MONTANA STATE UNIVERSITY

The inheritance of resistance of barley (Hordeum vulgare L.) to Rhynchosporium secalis (Oud.) J.J. Davis
by Moncef Mohamed Harrabi

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Plant Pathology
Montana State University
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Abstract:
Research was initiated to gain a better understanding of the inheritance of reaction to Rhynchosporium secalis (Oud.) Davis in some barley cultivars and lines that are components of the recurrent selection population (Rrs-5). F2 plants resulting from different crosses were screened for seedling resistance to three isolates of R. secalis. Further evaluation of some F2 populations was done under disease conditions in the field to one isolate from Montana. Some of the culti-vars that were studied for inheritance of resistance were further evaluated in terms of their combining ability for yield and yield components.

Further studies were done to estimate the change in gene frequencies for resistance to scald after four cycles of recurrent selection. The total number of genes conditioning scald resistance is probably not as large as previously believed. Evidence was presented on the existence of a series of multiple alleles at the Rh-Rh3-Rh4 locus complex. Further evidence on the existence of resistance factors in susceptible cultivars was shown by crosses between susceptible cultivars. Transgressive segregation indicated the presence in barley of minor genes for scald resistance.

No significant build up in resistance between different cycles of recurrent selection was observed. This was attrib-uted to either the inability to combine multiple alleles in any single pure line or to insufficient natural disease infections at different nurseries. The probability, however, of selecting plants resistant to isolates representing a wide range of virulence types from the recurrent selection popula-tions is high.

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In dedication to:

my wife Hajer, my daughters Ons and
Maysem, my son Aymen, my father and mother.

THE INHERITANCE OF RESISTANCE OF BARLEY (<u>HORDEUM</u> <u>VULGARE</u> <u>L</u>.)

TO <u>RHYNCHOSPORIUM</u> <u>SECALIS</u> (OUD.) J. J. DAVIS

by

MONCEF MOHAMED HARRABI

A thesis submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Plant Pathology

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MONTANA STATE UNIVERSITY
Bozeman, Montana

March, 1982

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ACKNOWLEDGEMENT

I wish to express my sincere appreciation and thanks to Dr.
Eugene L. Sharp for his help, guidance and willing assistance during
the years of study and research, and in the preparation of this thesis.

I would like to thank Dr. John Martin, Dr. Raymond Ditterline,
Dr. Albert Scharen and Dr. David Sands for their help and advice
while serving on my graduate committee.

Appreciation is also extended to Dr. Harold Bockelman for his
patience and help.

I am also very grateful to the Government of Tunisia and the
USAID for sponsoring my studies at Montana State University.

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ABSTRACT

Research was initiated to gain a better understanding of the inheritance of reaction to <u>Rhynchosporium secalis</u> (Oud.) Davis in some barley cultivars and lines that are components of the recurrent selection population (Rrs-5). F_2 plants resulting from different crosses were screened for seedling resistance to three isolates of <u>R. secalis</u>. Further evaluation of some F_2 populations was done under disease conditions in the field to one isolate from Montana. Some of the cultivars that were studied for inheritance of resistance were further evaluated in terms of their combining ability for yield and yield components.

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Chapter 1

INTRODUCTION

Scald of barley (<u>Hordeum vulgare</u>, L.) caused by <u>Rhynchosporium</u>
<u>secalis</u> (Oud.) Davis is a destructive disease of barley that can
seriously limit yield in many humid and sub-humid growing areas. The
disease is considered of minor importance in the northcentral North
American spring barley area, but causes considerable damage along the
Pacific coast, Europe, Asi, the Middle-East and Australia.

Changes in agricultural practices (minimum tillage, continuous
cropping, etc.) have influenced the microclimate and resulted in more
residues favorable for the scald organism. Control of this disease has
been achieved mainly by genetic resistance. At least fifteen alleles
for scald resistance have been described, but their genetics are only
partially clarified. Starling et al. (1970) believed that scald
resistance genes were not numerous. They assumed that most of the
resistance genes are located at the Rh-Rh3-Rh4 locus complex on
chromosome 3. It is not known if these genes are alleles or closely
linked. Dyck and Schaller (1961) reported that the Rh-Rh3-Rh4 genes
were linked and not allelic. None of the recessive genes rh6, rh7
and rh8 have been tested for allelism against Rh-Rh3-Rh4, Rh3 or Rh5.
Rh9 has not been tested for allelism with any other locus.

If the number of genes for scald resistance is much smaller, then

developing pure line cultivars with long term resistance may be extremely difficult, if not impossible. As new genes for resistance have been incorporated into suitable agronomic cultivars, the pathogen has mutated resulting in breakdown of resistance, and thus a short term control (Jackson and Webster, 1976).

Recently resistance factors that are not specific in their action have been demonstrated in different host-pathogen interactions. The theoretical value of this type of resistance is that it operates indiscriminately to all pathogen races and provides a durable resistance. If genes controlling different components of this type of resistance can be identified then recurrent selection techniques represent a valuable tool in building quantitative resistance. The objectives of this study were to: (1) gain more understanding of the genetics of resistance of some barley cultivars, (2) measure changes in resistance in different cycles of recurrent selection to different isolates of Rhynchosporium secalis, and (3) determine the potential of some component cultivars of the recurrent selection population in terms of their combining ability to disease reaction, yield and yield components.

