# Infection Kinetics of *Erysiphe graminis* f. sp. *hordei* on Barley with Different Alleles at the *Ml-a* Locus

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## ABSTRACT

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The infection kinetics were determined for race CR3 of *Erysiphe graminis* f. sp. *hordei* on six sets of isolines of barley. Lines with genes *Ml-a, Ml-a6*, or *Ml-a13* gave a final infection type 0 whereas lines with genes *Ml-a7(Mu), Ml-a7(LG)*, or *Ml-a10* gave final infection types of 1–2 or 2. The percent elongating secondary hyphae (ESH) ranged from 8 to 21 at 28 hr after inoculation. A significant difference in the percent ESH was found between three groups of genes: (*Ml-a, Ml-a6, Ml-a13*), *Ml-a7(Mu)*, and [*Ml-a10, Ml-a7(LG)*]. A significant difference was also found in the rate of development of ESH between *Ml-a7(LG)* and the other five genes examined, suggesting a difference in specificity. It is proposed that the gene in cultivar Long Glumes at the *Ml-a* locus conferring resistance to race CR3 of *E. graminis* f. sp. *hordei* be designated *Ml-a15*.

Genes in plants for resistance to pathogens are commonly identified by the reactions produced when the plants are inoculated with a set of races of the pathogen. Final infection type is the usual criterion for evaluation of interactions. It has also been possible to determine the effects of host and parasite genes during the first few hours of interaction of *Erysiphe graminis* f. sp. *hordei* with barley (*Hordeum vulgare*) (5,9). It has been possible to study the effects of the host and parasite genes on early interactions because procedures have been developed to get high infection efficiency and synchronized development of the pathogen during the early stages of pathogenesis (4). The infection kinetics of *E. graminis* represent a very sensitive assay of the effects of host and parasite genes on the development of the parasite and the response of the host.

A number of the *Ml* genes in barley for resistance to *E. graminis* f. sp. *hordei* appear to be alleles at the *Ml-a* locus (2). If a gene for resistance affects a distinct stage in the ontogeny of the interactions between host and parasite, one may expect an allele of that gene to affect the same stage of the interactions. Different alleles would be expected to produce the same gene product, although their products would have different specificities. The different specificities may account for the intensity of the interaction, but the time of expression should be the same (1). If the different alleles at the *Ml-a* locus are separate but closely linked cistrons, the different genes might be expected to affect unique stages in the ontogeny of interactions.

The objective of the research reported here was to determine the effect of six *Ml-a* "alleles" on the process of primary infection and final infection type.

## MATERIALS AND METHODS

The six isogenic paired barley lines and barley cultivar Manchuria (Table 1) were obtained from J. G. Moseman (6). The paired lines were developed by crossing each of six resistant lines with Manchuria, backcrossing the resistant progeny to Manchuria for three generations, selfing the heterozygotes for 13–15 generations, and selecting homozygous-susceptible and homozygous-resistant lines for increase and use. Cultivar Manchuria was used as the susceptible control. The designation for each line is given in Table 1.

Culture CR3 of *Erysiphe graminis* DC. Mérat f. sp. *hordei* is avirulent on each of six host lines with dominant *Ml-a* genes. CR3, therefore, is considered to have the corresponding *P* gene for each of the six *Ml* genes. Culture CR3 was propagated as described previously (5).

Individual seedlings 5–6 days old were inoculated by the rolling method (8). A reasonably uniform distribution of approximately 100–200 single conidia per cm length of leaf was obtained. Following inoculation, the plants were kept under environmental conditions that favor high infection efficiencies (70% on Manchuria) and give reasonably synchronous development of the parasite (9). At a given hour after inoculation, a 1-cm section of a leaf was examined under a light microscope (×250). Parasite units with secondary hyphae longer than 10 μm were classified as elongating secondary hyphae (ESH). The percentage of ESH was recorded. The percent ESH was used as a measure of infection efficiency since it has been shown that the formation of ESH is dependent on the establishment of haustoria in the host epidermal cells (4). Each determination was replicated at least four times with at least 80–100 spores observed in each determination.

