AN ABSTRACT OF THE THESIS OF

___Crystal A. Rose___ for the degree of ___Master of Science___

in ___Crop Science___ presented on ___March 22, 1985___ .

Title:_The Inheritance of Stem Rust (Puccinia graminis graminicola)

Resistance in Perennial Ryegrass (Lolium perenne).

Abstract approved: Redacted for privacy

Three stem rust Puccinia graminis graminicola, resistant sources
of perennial ryegrass, Lolium perenne, 4A, 48A, and 77A, were crossed
in a partial diallel design with one susceptible source, MP-2,
resulting in six crosses excluding reciprocals. The F1's and cloned
parental populations were inoculated in the field and rated on a stem
rust scale of 1-9 with 9 best (immune), once a week for five weeks.
Maturity stage ratings of 1-6 with 1 during the vegetative stage and 6
at full maturity were also taken once a week.

Maturity stages were not highly correlated with stem rust
reactions except during the bootstage and early anthesis. Genetic
differences were found between parental sources for stem rust
reactions. Frequency distributions based on stem rust reactions among
the F1 progeny populations suggested that resistance was
quantitatively inherited with minor and possibly some major genes
acting in an additive manner. Transgressive segregation toward

susceptibility and slow rusting types were evident.

Broad sense heritability estimates for stem rust resistance varied depending on the particular cross. The estimates suggested that there is considerable genetic variability for resistance between the parental lines. Selection would be more effective in the bootstage stage prior to anthesis when the heritability estimates were found to be higher. As the inoculum increased in the later stages of plant development, the resistance appeared to be overcome by the spore load.

Seedling stem rust reactions were found to differ from adult plant reactions in the F1 generations of the six crosses in this study. In the F2 generation with open pollination of the F1 parents a gradient of percent susceptible seedlings occurred. Results of this study indicate a potential for developing stem rust resistant varieties with several minor and some major genes for increased longevity of resistance.

The Inheritance of Stem Rust (Puccinia graminis graminicola)
Resistance in Perennial Ryegrass (Lolium perenne).

by

Crystal A. Rose

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed March 22, 1985

Commencement June, 1985

APPROVED:

Redacted for privacy

Professor of Agronomy in charge of major

Redacted for privacy

Head of Department of Crop Science

Redacted for privacy

Dean of Graduate School

Date thesis is presented   March 22, 1985

Typed by   Crystal Rose

ACKNOWLEDGEMENTS

I would like to express my deep appreciation to Dr. Warren E. Kronstad, my major professor, for his invaluable advice, encouragement, interest and influence during my course work and preparation of this thesis.

I want to extend my gratitude to Dr. William A. Meyer, my adjunct professor, for his expertise, unending support and assistance with this research. The financial assistance, nursery space, supplies, equipment and efforts of Pure Seed Testing Inc. and its employees are also greatly appreciated.

I sincerely thank Dr. R. Petersen and Tom Cook for serving on my graduate committee and reviewing the manuscript.

I wish to thank my parents, Bill and Betty Rose and the rest of my family and friends, for their advice, support and prayers.

I would also like to thank my fiance, Mark Fricker, for his patience and help throughout my graduate studies.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

APPENDIX TABLES

The Inheritance of Stem Rust (Puccinia graminis graminicola)
Resistance in Perennial Ryegrass (Lolium perenne).

INTRODUCTION

Perennial ryegrass, Lolium perenne, is a cross-pollinated species
with 14 chromosomes. It is a cool-season, bunch grass well adapted to
maritime climates having mild winters and cool, moist summers, noted
in New Zealand, the British Isles and the coastal areas of Washington,
Oregon, and northern California. Many new varieties of perennial
ryegrass have been specifically developed for either turf or forage
and are being used throughout the United States. Stem rust, Puccinia
graminis graminicola (Smiley, 1983), is a serious problem in the seed
production of perennial ryegrass in western Oregon. Farmers must
apply fungicides April through July to prevent seed yield losses of up
to 93% (Meyer, 1982).

NATURE OF DISEASE

Stem rust is a macrocyclic, heteroecious fungus whose pustules
rupture the epidermis of stems, leaves, and eventually seed heads.
This fungus can penetrate closed stomatas (Hooker, 1967). The
alternate host is the barberry bush, Berberis vulgaris which has not
been successfully eradicated. Infected foliage serves as the
overwintering site for the mycelium and urediniospores in areas of
mild climates. This has been observed in the Willamette Valley of
western Oregon. The rust colored urediniospores reproduce asexually

all spring, summer and fall, with new cycles occurring every two
weeks.   Black teliospores may also over-winter on senescing leaf
tissue of the perennial ryegrass, they germinate in the spring to form
basidiospores which are blown through the air to infect the alternate
host. On the barberry bush the sexual pycnia stage occurs producing
aecia. Aeciospores are carried by the wind to the ryegrass plant.
These penetrate the stems and leaves through the stomata and the
mycelium then grows intercellularly. Hyphae with one urediniospore at
the tip of each, form just below the epidermis completing the life
cycle (Smiley, 1983; Agrios, 1978).

At this time no races of stem rust that infect perennial ryegrass
have been identified. Occurrences of new races of stem rust are
common in other crop species and are expected to be found in perennial
ryegrass (Meyer et al., 1984, in press). Prior to 1983 all of the
commercial turf-type perennial ryegrass cultivars were susceptible to
stem rust. Stem rust is prevalent in many areas of the United States
including Oregon, California, Washington, Pennsylvania, Nebraska, and
New Jersey (Funk, 1984) and in France (van Wijk 1981) and in New
Zealand (Armstrong and Rumball, 1976). In New Zealand stem rust is
becoming more of a problem as more later maturing varieties of
perennial ryegrass are being used (Latch, 1984).

In studies in western Oregon, three sources of rust resistance
were reported and several stem rust resistant varieties were developed
with these sources and tested in Oregon, New Jersey, and France
(Meyer, 1982; Meyer et al., 1984, in press). Because chemical control
of stem rust is expensive, genetic resistance is a desirable solution
to control this disease.

OBJECTIVES

The objectives of this study involving stem rust in perennial ryegrass were the following: 1) to determine if there is an association of disease reaction and plant maturity; 2) to determine the nature of inheritance by looking at frequency distributions of heterogeneous F1 populations; 3) to study slow rusting types over time in relation to parents, F1's, and the association between F1's and parents; 4) to determine if seedling reactions could be used to predict adult plant resistance.

<u>LITERATURE REVIEW</u>

NATURE OF RESISTANCE

The simplest mode of inheritance is a single or major gene
(Parlevliet and Zadoks, 1977). Most hosts of nearly all rust fungi
species have resistance due to a single, completely dominant (Allan et
al., 1966; Hagan and Hooker, 1965; Knott and Anderson, 1956),
partially dominant (Luke et al., 1975; Reifschneider and Arny, 1983),
or recessive gene (Berg et al., 1963). Genetic studies have also
revealed two or more genes which may be dominant, recessive, or a
combination of dominants and recessives acting either independently or
through some form of gene interaction (Berg, 1963). These monogenic
and oligogenic types of resistance have been incorporated as major
gene (Flor, 1971), or vertical resistance (van der Plank, 1968) to
give temporary control of rusts. Characterized by a hypersensitive
reaction in the host (Heath, 1976), segregates usually fall into
discrete, discontinuous classes and frequently fit genetic ratios
(Hooker, 1967).

Another type of resistance in contrast to the hypersensitivity-
based infection types, has quantitative variation where the rust
reaction in segregating populations is continuous, between and
sometimes beyond the resistant and susceptible parent. Quantitatve
variation in a character usually means that its expression is under
the control of many gene loci called polygenes (Hooker, 1967). A
polygenic character is not just a character conditioned by many genes.

It is a character conditioned by many genes each with a minor effect
(or, alternatively, has an effect large in relation to nonheritable
variation) van der Plank, 1982. Continuous variation does not
necessarily mean that the resistance is polygenically inherited. van
der Plank, 1982, describes the following four models of continuous
variation that are not polygenic in origin: 1) the oligogene model is
where a few genes contribute unequal effects resulting in a normal
distribution; 2) the linked-loci model postulates unequal effects of
gene blocks; 3) the monogene model has high nonheritable variance
caused by environmental variance which contributes to the continuous
distribution; and 4) the composite model which is a more realistic
mixture of the above models. Continuous phenotypic variation of
resistance is probably determined by a large number of genes with
small effects and a few genes with large effects, and by environmental
variance.

Additive genetic variance, not the number of genes seems to be
what determines the continuous variation of resistance. This type of
variance has been associated with continuously variable resistance and
is implied in the van der Plank models. Additive variance results in
some progeny resembling their respective parents. If all of the
variance is additive, the mean reaction of the F1 hybrid will fall
midway between its parents. Alternately, if dominance is complete or
nonadditive genetic variance is involved, the hybrid will resemble one
parent. According to van der Plank (1982), the general concept of
polygenic resistance can be replaced with the concept of mainly
additive resistance.

Reports of polygenic inheritance for rust resistance are less

frequent. In a field study of the inheritance of resistance in maize to Puccinia sorghi the segregating population of the F2 generation, had continuous variation (Hooker, 1962). The plant rust reactions ranged from the susceptible to the resistant parent, with the mean score of the F2 population near the average score of the parents suggesting that many genes condition the resistance. In some wheat varieties a slow-rusting reaction is apparently under polygenic control, being comprised of a number of linked genes resulting in the slow development of stem rust (Skovmand et al., 1978a). In a field study involving perennial ryegrass by Wilkins, (1975) resistance to crown rust, P. coronata Corda, was found to exhibit a continuous variation in ten of twelve genotypes with the inheritance conditioned by a large number of genes functioning in an additive manner. In Italian ryegrass, Wilkins (1974), found that ten parents had resistance to crown rust and Rhynchosporium orthosporum Caldwell, with resistance being controlled by a large number of genes exhibiting an additive type of genetic variance.

There are some reports of a few genes exemplifying continuous variation. Work done on resistance to blister rust, Cronartium ribicola in pine, Pinus monticols by Hoff and McDonald (1980), classified progeny according to the number of infection spots per linear meter of needle. The distribution of seedlings in the progeny was normal but slightly skewed with most of the seedlings giving an intermediate reaction. Heritability estimates were low and the data were best fitted by assuming monogenic inheritance. Kim and Brewbaker (1977) used two maize inbreds highly resistant to rust caused by P. sorghi and crossed them with susceptible inbreds. They regarded

general resistance as having a reduction in the number and size of lesions. It was estimated that one of the resistant inbreds, Oh545, had two gene pairs conditioning resistance. The other resistant inbreds averaged 1.3 gene pairs, indicating one pair had a major effect and others had minor effects. In Red Rustproof oats horizontal resistance to crown rust was found to be controlled by 2.16 genes showing slight partial dominance for susceptibility (Luke et al., 1975). In some cases resistance formerly attributed to polygenes have been found to be conditioned by major genes (Flor, 1971).

In several studies with perennial ryegrass, it appears that resistance to crown rust in some populations is polygenic while in others it is controlled by a few major genes (Kopec et al., 1983; Wilkins, 1974; Wilkins, 1975). Studying crown rust resistance in tall fescue, Festuca arundinacea, Wofford and Watson (1982) also found that different populations have different gene systems.

Polygenically inherited resistance has been found to be highly heritable. In 45 crosses of maize, the broad-sense heritability was over 85% (Hooker, 1967), and slow rusting resistance to stem rust in wheat had a narrow-sense heritability of 80% (Skovmand et al., 1978b). Luke and associates (1975) found that horizontal resistance to crown rust in oats has a broad-sense heritability value of 87%.

Stability or longevity of resistance, is where genes are not easily overcome by genetic changes in the pathogen. This depends on the degree of diversity of resistance genes and in their effect, frequency and distribution over the individuals in the host population. In natural populations no single host genotype is fully susceptible nor fully resistant to all pathogen genotypes, conversely,

a pathogen genotype is neither fully virulent or avirulent to all host genotypes. Every resistant gene, although its corresponding virulent gene is present at a certain frequency, contributes to the average degree of resistance of the population through its effect on the individual plant times its effective gene frequency in the population (Parlevliet and Zadoks, 1977). In modern crops this is different because genetic homogeneity of crops has allowed pathogens to develop more specialized virulent genes.

Resistance to disease based on veritical or major genes, is usually subject to erosion as has been evident in cereals (Hooker, 1967). The large genetic variability and adaptability of the rust fungus has caused epiphytotics to erupt via the wide spread and distribution of new rust biotypes virulent to currently grown varieties (Stakman, 1964). In maize near total disease control has been achieved with no reports of any widespread epiphytotics. This was done without race surveys, though races are present in nature and can be identified. Control was achieved without the use of genes for hypersensitivity-based resistance and yet these genes are well represented in the species (Hooker, 1967). Maize utilizes a polygenic or generalized type of resistance expressed in the form of low infection intensity and conditioned by many genes, which is opposite of the traditional major gene or specific type of resistance commonly used in cereals (Hooker, 1967).

Stability is an important component of disease resistance, especially in perennial species such as perennial ryegrass. In theory, multigene resistance offers increased stability because the host has an increased buffering capacity against the pathogen (Kopec

et al., 1983). A polygenically inherited resistance is likely to be relatively uniform in reaction to different pathogen populations and an improvement in stable resistance in comparison to major gene resistance (Wilkins, 1974).

