Primary Infection by Erysiphe graminis f. sp. hordei of Barley Mutants with Resistance Genes in the ml-o Locus

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ABSTRACT

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Ten barley mutants and one barley line, each with an independently arisen resistance gene in the ml-o locus, and some susceptible cultivars were inoculated with *Erysiphe graminis* f. sp. *hordei* conidia suspended in a fluorochemical liquid. The germination percentage of the conidia was not affected by the hosts. An average of about 60% of the germinated conidia formed haustoria, 50% formed hyphae, and 40% formed mildew colonies on the susceptible hosts. In contrast, only about 0.7% formed haustoria, 0.4% formed hyphae, and 0.2% formed colonies on the resistant hosts, on which the great majority of haustoria were located in the subsidiary cells next to the guard cells. Secondary haustoria were formed in susceptible hosts by 84% of the conidia that formed primary haustoria. In resistant hosts, the percentage was as high as 36, and they were almost exclusively formed in epidermal cells adjacent to invaded subsidiary cells. This suggests that the adjacent epidermal cells become less resistant, allowing colony development. The frequency of colonies differed significantly among the resistant barleys; this could be ascribed to differences in the genetic background. Pathogen cultures derived from the occasional colonies on the resistant barleys were avirulent on ml-o resistant barleys. The 11 *ml-o* resistance genes all affected the primary infection and the formation of mildew colonies, equally.

Additional key words: Hordeum vulgare.

Several induced mutants of barley (*Hordeum vulgare* L.) and some barley lines from Ethiopia have recessive, allelic genes that condition resistance to the powdery mildew fungus, *Erysiphe graminis* DC. ex Mérat f. sp. *hordei* Marchal (7, 10, 12, 15, 26, 27). These genes are in a locus designated ml-o (7) on the barley chromosome 4 (12, 13). The resistance of these mutants has been proved in all of the many tests with cultures, races, or populations of *E. graminis* f. sp. *hordei* from most parts of the world (6, 11, 16, 24, 28). The mutants are further characterized by producing the same infection type designated 0/(4); i.e., they are resistant with infection type 0 except for a few sporulating colonies (6, 10, 23, 27). Our preliminary observations indicated that the frequency of occasional colonies was higher on some mutants than others, which suggested that different *ml-o* genes conditioned quantitatively different infection types.

The primary infection of barley by *E. graminis* f. sp. *hordei* consists of a number of morphologically identifiable stages of development: germination of conidia; formation of appressoria; penetration of the host cell wall; formation of haustoria in the host cells; and formation of one or two hyphae [designated "elongating secondary hyphae" by Ellingboe and co-workers (18, 19, 20)] on the host surface that are capable of initiating secondary and tertiary penetrations (4). Ellingboe (4) and Ellingboe and Slesinski (5) suggested that host resistance genes in different loci affect the development of avirulent pathogen cultures at different stages, whereas different alleles in one locus affect the same stage, but possibly with different intensities.

The methods developed by Ellingboe and coworkers (18, 19, 20) for examining the primary infection of barley by *E. graminis* f. sp. *hordei* were applied in the present study to ten induced mutants of barley and one spontaneous barley with allelic resistance genes in the ml-o locus. The objectives were: (i) to determine the stage (s) in the primary infection affected by the *ml-o* resistance genes; (ii) to disclose possible differences in the effect of the 11 *ml-o* resistance genes; and (iii) to study the origin of the occasional mildew colonies that develop on barley with *ml-o* genes for resistance.

MATERIALS AND METHODS

Barley material.—The ten powdery mildew-resistant mutants and their six susceptible mother lines of spring barley were: Risø 5678 (C. I. 15219), Risø 6018 (C. I. 15220), Risø 7085 (C. I. 15622), and Risø 7372 (C. I. 15221) from cultivar Carlsberg II (C. I. 15218) (10, 15); M 66 (C. I. 15217) from cultivar Haisa (C. I. 9855) (8); H 3502 (C. I. 15223) from cultivar Probstdorfer Vollkorn (C. I. 15222) (9); SR 1 (= Refoma) and SR 7 from cultivar Foma (27); M.C. 20 (C. I. 15225) from cultivar Malteria Heda (C. I. 15224) (6); and SZ 5139b (C. I. 15227) from cultivar Diamant (C. I. 15226) (26). The C. I. numbers are