Chapter 2

LITERATURE REVIEW

The Pathogen: Rhynchosporium secalis

    Barley leaf scald caused by the pathogen Rhynchosporium secalis
was first described by Oudemans in Holland in 1897 who named it
Marsonia secalis (Oudem). In 1900, Heinsen, in Germany, put the fungus
in a new genus Rhynchosporium because of the beaked one-septate spores,
and named it Rhynchosporium graminicola Heinsen. Formal descriptions
were later provided by Saccardo who credited authority for the genus
Rhynchosporium and R. graminicola to Heinsen. In 1922, Davis, in the
United States, named it Rhynchosporium secalis (Oudem) J. J. Davis and
it is by this name that the fungus is presently known. Caldwell (1937)
described the fungus in the following manner: "

>   Parasitic, producing spots on leaves; sterile mycelium
> sparse in mesophyll of host; mycelium subcuticular at first,
> later developing into a superficial fertile stroma more or
> less covering the leaf spot; conidiophores absent; conidia
> one-septate, hyaline, sessile on cells of fertile stroma.

The genus, Rhynchosporium, would fall in the classification, Monili-
aceae Hyalodidymae, Micronemeae of the imperfect fungi. The conidia of
several isolates from the host and from culture show a relatively
homogeneous shape and size. The conidia length and diameter are 15.8
± .21 and 3.3 ± .05 micrometers respectively (Caldwell, 1937). One-
celled conidia in which the septum had not formed had only one nucleus,
while two-celled spores had a nucleus in each cell (Caldwell, 1937).

Since R. secalis is uninucleate, heterokaryotic phenomena probably do not apply. Variations in the fungus must occur through mutations, parasexual cycle or even environmentally-induced changes. A population of R. secalis, made up by mixing four races in equal frequencies, has resulted in 18 races after only two successive disease cycle on a susceptible host (Jackson and Webster, 1976). This clearly demonstrates that the natural gene pool of R. secalis is probably very large. Caldwell (1937) had no success in obtaining the perfect stage of the fungus. Skoropad and Grinchenko (1957) discovered microconidia suggesting the possible existence of a sexual cycle. A few years later Ali (1972) also observed microconidia being exuded from flask-like mycelial branches but these microconidia failed to germinate and probably were spermatia.

The major source of primary inoculum appears to be from infected plant debris (Caldwell, 1937; Skoropad, 1960; Ayesu-Offei and Carter, 1971). Heinsen (1901) noted the saprophytic capability of the fungus in the greenhouse where its viability was maintained for a period of 15 months. This finding was also supported by Bartel (1929) who reported a six month survival of the fungus in infested soils. Survival of the fungus under saprophytic conditions in the soil has not been substantiated by later authors (Caldwell, 1937; Skoropad, 1962). The information on seed-borne inoculum is contradictory. Caldwell (1937) used barley seed from severely infected plants in soils with no

previous scald history. No scald infections appeared in his trials.
This perhaps does not indicate clearly that the fungus is not seed-
borne since infected plant fragments may accompany the seed. However,
Reed (1957) has shown that the fungus can be seed-borne. The ease
of the spread of the disease to new barley areas suggests that there
is some means of long-range dissemination of R. secalis. There is no
evidence that volunteer barley can serve as a source of inoculum
(Reed, 1957; Ali, 1974; Caldwell, 1937). Like most leaf-infecting
organisms, R. secalis overwinters as dormant mycelium on barley residues
(Skoropad, 1959).

Symptoms. Rhynchoporium secalis affects any parts of the leaves
including the leaf sheath producing spots of irregular shape (Brooks,
1928; Reed, 1957; Ali, 1974; Caldwell, 1937). In addition, the barley
auricles are commonly attacked, perhaps because of the tendency for
water to collect in this region (Brooks, 1928). Infection in early
stages occurs as bluish-gray lesions with a water-soaked appearance.
As invasion advances, the center of the lesions usually dry out and
become light gray to grayish white with a dark brown edge. Lesions may
develop separately and then coalesce or may develop progressively
along the leaf. Symptoms under greenhouse conditions vary somewhat.
Usually there is no definite lesions except color change of the leaf
which becomes light gray to gray-green. This phenomena is observed in
most cases. Tissue collapse takes place quickly and drying occurs

shortly after. Smith (1938) and Skoropad (1957) observed conspicuous
scald lesions on the chaff of the grain. The authors both suggested
that seed may serve as a source of primary inoculum. Shipton et al.
(1974) reported that floral bracts awns and pericarp are readily
infected. This is perhaps a further evidence of possible seed trans-
mission of the fungus. Hypersensitive reaction which resulted in the
formation of small dark-brown patches on the leaves has been reported
by Ayesu-Offei and Clare (1971).

Pathological histology. The pathogen-host tissue relationship
has been studied by few workers. Condia of R. secalis germinate from
one or both cells in 12 hours at 13 C forming short germ tubes and
appressoria (Shipton et al., 1974; Ayesu-Offei and Clare, 1970;
Caldwell, 1937). The first germ tube usually arises from the large
cell and the second came from the other cell. In some cases two germ
tubes developed from a single cell. The germ tubes are septate,
usually about 0.8 micrometers in diameter and 20.30 micrometers in
length (Ayesu-Offei and Clare, 1970). After their formation some
germ tubes enlarge to form appressorium and penetrate the cuticle
within a period of 24 hours after inoculation (Ayesu-Offei and Clare,
1970). The authors further stated that appressoria was not essential
for penetration and the penetration does not occur through stomata as
reported by Bartels (1928) but occurs directly as has been reported by
Caldwell (1937). Enzymatic degradation of the cuticle may provide the

initial penetration. Following penetration, the infection hyphae grows

rapidly between the cuticle and the epidermis. This is followed by a

subcuticular mycelium growing between epidermal cells and branching

profusely (Shipton et al., 1974). The subcuticular hyphae imparts a

grayish cast to the infected area. Mesophyll cells beneath subcuticular

hyphae soon collapse, possibly due to toxic substance(s) produced by

the hyphae. Cell disruption may be caused by a toxin, Rhynchosporoside,

one of the 1-0-α cellobiosides of 1,2. Propanediol (Beltran et al.,

1980). The fungus may be responsible for the increase in the perme-

ability of host cells so that the concentration of nutrients in the

free space is increased for use by the fungus (Jones and Ayres, 1972).

