Conservation of powdery mildew resistance genes in three composite cross populations of barley
by Guy De Smet

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Plant Pathology
Montana State University

Abstract:
The barley Erysiphe graminis f.sp. hordei host-parasite system was used as a model to evaluate the potential of barley composite cross populations for conservation of disease resistance. The objective was to determine if increases in resistance to powdery mildew could be detected over periods of time in composite cross populations developed in California, where the disease might have had a selective influence on the populations, and the same populations grown in Montana, where no selective influence of powdery mildew was expected. Four isolates of E. graminis f.sp. hordei were used to monitor the frequencies of specific mildew resistances through early, intermediate and late generations of three composite cross populations (CC II, CC V, CC XII) grown at Davis, California, and Bozeman and Moccasin, Montana.

Following artificial inoculation with the powdery mildew cultures in a controlled environment, the barley seedlings were classified as resistant or susceptible according to their reaction types. The number of resistant seedlings was computed for each treatment combination in each replication and converted to a percentage form, on which statistical analyses were performed.

Changes in frequencies of plants resistant to the four isolates were observed between generations in all populations from the three locations. Increases in the frequency of resistant plants were detected by inoculations with culture 59.11 in CC II from Bozeman and CC XII from Bozeman and Moccasin, and by inoculations with cultures 63.12 and MT from the California CC XII. The largest increases in frequency of resistance were detected by inoculations with culture 59.11. CC XII revealed the most and largest increases in resistance of the three barley populations. Trends in the frequencies of resistance are discussed in relation to selection pressure applied by powdery mildew. It is suggested that associations with gene complexes other than resistance to E. graminis might help to explain the increased resistance observed in these studies.

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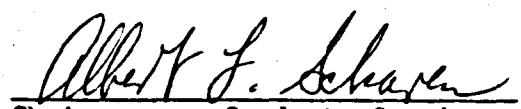
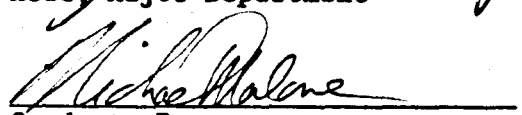
Approved:



Chairperson, Graduate Committee

Head, Major Department



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ABSTRACT

The barley Erysiphe graminis f.sp. hordei host-parasite system was used as a model to evaluate the potential of barley composite cross populations for conservation of disease resistance. The objective was to determine if increases in resistance to powdery mildew could be detected over periods of time in composite cross populations developed in California, where the disease might have had a selective influence on the populations, and the same populations grown in Montana, where no selective influence of powdery mildew was expected. Four isolates of E. graminis f.sp. hordei were used to monitor the frequencies of specific mildew resistances through early, intermediate and late generations of three composite cross populations (CC II, CC V, CC XII) grown at Davis, California, and Bozeman and Moccasin, Montana.

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INTRODUCTION

Bulk populations have often been praised as reservoirs of genetic variability, assuring them a wider adaptibility under varying and stress environments. Composite cross populations of spring barley (Hordeum vulgare L.) are such "mass reservoirs" containing considerable variability and are suitable both as a means of deriving superior recombinants and for maintaining the variability for further improvements. Barley composite cross (CC) populations, synthesized as early as 1927 have been allowed to reproduce by their natural mating systems in California and Montana, without conscious selection. Evidence for yield improvements, resulting from natural selection of the "fittest" genes and associations, has been shown (25, 29, 65, 77).

Some authors have suggested that plant mixtures might be useful for pest and disease control through host resistance (26, 78). Another improvement in composite cross populations could be the accumulation of genes to obtain higher levels of resistance to pests and diseases. Because composite cross populations are managed so that natural interactions may take place between host and pathogen, it is expected that alleles governing disease resistance will reach frequencies determined by natural selection. But aside from one study on the conservation of scald resistance in barley composite cross populations, this aspect of bulk population use has not been

investigated. In a study of barley scald disease, caused by Rhynchosporium secalis (Oud.) Davis, Jackson et al. (26) found that the frequency of resistant individuals to three of the four isolates of the fungus studied increased significantly in later generations of CC II, whereas little change occurred in the frequency of resistant plants in CC V and CC XII. However, resistance to each isolate was maintained through the latest generations tested of each population.

Erysiphe graminis f.sp. hordei is an obligate parasite and is present all over the world wherever barley is grown. It is highly variable in pathogenicity. The occurrence of powdery mildew is not consistent in the early stages of the barley crop development at Davis, California. Barley powdery mildew is more consistent in the months of January and February and it is then that selective influence from this organism on barley at Davis can be expected (C.W. Schaller, personal communication). In Montana, powdery mildew occurs regularly early in the season. Usually the infection is of short duration and light intensity. Therefore, no selective influence can be expected from the powdery mildew fungus on the CC populations grown at Bozeman and Moccasin, Montana. It was postulated that the frequency of resistant plants would increase from the early to the later generations of the composite, given enough selection pressure exerted by respective races of the pathogen.

The objective of this study was to determine if increase in the frequency of resistant plants to four isolates of powdery mildew could be detected in the populations developed in California as compared to those grown in Montana.

Four isolates of E. graminis f.sp. hordei were used to monitor the frequencies of the corresponding specific resistances in early, intermediate and late generations of three barley composite cross populations grown at three locations.

Variations of resistance to the powdery mildew cultures and conservation of resistant genotypes throughout several generations of each composite cross population are reported in this paper.

LITERATURE REVIEW

The Composite Cross Populations

A survey of recent literature in genetic conservation, phytopathology and crop ecology shows that bulk populations are often praised as reservoirs of genetic variability that assures them wider adaptability under varying or stress environments (41, 77). The term "bulk population" means any population of genotypes that is synthesized by mixing or employing one or more cyles of hybridization and is propagated or used without any artificial selection (29).

A Composite Cross population is such a bulk population, and its use provides one with a breeding method that takes advantage of an initial pool of genetic variability, with natural selection favoring the individuals that are more fit in the competitive sense and/or more fecund, thus evolving toward higher yielding ability (29). Composite cross breeding is also very attractive since a minimum of effort, space and expertise is required (25).