Forage and turf grasses are generally self sterile, cross pollinators and being synthetic varieties are restricted populations of practically unique individuals, hence varieties may contain large numbers of resistance genes and gene combinations (Wilkins, 1975). Knowledge of the inheritance of rust resistance, qualitative or quantitative not only facilitates resistance breeding but provides an indication of the likelihood of serious erosion due to changes in the pathogen population.

The maturity stage of plants seems to have an effect on rust resistance as is evident in several studies. Skovmand et al., (1978b) found an association between later maturing varieties and slow rusting. The mature or adult plant resistance may condition resistance to a large number of races to which a variety is susceptible in the seedling stage (Hooker, 1967). Middle-age resistance is the main component of the horizontal resistance as a whole. This phenomenon of middle-age resistance and adult plant resistance is common in wheat, barley and oats for several types of rust (van der Plank, 1982). Gassner and Kirchhoff (1934) noted that in these same cereals with incomplete resistance a wave of resistance just before tillering and flowering is followed by a wave of susceptibility in old plants and the older parts of the plants. This wave of susceptibility in older plants is first seen in leaves, in the middle of the stems and later in the top leaves. Ohm and Shaner

(1976) found that growth stage affects expression of the component of slow rusting. Latent period was longest and pustule size the least on plants inoculated with leaf rust at growth stages between flag leaf emergence and flowering. Pustule size was the smallest just after flowering. Presenescence resistance or adult plant susceptibility is manifested as slow rusting which declines after flowering. Ohm and Shaner point out that even though slow-rusting resistance declines with senescence, it may nevertheless provide enough protection to the crop before and during flowering to prevent significant loss of yield from leaf rust.

In many breeding programs screening seedlings for resistance to rust is used to eliminate any plants that are susceptible. In many crops this technique is valid, but in some cases discarding susceptible seedlings may be a mistake. Allan and associates (1966), found in the case of seedling and adult plant reaction of wheat to stripe rust, some crosses had an association between adult and seedling reactions, while some lines resistant as adults were susceptible as seedlings. Apparently some loci conditioning adult resistance in the field do not influence seedling resistance in the greenhouse. Seedlings of plants possessing horizontal resistance often are relatively susceptible to pathogens, especially the plant rusts (Flor, 1971). Martin and associates (1979) found that the seedlings of particular wheat lines and cultivars may exhibit slow rusting. Reifschneider and Arny (1983), also found that screening for resistance to eyespot, Kabatiella zeae, at the seedling stage in maize is not correlated with adult plant resistance.

MATERIALS AND METHODS

During the summer of 1983 four perennial ryegrass parent clones were crossed in a partial diallel design resulting in six crosses excluding reciprocals. Three of the parents were stem rust resistant sources based on nine years of evaluation (Meyer, 1983). The resistant sources 4A and 48A were collected in 1975 from old turf areas in St. Louis, Missouri, and 77A was collected in Washington D.C. They will be referred to as Parents A, B, and C respectively throughout this paper. The susceptible parent, MP-2, referred to as Parent D, was obtained from a turf area in Washington D.C. of Belle perennial ryegrass selected for insect resistance.

Seed from each cross was planted and inoculated with stem rust spores two weeks after emergence. Stem rust infected perennial ryegrass leaves taken from several surrounding areas and sources were chopped and mixed in water to make a spore suspension of approximately 1000 spores/ml, determined by a microscopic count. This solution was misted onto the seedlings which were then kept in a moist chamber for three days and nights.

FIELD STUDY

Sixty seedlings from each cross were transplanted to the field in 5 1/2 cm. peat pots on October 19, 1983. The F1 progeny were space planted 60 cm. apart with 60 cm. between rows in a sandy silt loam soil of the Willamette series near Hubbard, Oregon in a randomized

block design with four replicates. Each replicate contained fifteen progeny from each cross. Twenty-seven vegetative clones were taken from each parent plant and randomly planted among the first three replicates of the progeny. Seven plants of the 4A parent and six plants of the susceptible, MP-2 parent died during the winter creating unequal numbers. Standard fertilization and weed control practices for commercial ryegrass production were used. All plants were fertilized with 50 kg of actual nitrogen per hectare in November and 40 kg of actual nitrogen per hectare in March and 45 kg of actual nitrogen per hectare in May. Weeds were controlled with 2,4-D at .56 kg per hectare and Dicamba at .23 kg per hectare rates.

On May 17, and June 12,1984, the plants were inoculated with 10-15 chopped blades of stem rust infected perennial ryegrass foliage obtained from the surrounding area dropped in the middle of each plant. From May through July the F1 progeny from each cross and the cloned parents were rated five times weekly using a visual estimate based on the percentage of rust pustules on the foliage of each plant. The scale of 1-9 was used to denote reaction patterns of perennial ryegrass to stem rust and is presented as follows:

Stem Rust Rating Scale

Score	Description
9	No rust.
8	Trace of foliar infection, 1-3 stems infected.
7	Up to 10% of the stems, leaves and seed heads infected.
6	10-30% of the stems, leaves and seed heads infected.
5	30-50% of the stems, leaves and seed heads infected.
4	50-70% of the stems, leaves and seed heads infected.
3	70-85% of the stems, leaves and seed heads infected.
2	Stems and seed heads dead, a few green leaves.
1	Whole plant dead.

These rating values represent a relative estimate of the percent of foliage infected with stem rust pustules, and not a reaction type. A scale of 1-9 with 9 best was also used by Meyer (1984, in press). Each plant was also rated for maturity stage on a scale of 1-6 to describe the life cycle of perennial ryegrass as follows:

Maturity Rating Scale

Score	Description
1	Vegetative stage
2	Bootstage
3	Full emergence of spike
4	Anthesis
5	Two weeks after anthesis
6	At harvest

Both of these ratings were taken once a week on the following dates June 18, June 27, July 3, July 10, and July 17, 1984. The approximate corresponding growth stages with these dates are bootstage, early anthesis, anthesis, two weeks after anthesis, and at harvest.

SEEDLING STUDY

On August 7, 1984 seed was harvested from plants of the most diverse crosses AXD and BXD for stem rust reaction. A representative of each rating of the stem rust scale, except a plant rating 1, was harvested from the F1 progeny. The F2 seed from these plants was planted on August 15, 1984 and two weeks after emergence the seedlings were inoculated with stem rust spores collected from the surrounding area. Severely infected leaf blades were chopped and mixed with water to produce a spore suspension which was misted onto the seedlings in a water solution. The plants were kept in a moist chamber for three days and nights. Fifty seedlings from each plant were studied by rating the spore reaction type, based on the following scale by E.C. Stakman et al. (1962).

Infection type	Reaction class
0	IMMUNE-No uredia nor other indications of infection
0;	NEARLY IMMUNE-No uredia, but hypersensitive flecks present
1	VERY RESISTANT-Uredia minute; surrounded by distinct necrotic areas
2	MODERATELY RESISTANT-Uredia small to medium; usually in green islands surrounded by a decidedly chlorotic or necrotic border
3	MODERATELY SUSCEPTIBLE-Uredia medium in size; coalescence infrequent; no necrosis, but chlorotic areas may be present, especially under unfavorable growing conditions
4	VERY SUSCEPTIBLE-Uredia large, and often coalescing; no necrosis, but chlorosis may be present under unfavorable growing conditions

The number of resistant and susceptible seedlings of each cross was counted in the three-leaf stage on September 28, 1984, approximately two weeks after inoculation.

STATISTICAL ANALYSIS

A two-way analysis of variance was performed on stem rust and plant maturity means of the parents and F1 progeny for five dates. Because of the unequal number of parents, a nonorthogonal analysis of variance was used to determine significant differences between parents on an individual plant basis. The mean disease and maturity ratings, variances, and standard deviations were calculated at each rating date for the plant populations. Differences between grand means of each cross and parent were compared by using Fishers protected LSD. The relationship between maturity stages and rust reactions were computed. Broad sense heritability estimates were obtained on a single plant basis using the variances of the cloned parental and segregating F1

populations as suggested by Burton (1953).

$$\frac{F1 - (P1 + P2/2)}{F1} = \frac{VF1 - VE}{VF1} = \frac{VG}{VG + VE} = h^2$$

<u>RESULTS</u>

To determine the nature of inheritance of stem rust resistance in perennial ryegrass a uniform disease pressure was created through artificial inoculations resulting in an epidemic common to those observed in the Willamette Valley of Oregon. On July 3, 95% of the populations were infected and a week later on July 10, 97.5% of the plants in the study were infected with stem rust. The three resistant Parents A, B, and C are early while the D parent is slightly later in maturity.

ANALYSIS OF PARENTS AND F1 POPULATIONS

No differences in parent maturity stages were observed except on June 18 (Appendix Table 1). However, when Fishers protected LSD was employed no differences were found for maturity stage between any of the parents even on June 18 as can be observed in Table 1. Differences between the parents for stem rust reactions were found at all five dates when rust ratings were taken (Appendix Tables 2-6). No significant differences in maturity stages for the F1 populations at any of the rating dates could be determined. Differences between F1 populations were found on June 18 and July 3 for stem rust reactions (Appendix Tables 7-11).

RELATIONSHIP OF MATURITY STAGES AND STEM RUST REACTIONS

Even though no differences were found for maturity stages of the parents or F1 populations, significant associations were noted for maturity stage and stem rust reactions. In Table 2 correlation and coefficient of determination values for these two variables are presented with parent and F1 populations combined. On June 18 the highest correlation and coefficient of determination values were -0.637 and 0.40 respectively, followed by -0.52 and 0.27 respectively, noted on July 3. The remaining dates had low correlation values between these variables.

TWO-WAY FREQUENCY TABLE OF PARENTS AND F1 POPULATIONS

Table 3 represents a two-way frequency table with totals, means, and standard deviations of the parent and F1 population stem rust reactions on July 10, 1984. This date was selected because the largest differences were observed in reactions between the resistant and susceptible parents. Similar tables for the other rating dates can be found in Appendix Tables 12-15. Differences in the F1 generations was evident for several characteristics such as rust reaction, plant height, color and tiller density in each cross. For rust reaction differences, all of the frequency distributions of the F1 populations showed transgressive segregation which was skewed toward susceptibility. This was especially evident in the resistant X resistant crosses, AXB, BXC, and AXC.

Table 1. Mean values for maturity stages of parents at five rating dates.

Parents	June 18	June 27	July 3	July 10	July 17	Grand Mean
A	3.26a 1/	4.04	4.26	5.15	5.70	4.48
B	3.11a	3.76	4.06	4.71	5.43	4.21
C	3.26a	4.04	4.26	5.00	5.48	4.40
D	2.84a	3.66	3.99	4.71	5.41	4.12

1/ LSD at the .05 probability level = 0.69 for the June 18 date. Means with a letter in common are not significantly different.

Table 2. Correlation and coefficient of determination values of stem rust reactions and maturity stages involving four parents and six resulting F1 populations measured at five dates.

X Maturity	Y Stem rust reaction	r		r^2
June 18	June 18	-0.637	*	0.40
June 27	June 27	-0.307	*	0.09
July 3	July 3	-0.520	*	0.27
July 10	July 10	-0.201	*	0.04
July 17	July 17	-0.259	*	0.07

* Significant t value at the 5% probability level.

Table 3. Two-way frequency distributions, means, and standard deviations of stem rust ratings of F1 and parent populations taken on July 10.

F1's and Parents	Stem Rust Rating Scale [1]									Total	Mean	S.D.
	1	2	3	4	5	6	7	8	9			
A	0	0	0	0	3	6	3	8	7	27	7.37	1.39
B	0	0	0	2	9	3	4	1	1	20	5.80	1.32
C	0	0	3	2	7	8	3	3	1	27	5.70	1.56
D	0	11	0	2	2	5	1	0	0	21	3.67	1.91
AXB	0	2	8	12	13	15	4	6	0	60	5.12	1.57
BXC	3	11	5	4	8	10	12	6	1	60	4.95	2.25
BXD	4	13	6	7	10	13	2	3	2	60	4.33	2.12
AXC	4	9	3	4	12	11	7	8	2	60	5.07	2.27
CXD	6	13	7	8	14	5	3	4	0	60	3.97	2.01
AXD	2	2	5	15	16	14	4	1	1	60	4.83	1.53
Total	19	61	37	56	94	90	43	40	15	455	4.93	1.90

[1] Stem rust scale based on visual estimates of area of foliage and seed heads infected.

The AXC and BXC crosses had several susceptible progeny rating 1's and 2's suggesting genetic differences between Parent A and C and Parent B and C. When the mean values and corresponding standard deviations are compared, none of the F1 populations exceeded the most resistant Parent A.

FREQUENCY DISTRIBUTIONS OF PARENTS

Frequency distributions of stem rust reactions for each parent observed on July 10, are shown in Figures 1-4. Parent A had a mean rust rating of 7.37 (Figure 1). The frequency distribution shows that 88% of the Parent A clones had a score of 6 or better with 55% of those plants rating 8 or 9. For Parent B (Figure 2) 45% of the population rated a 6 or better for rust reaction. The mean rating values for the clones of Parent B is 5.86. Parent C had 56% of the population rating a 6 or better with a mean value of 5.71 (Figure 3). Only 28.5% of the susceptible parent D clones had a 6 or 7 rating with none in the 8 or 9 classes (Figure 4). Fifty-two percent of the population received a rating of 2 resulting in a mean value of 3.68 for the susceptible parent. Thus when the resistant and susceptible parents were compared large differences in stem rust reaction were present.