The authors reported no evidence of toxic metabolites produced by

R. secalis as opposed to the finding of Ayesu-Offei and Clare (1970)

and Beltran et al., (1980). No entry of the fungus through stomata

have been reported except by Bartels (1928). Ayres (1972) has observed

increased stomata opening in areas of the leaf colonized by the fungus.

Factors affecting infection and symptom expression. Host-pathogen

and environmental interactions are responsible for infection and colon-

ization of the host by the pathogen. Phenotypic variability in disease

expression is associated with host genotype, pathogen-genotype and

environmental factors at the time of interactions (Shipton et al.,

1974). Relative humidity as well as temperature appear to be the

limiting factors in successful infection (Shipton et al., 1974;

Caldwell, 1937; Brooks, 1928; Skoropad, 1962a and 1959; Fowler and

Owen, 1971). Temperatures around 20 C are the most favorable for

conidia production and germination (Caldwell, 1937; Shipton et al.,

1974; Skoropad, 1957 and 1962; Reed, 1957). Ayrea and Owen (1970)

reported failure of conidial germination in lesions due to the

presence of self-inhibitors. At high concentration (120,000 spores/

cm^3) spore germination does not cease. The inhibitor apparently feeds

back at high concentrations to inhibit its own production (Ayres and

Owen, 1970). Fowler and Owen (1971) reported that on intact plants

spore germination increased slightly with increasing concentration of

spores. Optimal germtube elongation occurs at a temperature range of

18 - 21 C. Temperatures above 30 C resulted in conidial rupture

(Caldwell, 1937). Reed (1957) observed the shortest germtube growth

at 7 C, and that the maximum growth rate of the R. secalis isolates

occurred at a pH of 5.2 which is close to the pH of 5.6 expressed in

barley leaf juices.

Skoropad (1957) observed that lesions appeared most rapidly when

inoculated plants were held in saturated air at 15 - 18 C for 48 hours

and then moved to a greenhouse held at about 24 C. Using both sus-

ceptible and resistant cultivars, Ali (1972) noted that at high

diurnal temperature regimes (18 C min/30 C max) certain isolates

lose the ability to infect hosts normally susceptible to them. Low

temperature (8 - 20 C) favored certain isolates while others are
enhanced at higher temperatures (15 - 24 C). Lesion development
proceeds normally at post-inoculation temperatures around 20 C and is
slowed down or ceases at low temperature (6 - 12 C) and at high
temperature (24 C) (Shipton et al., 1974).

Few experiments have been conducted on the influence of host stage
of development on symptom expression. Ali (1972) reported that geno-
type age may influence symptoms. Greater symptom expression occurs at
anthesis probably due to tissue senescence.

Physiological races. Many authors have shown the existence of
races of Rhynchosporium secalis (Shipton et al., 1974; Schein, 1958
and 1960; Reed, 1957; Caldwell, 1937; Williams and Owens, 1973; Owen,
1958 and 1963). No specialization of isolates into races from barley
was recognized until 1955, although Sarasola and Campi (1947) in
Argentina have differentiated four races. Riddle and Suneson (1948),
in field trials at Davis, California observed no evidence of physio-
logical races. Later Schein (1958) found five races based on their
ability to attack the barley cultivars Wisconsin winter x Glabron,
Brier, Hudson, California 1311, Atlas 46 and Turk. Schein (1960),
working with eight isolates from different parts of the U.S., differ-
entiated seven races which he designated U.S. 1 to U.S. 7. Later,
Dyck and Schaller (1961a) identified two additional races, U.S. 8 and

U.S. 9. Kajiwara and Iwata (1963) reported the existence of ten races in Japan. Reed (1957) showed the presence of races of R. secalis in Canada and the U.S., however Skoropad (1960) found no clear evidence of pathogenic races in Canada. Williams and Owen (1973) tested 122 single spore isolates of R. secalis collected from Britain on 12 cultivars of barley and were able to find two distinct races which they called U.K. 1 and U.K. 2. Earlier Owen (1963) working with ten British isolates demonstrated variability within R. secalis but could not distinguish any races present at that time. Recently Ceoloni (1980) in Italy, using 13 barley cultivars with known genes for specific resistance to scald, was able to differentiate 17 races. Atlas and Atlas 46 were resistant to all Italian isolates. This clearly demonstrates the difference between the U.S. and the Italian races. In California, both Atlas and Atlas 46 are very susceptible (Jackson and Webster, 1975). Further, the Italian races appear to be different from those reported by Owen (1963) in Britain. La Mesita, resistant to Owen's isolates, was susceptible to ten of the Italian races.

Ali (1972) found 15 biotypes from a collection of 35 isolates from Western Australia. The author studied the performance of these 15 biotypes under summer and winter conditions and was able to detect marked differences in host response, suggesting that environmental conditions at the time of testing may influence the interpretations.

Kajiwara and Iwata (1963) found that substrate composition and culture age can influence virulence. Hansen and Magnus (1973), working with 11 barley cultivars, found 11 different virulence genes in Norway. The virulence genes r8, r9 and r10 were most predominant in Norway.

