Induced Morphological and Virulence Variants of the Obligate Barley Pathogen *Erysiphe graminis* f. sp. *hordei*

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ABSTRACT

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A mutagenesis procedure desig. ed to limit contamination was developed for *Erysiphe graminis* f. sp. *hordei*, an obligate pathogen that causes powdery mildew disease on barley. Gnotobiotic cultures of *E. g. hordei* were established on barley leaf sections placed on an agar medium in petri dishes. To mutagenize *E. g. hordei*, leaf sections with 3-day-old cultures were transferred to an agar medium containing ethyl methanesulfonate for 12–14 h. Seven days after mutagen treatment, the cultures were screened for morphological mutants. Among the classes of variants observed were those with red conidia and hyphae, round conidia, prematurely germinating conidia, and altered colony morphology. Based on the occurrence of red variants, the mutation frequency after exposure to ethyl methanesulfonate was estimated to be $1\text{–}2 \times 10^{-4}$. As a means of further analyzing host-pathogen interactions in the powdery mildew disease, mutants of *E. g. hordei* with increased virulence were generated with this mutagenesis protocol. A number of variants showing minor increases in virulence were recovered, but no fully virulent variants were observed. The mutation in one weakly virulent isolate was inherited as a simple, nuclear factor.

Additional keywords: avirulence, *Blumeria*, gene-for-gene hypothesis, *Hordeum vulgare*.

The gene-for-gene hypothesis states that for highly specific race-cultivar interactions an incompatible relationship occurs when a host carrying a dominant resistance allele interacts with a pathogen having a dominant avirulence allele (11,14). Other combinations involving recessive alleles for susceptibility in the host or recessive alleles for virulence in the pathogen lead to the establishment of compatible interactions and disease. An important feature of the hypothesis is that there is a one-to-one relationship between a resistance locus in the host and an avirulence locus in the pathogen, and the interaction between a host and a pathogen is very specific. That is, a given resistance allele in the host can confer resistance only to a pathogen carrying the complementary avirulence allele. A second feature of the hypothesis is that resistance and susceptibility and avirulence and virulence are treated as qualitative traits. Genes that exert minor, quantitative effects on host-pathogen interactions are generally not considered in the context of the gene-for-gene hypothesis, although, Ellingboe (11) has argued that minor genes affecting race-cultivar-specific interactions can be considered under the rubric of the gene-for-gene hypothesis. A number of exceptions to this general pattern of race-cultivar-specific interactions have been observed. Examples of recessive resistance alleles (2,53) and of dominant virulence alleles (22) have been found. In addition, modifiers of avirulence (29,30,35) and of resistance (10,31,33) occur, and cases of complementary genes conditioning resistance have been reported (38,52). Thus, the one-to-one relationship is not universal in race-cultivar-specific interactions.

However, in the powdery mildew disease of barley (*Hordeum vulgare* L.), host-pathogen interactions generally conform to the features of the gene-for-gene hypothesis (41). For the barley *Ml-a* resistance locus in particular, resistance alleles are either dominant or semi-dominant in action (53). The dominance relationships of the avirulence alleles cannot be tested because the powdery mildew pathogen, *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal (=*Blumeria graminis* (DC.) E.O. Speer f. sp. *hordei*; 23) is haploid throughout most of its life cycle (32). The results of genetic crosses between races of *E. g. hordei*, which are avirulent or virulent on barley genotypes with *Ml-a* resistance, suggest that the gene-for-gene hypothesis is a good model of these interactions (39–41).

The gene-for-gene hypothesis is based on the results of genetic analyses of pathogen virulence and host resistance (12,13). However, the interpretation of data from genetic analyses of avirulence can be difficult if the genotypes of the parents are not fully characterized, as is often true of natural isolates recovered from the field (8). Also, the possibility that closely-linked genes control a character is difficult to exclude by genetic analysis alone (36). Mutational analysis provides an alternate means of identifying genes controlling avirulence.

Historically, mutational analysis has been an important tool for the study of biological phenomena. When coupled with molecular techniques, such as transposon tagging or complementation following transformation, mutants have provided an important source of materials for identifying and characterizing genes that control biological processes. However, mutational approaches, transposon tagging, and transformation have not been widely exploited for the study of plant-fungus interactions because most fungal plant pathogens do not lend themselves to these methods. For example, many fungal plant pathogens are multinucleate, heterokaryotic, or lack a known sexual cycle. *E. g. hordei* is in many ways an ideal candidate for mutational analysis of pathogen-host interactions; it is mononucleate, has a heterothallic sexual cycle, and is haploid throughout most of its life cycle (32). A major disadvantage of working with *E. g. hordei* is that it is an obligate pathogen and cannot be cultured axenically.

One prediction of the gene-for-gene hypothesis is that the inactivation of a dominant avirulence allele by mutation will produce a fully virulent mutant. Such a loss-of-function mutation should

be easy to recover relative to a gain-of-function mutation in a recessive virulence allele. Spontaneous mutations to virulence have frequently been suggested as the source of new virulent races that appear in nature after the introduction of cultivars with novel resistance alleles (8,48,62). In a greenhouse, Watson was able to isolate spontaneous virulence and spore-color variants of *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (58,59). For *E. g. hordei*, only one spontaneous mutant to virulence has been documented on barley lines with *Ml-g* resistance (25). In a screen of about 10^8 conidia, Torp and Jensen (57) were unable to find any spontaneous mutants of *E. g. hordei* once contaminants were identified. In addition to these reports, there are several examples in the literature of avirulent races of fungal pathogens that have been successfully mutated to virulence following chemical mutagenesis (5,18–20,28,37,54–56) or ionizing radiation (16,17,49,50). For those few fungal mutants that were analyzed genetically, the virulent phenotype was demonstrated to be a heritable trait caused by one mutation, thus confirming the gene-for-gene hypothesis (17).

Concern was raised in some of these studies (16) and by Torp and Jensen (57), whether true mutants, rather than contaminants, were isolated during these mutagenesis treatments. For most fungal pathogens, the number of genetic markers is very limited, so that the genotype of potential mutants cannot be confirmed (8,47). We set out to develop a mutagenesis procedure for *E. g. hordei* that addresses this concern by using gnotobiotic cultures growing on leaf sections in petri dishes. The efficacy of the procedure was verified by the recovery of morphological variants. We then used mutational analysis of virulence in *E. g. hordei*, as an alternate to genetic crossing, to test the gene-for-gene hypothesis as a model for barley-*E. g. hordei* interactions.

MATERIALS AND METHODS

Plant materials and fungal cultures. Race CR3 of *E. g. hordei* was obtained from R. Wise, U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS), Iowa State University, Ames; race 59.3 was received from J. G. Moseman (retired), USDA-ARS, Beltsville, MD; and race NC1 was isolated in East Lansing, MI in 1985. Because the mating type alleles have not been previously named, we have arbitrarily designated races CR3 and NC1 as *MAT1-1* and race 59.3 as *MAT1-2* as suggested by Yoder et al (63). Barley isolines, Manchuria (CI-2330, with the *ml-a* allele), CI-16137 (*Ml-a*1), CI-16138 (*ml-a*), and CI-16141 (*Ml-h*) were generously provided by J. G. Moseman (43). Barley isolines CI-16137 (AlgR) and CI-16138 (AlgS) are 99% homologous (43).