FREQUENCY DISTRIBUTIONS OF F1 POPULATIONS

Bar graphs of the frequency distributions for the F1 populations are presented in figures 5-10 for data taken on July 10, 1984. Figure

Fig. 1. Frequency distribution of stem rust reaction for clones selected from parent A on July 10.

Fig. 2. Frequency distribution of stem rust reaction for clones selected from parent B on July 10.

Fig. 3. Frequency distribution of stem rust reaction for clones selected from parent C on July 10.
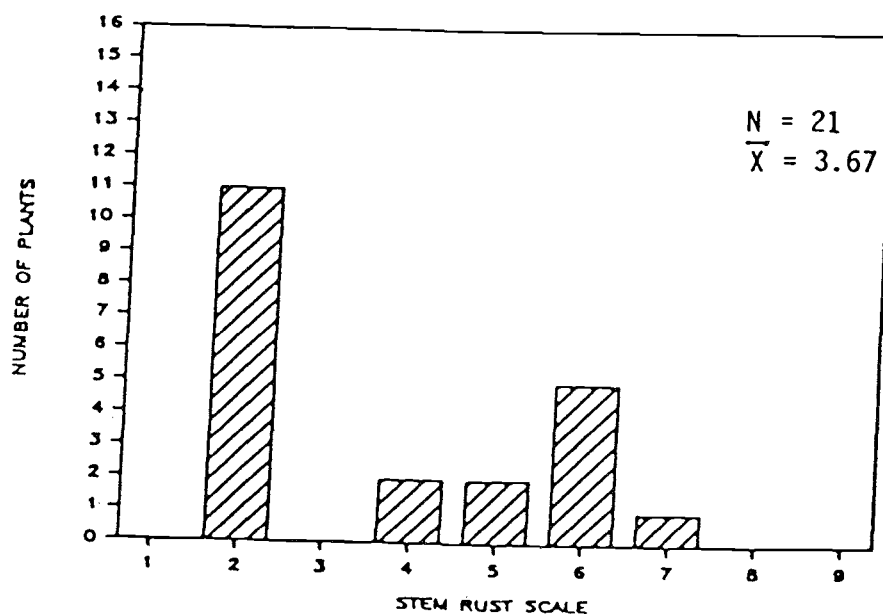
Fig. 4. Frequency distribution of stem rust reaction for clones selected from parent D on July 10.

5 shows a normal distribution of the progeny from the two rust resistant sources A and B. The population mean is 5.12 which is the highest when compared to the other F1 populations. Progeny from BXC suggest a bimodal distribution with two peaks, one at 2 and the other at the 7 rating class. The population mean of 4.95 was the lowest of the resistant crosses. In the AXC cross (Figure 7) normal distribution is suggested, however there appears to be a peak at the 2 rating class.

The resistant X susceptible cross of AXD has a normal distribution with a mean value of 4.83. Cross BXD appears to also be bimodally distributed with a peak at 2 and at 6 rating classes. The population mean was 4.33 (Figure 9). Progeny from CXD had the lowest population mean of 3.97. Peaks can also be observed at the 2 and 5 rating classes (Figure 10).

STEM RUST REACTIONS OVER TIME

Stem rust reactions over time were studied by plotting mean values of each parent and F1 population at each rating date. The parents are presented in Figure 11. Parent A was the most resistant parent having a mean rating of 9, on June 18. This was four weeks after the first field inoculation. The average dropped to a 7.85 after two weeks, with approximately 4% of the foliage infected. At harvest, July 17, 1984, the level of resistance had dropped to 6.59 with approximately 14% of the foliage infected. The B and C parents were similar in their rust reaction patterns over time. Parent B's mean ratings were slightly better than Parent C. Over time the
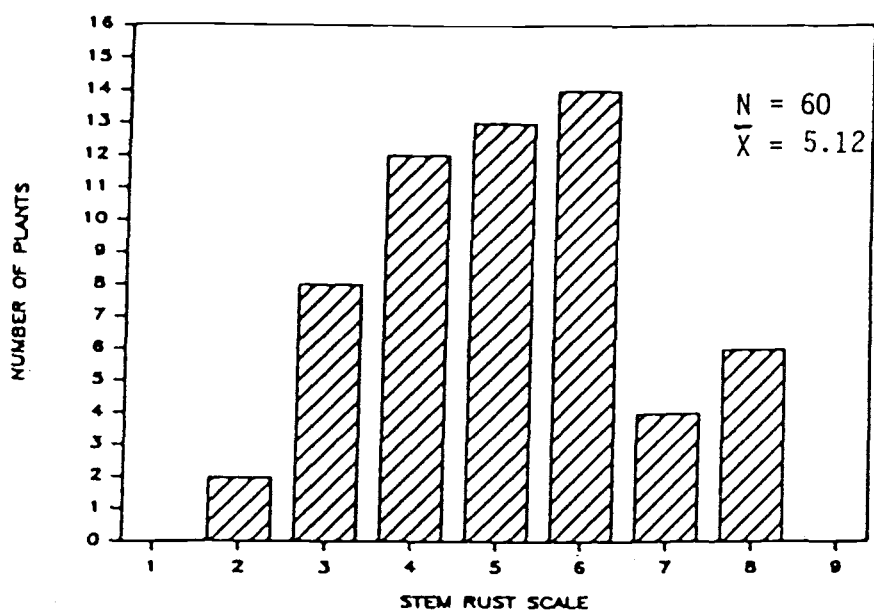
Fig. 5. Frequency distribution of stem rust reaction for F1 progeny from parents A and B on July 10.
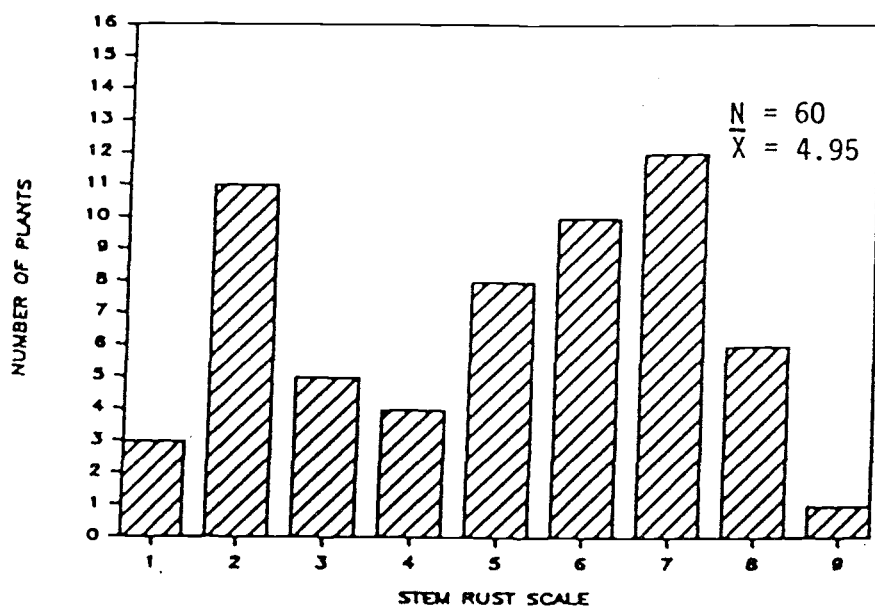
Fig. 6. Frequency distribution of stem rust reaction for F1 progeny from parents B and C on July 10.
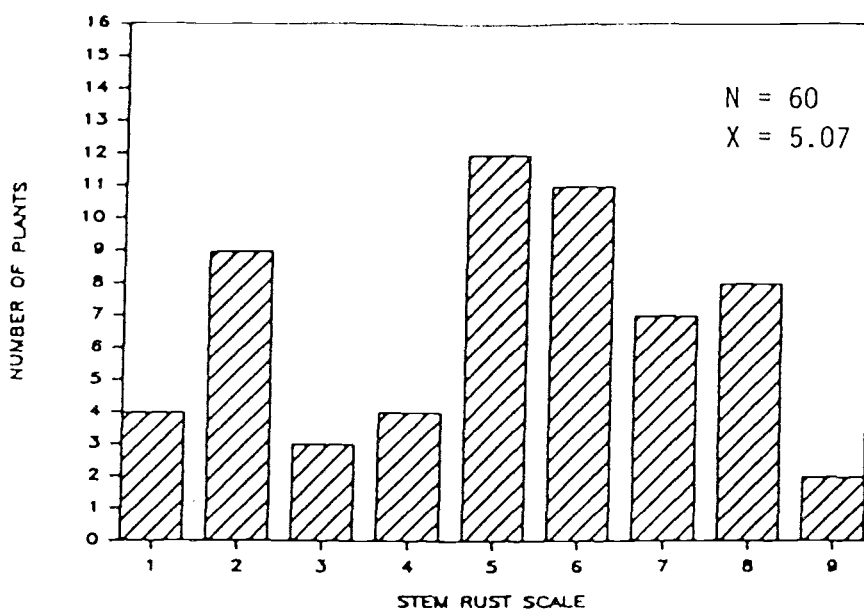
Fig. 7. Frequency distribution of stem rust reaction for F1 progeny
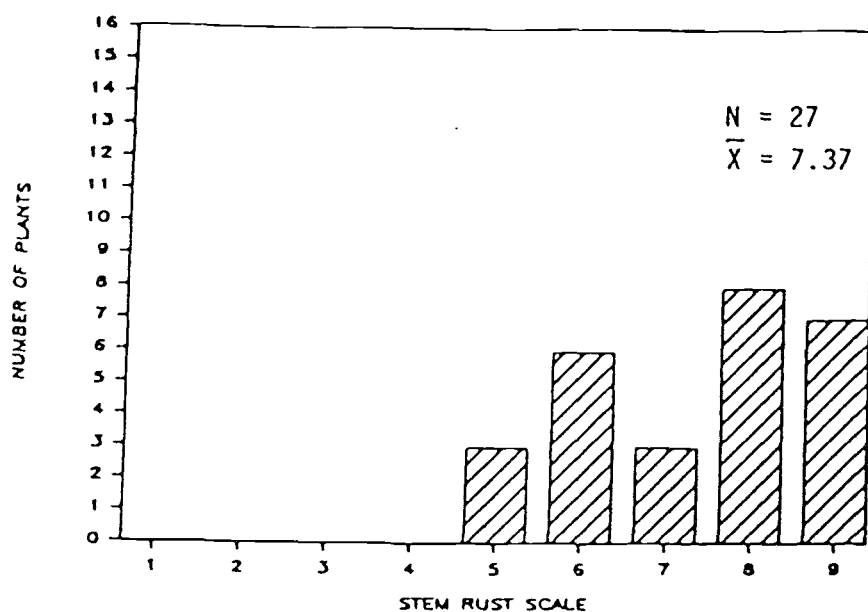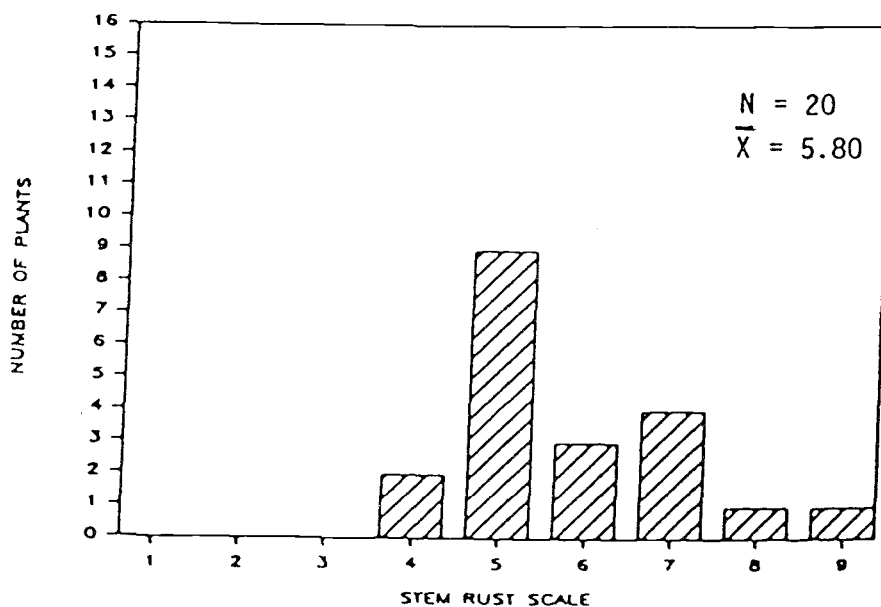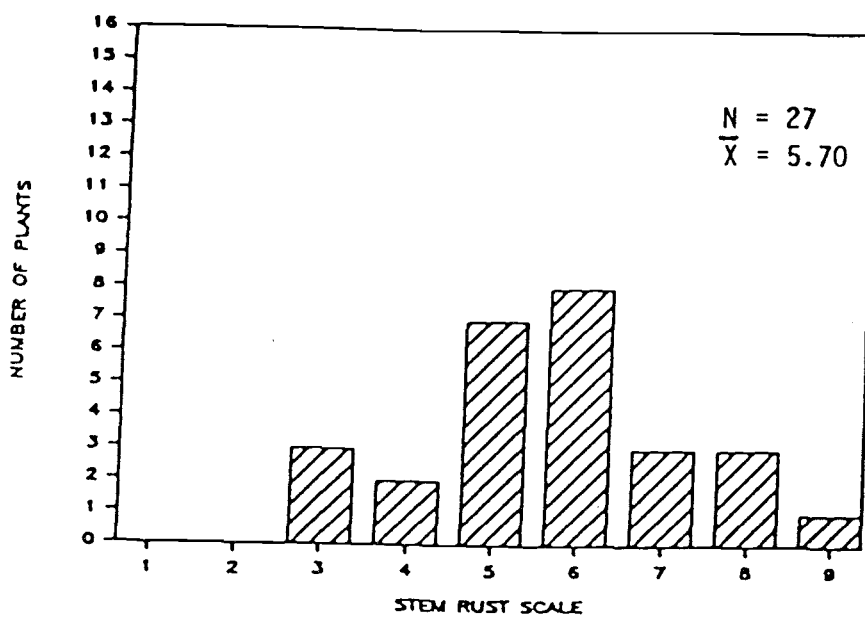from parents A and C on July 10.
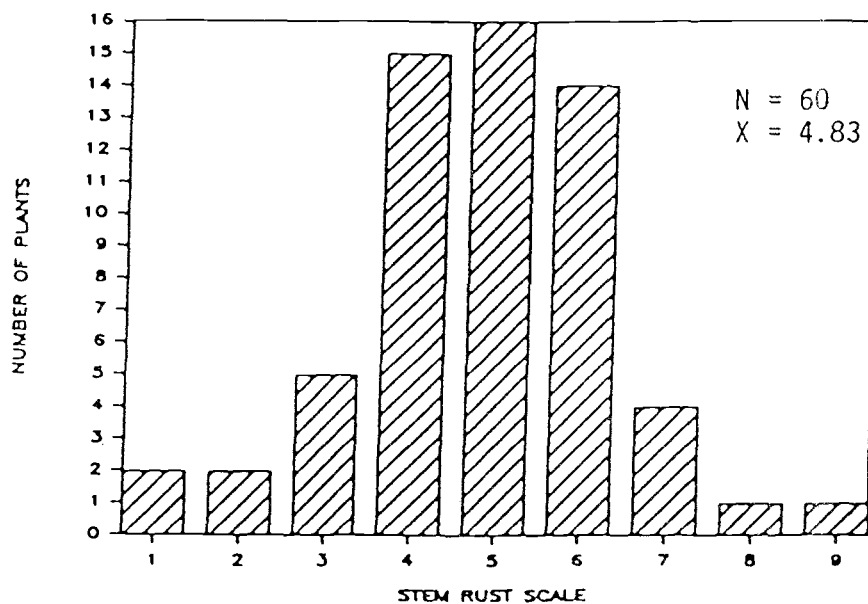
Fig. 8. Frequency distribution of stem rust reaction for F1 progeny
from parents A and D on July 10.
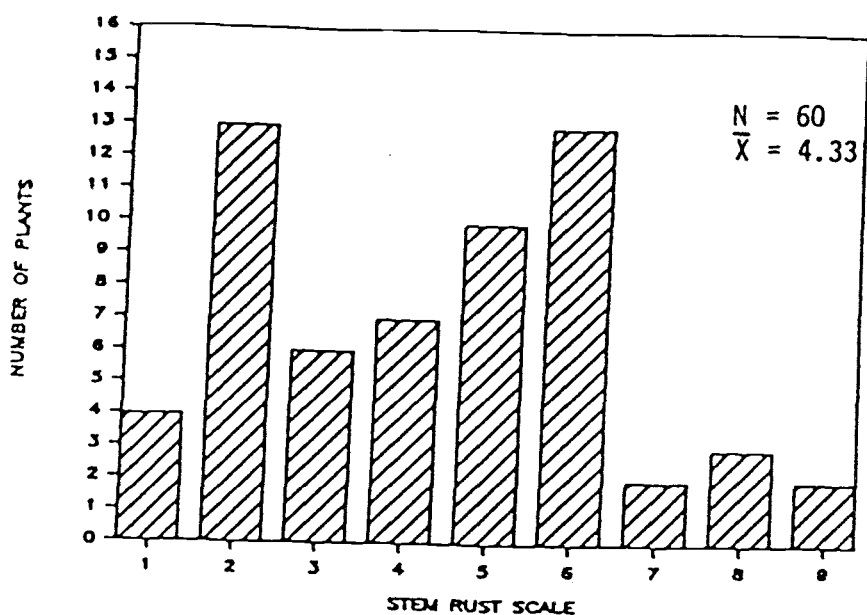
Fig. 9. Frequency distribution of stem rust reaction for F1 progeny
from parents B and D on July 10.
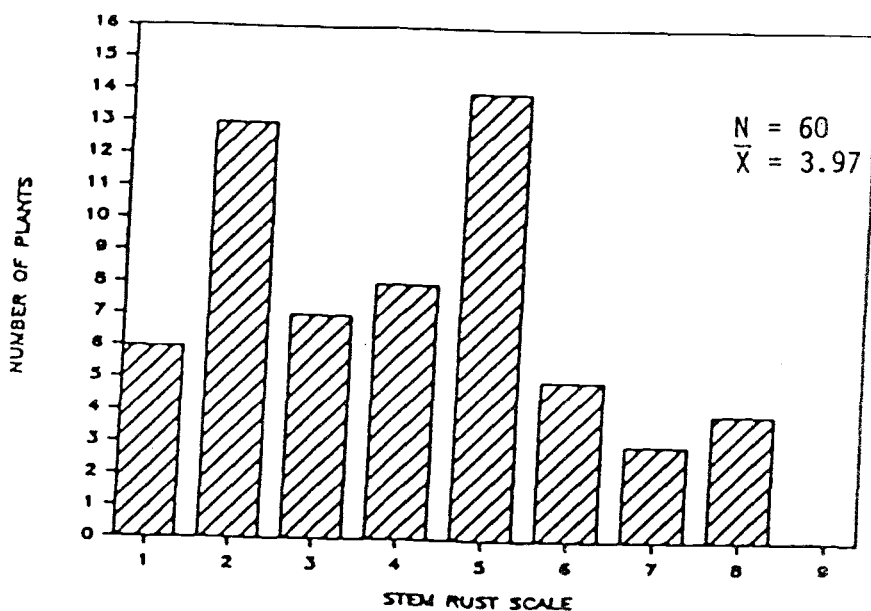
Fig. 10. Frequency distribution of stem rust reaction for F1 progeny
from parents C and D on July 10.