Initial Make-up and Propagation

Through the diligent efforts of H. V. Harlan, G. A. Wiebe, C. A. Suneson, R. W. Allard, E. A. Hockett, and others, we now have in barley a large series of populations originating from composite

crosses involving various schemes of initial hybridization among a diverse set of lines.

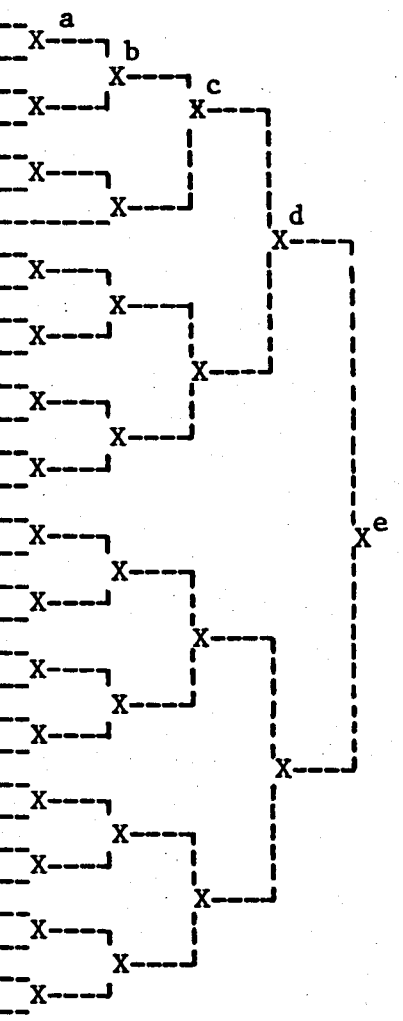
The barley populations used for the present studies were three composite cross populations, called Composite Cross II (CC II), Composite Cross V (CC V), and Composite Cross XII (CC XII).

Composite Cross II (C.I. 5461) was synthesized in 1927 by Harlan and Martini from 28 cultivars representing the major barley growing regions of the world (21). The population was created by pooling equal amounts of seed harvested from F1 plants from the 378 possible intercrosses among the parents and it has been propagated in large plots in Davis, California (21, 79, 80), each year under normal agricultural conditions. It was also grown in Montana, but not on a yearly basis.

CC V (C.I. 6620), also developed by Harlan, differs from CC II in both parentage and method of synthesis. The population was developed by pair crossing between 30 cultivars (all six row types) and their progeny to give a single multiple hybrid. The 30 parents, including 11 in common with the parents of CC II, were crossed in all possible pairs, the resulting F1 hybrids again pair crossed, and the cycle repeated until a single grand F1 hybrid was obtained. The population was initiated from selfed seed produced by the grand hybrid in 1941 (79, 80), (Table 1). It has since been propagated in Davis, California, in the same fashion as CC II. It was also

Table 1. History of the initial composition of composite Cross V, C.I. 6620 (unpublished information, Dr. G. A. Wiebe).

C. I. No.	Cultivar
975	Baker
1330	Pannier
4118	Atlas
1367	Vaughn
3556	Minia
1311	Flynn
6299	New ZZ
4166	Afghan I
708	Black Algerian
2130	Stavropol
4019	Lioness
936	Trebi
6298	Old ZZ
261	Club Mariout
6083	Good Will
6366	Afghan II
4019	Lioness
6109	Velvon
1179	Algerian
1256	Arequipa
5888	Rikote
206	Han River
14119	Abate
6265	Ezond
937	Sandrel
14061	Bonfarik
14065	Parla
3387-8	Maison Carre
2238	Lion
6626	Coaston
5267	Peatland



[a] Parents crossed at Aberdeen, Idaho, in 1937
[b] F_1s crossed at Sacaton, Arizona, 1938
[c] " " " Aberdeen, Idaho, 1938
[d] " " " Aberdeen, Idaho, 1939
[e] " " " Aberdeen, Idaho, 1940

grown in Montana, again, with some irregularity and not always on a yearly basis.

CC XII (C.I. 6705) was developed by G. A. Wiebe and was derived from intercrossing 25 parents. These 25 cultivars, 21 in common with those in CC V and nine in common with those in CC II, were combined through a series of four pairings of F1 plants. In 1940, the residual was backcrossed to the F1 of Atlas x Vaughn (Table 2). Suneson (77) cited:

> "The addition of this final cross is of special interest because of the wide difference in survival ability of Atlas and Vaughn, and the unusual record of an Atlas x Vaughn bulk population which produced four commercially grown varieties Arivat, Beecher, Glacier and Gem."

