviour of Their Corresponding BC_1F_3 Bulks Tested with Race EA8.

Genotype of Resistant Parent	Genotype of Susceptible Parent
AABBCCDD	aabbccdd

BC_1F_1 Genotypes	Breeding Behaviour of BC_1F_2 Lines Tested with EA4	Breeding Behaviour of BC_1F_3 Bulks Tested with EA8
AaBbCcDd	Seg	Seg
aaBbCcDd	Seg	Seg
AabbCcDd	Seg	Seg
AaBbccDd	Seg	Seg
AaBbCcdd	Seg	Seg
aaBbccDd	Seg	Seg
aaBbCcdd	Seg	Seg
AabbccDd	Seg	Seg
AabbCcdd	Seg	Seg
AaBbccdd	Seg	Homo. Susc.
Aabbccdd	Seg	Homo. Susc.
aaBbccdd	Seg	Homo. Susc.
aabbCcDd	Homo. Susc.	Seg.
aabbccDd	Homo. Susc.	Seg.
aabbCcdd	Homo. Susc.	Seg.
aabbccdd	Homo. Susc.	Homo. Susc.

reaction to race EA4 and would be homozygous susceptible to race EA4 would be $(3/4)^m$, where m is the number of BC_1F_2 lines that segregate for reaction to race EA4 and are also tested to race EA8. When a series of these BC_1F_2 lines, some of which are homozygous susceptible to race EA4 and some of which segregate for reaction to it are also tested to race EA8, the probability of failing to detect a BC_1F_2 line which would exhibit a dissimilar breeding behavior would be $(1/4)^n \times (3/4)^m$.