resistance of both parents broke down with about 55% of their foliage infected with stem rust. At harvest the mean value for Parent B was 4.70 while Parent C averaged 4.67. Four weeks after the first field inoculation the susceptible Parent D averaged 8.33, but dropped to 2.38 at harvest, with approximately 95% of the foliage infected.

A comparison of the six F1 progeny means over five rating dates is presented in Figure 12. The resistant X resistant crosses, AXB, AXC, BXC, had the highest average ratings at harvest. The BXC cross, however, did have some plants which were infected with rust early bringing the mean rating below the other two. The most resistant cross was AXB until the last rating date when it dropped below the other resistant X resistant crosses to a mean rating of 3.90. Among the resistant X susceptible crosses, AXD has the highest mean ratings being above some of the resistant X resistant crosses during the first three rating dates. The AXD F1 progeny at harvest were the most resistant of the resistant X susceptible crosses with a mean value of 3.47. Cross BXD had the lowest mean ratings on June 18, but CXD has the lowest mean scores for the rest of the rating dates especially on July, 17, when it dropped to 2.80.

Figures 13 to 18 depict line graphs comparing the mean rust reaction of each F1 population with its respective parents over the five dates. In all of the resistant X resistant comparisons, the F1 population means were below both parents. While in the resistant X susceptible crosses the F1 means were above the susceptible Parent D mean at the harvest date.

Figure 13 shows the difference between the Parents A and B and F1's in rate of disease development. The rust developed slowly in
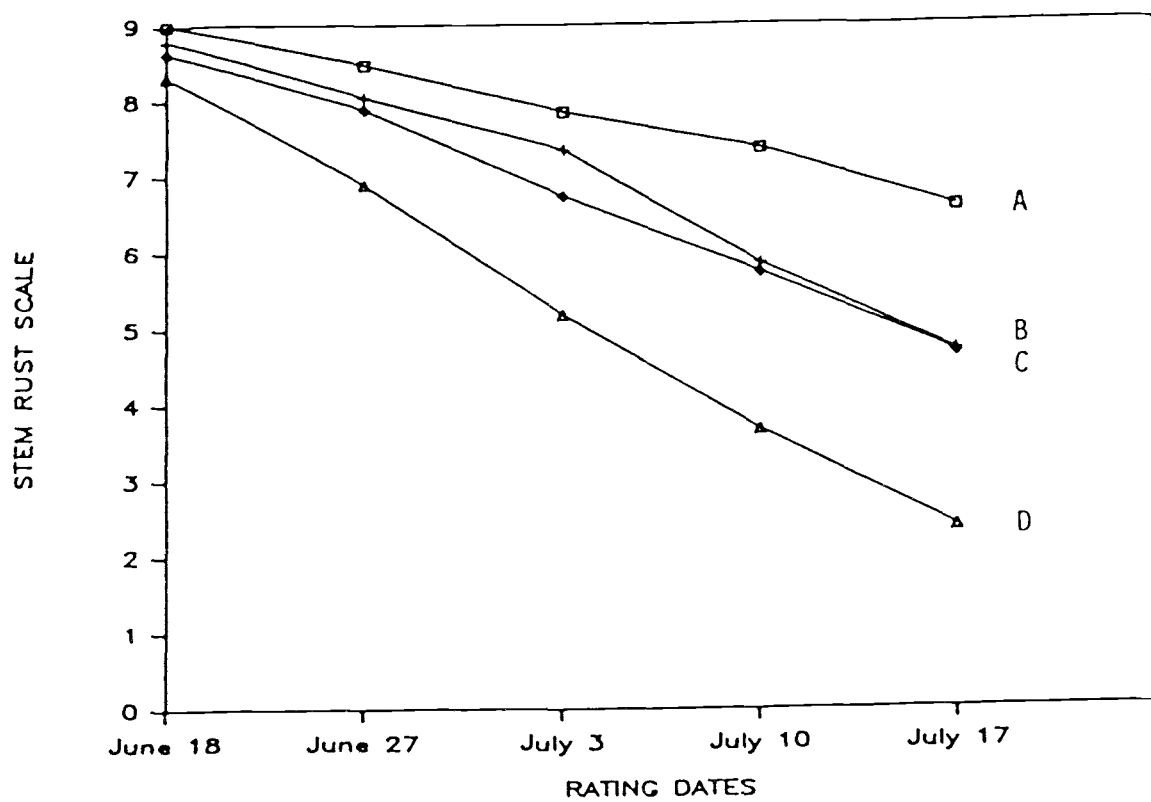
Fig. 11. Mean value of stem rust reactions for four parents (A, B, C and D) measured at five dates.
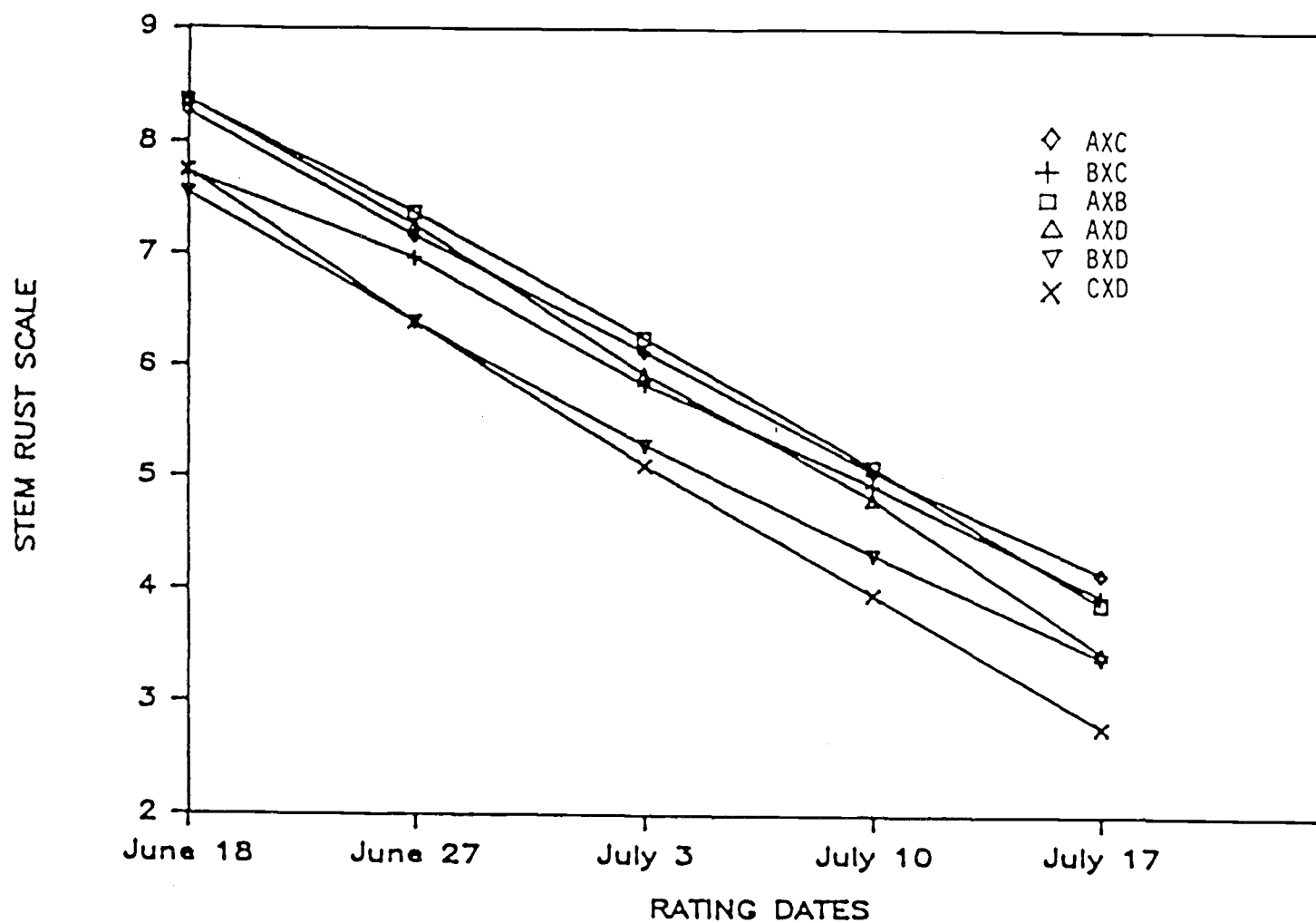
Fig. 12. Mean value of stem rust reactions for F1 progeny resulting from four parents (A, B, C, and D) measured at five dates.