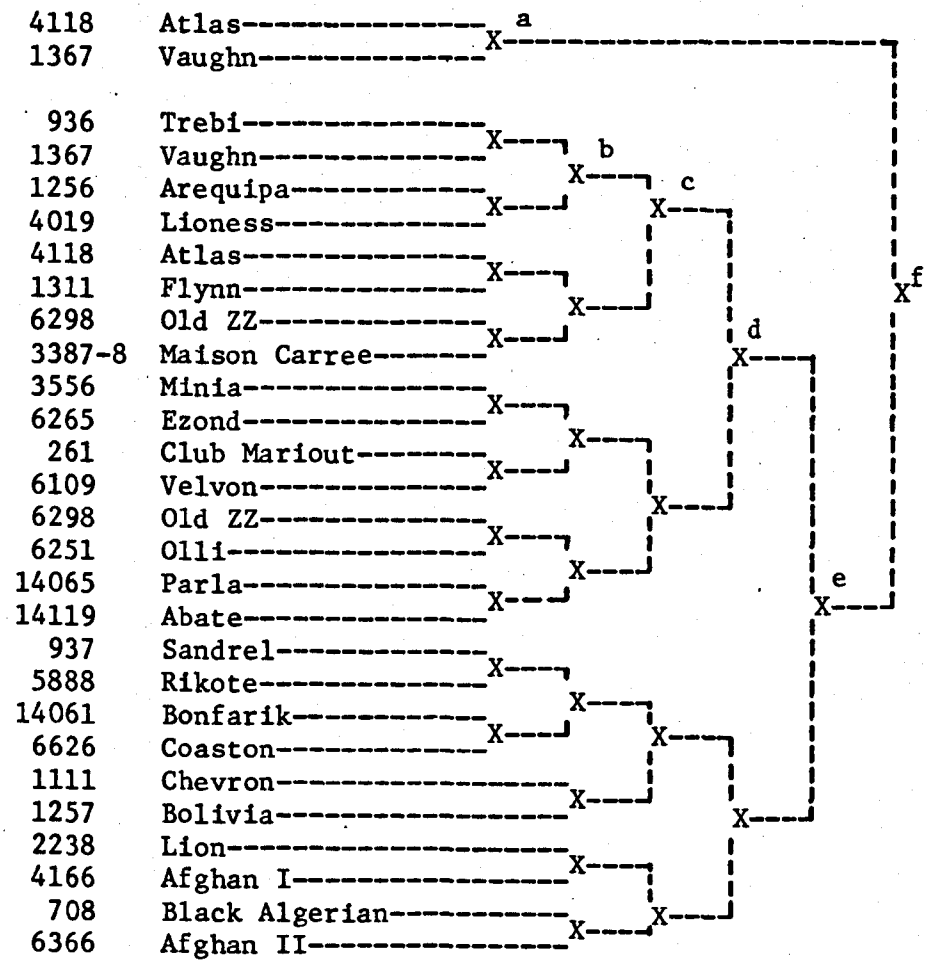
This population has also been grown in California and Montana.

Population Dynamics: The Composite Crosses

The Composite Crosses of spring barley are not new. They were developed in Davis, California, as early as 1927. The importance of using diverse germplasm in a breeding program and the need to retain variability in the population have been emphasized by many authors (41).

Diversification in populations of predominately autogamous species to provide protection against crop pests was advocated by Suneson (78). CC populations, or "mass reservoirs" containing considerable variability were suitable both as a means of deriving

Table 2. History of initial composition of composite Cross XII, C.I. 6725 (unpublished information, Dr. G. A. Wiebe)



a
b Parents crossed at Madison, Wisconsin, in 1937
c F_1s crossed at Arlington Greenhouse in 1938
d " " " Madison, Wisconsin, in 1938
e " " " Arlington Greenhouse in 1939
f " " " Madison, Wisconsin, in 1939
" " " Madison, Wisconsin, in 1940

superior recombinants and for maintaining the variability for further improvement (41). It was suggested that superior recombinants with increased resistance to pests and diseases have been formed and maintained in these populations. Disease control was achieved in these plant mixtures through host resistance. However, in only one study has this important aspect of bulk population use been investigated. Jackson, et al. (26) studied the conservation of scald resistance in three barley CC populations. Four isolates of Rhynchosporium secalis were used to monitor the frequencies of specific scald resistances through early, intermediate and late generations of CC II, CC V, and CC XXI. Resistance to each isolate was maintained through the latest generation tested of each population. Changes in the frequencies of plants resistant to particular isolates were observed between generations in all three populations. In CC II, resistance to three of the four isolates changed from relatively low to extremely high frequencies by the latest generation tested, F47.

Jain and Qualset (29) stated:

> "Many epidemiological features of pathogens have been considered in the theories of epidemics. The role of genetic diversity has often been postulated in terms of counteracting such features, but this field is still wide open to critical long-range studies."

So far, only observations of disease resistance in CC populations have been made (25, 29, 78). Bal, et al. (6) looked, among

others, at the characters for scald and net blotch (Pyrenophora teres) resistance in CC II. Suneson (77) studied the impact of a barley yellow dwarf virus infection on CC II in 1951:

> "Seven percent of the plants were killed, 38% produced less than 25 seeds per head, and only 27% produced more than 50 seeds per head. This was the first noted impact of this virus on this population, and probably resulted in the greatest differential survival encountered in 25 years."

No critical long-range studies have been conducted on any disease, except scald (26). The same is true for powdery mildew. Its interaction with CC populations has never been thoroughly investigated. Only Hockett, et al. (25) noted about their experiments on CC II: "Apparently the Davis F24 and F35 generations tested in our experiments did not develop disease resistance for powdery mildew or BYDV."

To further understand some of the mechanisms involved in these CC populations, some studies performed on aspects other than accumulation of resistance to pests and diseases will be reviewed. These aspects may reveal certain patterns of response within the populations, which, in turn, may explain the response of these host populations to selection pressures from diseases. Jain (27) stated:

> "The key issues raised at population level often relate to the analyses of form and amount of genetic variability, the mode and intensity of selection pressures, role of heterozygosity in the performance and evolutionary flexibility, adjustments in the genetic system, and the relationship between certain measures of productivity and stability."

The following topics are discussed: 1) Yield and adaptation studies, 2) Possible uses of the CC populations, and 3) Specific studies performed on the three CC populations used in this study.

Yield and adaptation are discussed because these factors showed how selective forces were acting upon CC populations, and how the populations were reacting to them. Adaptation also explains population stability.

Uses of CC populations are also presented because this topic raises questions on future population management schemes. The methods used to work the CC populations are largely dependent upon the results one wishes to obtain from the populations.

Not many aspects of population use for disease control have been investigated. More comparative studies need to be done for a better understanding of the genetics involved in population breeding.

Yield and Adaptation Studies

Studies on bulk yields of four CC populations of barley (CC II, V, XII, and XIV) were done by Suneson (77) and showed that the initial yield of each of the original CC populations was lower than that of the same check cultivar Atlas 46. Yields remained unchanged up to F6, after which they were improved, and surpassed the yield of the check by F20. Suneson also presented data from the yield

performance of random selections from different generations of CC II. None of the F2 selections from CC II exceeded the yield of Atlas 46, but of 50 F20 selections one outyielded Atlas 46 by an average of 37%. The three top selections from F24 gave an average of 56% yield increase over the check. However, from the F2 to F40 approximately a 50% yield gain resulted from natural selection of the "fittest" genes and associations (79). Hockett, et al. (25), however, did not find such large increases in their trials with CC II in the disease and high yield environment at Davis, California.