Gnotobiotic cultures of *E. g. hordei* were obtained by serial transfer of conidia to a susceptible barley cultivar. Races of *E. g. hordei* were routinely grown on selective barley lines: race 59.3 on CI-16141, race NC1 on AlgR, and race CR3 on Manchuria. Cultures of *E. g. hordei* were maintained on axenic leaf sections of barley in petri dishes containing 2 mM $Ca(NO_3)_2$, 1 mM benzimidazole, and 1.2% agar (CBA) (6) under standard conditions (34). Using aseptic techniques, conidia from 7-day-old cultures were transferred to new leaf sections with sterile, cotton-tipped wood applicator sticks (46).

Mutagenesis. Leaf sections from 9-day-old axenic Manchuria seedlings were inoculated with *E. g. hordei* as described above. Typically, 25–30 leaf sections were used. The inoculated leaves were incubated for 3 days on CBA medium. The leaves were then transferred aseptically to fresh plates of CBA containing 0.2% (v/v) ethyl methanesulfonate (EMS). The EMS was added to autoclaved medium once the agar solution had cooled to 50 C. After a period of 12–14 h on CBA-EMS, the inoculated leaf sections were transferred to CBA plates. When observed 10 days after inoculation, a 12-h exposure to EMS gave an approximately 50% reduction in the leaf surface area covered by fungal mycelium as compared to unmutagenized cultures. Mutagenized cultures were incubated for 7–10 days after transfer from the mutagen and were then examined for morphological variants with a Wild model M5A binocular dissecting microscope (E. Leitz, Inc., Rockleigh, NJ). In initial experiments, conidiophores that were morphologically abnormal or were derived from abnormal mycelium were propagated by excising a small piece of infected leaf that included the desired conidia. The variant culture on this leaf piece was propagated by transferring conidia to a leaf section of Manchuria. These cultures were grown under standard conditions (34). In later experiments, conidia from a mutagenized population, 7 days after EMS treatment, were transferred en masse to Manchuria leaf sections with sterile cotton-tipped applicators; the conidia from one leaf section treated with EMS were transferred to one fresh leaf section. These leaf sections were incubated under standard conditions for 7–10 days and then examined for variants. Hollomon and co-workers (5,28) have recommended this intermediate amplification step on a nonselective host as a means of eliminating mutants defective in those characteristics required for successful growth and development of the pathogen on a susceptible host. The amplification step has the added advantage that if one conidium fails to develop for any spurious reason, a sibling may develop to produce a colony. To ensure that sibling variants were not selected, only one well-isolated pustule per leaf section was used to propagate the variant on a fresh leaf section. This procedure was repeated until pure cultures of the variants were obtained.

To isolate mutants of race CR3 with increased virulence, conidia from the mutagenized cultures were transferred to axenic leaf sections of AlgR to select for variants with increased virulence. Transfers were made from the mutagenized cultures on one AlgS leaf section to one AlgR leaf section, with a new applicator stick used for each transfer. Only one variant from any AlgR leaf section was retained. In some experiments, the mutagenized conidia were transferred to the susceptible line, Manchuria, and incubated for 7–10 days before transfer to AlgR. With either method, cultures producing conidia on AlgR after 7 days of incubation were generally transferred to Manchuria to maintain the cultures. Variants that retained the increased virulence phenotype on AlgR were then tested for virulence on barley isolines with alternate resistance alleles (43). Each observation was based on two replicates and each experiment was repeated at least twice.

In a few cases, the mutagen N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) at a concentration of 10 μg/ml was substituted for EMS in the standard mutagenesis protocol (28). In an alternate MNNG mutagenesis treatment, the cut ends of detached, inoculated leaves were placed in solutions of 125 μg of MNNG per milliliter in test tubes (25 × 200 mm) and allowed to take up the mutagen solution through the transpiration stream over a period of several days (20). The third mutagen that was tested was ultraviolet irradiation (15,50). Two different methods were used. In one, freshly collected conidia were exposed to a germicidal lamp for varying lengths of time and then transferred to leaf sections of Manchuria in petri dishes on CBA medium. The cultures were placed in the dark for 24 h and then allowed to develop under standard conditions. At 7–10 days after ultraviolet irradiation, the cultures were examined for the presence of red conidiophores. In the second approach, 3-day-old cultures of *E. g. hordei* growing on Manchuria leaf sections were exposed to ultraviolet irradiation for varying lengths of time, incubated in the dark for about 24 h, and then returned to standard incubation conditions. These cultures were examined for the occurrence of red conidiophores 4–7 days after irradiation.

Genetic analysis. Morphological variants of race 59.3 were crossed to race NC1, and the virulence mutant, CR3-3, was crossed to race 59.3 (3,5,27). The crosses were performed on Manchuria plants that were grown for 6 wk in growth chambers with a 16-h photoperiod and a light intensity of 100 μE PAR m^{-2} s^{-1} provided by a mixture of incandescent and cool white fluorescent bulbs. The day and night temperatures were 18 and 16 C, respectively. Conidia from 7-day-old gnotobiotic cultures of the parental fungal lines were collected on waxed weighing paper, weighed and suspended in FC43, a fluorocarbon carrier (3M Co., St. Paul, MN), to a concentration of 20 mg fresh weight conidia per milliliter of FC43 immediately before use (4,5). The conidial suspensions of the two parental cultures were mixed in a 1:1 ratio, and 5-

μl aliquots were used to inoculate individual leaves. The two youngest, fully expanded leaves from two tillers on six separate plants were inoculated, for a total of 24 leaves per cross. As controls for each parental race, leaves from three plants were inoculated with one fungal race. The inoculated plants were grown in the growth chamber for an additional 4 wk and then permitted to dry for 2 wk. At the sites of inoculation, leaves with cleistothecia were removed from the plants, placed in manilla envelopes, and stored at 5 C for at least 1 mo.

Before the induction of ascospore release, the cleistothecia were surface-disinfested by rinsing for 1 min in 5% bleach (0.26% sodium hypochlorite) and washing twice with sterile deionized water (27). Cleistothecia were placed on a 25-mm MF filter disk with 0.8 μm pore size (Millipore Corp., Bedford, MA); and the MF disk with cleistothecia was supported on a 4.25-cm Whatman 1 filter paper disk (Fisher Scientific, Livonia, MI) saturated with sterile deionized water in a petri dish (60 × 15 mm) (3,27). The cleistothecia were incubated at 5 C for 10 days (44). The Whatman filter disk with MF filter and cleistothecia was then placed in a petri dish (60 × 15 mm) lid over Manchuria leaf sections on CBA. The cleistothecia were incubated for 9 days with standard fungal growth conditions; the leaf sections below the cleistothecia were changed every 3 days. To keep the filter paper moist, sterile deionized water was added as needed. The leaf sections were incubated for an additional 7–10 days to permit the ascospore-derived colonies to develop.

Sixteen F1 ascospore progeny were isolated from a cross between mutant CR3-3 and race 59.3 and were subjected to three rounds of single-colony isolation on Manchuria. Leaf sections of AlgR and AlgS were inoculated with conidia of the progeny or the parent races of *E. g. hordei* and incubated for 7 days before assessing virulence. The progeny were also tested on CI-16141, which can be used to differentiate race 59.3 from race CR3. The mating type was tested by separately co-inoculating each progeny culture with either race 59.3 (*MAT1-2*) or race NC1 (*MAT1-1*) on leaf sections of Manchuria placed on 1.2% agar containing 0.2 M sucrose. Race NC1 was chosen for this purpose because we have found that a greater number of cleistothecia are produced in crosses between NC1 and 59.3 than between CR3 and 59.3. Successful mating, as characterized by the production of cleistothecia and/or the thick, white, aerial mycelium that is typically produced before cleistothecia formation, was evaluated at 2 wk after co-inoculation with the two races of opposite mating type. Although cleistothecia are formed on leaf sections in petri dishes, no ascospore progeny have been recovered from these cleistothecia to date.