accession numbers in the Small Grains Collection of the U.S. Department of Agriculture, Beltsville, Maryland, USA. These ten mutants each have one recessive resistance gene induced by radiation or chemical treatments. The ten genes originate from independent mutational events; they are functionally allelic in locus ml-o, and are designated *ml-o 1* through *ml-o 10* (or *Reg 6a* through *Reg 6j*) (15). We also used a spring barley line from Ethiopia, Grannenlose Zweizeilige HOR 2937 (G.Z. 2937), which has a spontaneously-arisen *ml-o* allele (12), designated *ml-o 11* or *Reg 6k* (15). The HOR number is the accession number in the cereal collection in Gatersleben, GDR. Further, the following four homozygous resistant lines from crosses were used: Risø 5678/3* Carlsberg II (R), M 66/3* Carlsberg II (R), SR 1/3* Carlsberg II (R), and G.Z. 2937/1* Carlsberg II (R). The symbol, 3*, refers to one cross and two backcrosses; 1*, to one cross to Carlsberg II. The barley cultivar Manchuria (C. I. 2330) was also used.

The barley seedlings to be inoculated were raised in 10-cm diameter pots with a 1:1 mixture of K-soil (a commercial peat-rich soil with fertilizer added) and sand in a growth chamber at a light intensity of about 10,000 lux and a temperature of 15 ± 1 C in 16-hr day periods alternating with 8-hr night periods at 13 C; the relative humidity was 65 ± 5%. The seedlings were 8 or 9 days old when inoculated.

Powdery mildew culture.—Culture CR3 of *E. graminis* f. sp. *hordei* was used. It was maintained on seedlings of the barley cultivar Carlsberg II in a growth chamber at a light intensity of about 6,200 lux and a relative humidity (RH) of about 65% in 17-hr day periods alternating with 7-hr night periods at about 75% RH; the temperature was about 17 C. The conidia used for inoculation were from 7-day-old colonies; 6 hr before use the plants were shaken gently to remove old conidia.

Inoculation and incubation.—The conidia were suspended in a fluorochemical liquid (Fluorochemical FC-43 from 3M Company, Chemical Division, St. Paul, MN 55101 USA) (2); a high density (about 7.0×10^5 conidia per ml) was used unless stated otherwise. The suspension was sprayed onto the barley seedlings with an atomizer; a solenoid valve controlled by a timer maintained a constant air-pressure in the atomizer for a given period of time, usually 4.68 seconds, during which one pot with barley seedlings was rotated six times on a turntable below the nozzle (K. Mortensen, *unpublished*). Using this method conidia were deposited singly on the barley leaves. The density usually averaged 200-500 conidia per cm^2 leaf area.

The inoculated seedlings were placed in a growth chamber with optimal conditions for obtaining a high infection efficiency and synchronous development of the pathogen (18, 19, 20). For the first hour after inoculation the seedlings were kept in the dark at 17 C and 100% RH; thereafter, they were kept at 22 C, 65% RH, and a light intensity of 3,800 lux (fluorescent: 3,700 lux, incandescent: 100 lux) except for a dark period from the

TABLE 1. Germination and formation of hyphae of *Erysiphe graminis* f. sp. *hordei* on six susceptible barley cultivars; and on ten barley mutants and one spontaneous barley line with genes for resistance in the ml-o locus

Barley cultivar, mutant, or line	Conidia			
	Germinated		Forming hyphae	
	(no.)	(%)	(no.)	(%)[a]
Carlsberg II	4,143	75.3	2,209	53.32
Risø 5678	625	74.2	1	0.11
Risø 6018	779	69.9	2	0.37
Risø 7085	745	80.4	4	0.37
Risø 7372	599	71.0	1	0.12
Haisa	1,057	78.4	515	38.05
M 66	1,053	77.5	11	0.83
Vollkorn	661	79.0	470	55.17
H 3502	744	77.2	3	0.28
Foma	1,638	75.5	633	44.93
SR 1	2,326	70.5	5	0.27
SR 7	1,967	72.6	4	0.18
Malteria Heda	1,494	71.9	487	40.48
M.C. 20	1,954	73.2	9	0.50
Diamant	1,367	72.9	669	58.47
SZ 5139b	1,699	72.9	19	1.08
G.Z. 2937	1,888	76.3	11	0.68
Total, six cultivars	10,360	75.0	4,983	48.10
Total, ten mutants	12,491	73.2	59	0.45

[a]ercentage of germinated conidia. The figures in the individual experiments are corrected to conform with the average percentage of Carlsberg II in all experiments.