While Suneson's (77) results showed that populations did not improve until the F7 generation, Rasmusson, et al. (65) observed a rapid improvement in yield over six years in a composite population obtained by mixing seed from 6,000 entries in the world collection. The difference may lie in the numbers of initial cultivars or parents and superiority of the base population. The presence of many inferior segregates in the early generations may slow down initial improvement (41).

Harlan and Martini (21) found that the less adapted cultivars were rapidly eliminated in a specific environment. Under natural selection, genotypes with specific adaptations were at a disadvantage and were gradually eliminated from the population. Widely adapted genotypes were favored (41).

Jain and Qualset (29) also studied the population structure and evolutionary dynamics of several composite crosses. By scoring the genotypic frequencies at several marker loci during successive generations, a variety of selective changes that occurred during many years of cultivation were shown. They stressed the importance of the number of macro- and micro-environments to be used in either the propagation or evaluation of the CC populations, since the adpative role of genetic variation is the central issue. Hockett, et al. (25) also emphasized this point in their study on CC II in three different environments.

Uses of Composite Cross Populations

One of the attractive aspects of CC breeding is that benefits are expected, through low cost maintenance of populations, from the interplay of recombination and natural selection. Recombination ensures the appearance of new genotypes in the first few generations of bulk propagation. In the absence of any mechanism for prolonging heterozygosity, the majority of genotypes in such a population is expected to be homozygous by about F6 (30). Hockett, et al. said that new gene combinations would be fixed by F8 (25). Suneson (77) then suggested that 15 generations of natural selection seem desirable. Thereafter, there can be repeated recourse to three breeding

methods: 1) Continued natural selection with prospects for significant gains in yield to accrue throughout a working lifetime. However, Lohani (41) reported that there is a limit beyond which CC populations might not improve for yield, depending greatly on the characteristics of the base population. 2) The use of cyclic hybrid recombinations with intervening natural selection to give a kind of recurrent selection. An example is the work currently in progress at MSU with recurrent selection populations of barley containing broad based resistance to several diseases. The use of male sterility in various ways offers a very useful tool for regulating the breeding structure of a population. 3) The use of conventional selection and testing. Some authors (29, 30, 39, 41) strongly advocate the use of selection and exploitation of the populations to overcome the very serious losses of desirable genes which have been observed in populations due to selective disadvantage. Tee and Qualset (29) proposed a population-management scheme in deriving pure line cultivars, based on the single-seed descent methods for maintaining variability. This method minimizes natural selection but still allows the breeding system to be varied for influencing population changes.

Other modifications to be adopted in a breeding program were suggested by Khalifa and Qualset (39), such as the use of wider

spacing to decrease the effect of competition, and early (F2) subdivision of the population into separate groups based on the characters known to be important in competition.

As Allard mentioned (personal communication) a CC population as a whole is a stable system, with a fantastic amount of genetic variability. The population in and of itself is not a breeding method, but rather a good way of conserving variability, and a good source of plant materials.

Many authors agree (25, 29, 30, 39) that CC population development should be an integral component of any breeding program, but only as a complement to other breeding methodologies which are more time efficient (25) than those associated with CC populations.

Jana, et al. (30) stated:

> ". . . the bulk-population breeding method is too slow despite its attraction in view of the economy of management and the danger of over indulgence in pure-line breeding."

Thus, a wise combination with other breeding methods may be more appropriate.

Some important considerations concerning each CC population, such as its initial synthesis, its fitness versus agricultural productivity relative to a theory of evolution, determine the methodology applied to a given population. Each of these considerations raises a series of questions, such as (29):

> "Is the performance of mixtures dependent on the number of lines and their relative proportions? Do gene interactions play an important role in selective changes, and, if so, are certain optimal levels of recombination needed to generate and then preserve linked gene complexes? How often do disease and pest occurrences show genetic diversity to be highly desirable?"

A scientific answer to any one of the issues raised depends on the accumulation of statistical tests on many comparable studies appropriately designed.

Some Specific Studies Performed on the Three Populations

CC II. CC II was the principal gene pool used for a thorough study on breeding methods by its originators (29). They used it to develop an evolutionary breeding method (77); to make studies on population dynamics (6); and to point to genetic diversity as a mechanism for control of diseases and insects (78). The Montana Agricultural Experiment Station has the same gene pool grown independently (79). Hockett, et al. (25) have used the composite cross II, developed by natural selection under three different environments (one in California and two in Montana) and tested in these and other diverse environments, to evaluate agronomic improvement and stability over several years. CC II, together with CC V, was also used in a study on biochemical properties of polymorphic esterases by Edwards (16), and by Allard, et al. (3, 37). Clegg, et al. (13) studied the dynamics of gametic frequency change in CC

II and CC V. Jackson, et al. (26) have used CC II and CC V in a study of the conservation of scald resistance. Since evidence was shown for correlations between different genetic loci in highly self-pollinating populations such as CC populations (13, 84), Muona, et al. (62) investigated possible correlations between the observed changes in resistance in CC II to different isolates of scald, the same as used by Jackson, et al. (26).

CC V. Even though 15 generations behind CC II, evolutionary developments have complemented the more advanced population and confirmed that striking yield improvements accrue over time (79). CC V was the principal barley resource material used by Allard and Jain in their studies of population dynamics (2, 28). Also, Lohani (41) described the results of his investigations in South Australia on the yield and adaptation of selections from various generations of CC V and their association with some physiological and agronomic characters. Studies on allozyme polymorphisms (84), mating system (38), and components of selection (14) in an experimental plant population, were also performed on CC V.

CC XII. CC XII was not used intensively in experimental studies. It closely resembles CC V, with the exception of a final backcross to the F1 of Atlas and Vaughn (Table 2). Suneson (79) ascribed the lack of studies on CC XII to a presence of fewer conventional marker genes. The California CC XII F26 embodies more

heterozygosity and other positive characters, and is more productive than the California CC II F40 and CC V F25 (77).