Disease rating scores. Disease rating scores (DR) for the various cultures of *E. g. hordei* were determined at 7 days post-inoculation by using a Wild M5A binocular dissecting stereo microscope at ×15–31 magnification. The scoring system was as follows: DR = 0, no visible pathogen growth; DR = 0–1, slight hyphal growth but no production of conidia, and weak or no visible necrosis of plant tissue; DR = 1, weak hyphal growth with conidia production, weak or no visible plant necrosis; DR = 2, moderate hyphal growth and conidia production, pronounced necrosis of plant tissue; DR = 3, substantial hyphal growth and conidia production, slight or no apparent necrosis of plant tissue, some chlorosis; DR = 4, luxuriant hyphal growth and conidia production, no apparent plant necrosis and little or no chlorosis (42). Each disease rating score is associated with a distinctive feature

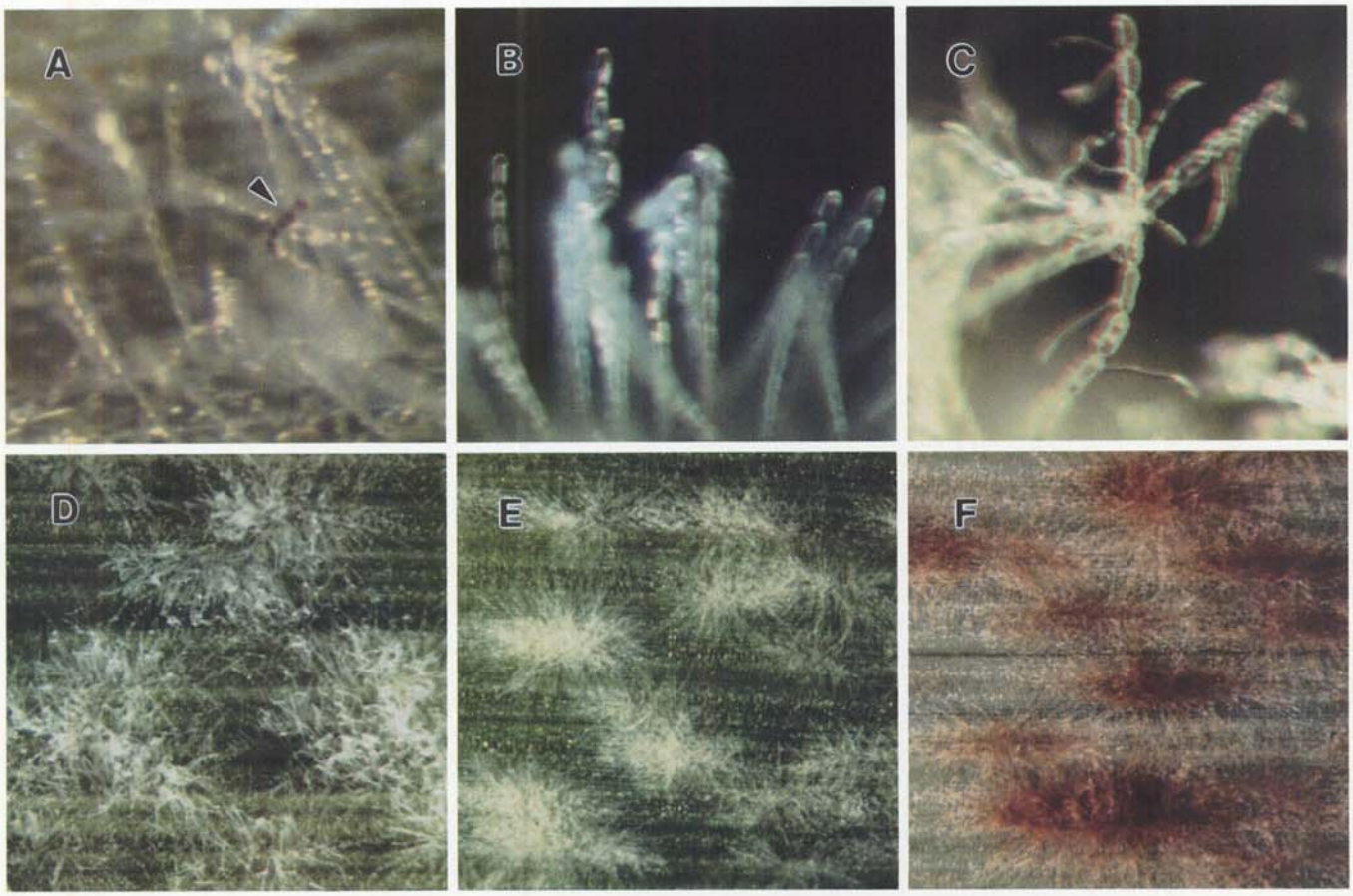

Fig. 1. Morphological variants of *Erysiphe graminis* f. sp. *hordei* recovered after treatment with ethyl methanesulfonate. **A,** isolated red conidiophore, indicated by an arrowhead, observed among normal hyaline conidiophores on a leaf section 7 days after treatment with the mutagen ethyl methanesulfonate. ×40. **B,** race CR3, a wild type isolate, with hyaline, oval conidia, and hyaline hyphae. ×40. **C,** VivJS1 variant of race CR3. Germ tubes have emerged from the terminal few conidia of the conidiophores. ×40. **D,** race CR3, the wild type isolate. ×9.6. **E,** DgmJS2 variant of race 59.3 with compact mycelium and delayed conidiophore development. ×9.6. **F,** RedJS2 variant of race CR3 with orange-red conidia and hyphae. ×9.6.

(e.g., conidia production, plant necrosis), which reduces the subjectivity inherent in this scoring system. The fully resistant barley line, AlgR, gives a DR of 0 and the fully susceptible lines, AlgS and Manchuria, give a DR of 4 following inoculation with race CR3 of *E. g. hordei*. All virulence tests were performed on the abaxial surface of barley leaf sections in petri dishes containing CBA medium (34). Under these conditions, we have found that the disease reaction of race CR3 on each barley isoline was identical to the disease reaction reported on intact plants growing in greenhouses or growth chambers (43).

Throughout this paper, we have used the terms avirulent and virulent as descriptive terms to indicate the ability of a given isolate of *E. g. hordei* to grow on a specific genotype of barley. Avirulent isolates are those that cannot complete the asexual infectious cycle on a specific host genotype, while virulent isolates can. Among the virulent isolates, there are a broad range of virulence levels from weakly virulent isolates producing only a few small colonies to highly virulent cultures producing luxuriant colonies that are readily visible by eye.

Low temperature scanning electron microscopy. Seven days after inoculation with either race 59.3 or variant RdcJS2, leaf sections were frozen in a liquid nitrogen slush, etched, coated with gold, and then viewed at 15 kV in a JSM35Z scanning electron microscope (JEOL, Tokyo, Japan) on a cryogenic stage cooled to −95 C (34).