6th to the 20th hr after inoculation.

Necoloidine peels.—Thirty-six hr after inoculation the first true leaf of each barley seedling was dipped in 'Necoloidine' solution (Stanvis), No. 36059 (BDH Chemicals Ltd., Poole, England) and allowed to dry. The conidia, germ tubes, appressoria, and hyphae on the leaf surface became embedded in the Necoloidine film. The film was peeled off the upper leaf surface and mounted on slides in lactofuchsin [0.1 g Säurefuchin (Rubin 5), No. 7629 (E. Merck AG, Darmstadt, FRG) in 100 ml lactic acid; mixed with 100 ml Water Mounting Medium, No. 95300 (Gurr, High Wycombe, Bucks., England)], which stains the fungal protoplasm. About 1 cm^2 peel from each plant was examined under a light microscope with phase-contrast and ×125 magnification. Counts were made of the number of nongerminated conidia, the number of germinated conidia without hyphae, and the number of germinated conidia with a hypha. A conidium was regarded as germinated if the germ tube had started to form an appressorium; a hypha was considered present if it was more than 12 μm in length (approximately the width of a conidium).

The 17 barley entries examined by this technique were divided into four series: (i) Risø 5678, Risø 6018, Risø 7085, and Risø 7372; (ii) Haisa, M 66, Vollkorn, and H 3502; (iii) Foma, SR 1, SR 7, Malteria Heda, and M.C. 20; (iv) Diamant, SZ 5139b, and G.Z. 2937. Carlsberg II was included in all four series as a susceptible check. Each series comprised four pots (replications) each with one plant of each barley entry in the series. The four pots were inoculated successively. Each series was sown in two independent experiments, which thus gave eight plants per entry.

Epidermal strips.—Forty, 65, or 90 hr after inoculation epidermal strips were removed from the lower surface of the first true leaf and mounted on slides in lactofuchsin. A strip of about 1 cm^2 from each plant was examined under a light microscope with phase-contrast and ×200 or ×700 magnification. The number of germinated conidia was counted and grouped according to the presence or absence of a haustorium, and whether the primary apressorial lobe was located on an epidermal cell or on a subsidiary cell next to a guard cell. The presence of secondary haustoria was recorded when the epidermal strips were removed 65 or 90 hr after inoculation.

The 12 barley entries examined by this technique were divided into two series: (i) Risø 5678, Risø 6018, Risø 7085, Risø 7372, M 66, and H 3502; (ii) SR 1, SR 7, M.C. 20, SZ 5139b, and G.Z. 2937. Carlsberg II or Manchuria were included as susceptible checks. Each series was examined in two independent experiments. The first experiment with both series was examined 40 hr after inoculation; the second experiment in series one was examined 90 hr after inoculation, and that in series two 65 hr after inoculation. Each experiment comprised two pots (replications) each with one plant per barley entry, which thus gave four plants per entry.

Frequency of mildew colonies.—Seedlings of the 15 resistant entries examined were inoculated with the usual density of conidia, whereas Carlsberg II was inoculated with the suspension diluted to about one-tenth. The number of conidia deposited per square centimeter, barley leaf was estimated by counting the number of conidia deposited on three sets of four 15-mm-wide glass slides (simulating barley leaves) exposed during inoculation of the barley plants. The germination percentage of the conidia was determined from Necoloidine peels removed about 36 hr after inoculation from one plant of each of four barley entries. The inoculated plants were kept in a growth chamber at 17 C, 65% RH and a light intensity of 5,900 lux, except for a dark period in a moist chamber with 100% RH for the 1st hour after inoculation and a dark period from the 6th to the 20th hour after inoculation.

Twelve days after inoculation the mildew colonies (those visible with the naked eye) on both sides of the first true leaf were counted on 12 plants per entry in each of two replications. The experiment was repeated so that there were 48 plants per entry.