Epidemiology. Several investigators have reported on the persistence of R. secalis as mycelium in barley debris (Skoropad, 1960; Caldwell, 1937; Reed, 1957; Shipton et al., 1974; Ayesu-Offei and Carter, 1971). Skoropad (1966) reported that scald lesions retained their ability to produce conidia for up to 340 days, depending on environmental conditions. In earlier studies, Skoropad (1960) suggested that after development of the first lesion, secondary inoculum was dispersed by wind-born rain splash. He further found that conidia are most abundant during rainstorms and that they are clustered in groups of three to ten, indicating transport of conidia in water droplets. Ayesu-Offei and Carter (1970) found that sporulation occurred most abundantly when free moisture is present. They further observed that fewer conidia were trapped under dry but windy conditions. Wind tunnel experiments indicated that conidia were trapped under dry, windy conditions. These experiments indicated that conidia are released and dispersed mainly as a result of water splash and not due to wind alone (Ayesu-Offei and Carter, 1970). In addition, they found that conidia are released at any time of the day or night, which is in contradiction to the finding of Ozoe (1956) who claimed that the

number of conidia of <u>R. secalis</u> in the air is greater in the day time.

Disease spreads from plant to plant and pockets of infection may appear as far as nine meters from the nearest source of inoculum (Reed, 1957; Ayesu-Offei and Carter, 1970). No evidence was found indicating spread of <u>R. secalis</u> by insects.

<u>Economic importance.</u> Scald, incited by <u>Rhynchosporium</u> <u>secalis</u> is a common foliage disease of barley in many parts of the world. Frank (1897) first reported the disease in Germany and recognized it to be of a major economic importance, especially when the plants were attacked before heading. The disease was first mentioned in the United States in 1917 (Caldwell, 1937). Today scald is known to occur in Northern Europe, United States, Canada, England, Middle East, North Africa, Mexico, Argentina and Peru. The first epidemic in the United States was reported in the interior valleys of California (Caldwell, 1937). Not only have seasonal conditions favored the disease development and outbreaks, but also the changes in agricultural practices (minimum tillage, sprinkler irrigation, close rotations, combine harvesters, etc...) have contributed significantly (Shipton et al., 1974).

Wiebe, as reported by Caldwell (1937) estimated yield losses up to 15 percent based on comparative yields of susceptible and resistant varieties during epidemic and non-epidemic years. In Wisconsin, scald completely destroyed spring barley plots when infection occurred early

in the season (Caldwell, 1937). In England, yield losses between 35

and 40 percent have been reported (Shipton et al., 1974). James et al.

(1968) have found that yield reduction occurs mainly through reduction

in kernel weight. If infection occurs early in the season, number of

tillers per plant may also be reduced.

In an extensive study between yield loss and disease caused by

R. secalis, Clives et al. (1968) compared yields from plants sprayed

with fungicide with those from unsprayed plots. They further compared

yields of cultivars with varying susceptibility level to the disease.

Yield loss was found to be equivalent to two-thirds of the percentage

of the flag-leaf area visibly infected or one-half of the infected

area on the second leaf. Clives et al. (1968) used the average of

these two estimates to predict yield loss.

Inheritance of Resistance

Losses caused by R. secalis stress the need for the development

of cultivars resistant to the scald organism. In order to facilitate

this objective in a breeding program, a thorough understanding of the

manner of inheritance of reactions to the disease becomes a necessity.

The most extensive and earlier work on the genetics of scald

resistance was reported by Dyck and Schaller (1961). They reported

five genes for resistance and designated them as Rh2, Rh3, Rh4, $Rh4^2$

(an allela of Rh4) and Rh5. These workers found a single dominant

gene (Rh2) in Atlas. Atlas 46 has a second gene (Rh3) in addition to

the Rh2. Atlas 46 is a derivative of a cross between Atlas and Turk. Turk was found to have two dominant genes (Rh3 and Rh5) by Riddle and Suneson (1950) and Dyck and Schaller (1961a). Other workers reported Turk as having only one gene (Evans, 1969; Baker and Larter, 1963; Wells and Skoropad, 1963; Starling et al., 1971; Ali, 1975a). Ali (1975b) found that the gene in Turk is allelic or closely linked to that of La Mesita.

La Mesita has been reported to possess one dominant gene Rh4 (Riddle and Briggs, 1950; Dyck and Schaller, 1961; Starling et al., 1971). Habgood and Hayes (1971) found that La Mesita contains two genes (Rh4 and Rh10). Ali (1975b) also reported on the existence of two genes in La Mesita, one of them probably being the same as Rh4 found is Osiris reported by Dyck and Schaller (1961). Baker and Larter (1963), in evaluating F_2 and backcross families, found that Jet and Steudelli each had two temperature sensitive complementary recessive genes designated rh6 and rh7. Temperatures greater than 25 C induced a susceptible reaction in Jet and Steudelli. Thus, that the usefulness of these two varieties in a breeding program is limited due to the temperature sensitivity of their resistant genes. Resistance of both Abyssinian (CI668) and Kitchin is controlled by a single gene that shows incomplete dominance and designated Rh9. This gene confers full resistance only in a homozygous condition. In an early study Riddle and Briggs (1950) have found that both Trebi and Modoc had a

dominant and a recessive gene for resistance to scald. This is
possible since Trebi was one parent of a composite cross from which
Modoc was derived. The dominant gene is identical to the one in
La Mesita (Riddle and Briggs, 1950). These authors also reported that
Turk has two dominant genes, one of which is similar to the one present
in La Mesita, Modoc and Trebi. Other workers have reported the
existence of only one gene in Turk (Baker and Larter, 1963; Evans,
1969; Wells and Skoropad, 1963). Habgood and Hayes (1971), on the
other hand, found one dominant and one recessive gene in Turk.

Dyck and Schaller (1961a) observed that Osiris contains only one
gene which is dominant and probably the same as the one found in La
Mesita (Rh4). Wells and Skoropad (1963), however, reported that
Osiris contains one gene Rh3 similar to the one found in Atlas 46.
Furthermore, Ceoloni (1980), working with Italian isolates of R.
secalis, reported that the genes of La Mesita and Osiris are different.
Habgood and Hayes (1971) suggested the presence of an additional
recessive gene (rh6) in Osiris when compared to La Mesita. These
workers reported that this gene acts as a "neutral" one in cultivars
other than Jet in which it was originally found. Thus Osiris may
contain two dominant genes (Rh 4 and Rh10) and one recessive gene
(rh6) (Hapgood and Hayes, 1971).