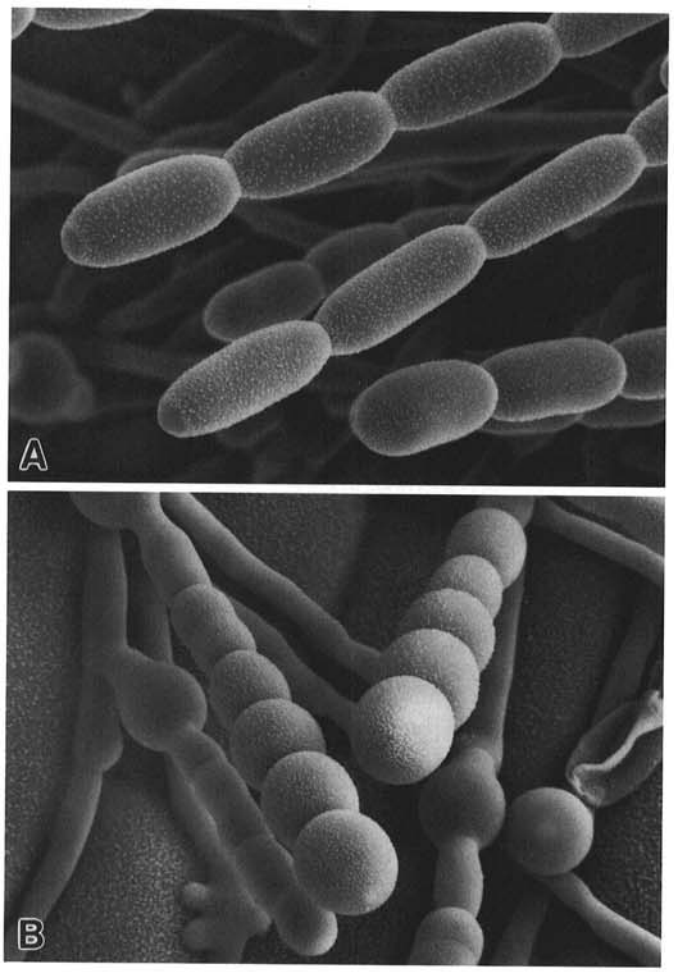

Fig. 2. Scanning electron micrograph of wild type and variant *Erysiphe graminis* f. sp. *hordei* isolates. **A,** Race 59.3, a wild type isolate, with oval conidia. ×800. **B,** RdcJS2, a variant derived from race 59.3, with round conidia. ×800.

RESULTS

Mutagenesis protocol and the recovery of morphological variants. The effectiveness of the mutagenesis procedure was immediately recognized by observation of the cultures 7–10 days after treatment with EMS. Red conidiophores were easily recognized against a background of hyaline, wild type conidiophores, and mycelia (Fig. 1A). A variety of morphological variants were isolated (Fig. 1; Fig. 2; Table 1). These included three isolates with red conidia and hyphae (Red$^-$), a variant with conidia that germinate while still attached to the conidiophore (Viv$^-$, *viv*iparous), two variants with altered colony morphology (Dgm$^-$, *d*ensely *g*rowing *m*ycelium), and three isolates with round conidia (Rdc$^-$, *r*oun*d* *c*onidia). The Rdc$^-$ isolates of race 59.3 and the VivJS1 variant grew as well as the wild type isolates. The Red$^-$ variants grew more slowly, requiring 9 days to advance to the same stage development as 7-day-old wild type cultures. In addition, the Red$^-$ variants could be maintained only on the adaxial leaf surface. RdcJS1, the race CR3 variant with round conidia, grew poorly and was eventually lost. Whether the weak growth of this variant is due to the mutation causing the altered morphology or to a second mutation is unknown.

Growth of *E. g. hordei* is confined to the epidermis and all mycelial growth occurs on the plant surface; thus, visual inspection of pathogen growth and development is relatively easy. Although the Red$^-$, Viv$^-$, and Dgm$^-$ variants have distinct colony morphologies that can be detected by eye, these variants cannot be detected without the aid of a microscope in a background of predominantly wild type mycelium and conidiophores. With a stereo dissecting microscope, the cultures growing on leaf sections in petri dishes could be observed in a nondisruptive manner. Thus, the use of inoculated leaf sections had the added advantage that mutant cultures could be readily identified.

In preliminary experiments, cultures of race CR3 were treated with MNNG. When high concentrations of this mutagen were used (20), substantial damage to the host leaves occurred, and the cultures of *E. g. hordei* did not develop. At lower concentrations of MNNG (28), red and virulence variants were recovered at a similar frequency to that observed following mutagenesis with EMS. Ultraviolet irradiation did not appear to be an effective mutagen for *E. g. hordei*, even at doses causing 80–90% lethality. Because EMS proved to be a satisfactory mutagen, we did not pursue experiments with other mutagens.

Because they are relatively easy to observe during the primary

TABLE 1. Morphological variants of *Erysiphe graminis* f. sp. *hordei* that were recovered after mutagenesis with ethyl methanesulfonate

Variant	Parental race	Wild type phenotype	Mutant phenotype
RedJS1	CR3	Hyaline conidia and hyphae	Red conidia and hyphae
RedJS2	CR3	Hyaline conidia and hyphae	Orange-red conidia and hyphae
RedJS3	59.3	Hyaline conidia and hyphae	Orange conidia, red conidiophore mother cell
VivJS1	CR3	Germination inhibited until conidium separated from conidiophore	Conidium germination before release from conidiophore
DgmJS1	CR3	Diffuse mycelial growth	Compact colony; conidiation delayed
DgmJS2	59.3	Diffuse mycelial growth	Similar to DgmJS1
RdcJS1	CR3	Oval conidia; conidiophores elongate indefinitely	Round conidia; conidiophore elongation ceases at about eight conidia
RdcJS2	59.3	Oval conidia; conidiophores elongate indefinitely	Round conidia; normal conidiophore elongation
RdcJS3	59.3	Oval conidia; conidiophores elongate indefinitely	Similar to RdcJS2

screen, the frequency of Red$^-$ variants was used to provide an estimate of the mutation frequency. To estimate the number of hyphal cells exposed to EMS, the number of colonies on 30 leaf sections was determined 5 days after inoculation and 2 days after treatment with EMS. At this time, mycelial growth was limited, and individual colonies could be distinguished. The mean number of colonies per leaf was 105 (SD = 58). At the time the leaves are transferred to the mutagen, each of these colonies would have had an average of 130 cells (26). About one third of these hyphal cells will produce a conidiophore. Taking these estimates together (number of conidiophores per colony, number of colonies per leaf, and number of leaves), approximately 136,000 conidiophores would survive the mutagenesis treatment. A total of 21 red conidiophores were observed. Therefore, the mutation frequency was approximately 1:6,500 conidiophore-producing cells. In a repetition of this experiment in which 22 red conidiophores were detected on 30 inoculated leaf sections, the mutation frequency was estimated to be 1:5,200 conidiophore-producing cells. Routinely, about 10 red conidiophores are observed in mutageneses consisting of 25 infected leaf sections, giving a slightly lower mutation frequency of one per 11,000 conidiophore-producing cells. It is possible that Red$^-$ variants arise from mutations in one of two genes, as is the case for red-colored mutants of *Neurospora crassa* and *Saccharomyces cerevisiae*, which are defective in adenine synthesis (9,51). If this is also true for *E. g. hordei*, the mutation frequency after treatment with EMS would be roughly 1:10,000 to 1:22,000.