TABLE 2. Formation of haustoria of *Erysiphe graminis* f. sp. *hordei* in epidermal cells and subsidiary cells on one susceptible barley cultivar; and on ten barley mutants and one spontaneous barley line with genes for resistance in the ml-o locus

Barley cultivar, mutant, or line	Epidermal cells			Subsidiary cells		
	Germinated conidia (no.)	Haustoria		Germinated conidia (no.)	Haustoria	
		(no.)	(%)[a]		(no.)	(%)[a]
Carlsberg II	628	370	58.92	45	38	84.44
Risø 5678	676	2	0.30	47	16	34.04
Risø 6018	1,881	0	0	78	9	11.54
Risø 7085	777	0	0	41	16	39.02
Risø 7372	889	0	0	35	9	25.71
M 66	1,363	0	0	61	17	27.87
H 3502	1,361	2	0.15	69	4	5.80
SR 1	1,287	1	0.08	43	0	0
SR 7	1,814	0	0	84	3	3.57
M.C. 20	1,288	0	0	57	2	3.51
SZ 5139b	439	2	0.46	23	2	8.70
G.Z. 2937	1,230	9	0.73	78	17	21.79
Total, ten mutants	11,775	7	0.06	538	78	14.50

[a]Percentage of germinated conidia: overall for Carlsberg II, 60.6; for the ten mutants, 0.69.

An experiment with an increasing amount of inoculum comprised 24 plants per entry. The barley leaf area was calculated from measurements of the width and the length of the leaves.

RESULTS

Germination of conidia and formation of hypha.—The germination percentage of the conidia determined from the Necoloidine peels was 70-80% on all 17 barley entries (Table 1). Analyses of variance did not disclose statistically significant differences ($P = 0.05$) between the susceptible cultivars and the resistant mutants, or among the cultivars and among the mutants (the coefficient of variance was 6.73).

The percentage of the germinated conidia forming hyphae on Carlsberg II varied between the different series and experiments; however, the variation among the four replications within each series and experiment was very small. The percentages of conidia with hyphae (Table 1) are therefore corrected to conform with the average percentage on Carlsberg II in all the experiments, 53.32%. The percentages on the six susceptible cultivars varied; however, analyses of variance indicated no statistically significant differences ($P = 0.05$) among them (the coefficient of variance was 15.31). The percentages on the eleven resistant lines were considerably lower (Table 1); they varied from 0.11 on Risø 5678 to 1.08 on SZ 5139b. Analyses of variance were not possible in this case due to the small number of conidia that formed hyphae.