Parent A as the mean ratings dropped very little over time. Parent B although considerably below Parent A did exceed the F1's at all dates. In Figure 14 Parents B and C were similar in reaction patterns over time. Figure 15 shows the F1 means of the AXC cross as being close to and below Parent C.

In the resistant X susceptible cross of AXD (Figure 16) the difference between the resistant Parent A and susceptible Parent D can be seen. The F1 progeny means fall between both parents and were similar to the most susceptible parent at all rating dates. The BXD comparison in Figure 17 shows the F1 progeny means below both parents for the first two dates and being above the susceptible Parent D for the last three dates. A similar phenomenon was noted with the CXD comparison in Figure 18, but the F1 progeny were closer to Parent D as they became more infected in the last two dates.

HERITABILITY ESTIMATES

The broad sense heritability estimates were calculated for the F1 progeny rust reactions on June 27, July 3, and July 10 dates and are presented in Table 4. The highest estimates for stem rust reactions were assessed for the BXC and AXC progeny with values of 65% and 70% respectively. A heritability estimate of 54% was found for the other resistant X resistant cross AXB. The highest heritability values for resistant X susceptible crosses BXD and CXD were 52% and 55% respectively. A lower estimate of 49% was noted in the AXD progeny. These values all were all lower at the later rating dates of July 3 and July 10.
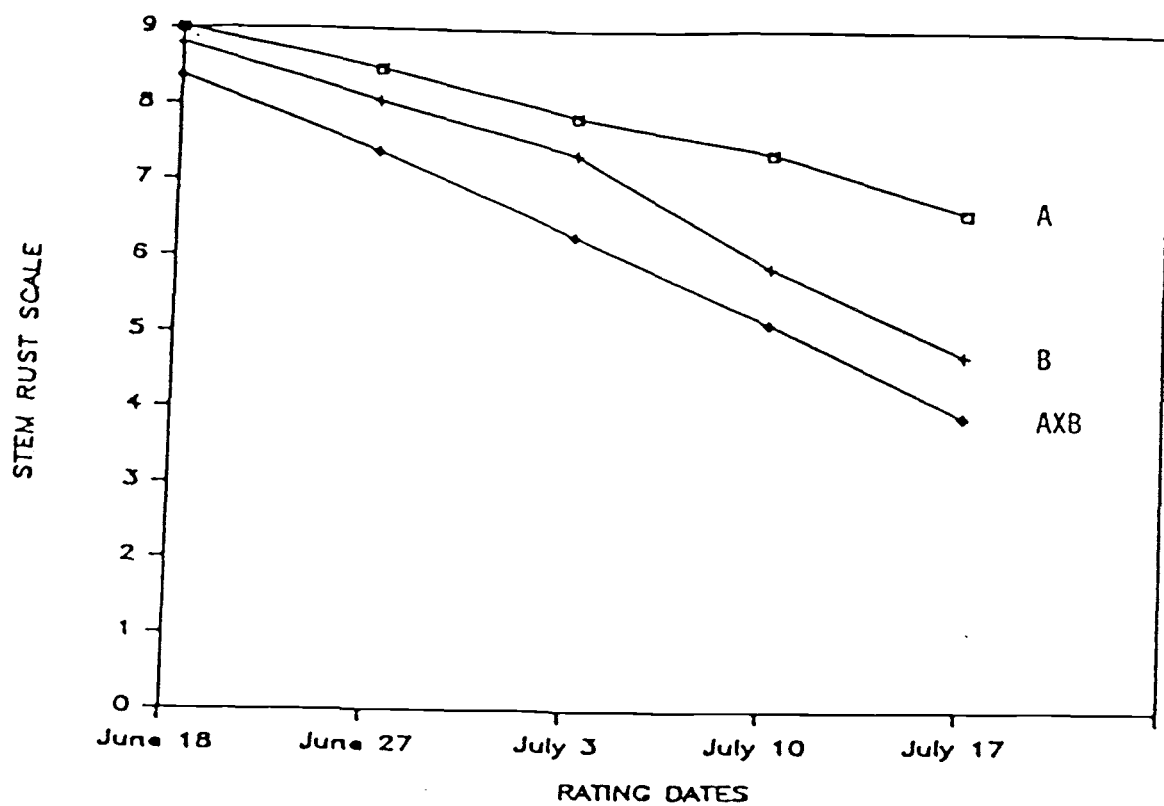
Fig. 13. Mean value of F1 progeny and parental clones A and B for stem
         rust reactions at five dates.
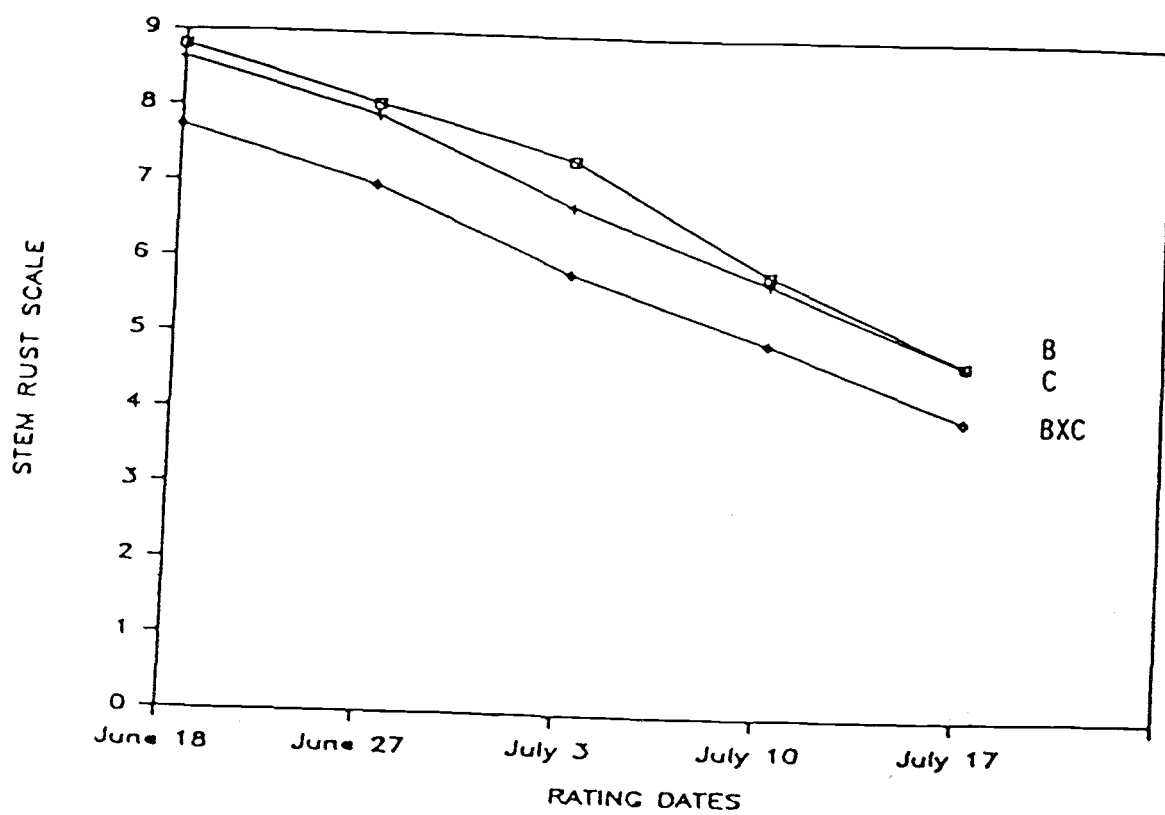
Fig. 14. Mean value of F1 progeny and parental clones B and C for stem rust reactions at five dates.
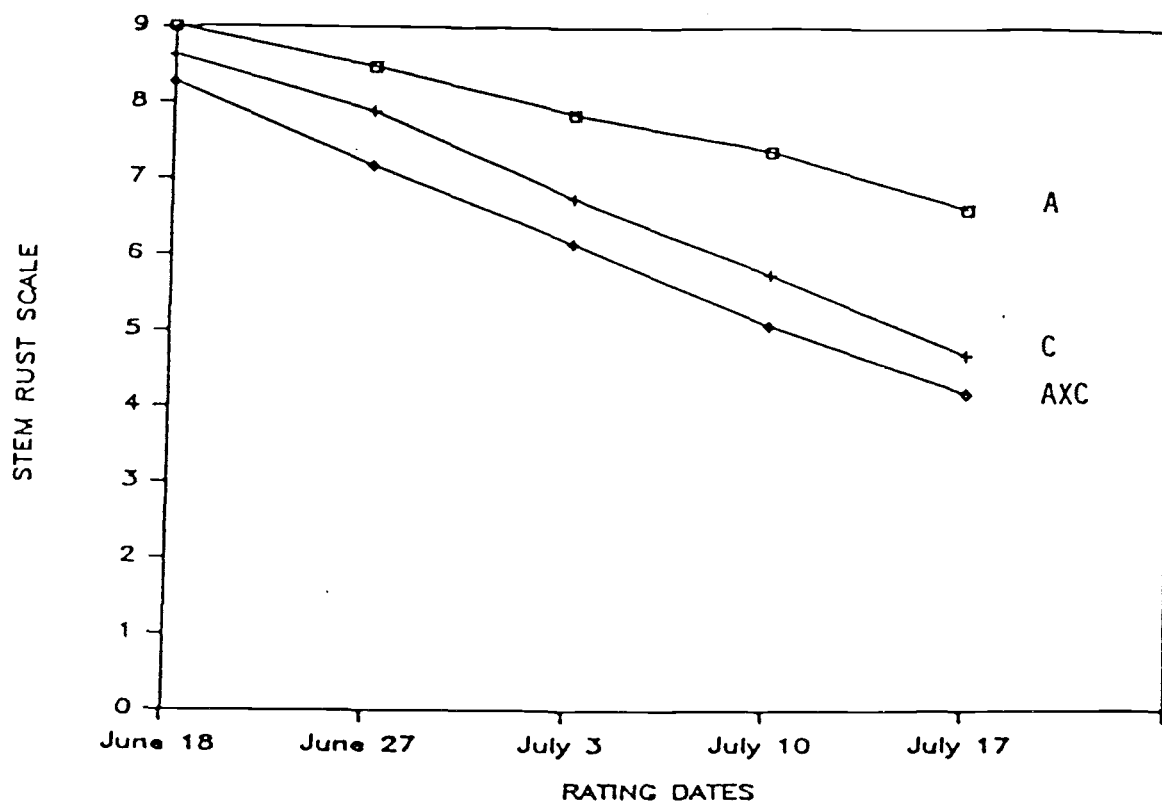
Fig. 15. Mean value of F1 progeny and parental clones A and C for stem
rust reactions at five dates .
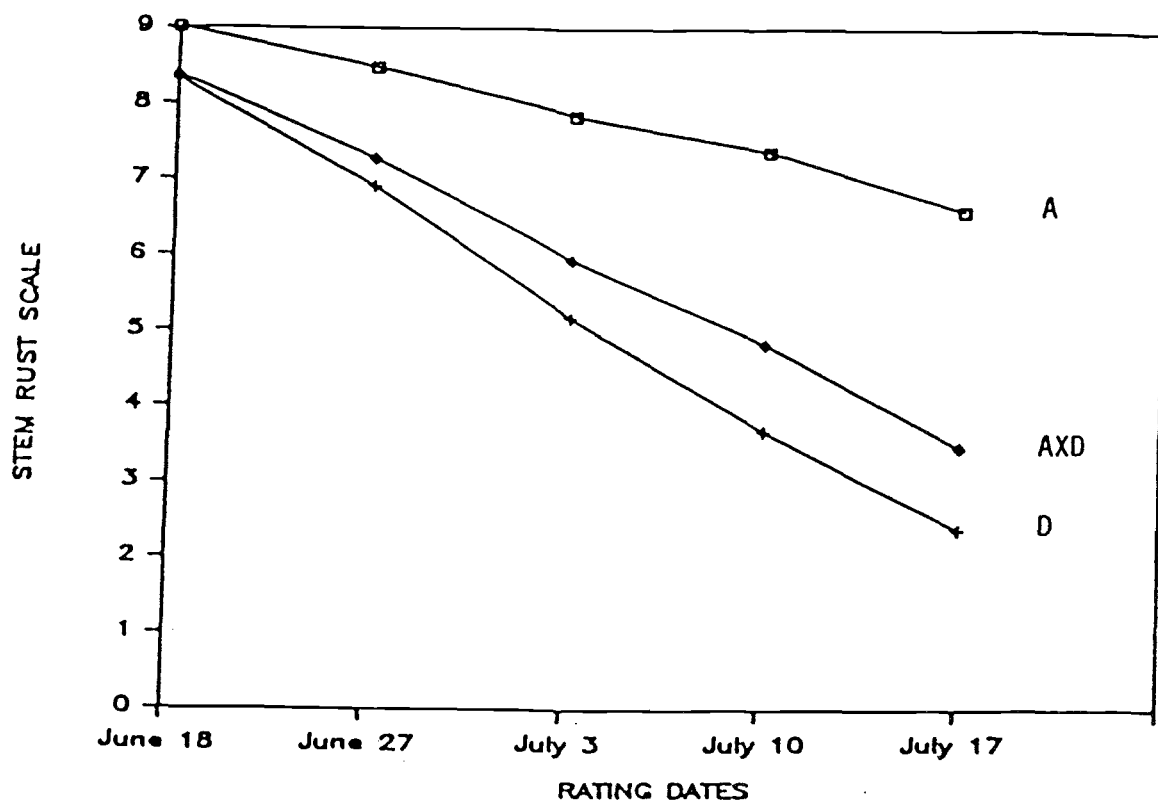
Fig. 16. Mean value of F1 progeny and parental clones A and D for stem
rust reactions at five dates.
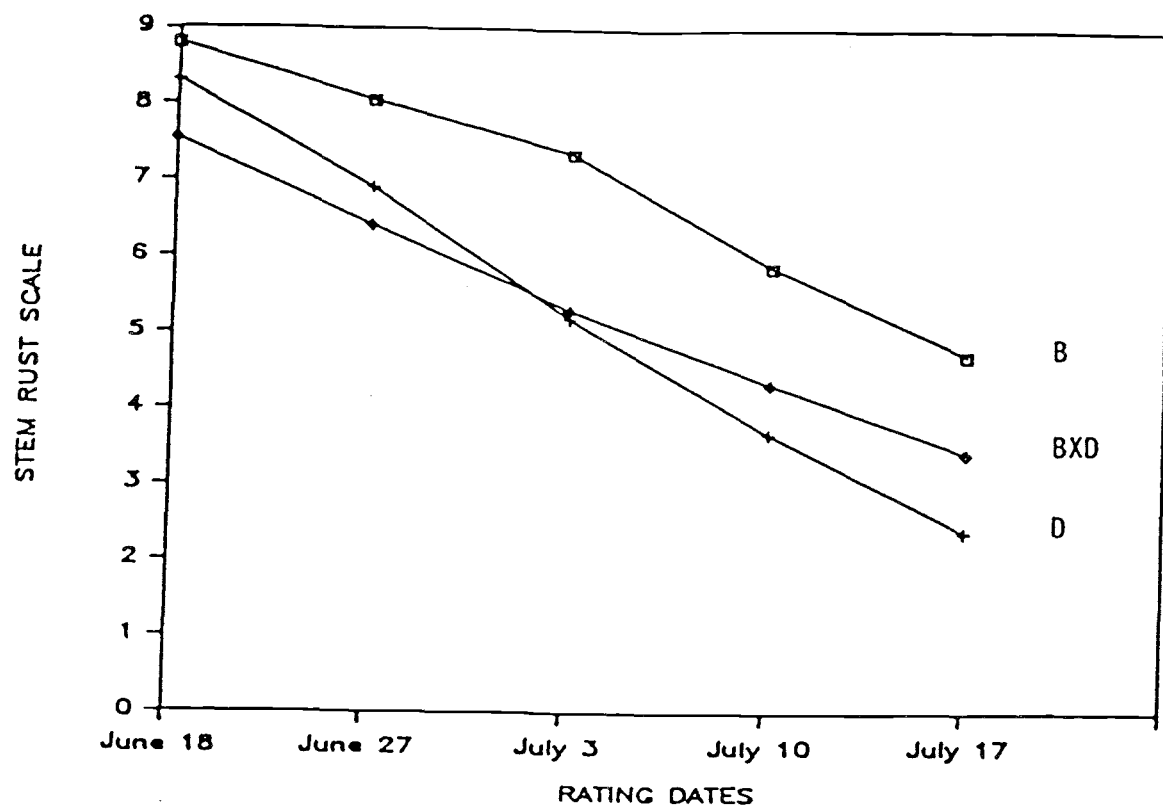
Fig. 17. Mean value of F1 progeny and parental clones B and D for stem rust reactions at five dates .
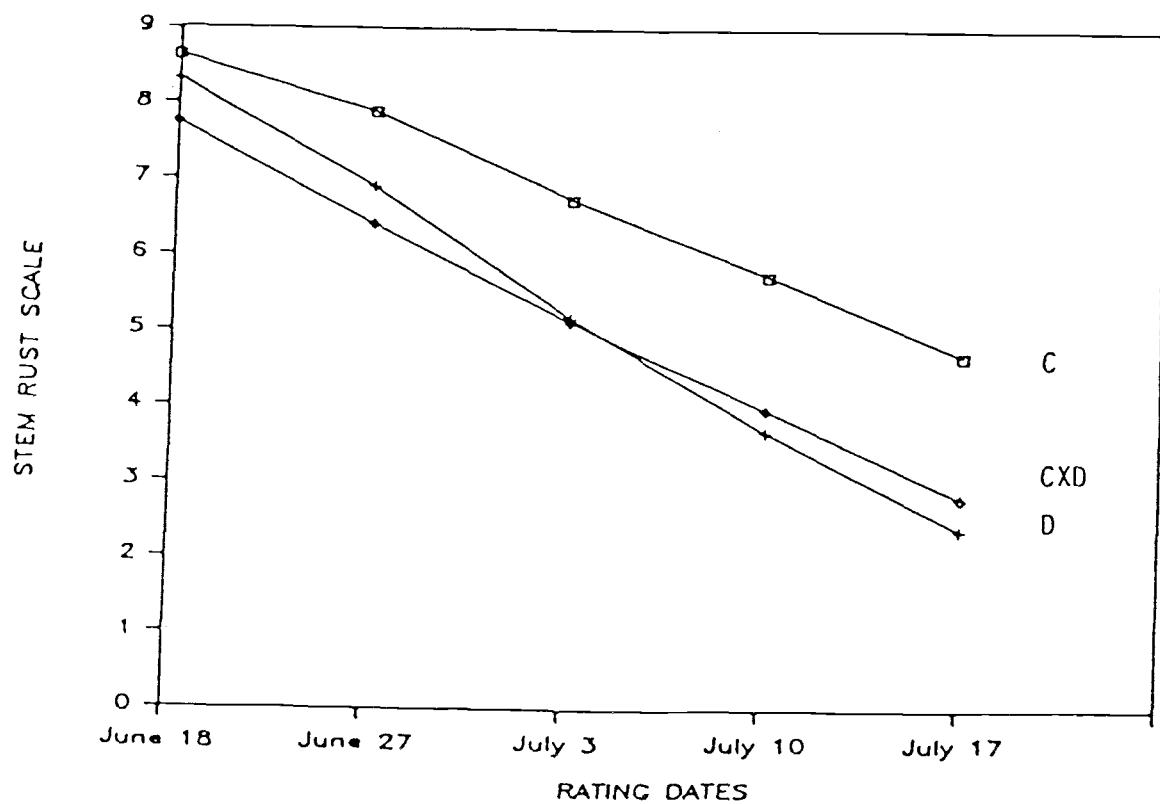
Fig. 18. Mean value of F1 progeny and parental clones C and D for stem rust reactions at five dates.