Cultures of race CR3, which were not exposed to EMS, were examined to assess the spontaneous mutation frequency. On eight different occasions, five heavily infected leaf sections were screened 7 days post-inoculation for red conidiophores. No red conidiophores nor any other spontaneous morphological variants were observed. The number of actual conidiophores scored was higher in the unmutagenized cultures, because the survival rate is about twofold greater in the absence of mutagen treatment. Additional evidence that such mutations occur rarely in the absence of a mutagen is provided by the fact that no morphological variants have been observed during normal culturing of this obligate pathogen, which is routinely transferred weekly. Thus, the spontaneous mutation frequency must be substantially lower than the mutation rate observed after treatment with MNNG at 10 μg/ml or with EMS.

Genetic analysis was attempted with the variants RedJS3, RdcJS2, and RdcJS3. All three retained their ability to mate with race NC1, as indicated by the formation of cleistothecia, suggesting that the variants retained the *MAT1-2* genotype of the parental race 59.3. However, in all three cases, no ascospore progeny were recovered even though ascospores were visible when the cleistothecia were crushed open. Thus, we have not been able to test the heritability of the morphological variants. To determine whether the low recovery of progeny was a feature of the variants or inherent to crosses between races NC1 and 59.3, we compared the recovery of progeny from NC1 × 59.3 crosses to the recovery of progeny from cleistothecia collected in the field. Progeny were recovered from both the NC1 × 59.3 cleistothecia and the field cleistothecia. Therefore, the inability to recover ascospore progeny from the crosses with the variants is likely due to the mutation(s) carried by these lines.

TABLE 2. Recovery of variants of race CR3 of *Erysiphe graminis* f. sp. *hordei* with increased virulence or with red conidiophores after mutagenesis with ethyl methanesulfonate

Experiment number[a]	Number of variants with DR = 1 on AlgR[b]	Number of Red$^-$ variants[c]
1	8 (3)[d]	7
2	9 (6)	11
3	10 (3)	8

[a] Each experiment consisted on 25 inoculated leaf sections.
[b] Race CR3 is avirulent on barley line AlgR; disease rating is 0 (DR = 0). This race rarely produced conidiophores on AlgR. Variants with a DR = 1 show weak hyphal growth and limited conidiophore production on AlgR.
[c] Race CR3 forms hyaline conidia and hyphae; Red$^-$ variants produce red- or orange-colored conidia and hyphae.
[d] Number observed (number recovered for further analysis).

Virulence variants. After EMS mutagenesis of race CR3, variants with marginal increases in virulence on AlgR were consistently found. These variants were termed class I variants. Disease rating scores of 0–1 or 1 were common among the more than 100 variants identified in the various screens for mutants with increased virulence. For any individual mutagenesis experiment, the number of variants with a DR of 1 closely approximated the number of variants with red conidiophores (Table 2). No highly virulent (DR = 3 or 4) variants of race CR3 were observed. Because variants with red conidiophores occur at a frequency of $1\text{–}2 \times 10^{-4}$, and no fully virulent variants were observed among more than 100 weakly virulent variants, mutations to full virulence occur at a frequency of $<1\text{–}2 \times 10^{-6}$ and, thus, are extremely rare. Weakly virulent variants were isolated, amplified on Manchuria, and retested on AlgR. Roughly 50% of the variants retained the altered virulence phenotype on AlgR. After reselection, all variants could be stably propagated on a susceptible host in the absence of selection, suggesting that the change giving the increased virulence was mitotically stable.

We believe that these variants were not contaminating cultures of *E. g. hordei*, because the variants showed the same mating type and, in most cases, the identical virulence patterns on 21 barley lines as the wild type, race CR3. Even though a common Michigan race, race NC1, shows a highly virulent phenotype on AlgR (DR = 4), no fully virulent isolates were recovered, indicating that the use of the gnotobiotic cultures was successful in preventing contamination.

The effect of amplifying the mutagenized cultures on a susceptible barley line before selection on the resistant host was examined. Mutagenized conidia were either inoculated directly onto AlgR leaf sections or inoculated first onto leaf sections from a susceptible barley line to amplify the mutagenized population before inoculating AlgR leaf sections. Again, only variants with modest increases in virulence were recovered.

The disease rating scores of the class I variants were determined on 21 congenic barley lines, which differ from each other by the powdery mildew resistance allele at one locus (43). The increase in virulence of the different variants was generally limited to AlgR, although, for some variants, virulence levels increased on more than one barley line (Table 3). In no case did virulence levels increase to a disease rating score of greater than 2 on any resistant isoline (Table 3); nor did any variant become avirulent on any of the 11 susceptible isolines in the Moseman collection (data not presented). In addition, the disease rating scores of the class I variants on barley lines CI-16139 (*Ml-g*), CI-16141 (*Ml-h*), CI-16143 (*Ml-k*), and CI-16145 (*Ml-p*) were identical to the scores of the wild type, race CR3 (data not presented).

One of the EMS-derived variants, race CR3-3, was selected for genetic analysis. Race CR3-3 differs from the wild type, race CR3, only in its virulence on AlgR (Table 3). Sixteen progeny from a CR3-3 × 59.3 cross were isolated and tested for mating type and for virulence on AlgR and on CI-16141. Six of 16 progeny showed increased virulence on AlgR, similar to the CR3-3 parent, whereas the remaining 10 progeny resembled the avirulent wild type, race CR3. These data are consistent with a 1:1 ratio ($P > 0.25$) predicted for a single nuclear gene with two alleles segregating in a haploid fungus. As an independent test to show that the crosses were successful, the progeny were also tested for mating type. Eight progeny had the Mat1-1 mating type and eight had the Mat1-2 mating type. Thus, mating type was also inherited in a 1:1 ratio ($P > 0.90$). No linkage was observed between the *MAT1* locus and the mutation affecting virulence. The disease reaction on CI-16141, which carries the *Ml-h* resistance allele and differentiates race 59.3 (DR = 4) from race CR3 (DR = 2), was also tested. Among the 16 progeny, five gave a DR of 2 and 11 gave a DR of 4 on CI-16141. These data are consistent with the hypothesis that two unlinked genes controlling virulence on CI-16141 assort in this cross and that

only one of the four possible combinations of alleles at the two genes gives a phenotype with a DR of 2 ($P > 0.5$).

After mutagenizing race CR3-3, five variants, the class II variants, with a second increase in virulence on AlgR, were recovered (Table 3). Again, no fully virulent isolates were observed. Among the second group of virulent isolates, similar increases in virulence were observed on barley isolines with the resistance alleles *Ml-a7*, *Ml-a13*, and *Ml-a15* (Table 3). On barley lines with resistance alleles at alternate loci (i.e., CI-16139 [*Ml-g*], CI-16141 [*Ml-h*], CI-16143 [*Ml-k*], CI-16145 [*Ml-p*]), the virulence of the class II variants was identical to the virulence pattern of the parental race, mutant CR3-3, and of the wild type, race CR3 (data not presented).