The Fungus

The Disease Organism

Powdery mildew fungi are obligate parasites which infect a wide range of host plants throughout the world. The extensive literature on powdery mildew of cereals and various grasses caused by the fungus Erysiphe graminis DC., indicates that this organism is of considerable economic importance in many parts of the world. In Europe, it is one of the main diseases causing enormous losses. Powdery mildew, with scald and barley stripe, are the most destructive diseases of barley in California (69). While not as spectacular as barley stripe or such diseases as stem rust (Puccinia graminis f.sp. tritici) and bunt (Tilletia spp.) of wheat, the widespread distribution and annual occurrence of powdery mildews make them a potential threat to barley production.

The classification of the powdery mildews is as follows:

Class:	Ascomycotina
Subclass:	Eusacomycetes (asci are produced in an ascocarp)
Series:	Plectomycetes (having a closed ascocarp or cleistothecium)
Order:	Erysiphales (obligate parasites)
Family:	Erysiphaceae (mycelium on the surface of the host plant)
Genera:	The genera are distinguished from each other by the number of asci they produce per cleistothecium, by the morphology of hyphal appendages growing out of the wall of those cleistothecia, and by host specialization.

The genus Erysiphe has more than one ascus per cleistothecium. The complete name of powdery mildew on barley is Erysiphe graminis DC. f.sp. hordei Em. Marchal (51).

Barley powdery mildew is an obligate parasite with superficial growth. It never invades the tissues of the host, but sends out specialized structures, haustoria (7), into the epidermal layer for feeding purposes. Its mycelium is heterothallic: an antheridium and ascogonium form the cleistothecium, which represents the overwintering or oversummering form.

The powdery mildew fungi, although they are common and cause serious diseases in humid areas, are even more common and severe in warm, dry climates because their spores can be released, germinate and cause infection at any relative humidity level in the air without need for the presence of a film of water on the plant surface (free moisture) (83). Once infection has begun, the mycelium continues to spread regardless of the moisture conditions in the atmosphere.

Life Cycle

The life cycle of Erysiphe graminis f.sp. hordei consists of: 1) a sexual stage and 2) an asexual stage.

The Sexual Stage

Cleistothecia produce asci, in which ascospores are formed. Cleistothecia, the survival structures, were produced by a heterothallic mycelium. The cleistothecia remain viable for a very long period of time (56). In a suitable environment (12, 20), cleistothecia crack open by a swelling of the contents (83). The asci are then thrown out and they discharge their ascospores. Usually eight ascospores are produced in each ascus. Ascospores are able to infect green living tissue immediately, but they can also survive some harsh environmental conditions and still be infective.

The Asexual Stage

The imperfect stage is characterized by the formation of conidia. The conidia of E. graminis f.sp. hordei belong to the form genus Oidium of the Fungi Imperfecti. The conidia are much more sensitive to environmental conditions than the ascospores. They cause secondary infections and are responsible for the spread of the disease (83).

Environment and Infection Process

Many investigations have been conducted upon the effects of temperature, relative humidity and light on the germination of powdery mildew conidia (12, 17, 18, 42, 54). Much emphasis has been placed on the effects of various environmental factors on the

progress of disease development, in order to characterize and define the events of the primary infection process and the genetic regulation of the events which occur during initial host-parasite interactions. Initially researchers (1, 10, 20, 44) studied environment and the biological systems on a quantitative basis. A quantitative approach reduced the variability in the results obtained, thereby improving the understanding of the establishment of a parasitic relationship (18, 21).

The sequential development of powdery mildew on the plant surface is divided into nine stages (61):

1. germination of conidia
2. production of "club-shaped" appressorial initials
3. maturation of appressoria
4. penetration of the cuticle and epidermal cells
5. formation of haustoria
6. formation of secondary hyphal initials
7. elongation of secondary hyphae (ESH)
8. initiation of additional infections, and
9. sporulation.

Each of these development stages differs in its requirement for, and sensitivity to, temperature, relative humidity and light (63, 64). When the optimum conditions for each stage are present, a high percentage of the parasite population undergoes these various development stages with increased synchrony at each stage, and a high infection frequency is obtained. High infection frequency means that a high percentage of conidia, placed on a plant surface, were able to form elongating secondary hyphae and show a compatible

relationship. The elongating secondary hyphae (ESH) are generally used as a criterion for the establishment of a functional, compatible host-parasite relationship, since only those parasitic units which form functional haustoria can form ESH (43).

Genetics of the Host-Parasite Relationship

In an incompatible relationship, genes for resistance in the host can affect powdery mildew strains differently: haustoria may not develop, or no ESH will form. An historical breakthrough came with Flor's gene-for-gene concept (29) and the quadratic check, as a general rule for many host-parasite relationships (18) (Figure 1).

Only in combining the two organisms and by examining the reaction type of the host as it responds to the infection of the pathogen is identification of either the host or the pathogen possible. An incompatible relationship is one in which a resistant host genotype interacts with the corresponding avirulent type of the pathogen. An advantage of this abbreviated graphic explanation is that neither ploidy nor heterozygosity alter the generalization that only corresponding P/R genotypes act to restrict disease development and do so regardless of the other corresponding gene pairs in the host-parasite genotypes (Figure 1).

The barley powdery mildew terminology, proposed by Loegering and used by Moseman (53), is used in this investigation (Figure 2).