These data show that the germination of the conidia is

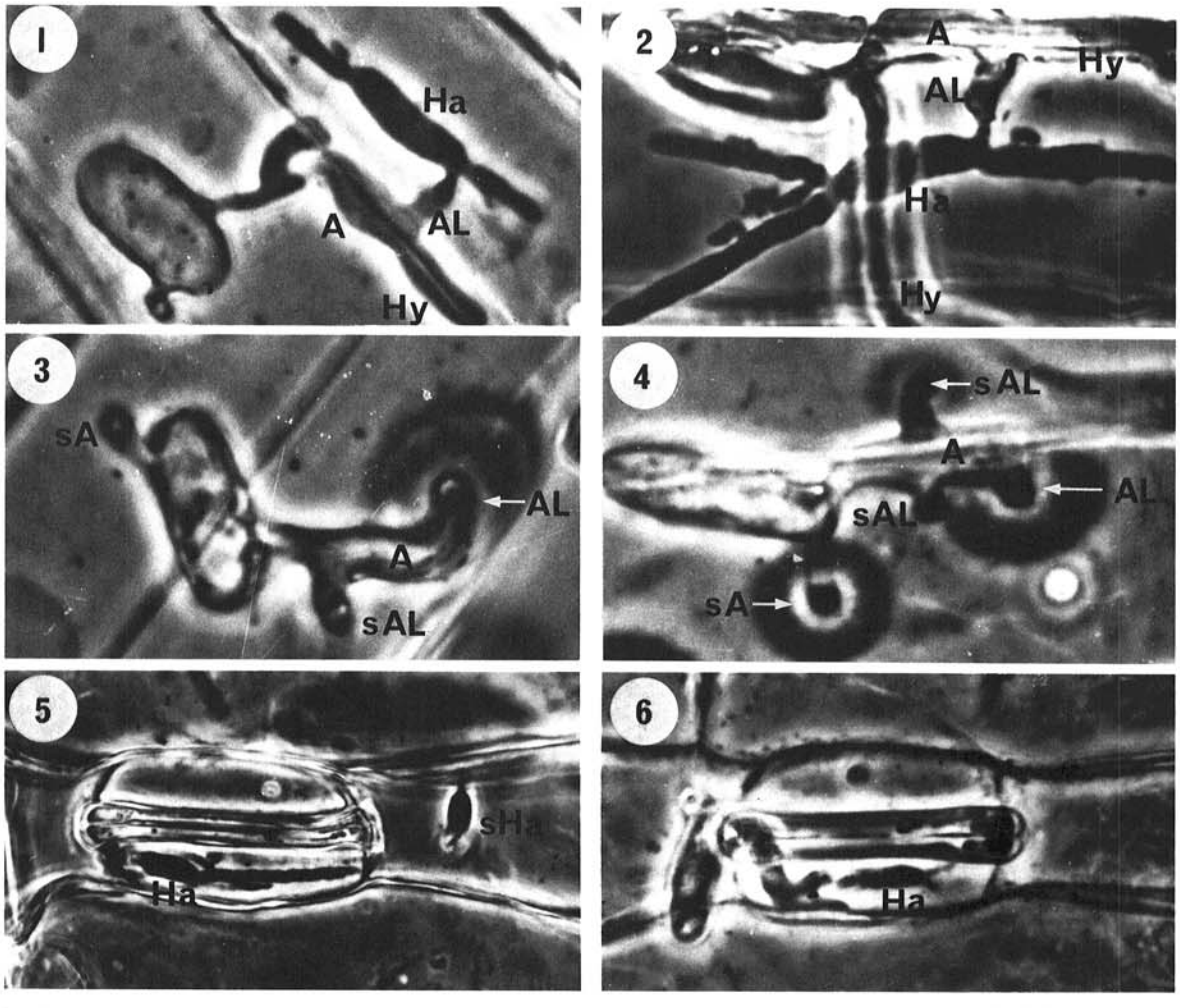

Fig. 1-6. Primary infection by *Erysiphe graminis* f. sp. *hordei* in barley. Epidermal strips stained in lactofuchsin and observed with phase-contrast optics at ×600 magnification: (1 and 2) compatible reaction of susceptible cultivar Carlsberg II at **1)** 26 hr and **2)** 36 hr after inoculation showing appressorium (A), appressorial lobe (AL), haustorium (Ha), and hyphae (Hy); (3 and 4) incompatible reaction of **3)** susceptible Carlsberg II and of **4)** resistant mutant Riso 5678 at 40 hr after inoculation showing secondary appressorial lobes (sAL), secondary appressorium (sA), and halos of "basic-staining material"; **5)** haustorium in subsidiary cell and secondary haustorium (sHa) in epidermal cell of resistant barley line G.Z. 2937 at 65 hr; and **6)** haustorium in subsidiary cell of Carlsberg II at 40 hr.

independent of the host genotype, and that the frequency of formation of hyphae on the six susceptible cultivars is not drastically influenced by the host genotype. The 11 resistance genes reduced the frequency of conidia with hyphae to about 1/100 of that of the cultivars.

Formation of haustoria.—Of the total of 14,294 germinated conidia counted on the epidermal strips, 4.6% had the primary appressorial lobe located on subsidiary cells in which the frequency of haustorium formation was higher than in other epidermal cells (Table 2). This was much more pronounced on the 11 resistant entries than on Carlsberg II; i.e., the resistance genes reduced the frequency of haustoria to about 1/1,000 and 1/6 of that on Carlsberg II in normal epidermal cells and in subsidiary cells, respectively. Due to the small number of haustoria observed in the 11 resistant entries, possible differences among them could not be detected.

The overall percentage of conidia that formed haustoria on Carlsberg II was 60.6; that on the ten mutants averaged, 0.69, was about 1/100 of this value. This ratio as well as the actual percentages are in agreement with the figures for formation of hyphae, namely 53.3 and 0.45% (Table 1) for Carlsberg II and the ten mutants averaged, respectively. However, the data in Table 2 show that the resistance conditioned by the *ml-o* genes is much more efficient in epidermal cells than in subsidiary cells.