DISCUSSION

Due to their obligate lifestyle, rust and powdery mildew pathogens present unique problems for developing effective mutagenesis protocols. Mutagenesis treatments or selection schemes that significantly decrease the viability of the host will reduce the recovery of mutant isolates. In general, two approaches have been employed for mutagenizing obligate fungal pathogens. For those organisms with hardy spores, asexual spores were exposed to a mutagen and then used to inoculate either a susceptible or a resistant host. The recovery of stable, heritable, virulence mutants of *Melampsora lini* (Ehrenb.) Desmaz. has been reported using this technique (17). In addition, several authors have recovered stable virulence and spore color variants of obligate fungal pathogens (37,56). Conidia of *E. g. hordei* are unusually labile in aqueous solutions (4), and hence, do not lend themselves to direct treatment with chemical mutagens. Mutagenesis by ultraviolet irradiation is a convenient method that is widely used with other microbes (15,50). However, *E. g. hordei* proved to be refractive to mutagenesis by ultraviolet irradiation (28; *personal observation*). Thus, we adopted a method of exposing infected barley leaf sections to EMS as a means of mutagenizing *E. g. hordei*. By a similar method, virulence variants of *E. g. tritici* (20) and both virulence- and fungicide-tolerant variants of *E. g. hordei* (5,28) were recovered. In addition, virulence and spore color variants of *M. lini* and various *Puccinia* spp. have been isolated using the latter mutagenesis method (18,54,55).

In developing a method for mutagenizing *E. g. hordei*, we wished to minimize the recovery of contaminating cultures. The possibility of accidentally introducing a contaminating genotype into a screen for mutants with altered virulence is particularly acute for the rust and powdery mildew pathogens, which produce massive numbers of airborne asexual spores (16,57). For this reason, all manipulations in the protocol outlined in the Materials and Methods section were done by using sterile techniques, and all cultures were maintained on axenic leaf sections in petri dishes rather than on plants grown in greenhouses or growth chambers.

The fungal cultures were exposed to EMS 3 days after inoculation onto leaf sections, at a time just before the formation of conidial mother cells (26; *personal observation*). Thus, the cells that differentiate into conidial mother cells were mutated, and entire conidiophores with the same mutation were produced (see Fig. 1A). The frequency of mutation was estimated to be between $1–2 \times 10^{-4}$, a calculation that was based on the occurrence of red conidiophores. These variants are particularly useful for assessing the efficacy of a mutagenesis treatment because the red conidiophores are easily detected. When designing the mutagenesis protocol, a 50% survival rate was chosen as a suitable level of mutagen treatment so as to minimize the recovery of variants with multiple mutations (1). Although *E. g. hordei* can be crossed (27,32), backcrossing mutants several times to reduce the number of extraneous mutations in a mutant line requires a large investment of time and space and is not convenient.

The obligate growth habit of *E. g. hordei* limits the spectrum of mutant classes that can be recovered. For example, mutations resulting in auxotrophy for essential nutrients not supplied by the plant would be lethal. The selection of antibiotic resistance would necessarily be limited to compounds that do not affect the vitality of the host. However, there are a number of mutants that can potentially be recovered, including morphological mutants, fungicide-tolerant mutants, and mutants with altered host range. In this report, we demonstrated that morphological and virulence variants of *E. g. hordei* can be recovered (Table 1; Table 3; Fig. 1; Fig. 2).

Morphological mutants of *E. g. hordei* are valuable for a number of purposes. First, they demonstrate the effectiveness of this mutagenesis procedure and will potentially add to the genetic map of *E. g. hordei* (7,32). Also, the biochemical basis for the altered phenotype can be determined, increasing our understanding of the interaction between this pathogen and its host and, in turn, possibly suggesting novel targets for specific fungicides. Lastly, when a transformation protocol is developed, these mutants can be used as recipients in mutant-rescue experiments.

Once the mutagenesis protocol was demonstrated to be successful by the recovery of morphological variants, this procedure was used to isolate virulence mutants. According to the gene-for-gene hypothesis, avirulence and virulence are qualitative traits with distinctly different phenotypes. Therefore, a mutation in an avirulence allele is predicted to result in an inactive gene product, and thus, in a fully virulent mutant (20). The weakly virulent phenotypes of the variants described in this report were more typical of the phenotypes ascribed to minor genes, with the exception that the changes in virulence were specific to the barley line with the *Ml-a1* allele. A few variants (e.g., races CR3-7, CR3-9, CR3-10, and CR3-12) showed altered disease rating scores on more than one barley isoline. However, these additional

TABLE 3. Disease rating scores for race CR3 of *Erysiphe graminis* f. sp. *hordei* and for variants of race CR3 on barley isolines

Barley line	Resistance allele	Race CR3	Variant culture[a]															
			Class I											Class II				
			1	3	4	7	9	10	12	13	16	20	22	3C	3F	3G	3H	3J
AlgR	*Ml-a1*	0[b]	1	1	1	2	1	1	1	1	1	1	1	2	2	2	2	2
CI-16151	*Ml-a6*	0	–[c]	–	–	1	–	–	–	–	–	–	–	1	–	–	–	–
CI-16147	*Ml-a7 (Mu)*	0	–	–	–	–	2	1	2	–	–	–	–	1	1	1	1	–
CI-16149	*Ml-a10*	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CI-16155	*Ml-a13*	0	–	–	–	–	–	–	1	–	–	–	–	0–1	0–1	0–1	0–1	1
CI-16153	*Ml-a15*	0–1	–	–	–	–	–	–	–	–	–	–	–	1	1	1	–	2
AlgS	*ml-a*	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Manchuria	*ml-a*	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a]Class I variants, cultures CR3-1 through CR3-22, were derived from race CR3, and class II variants, cultures CR3-3C through CR3-3J, were derived from CR3-3 after mutagenesis with ethyl methanesulfonate.

[b]Disease rating scores are as follows: DR = 0, no visible pathogen growth; DR = 0–1, slight hyphal growth but no production of conidia, and weak or no visible necrosis of plant tissue; DR = 1, weak hyphal growth with conidia production, weak or no visible plant necrosis; DR = 2, moderate hyphal growth and conidia production, pronounced necrosis of plant tissue; DR = 4, luxuriant hyphal growth and conidia production, no apparent plant necrosis and little or no chlorosis (42).

[c]– Denotes a disease rating score identical to the wild type, race CR3.

changes in specificity may be the result of multiple mutations in these variants. Curiously, no mutations resulting in fully virulent phenotypes were found, despite the fact that variants with such mutations would be obvious in the selection used in these experiments. Our results differ from those reported in similar experiments with *E. g. tritici* (20) and *E. g. hordei* (28) in which highly specific, fully virulent variants were isolated.

Interestingly, all of the variants that showed increased virulence on barley lines other than AlgR did so on barley lines with resistance alleles, *Ml-a6*, *Ml-a7*, and *Ml-a13*. This pattern was maintained following either one or two rounds of mutagenesis. These same barley alleles could not be separated by recombination in an experiment to determine the fine structure of the *Ml-a* locus (61), suggesting that the alleles *Ml-a6*, *Ml-a7*, and *Ml-a13* are functionally related and distinct from other alleles that map to the *Ml-a* locus. The *Ml-a15* (=*Ml-a7* [LG]; 60) allele did not follow this pattern. This allele could be separated from the *Ml-a1* allele by recombination (61); however, all of the variants derived from isolate CR3-3 showed increased virulence on the isoline CI-16153 (*Ml-a15*).