Hansen and Magnus (1973), working with Norwegian isolates, clearly
showed that the resistance in La Mesita and Modoc was not conditioned

by identical genes. This appears to contradict the findings of Hapgood and Hayes (1971) who consider the genes for resistance in Turk, Atlas 46, Modoc, Osiris and La Mesita to be at the same locus. In an earlier study Riddle and Briggs (1950) found that the varieties La Mesita, Trebi and Modoc had a single dominant gene in common for scald resistance.

The gene rh8 in Nigrinudum has been reported by Wells and Skoropad (1963) and confirmed by Habgood and Hayes (1971). These last workers have also shown that the complementary recessive gene (rh7) in Jet is situated at the Rh locus. They amended the designation of rh7 reported by Baker and Larter (1963) to rh5. The rh6 was retained for the other gene which is present in Turk, Modoc and Osiria. A fourth recessive gene, rh11, was reported by Habgood and Hayes (1971) in CI4364 and CI4368. The results of these workers show that there are five alleles at the Rh locus; two are dominant Rh and Rh^2), two have complete dominance (Rh3 and Rh4) and one is recessive (rh5). Multiple alleles have also been shown at the Mla locus conditioning mildew resistance in barley (Moseman, 1966).

Dyck and Schaller (1961) have assigned Rh3 and Rh4 to linkage group 3. These authors have found that the Rh3 gene was linked to a gene-conditioning spike density with a recombination value of 14 ± 1.56 percent, and was closely linked with a gene for streaked seedlings; and that Rh4 was linked with a xantha seedling gene with an estimated

11.2 ± 1.21 percent recombination value.

The genetics of resistance of the cultivars mentioned above is not clear and more complicated than suggested by previous literature. Differences could be due to multiple alleles or to genes closely linked. The detection of certain genes that confer resistance and their identification with other previously described genes depends on the virulence of the genes in the genetic studies and also on the prevailing environmental conditions. It has clearly shown that the resistance of Jet and Steudelli is temperature sensitive and breaks down at temperatures above 25 C (Baker and Larter, 1963).

Fowler and Owen (1971) studied the mechanisms of resistance to R. secalis. They reported that the earliest point at which resistance was expressed was at penetration of the cuticle. Cutin thickness does not contribute to this resistance. Cutin acids, have been reported to be more prevalent in leaves of resistant strawberry to Sphaerotheca macularis (Fr.) Jaczev. than in leaves of susceptivle cultivars (Peries, 1962). Conidia of Erysiphe graminis germinated, and appressoria were formed at the same rate on all tested barley cultivars whether resistant or susceptible (White and Baker, 1964). Ayres and Owen (1971) found that host resistance did not affect germination or appressorium formation of R. secalis.

Recurrent Selection Populations

The prevalence of the disease and the extent of the damage it can

cause have necessitated the development of resistant cultivars. There are two major types of resistance that are of importance in breeding programs, race specific and race non-specific resistance.

Race-specific resistance has been extensively utilized in the past to produce extremely resistant cultivars (Evans and Griffiths, 1971). This approach presents serious disadvantages and often has resulted in "boom and bust" cycles. Atlas 46, a race specific resistant cultivar to California races of R. secalis, was released in 1947. Nine years later resistance broke down and Atlas 46 became susceptible. Today there is growing interest in resistance which is not race-specific. The winter wheat cultivar Crest, for example, has three minor genes conditioning resistance to stripe rust and has been cultivated for the past 15 years without loss of resistance (Sharp et al., 1976). Moro, on the other hand, with only one major gene for resistance to stripe rust lost its resistance a few years after its release.

The use of single major genes for resistance to any disease remains an attractive method for plant breeders because of ease of incorporation and selection. Breeding for race non-specific resistance however, is more difficult to achieve, specially if this type of resistance shows both continuous variation and a genotype-environment interaction (Evans and Griffiths, 1971).

Recurrent selection is an effective breeding method of accumulating genes and developing multigenic resistance (Barnes et al., 1971). It

was first proposed by Hayes and Gerber (1919) and has been used extensively to improve breeding populations of maize (Moll and Stuber, 1971).

Many recurrent selection methods have been proposed to improve breeding populations (Sprague and Eberhard, 1977). They require the selection of plants with superior genotypes from the population and the intermating of these selected individuals to form a new population. Thus recurrent selection gradually increases the frequency of favorable alleles. This increase depends on the ease by which superior individuals are identified and on the number of genes controlling a specific trait. Barnes et al., (1971) subjected two unrelated populations of alfalfa to bacterial wilt (Corynebacterium insidiosum (McCall) Jens). They were able to reduce the disease severity indices rather rapidly in one population which apparently possessed major genes. The disease severity reduction, however, was slower in the second population which probably contained minor genes.

The average rating for leaf feeding by the European corn borer (Ostricia nubilalis Hubner) was reduced from 5.4 to 2.9 (1 to 9 scale) in only three cycles of recurrent selection (Penny et al., 1967). An average reduction of 2.8 percent of ears with kernel damage due to the feeding of earworms (Heliothis zeae Boddie) per cycle of recurrent selection was reported by Zuber et al. (1971). Jenkins et al. (1954) used phenotypic recurrent selection to reduce

the disease rating to leaf blight (Helminthosporium turcicum Pass.)
from 3.3 to 2.1 (on a 0 to 5 scale) after three cycles. Stalk-rot
resistance (Diplodia zeae (Schw.) Lev.) was also improved by recurrent
selection (Jinahyon and Russell, 1969).