Secondary haustoria developed on Carlsberg II from 84% of the conidia with primary haustoria at 65 or 90 hr after inoculation. The percentage on the ten resistant mutants averaged was as high as 36%; this frequency is about 50 times higher than that for the formation of primary haustoria. Further, the secondary haustoria on the mutants were almost exclusively formed in epidermal cells adjacent to a subsidiary cell in which the conidium had formed the primary haustorium. This indicates that when a compatible relationship is established between a mutant host cell and the pathogen, the adjacent host cells become less resistant.

Morphology.—The 50 to 60% of the germinated conidia that established a compatible relationship with the susceptible Carlsberg II germinated with a narrow germ tube. It developed further in a broadened appressorium near the tip of which an appressorial lobe was formed. An infection peg below the appressorial lobe penetrated the host cell wall and a haustorium developed within the host cell. Subsequently, a hypha was formed on the tip of the appressorium, and usually a second near the base of the appressorium (Fig. 1 and 2). The remaining 40 to 50% of the germinated conidia, which did not establish a compatible relationship with Carlsberg II, failed below the primary appressorial lobe. They formed one and occasionally two secondary appressorial lobes on the side of the germ tube, and usually a new germ tube with a very small appressorium (secondary appressorium) from the conidium (Fig. 3). A haustorium rarely formed from a secondary appressorial lobe. A stained spot appeared below the unsuccessful appressorial lobe; the spot increased in diameter and formed a halo. The stained material observed was apparently "basic staining material" (3, 17). Basic staining material was never seen at appressorial lobes below which haustoria were formed. The host cells below the unsuccessful appressorial lobes appeared to be undamaged because we observed occasional host cells with unsuccessful appressorial lobes and with fully developed haustoria that had originated from other conidia. The morphology of the compatible and the incompatible reactions of the other five susceptible cultivars examined at a later date was similar to that of Carlsberg II.

The 99.3 to 99.5% of the conidia that were unable to establish a compatible relationship with the resistant

TABLE 3. Colonies of *Erysiphe graminis* f. sp. *hordei* that developed on one susceptible barley cultivar; and on ten barley mutants, one spontaneous barley line and four backcross populations with resistance in the ml-o locus

Barley cultivar, mutant, line, or backcross populations	Number of colonies[z]	
	Total observed	Per 100 germinated conidia
Carlsberg II	10,057	42.33
Risø 5678	384	0.11 a
Risø 6018	308	0.09 a
Risø 7085	422	0.12 a
Risø 7372	523	0.14 a
M 66	276	0.08 a
H 3502	248	0.06 a
SR 1	1,308	0.35 b
SR 7	1,591	0.38 b
M.C. 20	2,064	0.52 bc
SZ 5139b	1,997	0.58 cd
G.Z. 2937	2,815	0.85 e
Risø 5678/3* Carlsberg II (R)	265	0.07 a
M 66/3* Carlsberg II (R)	410	0.11 a
SR 1/3* Carlsberg II (R)	413	0.11 a
G.Z. 2937/1* Carlsberg II (R)	2,285	0.66 d
Total, ten mutants	9,121	0.24

[z]Values followed by different letters are significantly different ($P < 0.05$) according to Duncan's new multiple range test.

barley mutants developed secondary appressorial lobes, and halos of basic staining material were formed (Fig. 4). The morphology of the incompatible reaction was identical in the 11 resistant entries and was similar to that of the susceptible cultivars. When a haustorium was formed in a subsidiary cell we frequently observed a secondary haustorium in a neighboring epidermal cell (Fig. 5). The morphology of the compatible relationship established in the subsidiary cells was the same in the 11 resistant entries and similar to that of susceptible cultivars (Fig. 6).

Frequency of mildew colonies.—The percentage germination of the conidia in this experiment was 64.3 averaged. The total number of germinated conidia estimated per square centimeter of leaf area was 33 for Carlsberg II (Table 3), and 508 for the 15 resistant entries.

About 42% of the germinated conidia formed colonies on Carlsberg II; this corresponds to the expected figure based on percentage conidia forming haustoria (61%, Table 2) or hypha (53%, Table 1) multiplied by percentage conidia forming secondary haustoria (84%). Only about 0.24% of the germinated conidia formed colonies on the ten mutants averaged; this is close to the expected figure based on $0.69 \times 0.36 = 0.25$ or $0.45 \times 0.36 = 0.16$. This figure is roughly 1/200 of that for Carlsberg II.