There are several possible explanations for our inability to recover fully virulent mutants. The virulence gene complementary to the *Ml-a1* allele may serve a second vital function that cannot be eliminated without causing death (19,20). If this is true, only a limited number of mutations may result in a loss of avirulence without interfering with the putative vital function of the gene product. Analogous to herbicide-resistance mutations (24), mutations causing a change of function would be expected to be about 100-fold less frequent than mutations resulting in a loss of function. The partially virulent variants described in this paper are unlikely to have change-of-function mutations, because both red-colored and weakly virulent variants arose at a similar frequency (Table 2).

The possibility also exists that two or more closely linked genes are required to give a fully avirulent phenotype on barley lines with the *Ml-a1* allele. Such clustering of genes of related function has been reported for the genes controlling biosynthetic pathways (e.g., the *qa* gene cluster of *N. crassa*; 21) and for some of the avirulence genes found in *M. lini* (15,17). In genetic analyses of *E. g. hordei*, relatively few progeny were analyzed in crosses to determine the mode of inheritance of avirulence genes. For example, 49 (39) and 60 (45) progeny were examined in crosses to determine the genetic basis of avirulence on barley lines with the *Ml-g* and *Ml-k* resistance alleles and with the *Ml-a1* and *Ml-at* resistance alleles, respectively. These numbers are too low to permit discrimination between a hypothesis based on one gene from one based on two or more closely linked genes. With a multi-gene model of an avirulence locus, a mutation in one member of the locus may have a relatively minor effect on avirulence. The probability of inactivating more than one gene of the cluster to give a fully virulent phenotype would be very low with a mutagen like EMS, which induces primarily point mutations. If this hypothesis is correct, avirulence on barley lines with the *Ml-a1* resistance allele would likely be controlled by three or more genes, because no fully virulent isolates were found following two rounds of mutagenesis. This complex relationship in which several pathogen genes mediate avirulence on a barley line, which carries resistance alleles at only one resistance locus that is effective against race CR3, does not fit the typical gene-for-gene relationship.

With the data at hand, we cannot discriminate between the alternate explanations for our inability to recover fully virulent mutants. However, for race CR3 of *E. g. hordei*, it appears that avirulence on AlgR is not controlled by one, simple, dispensable gene. We did demonstrate the occurrence of a host-specific avirulence gene with a minor effect on the virulence phenotype. As Torp and Jensen (57) have suggested, even though mutations to full virulence appear to be very rare, the generation of asexual spores by *E. g. hordei* is so prolific that fully virulent isolates could arise in the field (62).

We chose to select for mutants with increased virulence, because we wished to evaluate the gene-for-gene hypothesis. Although technically more demanding, screens of mutagenized virulent isolates may yield avirulent mutants with informative properties (55). A broadly based collection of pathogen and host mutants with altered disease reaction properties would extend our understanding of host-pathogen interactions and provide genetic strains for future efforts to clone the genes that control these interactions in the powdery mildew disease.

LITERATURE CITED

1. Bos, C. J. 1987. Induction and isolation of mutants in fungi at low mutagen doses. Curr. Genet. 12:471-474.
2. Bennett, F. G. A. 1984. Resistance to powdery mildew in wheat: A review of its use in agriculture and breeding programmes. Plant Pathol. 33: 279-300.
3. Bronson, C. R., and Ellingboe, A. H. 1986. The influence of four unnecessary genes for virulence on the fitness of *Erysiphe graminis* f. sp. *tritici*. Phytopathology 76:154-158.
4. Bushnell, W. R., and Rowell, J. B. 1967. Fluorochemical liquid as a carrier for spores of *Erysiphe graminis* and *Puccinia graminis*. Plant Dis. Rep. 51:447-448.
5. Butters, J., Clark, J., and Hollomon, D. W. 1986. Recombination as a means of predicting fungicide resistance in barley powdery mildew. Proc. Br. Crop Prot. Conf. Pests Dis. 4:561-565.
6. Carver, T. W., and Phillips, M. 1982. Effect of photoperiod and level of irradiance on production of haustoria by *Erysiphe graminis* f. sp. *hordei*. Trans. Br. Mycol. Soc. 79:207-211.
7. Christiansen, S. K., and Giese, H. 1990. Genetic analysis of the obligate parasitic barley powdery mildew fungus based on RFLP and virulence loci. Theor. Appl. Genet. 79:706-712.
8. Day, P. R. 1960. Variation in phytopathogenic fungi. Annu. Rev. Microbiol. 14:1-16.
9. de Serres, F. J. 1956. Studies with purple adenine mutants in *Neurospora crassa*. I. Structural and functional complexity in the *ad-3* region. Genetics 41:668-676.
10. Dyck, P. L. 1982. Genetic inhibition of expression of resistance gene *Lr23* in wheat to *Puccinia recondita*. Can. J. Plant Pathol. 62:219-220.
11. Ellingboe, A. H. 1981. Changing concepts in host-pathogen genetics. Annu. Rev. Phytopathol. 19:125-143.
12. Flor, H. H. 1946. Genetics of pathogenicity in *Melampsora lini*. J. Agric. Res. 73:335-357.
13. Flor, H. H. 1947. Inheritance of reaction to rust in flax. J. Agric. Res. 74:241-262.
14. Flor, H. H. 1955. Host-parasite interaction in flax rust—Its genetics and implications. Phytopathology 45:680-685.
15. Flor, H. H. 1956. Mutations in flax rust induced by ultraviolet radiation. Science 124:888-889.
16. Flor, H. H. 1958. Mutation to wider virulence in *Melampsora lini*. Phytopathology 48:297-301.
17. Flor, H. H. 1960. The inheritance of X-ray-induced mutations to virulence in a urediospore culture of race 1 of *Melampsora lini*. Phytopathology 50:603-605.
18. Franzone, P. M., and Favret, E. A. 1982. Mutations for pathogenicity in wheat leaf rust *Puccinia recondita* var. *tritici* induced by ethylmethanesulfonate. Phytopathol. Z. 104:289-298.
19. Gabriel, D. W., Ellingboe, A. H., and Rossman, E. C. 1979. Mutations affecting virulence in *Phyllosticta maydis*. Can. J. Bot. 57:2639-2643.
20. Gabriel, D. W., Lisker, N., and Ellingboe, A. H. 1982. The induction and analysis of two classes of mutations affecting pathogenicity in an obligate parasite. Phytopathology 72:1026-1028.
21. Geever, R. F., Huiet, L., Baum, J. A., Tyler, B. M., Patel, V. B., Rutledge, B. J., Case, M. E., and Giles, N. H. 1989. DNA sequence, organization and regulation of the *qa* gene cluster of *Neurospora crassa*. J. Mol. Biol. 207:15-34.
22. Green, G. J. 1964. A color mutation, its inheritance, and the inheritance of pathogenicity in *Puccinia graminis* Pers. Can. J. Bot. 42:1653-1664.
23. Hanlin, R. T. 1990. *Blumeria* Golovin ex. E.O. Speer. Pages 24-25 in: Illustrated Genera of Ascomycetes. The American Phytopathological Society, St. Paul, MN.
24. Haughn, G., and Somerville, C. R. 1987. Selection for herbicide resistance at the whole-plant level. Pages 98-108 in: Biotechnology in Agricultural Chemistry. H. M. LeBaron, R. O. Mumma, R. C. Honeycutt, and J. H. Duessing, eds. American Chemical Society, Washington.
25. Hermansen, J. E. 1980. A spontaneous mutation in *Erysiphe graminis* f. sp. *hordei* for virulence to host gene *Ml-g*. Phytopathol. Z. 98:171-177.