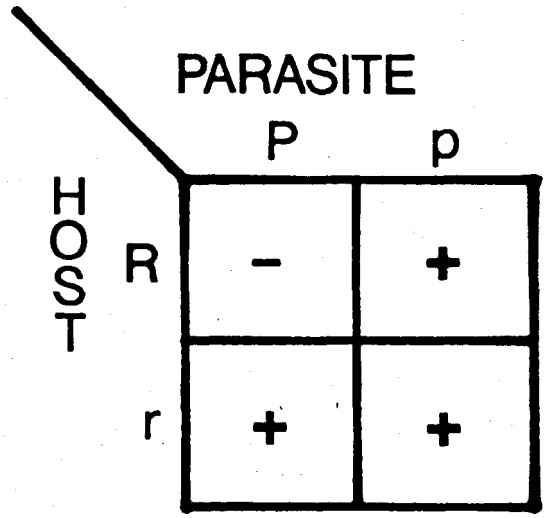


Figure 1. Host-parasite relationship in a quadratic check. Genes conditioning resistance (R) and avirulence (P) are dominant. Genes for susceptibility (r) and for virulence (p) are recessive. (+) = compatible relationship; (-) incompatible relationship. From Ellingboe (18)

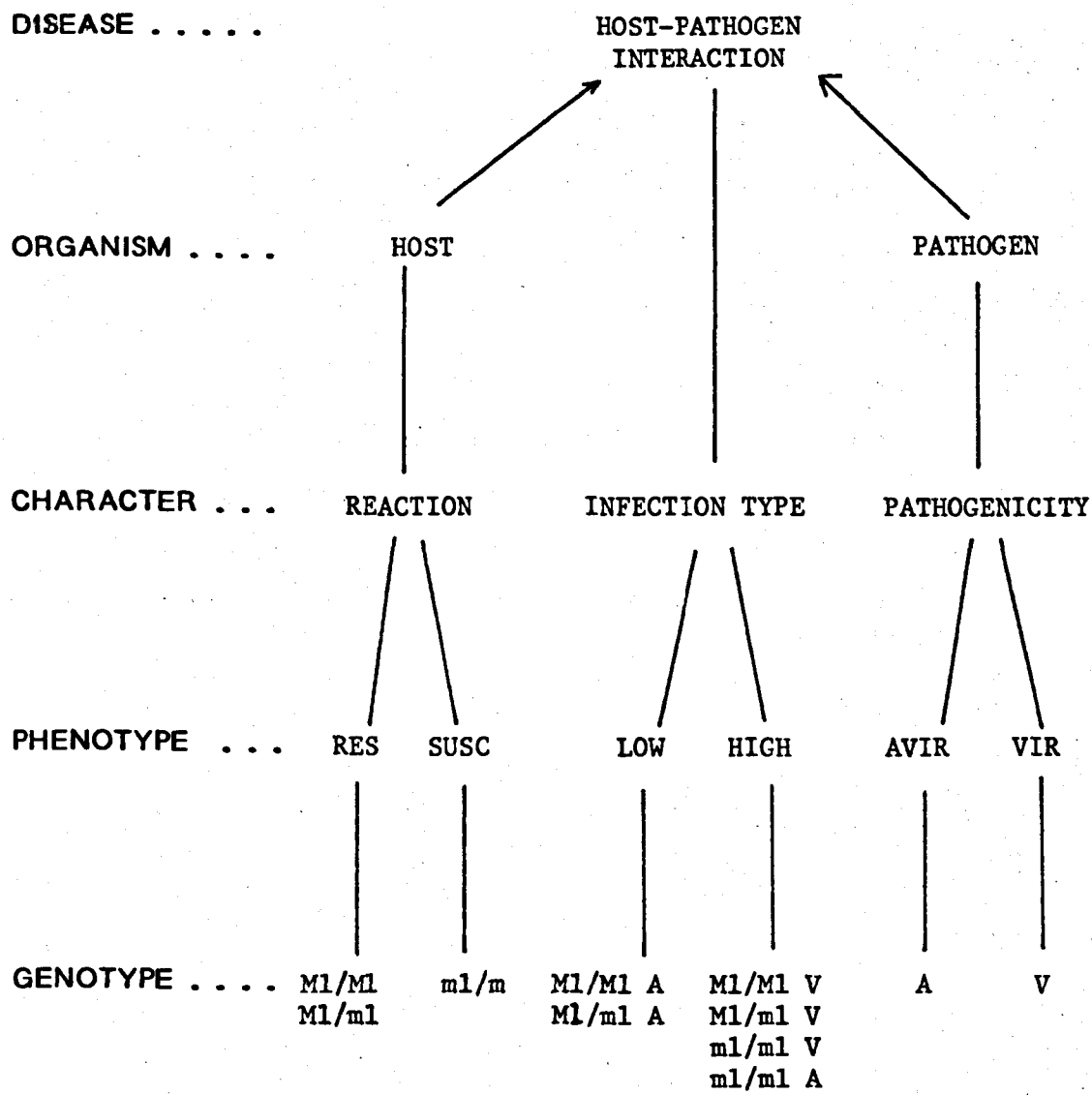


Figure 2. Diagram of how gene interactions between host (barley) and pathogen (Erysiphe graminis f.sp. hordei) result in the disease (barley powdery mildew). From Moseman, 1971 (53).

Genes

Some 40 genes for resistance to Erysiphe graminis f.sp. hordei are known in barley. Table 3 represents some well known and characterized resistance sources and genes in barley to powdery mildew (24). The significance and origin of the host gene symbols in Table 3 is as follows (52): 1) The letters Ml refer to genes conditioning the reaction of varieties to Erysiphe graminis f.sp. hordei (The abbreviation Ml indicates a gene in the dominant condition, and ml indicates a gene in the recessive condition.); 2) The letters and superscripts following the letters Ml refer to specific genes; and 3) the letters identify the locus and the superscripts number the specific gene at that locus.

Most of those genes are located on chromosome five of barley. Only the ml-o and Ml-g genes were found to be on chromosome four (35, 36, 51, 69, 70). The ml-o gene is also the only recessive gene known for powdery mildew in barley. For the 40 known genes, at least seven loci are recognized with at least 17 distinct alleles conditioning resistance (86).

Linkage Maps of Barley

All the known genes for resistance to powdery mildew are located on chromosomes four and five of barley. The linkage maps in barley by Tsuchiya (81, Appendix 1) show three genes for powdery

Table 3. Sources of resistance against Erysiphe graminis in barley. See text for explanation of the host gene symbols.