Phenotype recurrent selection was also used to improve quantita-
tive traits. Russell and Eberhart (1970) reported that additive
genetic variation for yield and other quantitative traits is sufficient
to obtain progress from recurrent selection. A yield increase in corn
of 5.2 q/ha has been reported by Eberhart et al. (1973a). In order to
achieve a positive genetic gain, plants with superior phenotypes must
be selected in the breeding population. Inbreeding may be a serious
problem in these populations but can be avoided when a sufficient
number of plants is selected after each cycle. Thus population size
needs to be taken into consideration.

The method has its greatest success in breeding cross-pollinated
crops. Its usefulness in self-pollinated species is limited because
of the difficulty in obtaining large number of crosses for recombina-
tions. This difficulty is circumvented in barley by utilizing genetic
male sterility to facilitate crossing. A number of male sterile genes
have been identified in barley (Hockett et al., 1968) since the first
one described by Suneson (1940). Suneson (1956) utilized genetic
male sterility in several composite crosses. He relied on natural
selection to improve population characteristics.

Chapter 3

MATERIALS AND METHODS

Inheritance of resistance

Materials. The barley tested for scald resistance consisted of
(a) a number of cultivars and strains of spring barley from different
areas of the world with known genes for scald resistance to strains from
the United States and Europe, (b) selected cultivars with good agronomic
characters and wide adaptation, (c) lines with unknown genes for resis-
tance to scald, (d) F_1 and F_2 lines from all possible crosses of some
selected lines. The parents and their C1 numbers are listed in
Appendix Table 1.

Inoculum preparation. The fungus was cultured on lima bean agar
in an incubation chamber at 18 C and eight hours of light. The Montana
isolate used in these studies originated from scalded plants collected
from Lewistown, MT. in 1975. The Tunisian (Tun.1), California (Ca.75)
and Morocco (Mor.25) isolates came from lyophilized cultures main-
tained in the refrigerator. Subsequent culturing was done by trans-
ferring spores to fresh lima bean agar plates with autoclaved Q-tips.
Cultures to be used as inocula were started on the same day that the
barley plants to be inoculated were planted and harvested two weeks
later. The method of Schein and Kerelo (1957) for isolation of the
disease organism was used.

The inoculum was prepared by flooding two week old plates with

distilled water and scraping the colonies with a microscope slide.
The resulting spore suspension was filtered through four layers of
cheese cloth to remove mycelial and agar fragments. A Levy-Hausser
counting chamber No. 508 (Hausser Scientific) was used to measure spore
concentration. The inoculum was standardized at 6×10^6 spores/ml and
applied with a DeVilbiss atomizer attached to a compressed air hose
(15-20 psi). Each flat in the greenhouse was sprayed with 25 ml of
spore suspension and transferred to a dark dew-simulation chamber kept
at 20 C for 24 hours, then returned to the greenhouse. Two weeks later
scald readings were recorded using a 0 to 3 scale. (0 = no visible
lesion, 1 - marginal lesions, 2 = small lesions not confined to leaf
margins and 3 = typical scald.)

F_2 progeny field studies. Two hundred F_2 seeds from each cross
and their parents were space planted at the Horticulture Farm at
Bozeman, MT. in May 1981. The individual plants were spaced 6 cm
apart in rows 6 m in length and 35 cm apart.

Inoculation was made in later afternoon with a Montana isolate
(Lew B77) a few weeks after seeding. All entries were sprinkler
irrigated before and after inoculation for five minutes to maintain a
humid environment. Inoculation was repeated two weeks later to assure
infection. Each row was sprayed with a spore suspension using a solo
mist sprayer (Solokleinmotoren GMBH, West Germany). A generally good
infection was obtained. Scald symptoms began to appear on the plants

in the middle of June. Scald readings were made in July and early

August using a disease intensity rating scale of 0 to 3 of resistant

(0), intermediate (1, 2) and susceptible (3).

F$_2$ progeny greenhouse studies. Each F$_2$ population was grown in

a metal flat (14" x 10" x 3") containing about 200 seeds. Both parents

of each cross were also seeded in the beginning of each flat. All

flats were kept in the greenhouse maintained at about 20 C. During

warm days temperature had risen to about 27 C. Two weeks after sowing

each flat was sprayed with 25 ml of spore suspension adjusted to about

6 x 10^6 sproes/ml and applied with a DeVilbiss atomizer attached to a

compressed air hose (15-20 psi). Flats were transferred to a dew-

simulation chamber with an air temperature of about 20 C for 24 hours.

The flats were then returned to the greenhouse. Two weeks later scald

readings were made.

Statistical procedures. In most instances 0, 1 and 2 reactions

were grouped together as resistant and a 3 reaction was classified as

susceptible. In other 0, 1 and 2 and 3 reactions were classified as

resistant, intermediate and susceptible, respectively. Chi-square was

used to determine if the observed classes fit a hypothetical genetic

ratio.

Recurrent selection population studies

A male sterile-facilitated recurrent selection population,

designated Composite Cross XXXVI, was developed to select for

broad-based resistance to scald, incited by <u>Rhynchosporium</u> <u>secalis</u> (Oud.) J. J. Davis. A description of this population, including parents, assembly, and the recurrent selection cycle appears elsewhere (Bockelman et al., 1980).

Five stages (representing four cycles of recurrent selection) in the development of C.C. XXXVI were chosen for use in this study, as follows:

Stage 1: Initial population after assembly.

Stage 2: Stage 1 after planting in a disease nursery at Bozeman (1976), inoculation with a Montana isolate of <u>R.</u> <u>secalis</u> (Lew B77), roguing of the susceptible plants (40%), and harvesting seed on the remaining male sterile plants.

Stage 3: Stage 2 after recombination nursery in Arizona, planting in a disease nursery at Bozeman (1977), inoculation (Lew B77), roguing of the susceptible plants (25%), and harvesting seed on the remaining fertile plants.