26. Hirata, K. 1973. Some aspects of the hyphal growth of the barley powdery mildew fungus, *Erysiphe graminis* f. sp. *hordei*. Shokubutsu Byogai Kenkyu, Kyoto 8:85-101.
27. Hollomon, D. W. 1981. Genetic control of ethirimol resistance in a natural population of *Erysiphe graminis* f. sp. *hordei*. Phytopathology 71:536-540.
28. Hollomon, D. W., Butters, J. A., and Clark, J. 1984. Genetic control of triadimenol resistance in barley powdery mildew. Proc. Br. Crop Prot. Conf. Pests and Diseases 2:477-482.
29. Ilott, T. W., Hulbert, S. H., and Michelmore, R. W. 1989. Genetic analysis of the gene-for-gene interaction between lettuce (*Lactuca sativa*) and *Bremia lactucae*. Phytopathology 79:888-897.
30. Jones, D. A. 1988. Genetic properties of inhibitor genes in flax rust that alter avirulence to virulence on flax. Phytopathology 78:342-344.
31. Jørgensen, J. H. 1988. Genetic analysis of barley mutants with modifications of powdery mildew resistance gene *Ml-a12*. Genome 30:129-132.
32. Jørgensen, J. H. 1988. *Erysiphe graminis*, powdery mildew of cereals and grasses. Pages 137-157 in: Advances in Plant Pathology. Vol 6. G. S. Sidhu, ed. Academic Press, London.
33. Kerber, E. R., and Green, G. J. 1980. Suppression of stem rust resistance in the hexaploid wheat cv. Canthatch by chromosome 7DL. Can. J. Bot. 58:1347-1350.
34. Klecan, A. L., Hippe, S., and Somerville, S. C. 1990. Reduced growth of *Erysiphe graminis* f. sp. *hordei* induced by *Tilletiopsis pallescens*. Phytopathology 80:325-331.
35. Lawrence, G. J., Mayo, G. M. E., and Shepherd, K. W. 1981. Interactions between genes controlling pathogenicity in the flax rust fungus. Phytopathology 71:12-19.
36. Lawrence, G. J., Shepherd, K. W., and Mayo, G. E. 1981. Fine structure of genes controlling pathogenicity in flax rust, *Melampsora lini*. Heredity 46:297-313.
37. Luig, N. H. 1978. Close association of two factors for avirulence in *Puccinia graminis tritici*. Phytopathology 68:936-937.
38. Martens, J. W., Rothman, P. G., McKenzie, R. I. H., and Brown, P. D. 1981. Evidence for complementary gene action conferring resistance to *Puccinia graminis avenae* in *Avena sativa*. Can. J. Genet. Cytol. 23:591-595.
39. Moseman, J. G. 1959. Host-pathogen interaction of the genes for resistance in *Hordeum vulgare* and for pathogenicity in *Erysiphe graminis* f. sp. *hordei*. Phytopathology 49:469-472.
40. Moseman, J. G. 1963. Relationship of genes conditioning pathogenicity of *Erysiphe graminis* f. sp. *hordei* on barley. Phytopathology 53:1326-1330.
41. Moseman, J. G. 1966. Genetics of powdery mildews. Annu. Rev. Phytopathol. 4:269-290.
42. Moseman, J. G. 1968. Reactions of barley to *Erysiphe graminis* f. sp. *hordei* from North America, England, Ireland, and Japan. Plant Dis. Rep. 52:463-467.
43. Moseman, J. G. 1972. Isogenic barley lines for reaction to *Erysiphe graminis* f. sp. *hordei*. Crop Sci. 12:681-682.
44. Moseman, J. G., and Powers, H. R. 1957. Function and longevity of cleistothecia of *Erysiphe graminis* f. sp. *hordei*. Phytopathology 47:53-56.
45. Moseman, J. G., and Schaller, C. W. 1962. Interaction of genes conditioning reaction of Algerian barley and pathogenicity of *Erysiphe graminis* f. sp. *hordei*. Phytopathology 52:529-533.
46. Nair, K. R. S., and Ellingboe, A. H. 1962. A method of controlled inoculations with conidiospores of *Erysiphe graminis* var. *tritici*. Phytopathology 52:714.
47. O'Dell, M., Wolfe, M. S., Flavell, R. B., Simpson, C. G., and Summers, R. W. 1989. Molecular variation in populations of *Erysiphe graminis* on barley, oats and rye. Plant Pathol. 38:340-351.
48. Roelfs, A. P., and Groth, J. V. 1980. A comparison of virulence phenotypes in wheat stem rust populations reproducing sexually and asexually. Phytopathology 70:855-862.
49. Rowell, J. B., Loegering, W. Q., and Powers, H. R. 1963. Genetic model for physiologic studies of mechanisms governing development of infection type in wheat stem rust. Phytopathology 53:932-937.
50. Schwinghamer, E. A. 1959. The relation between radiation dose and the frequency of mutations for pathogenicity in *Melampsora lini*. Phytopathology 49:260-269.
51. Silver, J. M., and Eaton, N. R. 1969. Functional blocks of the *AD1* and *AD2* mutants of *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 34:301-305.
52. Singh, R. P., and McIntosh, R. A. 1984. Complementary genes for reaction to *Puccinia recondita tritici* in *Triticum aestivum*. I. Genetic and linkage studies. Can. J. Genet. Cytol. 28:189-197.
53. Søgaard, B., and Jørgensen, J. H. 1984. Genes for reaction to *Erysiphe graminis hordei* (powdery mildew). Barley Genet. Newsl. 14:173-182.
54. Statler, G. 1985. Mutations affecting virulence in *Puccinia recondita*. Phytopathology 75:565-567.
55. Statler, G. 1985. Mutations to virulence and avirulence in *Melampsora lini*. Phytopathology 75:771-773.
56. Teo, C., and Baker, E. P. 1975. Mutagenic effects of ethyl methanesulphonate on the oat stem rust pathogen (*Puccinia graminis* f. sp. *avenae*). Proc. Linn. Soc., NSW 99:166-173.
57. Torp, J., and Jensen, H. P. 1985. Screening for spontaneous virulent mutants of *Erysiphe graminis* D.C. f. sp. *hordei* on barley lines with resistance genes *Ml-a1*, *Ml-a6*, *Ml-a12*, and *Ml-g*. Phytopathol. Z. 112:17-27.
58. Watson, I. A. 1957. Mutation for increased pathogenicity in *Puccinia graminis* var. *tritici*. Phytopathology 47:507-509.
59. Watson, I. A., and Luig, N. H. 1968. Progressive increase in virulence in *Puccinia graminis* f. sp. *tritici*. Phytopathology 58:70-73.
60. Wise, R. P., and Ellingboe, A. H. 1983. Infection kinetics of *Erysiphe graminis* f. sp. *hordei* on barley with different alleles at the *Ml-a* locus. Phytopathology 73:1220-1222.
61. Wise, R. P., and Ellingboe, A. H. 1985. Fine structure and instability of the *Ml-a* locus in barley. Genetics 111:113-130.
62. Wolfe, M. S., and Schwarzbach, E. 1978. The recent history of the evolution of barley powdery mildew in Europe. Pages 129-157 in: The Powdery Mildews. D. M. Spencer, ed. Academic Press, London.
63. Yoder, O. C., Valent, B., and Chumley, F. 1986. Genetic nomenclature and practice for plant pathogenic fungi. Phytopathology 76:383-385.