Genes for Resistance	Variety Adapted from Hermansen (24)	Variety Adapted from other sources (51,52,85)
Ml-a	Iso 1 R C.I.16137	Rabat C.I.4979 (50,53)
Ml-a2	Black Russian C.I.2202	(50)
Ml-a3	Ricardo C.I.6306	(50)
Ml-a4	No 22 C.I.13654	(55)
Ml-a5,Ml-a?[a]	Gopal C.I.1091	(55)
Ml-a6	Voldagsen 8141/44	1-Gatersleben Mut.501 C.I.13132 2-Hordeum spontaneum nigrum C.I.13130(51)
Ml-a7,Ml-a?	Visir	/[b]
Ml-a8	Heil's Hanna C.I.682	/
Ml-a9,Ml-a4	Mona	/
Ml-a10	Iso 12 R C.I.16149	/
Ml-a11	A222 C.I.11555	/
Ml-at	Atlas C.I.4118	(50,53)
Ml-a,Ml-at	Algerian C.I.1179	(50)
Ml-a?	/	1-Cebada Capa C.I.6193 2-Multan C.I.3401
Ml-c	Indian HOR 1657	/
Ml-g	Iso 2 R C.I.16139	Goldfoil C.I.928 (50,60)
Ml-a?,Ml-g	/	Palmella Blue C.I.3609
Ml-h	Weihenstephan 37/136	1-Wehenstephan St. 37/136 2-Chevron C.I.1111(60)
Ml-k	Iso 4 R C.I.16143	Kwan C.I.1016 (50)
Ml-n	Gatersleben Mut.411 C.I. 13131	1-Gatersleben Mut. 511 C.I. 13131 2-Nepal C.I.595 (50)
ml-05	Risø 5678 C.I.15219	/
Ml-(La)	Lofa Abed	/
Ml-(GM501), Ml-?	Gatersleben Mut.501 C.I.13132	/
Ml-p	Iso 5 R C.I.16145	Psaknon
?	/	Lyallpur BS C.I.3395
?	/	Lyallpur C.I.3403

[a] ? = unknown location of the resistance gene on the barley chromosome.

[b] / = not applicable.

mildew resistance in the short arm of chromosome five: Reg 1, Reg 4 and Reg 5 (Reg = resistance to E. graminis is the new notation for powdery mildew resistance genes on the chromosome maps). Tsuchiya (81) also mentioned some other genes for powdery mildew resistance associated with chromosome five: Ml-at, ml-d and Ml-nn. Following the linkage map of chromosome five by Jensen (32, Appendix II), all the known genes for resistance to powdery mildew on chromosome five are located on its short arm: Ml-at, Ml-a (= Reg 1), Ml-k (= Reg 4), ml-d, Ml-nn and Ml-p (= Reg 5). Therefore, the probability of close connection or linkage is great between these resistance genes for powdery mildew and the following genes and/or loci:

1 - Pa 4 = Rph 4, resistance to Puccinia hordei;

2 - Hor 1, Hor 2, Hrd C, Hrd D, Hrd E, the hordein series;

3 - Yr 4 = Rps 4, resistance to P. striiformis;

4 - Lys 4d, high lysine.

Jensen, et al. (33) studied the linkage of the hordein loci with the powdery mildew resistance loci on chromosome 5. The recombination percentage between Ml-k and Hor 1 was estimated to be 4.0 ± 1.3, between Hor 1 and Ml-a, 5.3 ± 1.1, and between Ml-a and Hor 2, 6.1 ± 1.2. This association renders the hordein loci very useful as marker genes (15). The other genes on the short arm of

chromosome five were not cited in the literature of the CC populations.

Bal, et al. (6) studied some character associations in CC II. Heading date, spike density and waxy characteristics were regulated by genes on the long arm of chromosome five. Suneson and Stevens (80) described the rapid decline of the black seed character (B-series on the long arm) in CC II after about 12-15 generations. Jain and Qualset (29) summarized the evidence on the nature of the selective forces in CC populations (Appendix III). However, no indications of linkage were found between these potential markers and any known genes on chromosome five.

Two genes for powdery mildew resistance are located on the short arm of chromosome four: Reg 6 (= ml-o) and Reg 2 (= Ml-g) (Appendix I). Other genes resident on the short arm of chromosome four which might form associations with powdery mildew resistance genes have been investigated. Bal, et al. (6) studied character association with plant height (min and br) and waxy character (cer). Haus (23) found a recombination percentage of about 5% between the brachytic 2 (br 2) gene and Ml-g. Suneson and Stevens (80) described the decrease of the hooded character (K- series) in CC II from the F4 to the F23. The K- series, however, are located on the long arm of chromosome four (Appendix I) and possible linkages with genes for resistance to powdery mildew are unlikely.

Tsuchiya (81) and Haus (23) also included lists of genes associated with chromosome four. The male sterile gene (msg 24v, msg 25r) and the bl- gene for aleurone color may show some association with ml-o and Ml-g. Harlan and Martini (22) found this aleurone color not to be related to yield. Jain and Qualset (29) summarized the evidence on the nature of selective forces in CC populations of barley (Appendix III).

Genetic Action

The genes for resistance do not appear to operate by inhibiting the initiation of penetration of the host by the appressoria of the parasite. On susceptible and resistant cultivars, spores or conidia can land on the plant surface, germinate and make a close contact between appressorium and cuticle. This is followed by the early appearance of a deposit adjacent to the inner surface of the epidermal wall below the point of contact. Since these deposits are similar in resistant and susceptible cultivars, apparently they are not the prime mechanism of resistance (75).

The resistance seems to depend on hypersensitive responses. Different responses are obtained in function of the genes for resistance present, and are sometimes initiated before the pathogen has entered the lumen of the cell (11). Ellingboe (17, 18) has proposed that contact between the pathogen and plasmalemma of the

host is necessary for a resistant response. Genes for resistance can stop the development of the pathogen upon contact with the host plasmamembrane. For example, in the presence of the Algerian gene, Ml-a, 95% of the parasitic units do not produce haustoria, as opposed to a compatible relationship, in which 90% of the spores will produce haustoria (17).