The percentage of conidia forming colonies varied from 0.06 on H 3502 to 0.58 on SZ 5139b (Table 3). The ten mutants could be categorized into two major groups in this respect. One group that comprised six mutants had percentages around 0.1; the second group that comprised four mutants had percentages from 0.35 to 0.58. The differences among the ten mutants were statistically significant ($P = 0.001$) and Duncan's new multiple range test verified the grouping (Table 3). Further, the spontaneously-resistant line, G.Z. 2937, formed colonies from 0.85% of the germinated conidia, which is significantly higher than the percentages for any of the induced mutants. These data may suggest that some of the *ml-o* resistance genes condition quantitatively different infection types. On the other hand, the data from the resistant lines derived from crosses do not support a conclusion of this nature. The low frequency of mildew colonies on the two mutants, Risø 5678 and M 66, did not change in frequency after crossings with Carlsberg II, whereas SR 1 and G.Z. 2937, each with a high frequency of colonies, showed a distinct reduction in frequency when crossed with Carlsberg II three times and once, respectively (Table 3). These figures show that the genetic background of the resistance genes has the decisive influence on the frequency of mildew colonies.

The relationship between the number of conidia deposited on the leaves and the subsequent number of mildew colonies was examined in a separate experiment. The mutants Risø 5678, M 66, and SR 1 were inoculated with a suspension having 5.2×10^5 conidia/ml in 3.12, 4.68, 6.24, and 7.80 sec, corresponding to 4, 6, 8, and 10 rotations of the turntable. Carlsberg II was inoculated in 1.56 and 3.12 sec with a suspension of 5.3×10^4 conidia/ml. The average germination percentage of the conidia was 82.5. The results (Table 4) show a positive and approximately linear relationship between number of germinated conidia and number of mildew colonies. The data for the three mutants are pooled because they showed the same relationship. As expected, SR 1 had a higher number of colonies per plant than the other two mutants. The average number of colonies developed per 100 germinated conidia was 0.12, not very far from that found previously (Table 3). These figures also show that mutant seedlings may erroneously be classified as susceptible when very large numbers of conidia are applied.

Virulence of Erysiphe graminis f. sp. hordei isolated from the occasional colonies on the resistant barley entries.—Over several years a total of 45 single-colony isolates were removed from seven of the resistant entries following inoculation with: a Danish powdery mildew population (9 colonies), culture A_6 (290) from Sweden (18 colonies), cultures 59.2 and 59.21 from Canada (11 colonies), culture 59.11 from USA (four colonies), and culture 67.62, which is a hybrid (21) between CR3 from USA, and 63.5 from Japan (three colonies). Conidia from each colony were transferred to seedlings of Carlsberg II or Manchuria. When these plants were heavily mildewed they were shaken over pots with seedlings of the resistant entry (from which the colony had originated initially), and of Carlsberg II or Manchuria. Ten of the isolates were used to inoculate seedlings of the ten mutants and G.Z. 2937 used in this study. Twelve to 16 days after inoculation the infection type was read and the number of colonies counted. In all 45 cases the susceptible cultivar had infection type 4 and produced 100 or more colonies on the first true leaf, whereas the resistant entries had infection type 0/(4) and produced no more than 10, usually less than five, colonies per leaf. In a few cases we attempted to maintain single-colony isolates on resistant plants by secondary infections from conidia produced in the occasional primary colonies, but without success. These results confirm earlier observations (10, 27) that the occasional colonies on the resistant entries do not represent isolates with an increased virulence.

TABLE 4. Number of colonies of *Erysiphe graminis* f. sp. *hordei* that developed on one susceptible cultivar; and on three barley mutants (Risø 5678, M 66, and SR 1) with genes for resistance in the ml-o locus inoculated with increasing numbers of conidia

Barley cultivar and mutants	Germinated conidia per cm^2	Colonies per plant
Carlsberg II	19.0	58.04
	33.4	99.21
Three mutants	82.8	0.68
	288.4	3.39
	433.9	9.49
	702.2	16.11

DISCUSSION

The present experiments were done under controlled conditions in growth chambers and according to the prescriptions for obtaining a high infection efficiency and synchronous development of the pathogen. In spite of these precautions we obtained lower percentages of germinated conidia, and of formation of haustoria and hyphae than desired (4), and greater variation in the

percentage between different experiments. The variation within each inoculation date was small, however, and comparisons of the data obtained with the different techniques were in good agreement.