SEEDLING STUDIES

During the summer of 1983 the F1 seedlings from which adult plant ratings would eventually be taken, were inoculated. In the BXD cross, 23 out of 50 seedlings were classified as being susceptible (Table 5). The adult rust reaction average was 3.13 for the 23 susceptible seedlings indicating that plants susceptible as seedlings were also relatively susceptible as mature plants. However, three of the susceptible seedlings received resistance ratings of 7's and an 8 at maturity. The majority of the other susceptible F1 seedlings for the other crosses were susceptible as adults, except for a few plants being moderately resistant or 6's on the rating scale.

In 1984 F2 seedlings from F1 plants representing different reaction types were inoculated and scored according to the scale developed by Stakman, (1962). The results from the AXD cross shown in table 6, did not exemplify a distinct gradient. A plant rating a 2 produced 26% susceptible seedlings. The percent of susceptible seedlings decreased as each F1 adult plant rating was more resistant. There were no F1 plants at harvest rating an 8, but one plant was immune with a 9 rating. This plant produced as many susceptible seedlings as a plant rating a 3.

Table 4. Broad sense heritability estimates for stem rust reactions involving four cloned parental lines and six resulting F1 populations.

F1's	June 27	July 3	July 10
AXB	0.54	0.46	0.33
BXC	0.65	0.60	0.58
BXD	0.52	0.47	0.45
AXC	0.70	0.64	0.61
CXD	0.55	0.41	0.27
AXD	0.49	0.13	-0.21

Table 5. Contingency table comparing seven populations of susceptible F1 seedlings and their subsequent mature plant responses to stem rust.

F1	Adult plant stem rust ratings on July 17 1/									Total
	1	2	3	4	5	6	7	8	9	
AXB	Four infected seedlings died before maturity									
BXA	1	0	0	3	0	2	0	0	0	6
BXD	7	4	4	3	1	1	2	1	0	23
BXC	5	3	0	1	1	2	0	0	0	12
AXC	3	0	0	1	0	3	0	0	0	7
CXD	3	0	1	1	0	1	0	0	0	6
AXD	None of the seedlings were inoculated									
TOTAL	19	7	5	9	2	9	2	1	0	54

1/ Stem rust ratings at harvest on a scale of 1 to 9 with 9 best.

2/ x^2 = 25.72 No significant differences at the 5% probability level.

This variation could be due to segregation of the F2 progeny as a result of parents differing in genes governing stem rust resistance. Also, the low number of F2 plants studied could represent a relatively small proportion of the susceptible seedlings in relation to adult plant reactions.

Results of the F2 seedlings from BXD F1 adult plants ranged from 2 to 8 on the stem rust scale and are presented in Table 7. Depending on the mature plant reaction type of the F1's, the F2 seedlings ranged from 0, where the F1 plant rated an 8, to 64% susceptible for an F1 plant which rated a 2. In general there are more susceptible seedlings from the BXD cross than the AXD cross. A gradient exists in the BXD cross with susceptible F1 plants producing a higher percentage of susceptible F2 seedlings.

Table 6. Stem rust reaction classes and percent susceptible seedlings
of F2's from individual AXD F1 plants of known reaction
types.

F1 Mature Plant 1/ Stem Rust Rating	Reaction Classes 2/						% Susceptible Seedlings
	0	0;	1	2	3	4	
2	31	2	2	2	8	5	26%
3	34	6	0	1	5	4	18%
4	36	6	1	0	4	3	14%
5	40	4	0	0	1	3	9%
6	43	1	0	0	0	6	12%
7	43	4	2	0	1	0	2%
8	no plants available						
9	39	2	0	0	2	7	18%

1/ Stem rust rating of mature F1 progeny at harvest on July 17, 1984.
2/ Reaction classes of F2 seedlings, 0 to 1 are resistant; 2 is
moderately resistant; 3 and 4 are susceptible (Stakman et al.,
1962).

Table 7. Stem rust reaction classes and percent susceptible seedlings of F2's from individual BXD F1 plants of known reaction types.

F1 Mature Plant 1/ Stem Rust Rating	Reaction Classes 2/						% Susceptible Seedlings
	0	0;	1	2	3	4	
2	14	0	2	2	14	18	64%
3	21	2	3	3	11	10	42%
4	27	1	0	1	6	12	38%
5	40	1	1	0	5	3	16%
6	39	4	1	0	0	4	8%
7	37	3	1	0	2	6	16%
8	49	1	0	0	0	0	0%
9	no plants available						

1/ Stem rust rating of mature F1 progeny at harvest on July 17, 1984.
2/ Reaction classes of F2 seedlings, 0 to 1 are resistant; 2 is moderately resistant, 3 and 4 are susceptible (Stakman et al., 1962).