Stage 4: Stage 3 after recombination nursery in Arizona, planting in disease nurseries (1978) at Bozeman (inoculated Lew B77, rogued 10%), Fairfield, MT

(natural infection, rogued 5%), and Ft. Benton,
Mt (natural infection, rogued 5%), harvesting seed
on fertile plants and bulking (44, 44, 12% respectively)
along with seed (15% of total bulk) harvested from
disease nurseries (planted to seed of stage 2) in
Georgia (natural infection, fewer than 100 resistant
plants harvested), Maryland (natural infections, 20
resistant plants harvested, and Izmir, Turkey
(natural infection, fewer than 100 resistant plants
harvested.

Stage 5:   Stage 4 after 1) recombination nursery in Arizona;
2) addition of seed harvested from disease nurseries
(planted to seed of stage 3) at Woodland, CA (natural
infection, 150 resistant plants harvested) and Beja,
Tunisia (light infection, 200 agronomically better
plants selected) (16, 9% added respectively); and
3) planting in disease nurseries (1979) at Bozeman
(inoculated Lew B77, rogued 30%), Fairfield (natural
infection, rogued 5%), Ft. Benton (natural infection,
rogued 5%), Davis, CA (natural infection, rogued
70%), Suweon, Korea (natural infection, 200 resistant
plants and bulked (49, 4, 4, 25, 12, 6% respectively).

Seeds from the five stages were planted in a disease nursery at Bozeman, MT in 1980, using a randomized complete block design with four replications. Each plot in a replication contained about 50 plants from a stage which were grown in two, three meter rows. A 30 cm spacing was used between the rows. A spore suspension was streaked on lima bean agar and incubated for two weeks at 18 C. The agar with the fungus were comminuted with a blender. Inoculation was performed with the resulting spore suspension (about 6×10^6 spores/ml) using a Solo mist sprayer (SoloKelinmotoren GMBH, West Germany) four weeks after sowing with only one isolated from Montana (Lew B77).

Plants from the five stages were also grown in the greenhouse in metal flats. Four flats, each containing one replication of each cycle with approximately 100 seeds, were used. The greenhouse inoculations were done with three isolates representing a diversity in virulence. The isolates were from Montana (Lew B77), California (Ca. 75) and Tunisia (Tun.1) and were incubated as described above. The inoculum was adjusted to about 6×10^6 spores/ml. Each flat was sprayed with 25 ml of the spore suspension using a tip atomizer attached to an air hose. Flats were then placed in a dew-simulation chamber for 24 hours at 20 C. These flats were then returned to the greenhouse, maintained at about 20 C for two weeks, then readings were made. A disease rating scale of 0 to 3 was used: 0 = no visible lesions, 1 - marginal lesions only, 2 - small lesions not confined to leaf margins, and 3 = typical

scald lesions with total collapse of the leaf. Plants with 0, 1 and
2 infection types were pooled and classified as resistant. Fifty
plants in the field were tagged so that measurements of yield and yield
components could be made at harvest time. The frequencies of resistant
plants in each cycle were calculated along with analysis of variance
and correlation matrices.

In another study, about 200 plants were chosen at random from
stages 1, 3 and 5. Each plant was harvested separately and equal
amounts of seed from each plant was bulked. The resulting mixture
was seeded in Arizona. Two hundred spikes from each cycle were chosen
at random and harvested separately. Equal amounts of seed from the
200 spikes was bulked and seeded at the Horticulure Farm, Bozeman,
MT, in April of 1981. In September 1981 again 200 spikes from each
of the three stages were chosen at random and harvested separately.
Equal amounts of seed from each spike was bulked. Up to the third
generation no selection for scald resistance has been practiced and
at least 75 percent homozygosity has been achieved. About 200 seeds
from each stage were grown in metal flats and were inoculated with
three scale isolates Morocco (Mor.25), Tunisia (Tun.1) and California
75 (Ca.75). Inoculation technique and readings are similar to the
procedure described above. Since no selection in these three cycles
has taken plance and at least 75-80 percent homozygosity has been
obtained, the frequencies of resistant and suceptible plants, p and q

respectively, may be equal to the initial gene frequency we started with in these populations.

Combining ability analysis

Ten barley cultivars with known and unknown genes for resistance to scald were selected for this study. These parents and their CI numbers are shown in Appendix Table 3. The cultivars were not randomly chosen, but rather selected because of known high attributes for yield, adaptation and scald resistance. Thus, these cultivars will be defined as a population about which inferences will be made.

Crosses were made in the spring of 1980 between the ten parents and all possible combinations except reciprocals. The 45 crosses were grown in Arizona in the winter of 1981 and harvested in April. The F_2 seeds were brought back to Bozeman, MT and were grown along with their parents in a randomized complete block design with two replications at the Horticulture Farm, Bozeman, MT. About 100 seeds were space planted at 6 cm intervals within 6 m rows. A 35 cm spacing was used between the rows. Five plants from each replication were chosen at random and harvested separately and the following measurements recorded:

1. Total yield per plant in grams.

2. Number of seed bearing tillers per plant.

3. Average kernel weight obtained by randomly counting 100 seeds from each plant.

4. Average kernel number per spike was obtained by counting
   the total number of seeds per plant with an electronic
   seed counter and dividing by the number of tillers.

Average weather conditions prevailed throughout the growing season. Sprinkler irrigation was utilized a few times to improve infection conditions.

Statistical analysis. Analysis of variance were conducted separately for each character measured in the ten parents and their 45 F_2 progenies for the purpose of detecting true differences among the material. General combining ability effects were analyzed by regression methods.