A number of possible sources of variation or error can be excluded. Culture CR3 of *E. graminis* f. sp. *hordei* was checked for purity before and after the experiments by inoculating 10-15 seedlings of each of eight differential cultivars of barley with different resistance genes effective against CR3. In the first test, CR3 was pure; in the last test, we observed three colonies on the resistant differential cultivars. This suggests a frequency of contaminants virulent on one or more of the differential varieties of less than about 0.03%. The virulence of the three contaminants was not tested on the ml-o resistant barleys. It is most unlikely that any of the three random contaminants should be virulent on the ml-o resistant barleys because ml-o virulence has not been found in extensive tests comprising more than 100 individual pathogen cultures and field populations at more than 78 locations in 29 countries (6, 11, 16, 24, 28). The barley seed samples of each entry were from single, progeny-tested plants that had originated from a single plant; an exception was G.Z. 2937 that originated from a non-purified bulk sample. The very few admixtures or outcrosses found were discarded. The Necoloidine peels were taken from the upper surface of the barley leaves, whereas the epidermal strips were taken from the lower surface. This was done to ease handling and because prior observations revealed no differences between the two surfaces. We also checked whether the fluorochemical suspension medium could have any harmful effect; the germination percentage of the CR3 conidia and the formation of hyphae on Carlsberg II were the same in parallel inoculations made with conidia in a spore settling-tower and in a suspension.

In order to relate the present results to those obtained elsewhere, some experiments included the barley cultivar Manchuria, a susceptible check extensively used by others (3, 17, 19, 20, 22, 30). The results with Manchuria were identical to those with Carlsberg II. Further, culture 290 of race A_6, used extensively in Scandinavia (10, 11, 14, 27, 28, 29), gave results similar to those obtained with CR3.

The results in the present experiments are in agreement with earlier findings (30) of a lack of influence of the host genotype on the germination capacity of the conidia (Table 1). The host genotype affects the primary infection about 12-16 hr after inoculation when the infection peg has penetrated or nearly penetrated the host cell wall (1). It appears that the resistant reaction conferred by the *ml-o* genes is triggered when the pathogen is in the process of penetrating the host cell wall or comes into contact with the host plasmalemma. Further, the reaction is apparently established rapidly, because young haustoria were never observed below the unsuccessful appressorial lobes. The resistance is confined to normal epidermal cells in which it is highly efficient; the subsidiary cells are less resistant (or more susceptible) than other epidermal cells, even in susceptible cultivars (Table 2) (1, 17). The development of a haustorium in a cell of a ml-o resistant host affected the neighboring cells so that they became more frequently invaded by the pathogen. This phenomenon is probably analogous to the increased susceptibility of a non-host plant inoculated with a compatible pathogen before inoculation with a normally incompatible pathogen (25). The phenomenon could also be explained by assuming that the parasite unit, which has a compatible relationship with the ml-o resistant host (the primary haustorium), has an increased ability to overcome the resistance of the epidermal cell.

In spite of the highly efficient resistance of the normal epidermal cells, the ml-o resistant barleys can carry a number of colonies (Table 4) that must originate almost exclusively from primary infections in subsidiary cells. The number of colonies is, however, only about 1/200 of that developed on a susceptible cultivar such as Carlsberg II. Observations in greenhouses and the field (J. Helms Jørgensen, *unpublished)* have shown that the *ml-o* resistance genes are able to protect the plants from a powdery mildew epidemic.

The 11 *ml-o* resistance genes studied are known (i) to be noncomplementing alleles in one locus; (ii) to condition the same, unique infection type; and (iii) to confer the same, world-wide spectrum of resistance to *E. graminis* f. sp. *hordei*. The present study has shown (iv) that they affect the primary development of the pathogen at the same stage and with the same intensity, as predicted by Ellingboe (4) and Ellingboe and Slesinski (5). This may suggest that the 11 genes are identical. However, this is not the case, primarily because a recent recombination-study (14, and J. Helms Jorgensen, *unpublished*) has shown that some of the mutants have mutated in different sites within the ml-o locus; secondarily, because general experience with similar genetic situations in microorganisms (noncomplementing alleles of different origin) indicates that the alleles usually differ quantitatively in their function. Our inability to demonstrate functional differences among the 11 *ml-o* resistance genes may be ascribed to insufficient analytical methods.

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