DISCUSSION

Seed of many of the turf and forage varieties of perennial
ryegrass used in the United States are produced in the Willamette
Valley of western Oregon. When perennial ryegrass was first
introduced to this area in 1967 there was no evidence of stem rust
infections. Over the past several years however, the infection of
stem rust advanced to epidemic proportions which have caused seed
yields to decrease from approximately 1121 kg per hectare to 112 kg
per hectare. In the Willamette Valley severe stem rust epidemics have
been occurring for the past 10 years in commercial perennial ryegrass
fields. Stem rust resistant varieties have been developed within the
last six years for this area. Unfortunately little or no information
is available regarding the nature of inheritance of resistance to stem
rust in this crop species. The study of F1 populations derived from
crosses of 3 resistant sources and one susceptible source produced the
following observations.

RELATIONSHIP OF MATURITY STAGES AND STEM RUST REACTIONS

Past observations of perennial ryegrass fields in the Willamette
Valley have shown that late maturing susceptible varieties become
seriously infected with stem rust later in the season than early
maturing types. In general when late maturing varieties reach
anthesis they become infected with stem rust and at harvest both late
and early maturing susceptible varieties are covered with stem rust

pustules and produce light, poor quality seed.

No differences were found for maturity stages between the four parents used in this study. However, some correlations were found between maturity stages and stem rust reactions. On the first rating date of June 18, 40% of the change in stem rust reactions were associated with changes in maturity stages. On July 3 only 27% of the stem rust infection was due to maturity stage. Low correlation values were found for the other three rating dates. There were, of course, other factors influencing the rate of stem rust infection such as the environment and the level of resistance of the different cloned parental populations.. The low correlations were most likely due to the similarity of maturity stages of the parents. This could be classified as adult plant susceptibility where after anthesis the plants begin to senesce and become infected with stem rust. This type of resistance is evident in cereals as found by Skovmand et al. (1978b); Gassner and Kirchhoff (1934) and Ohmand Shanner (1976). Further studies should be made using parents that are significantly different in maturity if the relationship between stage of development and stem rust susceptibility is to be evaluated.

The similar maturity stages of the parents used in this study indicates that none of the plants escaped infection of stem rust due to maturity. The plants that were less infected with stem rust were genetically resistant.

FREQUENCY DISTRIBUTIONS OF PARENTS AND F1 POPULATIONS

In evaluating the populations for stem rust, frequency

distribution tables and bar graphs effectively portray the differences between F1 and cloned parental populations, and the amount and type of variation within each population.

Clear differences for stem rust reaction were found between the parents. The susceptible population representing Parent D was skewed toward the susceptible side of the scale. Parent A had the highest level of resistance with the population being skewed toward resistance. Parents B and C were in the middle of the scale with 50% of their population rating higher than a 6.

Transgressive segregation toward susceptibility was evident in all resistant X resistant crosses suggesting the presence of different alleles for susceptibility in the resistant parents. The presence of moderately susceptible and susceptible plants in these crosses are the result of genetic variability and indicate genetic differences between the resistant sources of A, B and C. These different genetic sources of resistance among parents could be incorporated through breeding to produce a more durable type resistance to stem rust in future varieties.

Bar graphs were used to provide insights into the type of resistance and mode of inheritance based on the type of variation or frequency distribution of each F1 population for stem rust reactions. In this heterogeneous cross-pollinated species there was evidence of both continuous variation and bimodal distributions. The AXB, AXD, and AXC crosses produced segregating progeny with near normal distributions indicating that many genetic factors are involved. Because Parent A is involved in all three cases, it could be contributing polygenic resistance via several minor genes. The AXC

cross had a second peak at the 2 rating, this could be due to major genes contributed by Parent C.

In a field study of resistance to crown rust in perennial ryegrass by Wilkins (1975), the resistance was also found to be distributed in a continuous variation with a large number of genes exhibiting additive type of genetic variance. The normal distributions involving the Parent A could be a result of additive genetic variance.

The BXC, BXD, and CXD crosses produced progeny which were bimodally distributed with a peak near 6 and a second near 2 in rust rating. Such bimodal distribution is again indicative of major genes undergoing segregation. Thus it appears that both Parents B and C may be contributing major genes for resistance. The genetic resistance to stem rust represented by the parents used in this study appears to be largely quantitatively inherited however. These three sources of stem rust resistance could be combined by incorporating both major and minor genes for resistance.

STEM RUST REACTIONS OVER TIME

Differences between the four parents for stem rust reactions were displayed over time. Parent A sustained a high level of resistance over all five dates while the other two resistant parents broke down to a moderately resistant level after three weeks and were further infected with rust up until harvest or the fifth rating date. Parent D's susceptibility was apparent as it developed a higher stem rust infection on the second date and dropped on the stem rust scale

dramatically there after.

Genetic differences of the resistant parents were inherited by the F1 progeny resulting in different rust reaction patterns of the F1 populations. Mean values for all of the F1 populations over five dates show the resistant X resistant crosses as having a higher degree of resistance which held up better over the five rating dates in the growing season. Cross AXC produced F1 progeny which had the highest rust resistant reaction mean at the last date because of the additive effect of different genes contributed by Parents A and C. Cross AXB had the lowest rust reaction for the resistant X resistant crosses perhaps due to the similarity of the parents in rust resistance. Cross BXC was different from the other resistant X resistant crosses, beginning with a low level of resistance but maintaining its resistance over time and at the last rating date it had the second highest mean rating. This could be due to minor genes from Parent B allowing some rust infection at earlier dates but hindering any further development or providing adult plant resistance. An interesting reaction occurred with the resistant X susceptible AXD F1 progeny had the same level of resistance as the F1's from resistant X resistant crosses initially and maintained the same level of resistance until the last rating date. Then the mean for the AXD cross dropped to the same level of resistance as the BXD cross. This early high level of resistance was due to the high level of resistance contributed by Parent A. BXD progeny began with the lowest mean rust reaction, but were the same as AXD progeny at the last dates, suggesting a slow rusting type of resistance imparted by Parent B.

A comparison of F1 populations with their parental stem rust

reactions over time portrays the relationship of each parent with its respective F1 population. Resistant X resistant F1 progeny were below both resistant parents because of the transgressive segregation toward susceptibility. AXC progeny means were high at the later rating dates while the other progeny means were dropping. This could be due to Parent C contributing different genes for resistance complimentary to that of Parent A resulting in F1 progeny with additional resistance. Resistant X susceptible F1 progeny means of the AXD cross were more resistant than Parent D means at all dates which shows a definite improvement in stem rust resistance in the first generation as a result of genetic influence from Parent A.

F1 progeny of crosses BXD and CXD, were below Parent D for the first two dates suggesting slow development of rust on Parent D early in the season possibly due to its slightly later maturity when compared to the F1 population. The BXD and CXD progeny were less infected during the last three weeks of the season than their susceptible parent. This depicts a slow rusting type of resistance, where early in the season the plants only have some stem rust infection due to minor genes for resistance. As the stem rust epidemic progresses the development of pustules on the resistant plants is slower than those on susceptible plants. This results in the resistant plants producing more healthy seed at harvest. In some wheat varieties this slow-rusting reaction has been reported to be under polygenic control (Skovmand et al., 1978a).

Stability or longevity of resistance, sometimes referred to as a durable type, depends on the degree of diversity of the resistance genes (Parlevliet and Zadoks, 1977). The quantitatively inherited

resistance found in this study should provide the needed genetic diversity to avoid erosion of resistance due to the development of new races of stem rust. The presence of many minor resistance genes provides a buffering effect increasing the chances for the variety to remain resistant over several years. Several stem rust resistant varieties have been developed from the three sources of resistance used in this study. Some of these resistant varieties have been grown in the Willamette Valley and have remained resistant for six years. These varieties have also been observed to remain resistant for four years in France, New Zealand and New Jersey (Meyer et al., 1984). This is evidence of a durable type of resistance because over those time periods and geographical locations several different races of stem rust were present and yet this resistance was maintained. The inoculum pressure in a perennial species, such as perennial ryegrass, is high because the urediniospores can overwinter on the plants producing inoculum each summer for the lifetime of the crop if environmental conditions are conducive for stem rust development.

HERITABILITY ESTIMATES

To provide additional information regarding the amount of genetic variability for stem rust resistance, broad sense heritability estimates were calculated for each of the crosses. Different heritability estimates between the F1 populations indicate that some parental combinations contributed more genetic variability for stem rust resistance. Results suggested that selection within the cross AXC followed by BXC and CXD would be the most promising. However,

even though the heritability estimates were relatively high across rating dates, the large number of progeny in the susceptible classes for each cross would reflect a low probability of success. What the heritability estimates do indicate is that there is considerable genetic variability for stem rust resistance. They also suggest that there are considerable genetic differences between the parental lines used in this study. Parent C appears to be genetically different compared to the other resistant parents resulting in high heritability estimates in crosses CXD, AXC, and BXC. Parent B contributed the second greatest amount of genetic variability in crosses BXC, BXD, and AXB. Parent A apparently had the least amount of genetic variability and only in crosses with Parents B or C did it have high heritability values. The AXD cross had the lowest heritability values. This was a result of low genetic variance in relation to the amount of environmental variance. In a breeding program the use of Parent A alone is not advisable because of low genetic variability for resistance. Even though Parent A seems to have the "best" resistance, with the highest stem rust ratings and longer stability over a growing season, it would be wise to incorporate this resistance into a program along with other resistant sources such as Parent C to increase genetic variability.

Perhaps the most significant feature of these results is the consistent decrease in heritability estimates over time. Broad sense heritability estimates were larger early in the growing season suggesting a greater amount of genetic than environmental variance in the earlier stages of the epidemic. With the increase in inoculum over time it appears that the resistance is being overcome by the

spore load. This occurs in polygenic resistance where plants do sustain some infection. Later in the season most of the plants may have a high percent of infection, but those with polygenic resistance should have normal seed. Thus effective selection for resistance must be made during early anthesis. This will vary with the year; however epidemics of stem rust generally coincide with higher temperatures which generally occur in late summer in the Willamette Valley. Selection on early dates, before anthesis, is also necessary when breeding a cross-pollinating crop such as perennial ryegrass to avoid pollination sources of susceptible plants.

SEEDLING STUDIES

In an effort to determine if seedling reactions could be used to identify mature plant resistance two studies were conducted. The first seedling study considered the relationship of seedlings and mature plant stem rust reactions. The F1 plants that were susceptible as seedlings were monitored throughout the growing season and at harvest their stem rust ratings were recorded. The stem rust reactions of mature F1 plants ranged from 1 to 8, while in the seedling stage those same plants were all susceptible. This indicates that seedling stem rust reactions were not correlated with adult plant stem rust reactions in F1 progeny derived from the four parents used in this study. Three of the BXD susceptible seedlings had resistant ratings of 7's and an 8 as adults. Similar results have been found in eyespot resistance in corn (Reifscheider and Arny, 1983). Stripe rust resistance in cereals was found to have some lines which were

resistant as adults but susceptible as seedlings (Allan et al., 1966).

Different stem rust reactions in the seedling and adult stages of one plant suggests two separate gene systems. One influencing the reaction pattern for the seedling while the second contributes the mature plant resistance. This is more evident when it is considered that many of the plants that were resistant as seedlings also broke down to 1's, 2's, 3's, and 4's as adult plants. Flor (1971) found that plants having horizontal or polygenic resistance to rust are relatively susceptible in the seedling stage. Observations of susceptible seedlings being resistant as mature plants indicates that a seedling screening process may not be warranted in a perennial ryegrass breeding program for stem rust resistance.

The second seedling study using F2 generations from given F1 plants depicted a gradient where more susceptible seedlings were produced than might be expected based on adult on adult plant reaction. An exception occurred when a resistant adult plant from the AXD progeny population produced a large percent of susceptible seedlings. In this study all of the F1 plants interpollinated randomly so the paternal effect can not be traced. Again it appears that seedling reactions and adult plant reactions are controlled by separate gene systems. The percent of susceptible seedlings found in the AXD F2 progeny was noticeably lower than those in the BXD F2 progeny. This is additional evidence of the high level of resistance contributed by the A parent.

BREEDING STRATEGY

A strategy for breeding quantitatively inherited durable
resistance to stem rust based on the results of this study for
perennial ryegrass would be as follows. Seedling screening procedures
would not be effective. After making crosses between moderately
resistant parental lines (rating 6 or 7) or resistant X susceptible
crosses with different sources for resistance, F1's rating 6's, and
7's before anthesis could be selected and interpollinated in a
polycross nursery. Polycross seed could then be used to start several
cycles of phenotypic recurrent selection for stem rust resistance and
other agronomic traits.

If qualitative resistance is desired, F1's rating 8's and 9's
should be selected and allowed to interpollinate. If F1 plants rating
6's, 7's, 8's, and 9's were selected and allowed to interpollinate the
effects of any minor genes would be masked by major genes in
subsequent generations. This would make it impossible to select for
quantitative resistance in subsequent cycles of selection. An
alternate procedure to incorporate both major and minor genes would be
to have two breeding programs. The first would be to select for
quantitative (6's, and 7's) and the second for qualitative (8's and
9's) resistance. Separate lines could be maintained with all other
characteristics similar except stem rust reactions. After several
cycles of phenotypic recurrent selection the two lines could be bulked
and planted for breeder seed. This would ensure the presence of minor
as well as major genes for stem rust resistance.