Chapter 4

EXPERIMENTAL RESULTS

Inheritance of Resistance

The cultivars used in this study, their CI numbers, and their
disease reactions to the three isolated of Rhynchosporium secalis used
are shown in Appendix Table 1. The Montana isolate (Lew B77) is less
virulent than the Tunisian (Tun.1) and the Moroccan (Mor. 25) isolated.
The last two isolates appear to possess different genes for virulence
as indicated by their reaction to Atlas 46 and Turk (Appendix Table 1).
Some of the cultivars used in this study have been tested previously
and assigned gene symbols by various workers (Appendix Table 2). The
other cultivars such as Steptoe and Gem were selected as parents for
this study because of their desirable characteristics and overall
performance under a wide range of environmental conditions.

Although such cultivars such as Trebi, Nigrinudum and La Mesita
show a susceptible reaction to some isolates of R. secalis they were
found to possess one or two recessive gene conditioning resistance
(Table 1). Most of the cultivars showed a digenic inheritance (Table
2). The two gene system varied in action according to the parents
used. Jet and Steudelli showed two recessive genes as indicated by
ratios not significantly different from 3:13 or 1:15. Jet gave a type
0 reaction to the Lew B77 isolate and a type 3 reaction to the Tun.1
and the Mor. 25 isolates. Steudelli was resistant to the Mor. 25

Table 1: Reaction of F_2 plants, resistant or susceptible, to two isolates of
Rhynchosporium secalis in the progeny of crosses involving Betzes[†].

Cross	Isolate	Resistant (type 0-2)	Susceptible (type 3)	Probability			
				1:3	4:12	3:13	7:9
Betzes x Gem (2)*	Tun.1	32	90	.83	.80		
Betzes x Trebi (3)	Tun.1	38	80	.09	.06		.01
Betzes x Atlas (2)	Tun.1	30	106	.49		.26	
Betzes x Nigrinudum (3)	Tun.1	42	94	.14	.10	.00	.00
Betzes x La Mesita (3)	Tun.1	31	111	.44	.54	.28	
Betzes x CI 3940 (0)	Mor.25	39	109	.78			

† Betzes is a susceptible cultivar
* Numbers in parenthesis indicate parent reaction

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Table 2. Reaction of F_2 plants, resistant or susceptible, to three isolates of _Rhynchosporium_ _secalis_ in the progeny of crosses involving Betzes[†].

Cross	Scald Isolate	Resistant (type 0-2)	Susceptible (type 3)	3:13	Probability 7:9	1:15	9:7	1:3
Betzes x Steudelli(1-2)*	Lew B77	26	126	.856				.03
Betzes x Steudelli (2)	Tun. 1	45	69		.368			
Betzes x Steudelli (0)	Mor. 25	7	146			.746		
Betzes x Jet (0)	Lew B77	33	153	.997				.03
Betzes x Jet (3)	Tun. 1	22	126	.451				.006
Betzes x Jet (3)	Mor. 25	0	120					
Betzes x Trebi (0)	Lew B77	95	64		.001		.487	
Betzes x CI668 (0)	Lew B77	26	110	.82				.138
Betzes x CI668 (0)	Tun. 1	20	127	.201				.002
Betzes x CI668 (0)	Mor. 25	13	132			.99		
Betzes x Gem (2)	Lew B77	81	78		.09		.116	
Betzes x Gem (2)	Mor. 25	21	131	.216				.002

Table 2. continued

	Scald Isolate	Resistant (type 0-2)	Susceptible (type 3)	Probability						
				13:3	3:1	9:7	7:9	1:15	15:1	3:13
Betzes x CI3940 (0)	Lew B77	130	19	.12	.01					
Betzes x CI3940 (0)	Tun. 1	82	29		.87					.04
Betzes x CI4354 (0)	Lew B77	126	26	.86	.03	.00				
Betzes x CI4354 (0)	Tun. 1	110	60			.04	.00			
Betzes x CI4354 (0)	Mor. 25	53	47			.52	.09			
Betzes x W.Hordeum(1)	Lew B77	64	75			.71				
Betzes x W.Hordeum(3)	Tun. 1	7	104					.87		
Betzes x Turk (0)	Lew B77	165	40	.64	.13					
Betzes x Turk (0)	Mor. 25	84	32	.01	.59					
Betzes x Turk (3)	Tun. 1	15	130					.02		.02
Betzes x Kitchin (0)	Lew B77	59	82				.65			
Betzes x La Mesita(0)	Mor. 25	4	109					.47		
Betzes x Atlas 46 (0)	Lew B77	182	24		.01				.01	

†Betzes is a universal susceptible cultivar
*Numbers in parenthesis indicate parental reaction

isolate but intermediate to the other two isolates.

When tested with the Lew B77 isolate the segregation of the F_2 population from Betzes x CI3940 fitted a 13:3 ratio, indicating that a dominant and a recessive gene conditioned resistance of CI3940 (Table 2). The same cross showed one recessive and one dominant to the Mor. 25 and Tun. 1 isolates respectively. The Lew B77 was able to identify both of these genes at the same time. This confirms the hypothesis that resistance of CI3940 is governed by two genes, one dominant, the othr recessive. On the other hand, two dominant genes appear to control resistance in CI4354 as shown by a ratio not significantly different from 9:7.

The segregation of the progeny from crosses involving La Mesita, Forrajera, Bey, Atlas 46 and CI4354 in response to inoculation with the three isolates are presented in Table 3. When crossed with the susceptible cultivars Betzes, Modoc and Trebi, all these resistant varieties gave F_2 segregations compatible with the hypothesis that resistance in each variety was conferred by one or two epistatic dominant genes since significant probability values were obtained based on one or two gene models. Atlas showed an incompletely dominant gene, however, resistance in Nigrinudum may be conditioned by one incompletely dominant gene or two recessive genes (Table 4). The genetic control of resistance in CI668 was examined by crossing this resistant cultivar to Betzes and other susceptible cultivars