Masri and Ellingboe (43) describe some resistant reactions for the following genes: 1) the Ml-a gene causes distortion of haustoria; 2) the Ml-g gene inhibits secondary infection; 3) the Ml-k gene causes collapse of host tissue; and 4) the Ml-p gene inhibits growth and sporulation. All these genes have different effects on the fungus at different stages of the fungal development. Other known genes for resistance to powdery mildew and their inheritance are described by several authors (8, 47, 48, 58, 59, 61).

For its survival, this obligate parasite must not kill the host cell. Compatibility or incompatibility can occur at any stage of infection by alteration in any one of the large number of physiological systems during the infection process. In fact, an infinite number of different mechanisms could be involved in incompatibility responses (9, 70).

Effect of Powdery Mildew on Barley

In an analysis of the effects caused by powdery mildew on barley, the following factors will be considered: 1) first appearance of the powdery mildew on the barley; 2) growth stage at which the barley crop is attacked; and 3) duration and severity of the infection.

Infection by powdery mildew results in gradual decline in vigor and growth. First, increased respiration is observed on infected plants, with a respiratory rate well above that of healthy plants of the same age. Reported oxygen consumption was from 250 to 650% more than with healthy plants (5, 67). This increased respiration eventually leads to the depletion of the carbohydrate reserves of the host, which can cause the vegetative growth of the fungus to slow down (4).

Smedegaard-Petersen (72) observed increased respiratory activity in highly resistant host-pathogen combinations with an incompatible relationship, where the host showed a hypersensitive reation. This incompatibility is associated with a number of biochemical and structural defense reactions. These require energetic and biosynthetic activities which may deprive the host of energy and ultimately lead to a lower yield (72).

Smedegaard-Petersen and Stolen (74) studied the effect of these energy requiring defense reactions on the yield and grain quality in a powdery mildew resistant barley cultivar. Grain yield was significantly reduced by seven percent and the kernel weight by four percent. There was a reduction of 11% in the yield of the grain protein.

Smedegaard-Petersen and Stolen (73) also presented an overview of the known changes in biochemical activity in incompatible combinations between barley and the powdery mildew fungus.

Schaller (68) studied the effects of powdery mildew on barley yield and its components: a) number of tillers per plant; b) number of kernels per spike; and c) kernel weight.

The cultivar Atlas and its isogenic Atlas 46, which was resistant to the powdery mildew cultures occurring in California, were planted in paired plots at several locations throughout California over a three-year period. No attempt was made to control or initiate disease development. In all three years, powdery mildew infection was widespread during the early stages of the barley development, causing considerable yellowing and defoliation of the young plants. Only in one out of the three years did late spring rains favor continuous development of the powdery mildew up to and including infection of the flag leaf. Symptoms then were abundant sporulation and leaf-yellowing. Under disease-free conditions no

difference was found in the yield of Atlas and Atlas 46. Powdery mildew infection of light intensity and short duration, present only in the early phases of the plant development in two out of the three years, reduced the yield of Atlas by 6.6 and 3.8%, and the number of kernel per spike by 14.9 and 8%, respectively. The average losses for the three years under heavy infection were 17.6, 14.0 and 8.1% of the total yield. The maximum reduction at any one location was 27% of the total yield. Continuous infection throughout the growing season resulted in an average reduction in kernel weight of 6.4% and in kernel number of 21.5%. Schaller (68) stated:

> "One of the most significant findings of this study was the measurable effect of limited infection occurring early in the season. Infection of the above intensity and duration is normally overlooked or ignored, and, since the plants fully recover and produce kernels of average weight, the resultant damage is not evident at harvest."

The effects of powdery mildew on the yield and yield components of several barley cultivars were studied by Scott, et al. (71) in a controlled environment. Pot-grown barley plants were exposed to infection by powdery mildew at different growth stages and for different periods of time. Powdery mildew infection was assessed in terms of percentage leaf area covered and at harvest the kernel weight per plant, kernel size and tiller number were recorded. Powdery mildew attacks up to growth stage (G.S.) 5.0 of the Feekes scale (40) reduced the number of fertile tiller up to

30% at harvest. These early mildew attacks also reduced the number of kernels per spike up to 11%, even when the disease was controlled during later stages of growth. Powdery mildew infection after G.S. 5% reduced mainly the kernel weight and the number of kernels per spike. At these later stages, losses were in the order of 6% in kernel weight and 13% in number of kernels per spike.

Johansen (34) reported the results of his four-year long experiments on spring barley varieties in Denmark. His barley plots were artificially inoculated with mildew after G.S. 5.0. He found an average yield loss due to mildew of 15.7%, varying from 10 to 22.5%.

Yield losses are largely dependent on the stage of plant development at which powdery mildew infection is initiated and on the subsequent duration and severity of infection. Studies on the physiology and development of the barley plant help explain these effects of early powdery mildew infection on the number of tillers per plant and the number of kernels per spike. Tillers and kernels primordia in barley are produced at an early stage, before G.S. 5.0. Infection during these phases of plant growth will then have deleterious effects on both the tiller and kernel primordia (71). Late powdery mildew attack reduces the kernel weight and this is normally explained as resulting from reduction in photosynthate at grain filling.

MATERIALS AND METHODS

The Composite Cross Populations

Random seed samples from early, intermediate and late generations of each CC population were obtained in 1979 from Dr. R. W. Allard of the University of California, Davis, and from Dr. E. A. Hockett of U.S.D.A. and Montana State University.

Since a minimum of 100 seedlings was needed for testing and reading one replication of each generation, the percentage germination of each seed sample was calculated. The number of replications needed for each generation was then adjusted accordingly so that about 300 readings were obtained for one generation-testing by each isolate of the powdery mildew fungus.

Seed from the following generations of each CC population from California and Montana was used in these experiments. The selection of early, intermediate and late generations of each CC population was made on the basis of even distribution between the generations as well as seed availability.

California

CC II (C.I. 5461) (Table 4). This population was grown at Davis, California on a yearly basis since its synthesis in 1927.