Parents A and C would provide a high level of resistance with different genetic factors. Because of transgressive segregation toward susceptibility a large proportion of the F1 population would be susceptible. Large populations should be used to select from and then maintained during recurrent selection to avoid inbreeding depression due to a narrowness of the gene base. If the stem rust resistant parent is an undesirable plant type, modified backcrossing could be used with several desirable plants as the recurrent parents. Again, different sources and relatively large numbers should be used to minimize inbreeding depression and narrowing of the gene base. Seed yield tests could also be included after a few cycles of selection to ensure that there are no inbreeding depression problems. The reaction patterns and the apparent diversity of different genetic sources of resistance observed in the parents used in this study indicate that the above strategies would be effective in developing stem rust resistant varieties of perennial ryegrass.

SUMMARY AND CONCLUSIONS

The objectives of this study involving the nature of resistance to stem rust in perennial ryegrass were: 1) to determine if there is an association between disease reaction and plant maturity; 2) to determine the nature of inheritance; 3) to study slow rusting types over time in relation to parents, F1's and the association between F1's and parents; and 4) to determine if seedling reactions are similar to adult plant reactions;

Three stem rust resistant sources and one susceptible parent were crossed in a partial diallel design resulting in six crosses, excluding reciprocals. Stem rust ratings and maturity ratings were taken for five weeks. Frequency distributions of heterogeneous F1 populations for stem rust reactions were obtained. Stem rust reactions were plotted over time for parents, F1's and a comparison of individual F1 populations with their respective parents. Correlations between maturity stages and stem rust reactions were computed for five dates. Seedling stem rust reactions were compared with adult plant reactions.

The following conclusions were drawn from the results of this investigation: 1) Differences between the parents for maturity stages were not found. Maturity stages were not highly correlated with stem rust reactions except during the bootstage and early anthesis. Further studies should be made using populations with greater maturity differences to effectively determine if there is a relationship between maturity stages and stem rust reactions.

2) Genetic differences for stem rust reactions were found between

the three resistant sources. Parent A had the highest level of resistance. Parent C differed genetically from Parent A making an attractive complimentary source for stem rust resistance. Parent B had stem rust reactions similar to Parent C. Parent A produced F1 progeny with normal distributions suggesting several minor genes for resistance. Parents B and C produced progeny with bimodal distributions indicating the presence of both minor and a few major genes imparting resistance. Transgressive segregation toward susceptibility was found in F1 progeny from resistant X resistant crosses again suggesting differences between the resistant sources. These genetic differences for stem rust resistance were found to be quantitatively inherited, involving both minor and major genes acting in an additive manner.

3) Studies of stem rust reactions over time displayed differences between parents, F1's and the relationship between F1's and parents. The high level of resistance found with Parent A was maintained over time . Parents B and C were similar in stem rust reactions over time. F1 progeny from Parents B demonstrated slow rusting characteristics by having high levels of infection early in the season and maintaining a relatively high level of resistance at the end of the season compared to plants that were not as infected early in the season. F1 progeny of resistant X resistant crosses were below both parents. The F1 progeny of resistant X susceptible crosses BXD and CXD were below both parents early in the season but due to slow rusting characteristics they had sustained less rust infection than the susceptible parent by harvest. The AXD progeny displayed higher levels of resistance over time in relation to the other resistant X susceptible crosses. This depicted

the high level of resistance contributed by Parent A.

The presence of a polygenic type of resistance involving minor genes and possible combinations with some major genes is suggested. This type of resistance could be used to produce varieties with durable resistance producing a buffering capacity against several potential races of stem rust. With the quantitative nature of inheritance and the diverse sources of resistance of the materials used provide strong evidence that stability can be achieved against this. Selection for stem rust resistance would be most effective during early anthesis in late June according to broad sense heritability estimates. The earlier rating dates had higher heritability estimates suggesting greater genetic variance for resistance. As the epidemic increased the inoculum load appeared to over come the resistance.

4) Seedling stem rust reactions were found to differ from adult plant reactions in the F1 generation. In the F2 generation with open pollination of the F1 parents a gradient of the percent susceptible seedlings occurred. Plants with a susceptible mature plant stem rust rating produced a high percent of susceptible seedlings while resistant plants generally produced a lower percent of susceptible seedlings. There may be separate gene systems responsible for stem resistance in seedlings and mature plants in perennial ryegrass. Therefore, a breeding program for stem rust resistance in perennial ryegrass that uses a seedling screening procedure may be discarding susceptible seedlings with genes for adult plant resistance.

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APPENDIX

Appendix Table 1. Analysis of variance for maturity stage of four
                   parental clones on June 18.

Analysis of Variance

Sources	df	MS	F
Replications	2	752.08	4.97 (NS)
Entries	3	1077.19	7.12 *
Error	6	151.19	
Total	11	512.99	

C.V.: 3.94%
 *: Significant at 5% level
NS: Not statistically significant

Appendix Table 2. Analysis of variance for stem rust reaction of four
                   parental clones on June 18.

Analysis of Variance

Sources	df	MS	F
Replications	2	179.08	0.63 (NS)
Entries	3	2436.75	8.51 *
Error	6	286.42	
Total	11	853.36	

C.V.: 1.95%
 *: Significant at 5% level
NS: Not statistically significant

Appendix Table 3.   Analysis of variance for stem rust reaction of four
                    parental clones on June 27.

Analysis of Variance

Sources	df	MS	F
Replications	2	904.08	0.38 (NS)
Entries	3	13639.78	5.76 *
Error	6	2366.53	
Total	11	5175.15	

C.V.: 6.20%
 *: Significant at 5% level
NS: Not statistically significant

Appendix Table 4.   Analysis of variance for stem rust reaction of four
                    parental clones on July 3.

Analysis of Variance

Sources	df	MS	F
Replications	2	2314.08	0.54 (NS)
Entries	3	40258.22	9.44 *
Error	6	4266.31	
Total	11	13727.33	

C.V.: 9.6%
 *: Significant at 5% level
NS: Not statistically significant

Appendix Table 5. Analysis of variance for stem rust reaction of four
                   parental clones on July 10.

Analysis of Variance

Sources	df	MS	F
Replications	2	2286.08	0.52 (NS)
Entries	3	68997.11	15.76 ***
Error	6	4378.19	
Total	11	21621.15	

C.V.: 11.70%
  *: Significant at 5% level
 **: Significant at 1% level
***: Significant at .5% level
NS: Not statistically significant

Appendix Table 6. Analysis of variance for stem rust reaction of four
                   parental clones on July 17.

Analysis of Variance

Sources	df	MS	F
Replications	2	698.25	0.29 (NS)
Entries	3	88472.56	36.73 ***
Error	6	2408.81	
Total	11	25569.73	

C.V.: 10.68%
  *: Significant at 5% level
 **: Significant at 1% level
***: Significant at .5% level
NS: Not statistically significant

Appendix Table 7. Analysis of variance for stem rust reaction of F1 progeny means on June 18.

Analysis of Variance

Sources	df	MS	F
Replications	3	2659.43	2.30 (NS)
Entries	5	5502.18	4.75 **
Error	15	1157.96	
Total	23	2298.20	

C.V. 4.25%
 *: Significant at 5% level
** : Significant at 1% level
NS: Not statistically significant

Appendix Table 8. Analysis of variance for stem rust reaction of F1 progeny means on June 27.

Analysis of Variance

Sources	df	MS	F
Replications	3	6742.00	2.23 (NS)
Entries	5	7526.00	2.49 (NS)
Error	15	3025.87	
Total	23	4488.87	

C.V.: 7.94%
 *: Significant at 5% level
NS: Not statistically significant

Appendix Table 9.   Analysis of variance for stem rust reaction of F1
                    progeny means on July 3.

Analysis of Variance

Sources	df	MS	F
Replications	3	11139.49	4.52 *
Entries	5	8611.54	3.49 *
Error	15	2466.12	
Total	23	4933.39	

C.V.: 8.60%
 *: Significant at 5% level
NS: Not statistically significant

Appendix Table 10.   Analysis of variance for stem rust reaction of F1
                     progeny means on July 10.

Analysis of Variance

Sources	df	MS	F
Replications	3	10418.04	3.46 *
Entries	5	8483.34	2.82 (NS)
Error	15	3009.81	
Total	23	5166.00	

C.V.: 11.60%
 *: Significant at 5% level
NS: Not statistically significant

Appendix Table 11. Analysis of variance for stem rust reaction of F1 progeny means on July 17.

Analysis of Variance

Sources	df	MS	F
Replications	3	8664.60	2.19 (NS)
Entries	5	9909.68	2.50 (NS)
Error	15	3957.50	
Total	23	5865.42	

C.V.: 17.38%
 *: Significant at 5% level
NS: Not statistically significant

Appendix Table 12.   Two-way frequency distributions, means, and
                     standard deviations of stem rust ratings on F1 and
                     parent populations taken on June 18.

F1's and Parents	1	2	Stem Rust Rating Scale 1/ 3	4	5	6	7	8	9	Total	Mean	S.D.
A	0	0	0	0	0	0	0	0	27	60	9.00	0.00
B	0	0	0	0	0	0	0	4	16	20	8.80	0.41
C	0	0	0	0	0	1	1	5	20	27	8.63	0.74
D	0	0	0	0	0	0	2	10	9	21	8.33	0.66
AXB	0	0	0	0	0	3	9	11	37	60	8.37	0.92
BXC	0	1	0	2	2	7	9	12	27	60	7.73	1.57
BXD	0	0	0	2	2	10	15	13	19	60	7.55	1.32
AXC	0	0	0	0	0	9	3	11	37	60	8.37	1.10
CXD	0	0	0	0	0	17	8	8	27	60	7.75	1.30
AXD	0	0	0	0	0	3	7	14	36	60	8.38	0.88
Total	0	1	0	4	3	50	54	88	225	445	8.15	1.17

1/ Stem rust scale based on visual estimates approximating area of
   foliage and seed heads infected.

Appendix Table 13.   Two-way frequency distributions, means, and
                     standard deviations of stem rust ratings on F1 and
                     parent populations taken on June 27.

F1's and Parents	1	2	Stem Rust Rating Scale 1/ 3	4	5	6	7	8	9	Total	Mean	S.D.
A	0	0	0	0	0	0	2	10	15	27	8.48	0.64
B	0	0	0	0	0	3	2	6	9	20	8.05	1.10
C	0	0	0	0	2	1	4	11	9	27	7.89	1.15
D	0	0	0	0	2	8	3	6	2	21	6.90	1.22
AXB	0	0	0	0	5	14	11	13	17	60	7.38	1.34
BXC	1	1	2	4	8	6	6	13	19	60	6.97	2.08
BXD	0	0	2	7	9	12	14	9	7	60	6.40	1.64
AXC	0	0	0	4	9	8	9	12	18	60	7.17	1.66
CXD	0	0	1	8	13	8	11	12	7	60	6.40	1.68
AXD	0	0	0	2	1	17	13	13	14	60	7.27	1.33
Total	1	1	5	25	49	77	75	105	117	455	7.13	1.63

1/ Stem rust scale based on visual estimates approximating area of
   foliage and seed heads infected.

Appendix Table 14. Two-way frequency distributions, means, and
standard deviations of stem rust ratings on F1 and
parent populations taken on July 3.

F1's and Parents	Stem Rust Rating Scale 1/									Total	Mean	S.D.
	1	2	3	4	5	6	7	8	9			
A	0	0	0	0	0	6	0	13	8	27	7.85	1.10
B	0	0	0	0	2	3	4	8	3	20	7.35	1.23
C	0	0	0	3	2	6	8	4	4	27	6.74	1.51
D	0	0	2	9	2	1	5	2	0	21	5.19	1.66
AXB	0	0	1	6	13	15	11	9	5	60	6.27	1.51
BXC	3	1	9	7	3	8	7	19	3	60	5.85	2.30
BXD	0	4	12	6	8	13	7	7	3	60	5.30	2.00
AXC	0	3	8	4	6	10	9	10	10	60	6.15	2.18
CXD	1	5	9	7	10	16	4	5	3	60	5.12	1.97
AXD	0	2	0	5	16	19	7	9	2	60	5.95	1.48
Total	4	15	41	47	62	97	62	86	41	455	6.00	1.95

1/ Stem rust scale based on visual estimates approximating area of
   foliage and seed heads infected.

Appendix Table 15. Two-way frequency distributions, means, and
standard deviations of stem rust ratings on F1 and
parent populations taken on July 17.

F1's and Parents	Stem Rust Rating Scale 1/									Total	Mean	S.D.
	1	2	3	4	5	6	7	8	9			
A	0	0	0	3	5	6	4	4	5	27	6.59	1.67
B	0	1	4	4	5	5	0	0	1	20	4.70	1.59
C	0	4	1	7	9	2	2	2	0	27	4.67	1.66
D	11	1	1	6	2	0	0	0	0	21	2.38	1.60
AXB	2	13	9	16	9	7	4	0	0	60	3.90	1.60
BXC	14	6	4	6	13	11	3	3	0	60	3.97	2.20
BXD	15	8	11	7	8	6	3	2	0	60	3.42	2.05
AXC	10	8	3	12	7	10	8	2	0	60	4.17	2.16
CXD	21	12	5	12	4	3	2	1	0	60	2.80	1.87
AXD	8	11	13	9	15	2	1	0	1	60	3.47	1.69
Total	81	64	51	82	77	52	27	14	7	455	3.85	2.07

1/ Stem rust scale based visual estimates approximating the area of
   foliage and seed heads infected.