Data for 24, 26, and 28 hr after inoculation were analyzed in a three-way mixed model analysis of variance (fixed lines and times, random replicates) for lines with dominant *Ml-a* genes. Time effects were analyzed by comparing slopes of regression lines of percent ESH on time. Data were compared by using Tukey's HSD test (Table 2).

TABLE 1. Six pairs of near isogenic barley lines and their reaction to culture CR3 of *Erysiphe graminis* f. sp. *hordei*[a]

| Isogenic lines | C.I. no.[b] | Gene conditioning reaction to CR3 | Infection type with CR3 |
|---|---|---|---|
| Algerian/4* (F14) Man. (R) | 16137 | *Ml-a* | 0 |
| Algerian/4* (F14) Man. (S) | 16138 | *ml-a* | 4 |
| Franger/4* (F15) Man. (R) | 16151 | *Ml-a6* | 0 |
| Franger/4* (F15) Man. (S) | 16152 | *ml-a6* | 4 |
| Durani/4* (F13) Man. (R) | 16149 | *Ml-a10* | 2 |
| Durani/4* (F13) Man. (S) | 16150 | *ml-a10* | 4 |
| Multan/4* (F15) Man. (R) | 16147 | *Ml-a7(Mu)* | 1–2 |
| Multan/4* (F15) Man. (S) | 16148 | *ml-a7(Mu)* | 4 |
| Long Glumes/4* (F15) Man. (R) | 16153 | *Ml-a7(LG)* | 1–2 |
| Long Glumes/4* (F15) Man. (S) | 16154 | *ml-a7(LG)* | 4 |
| Rupee/4* (F13) Man. (R) | 16155 | *Ml-a13* | 0 |
| Rupee/4* (F13) Man. (S) | 16156 | *ml-a13* | 4 |
| Manchuria | 2330 | *ml* | 4 |

[a] Table adapted from Moseman (6).
[b] C.I. = Cereal Introduction.

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## RESULTS

Barley lines with *Ml-a, Ml-a6*, or *Ml-a13* all gave an infection type 0 reaction following inoculation with CR3 (Table 1). The three lines with *Ml-a10, Ml-a7(Mu)*, or *Ml-a7(LG)* each gave an infection type 2 or 1–2. The infection type 0 signifies no macroscopically visible pathogen development on the leaf surface. The infection type 1–2 signifies a very small amount of macroscopically visible mycelial development. Infection type 2 signifies a moderate amount of macroscopically visible mycelial development with slight sporulation. Infection type 1–2 and 2 are both accompanied by visible host cell necrosis at the infection site, ie, a hypersensitive reaction. All of the host lines with the recessive *ml* genes gave an infection type 4, and were indistinguishable. Infection type 4 is characterized by abundant mycelial development and strong sporulation by the pathogen.

The kinetics of the formation of elongating secondary hyphae (ESH) on each of the seven susceptible lines with the recessive *ml* genes are given in Fig. 1. There were no significant differences among the seven host lines. The kinetics of the formation of ESH on each of the six resistant host lines with the dominant *Ml* genes are given in Fig. 2. All six *Ml* genes significantly reduced the percent ESH with respect to the susceptible member of each isoline pair (Figs. 1 and 2). The three host lines with genes *Ml-a, Ml-a6*, or *Ml-a13* gave lower percentages of ESH than the host lines with genes *Ml-a10, Ml-a7(Mu)*, or *Ml-a7(LG)* (Table 2). The first three had infection types 0, the latter had infection types 2 or 1–2.

There was a significant difference in the slope of the regression for host lines with *Ml-a7(LG)* and *Ml-a7(Mu)* during the first 28 hr after inoculation (Fig. 2). A maximum of 13% ESH was obtained on the host line with *Ml-a7(Mu)*. This was significantly different from a maximum of 21% ESH which was obtained on the host lines with *Ml-a7(LG)*. In an additional experiment, the percent ESH was recorded from 24 to 36 hr after inoculation (Fig. 3). An analysis of variance with a Student's *t* test for data collected at 28, 32, and 36 hr after inoculation showed a significant ($P<0.001$) difference between *Ml-a7(Mu)* and *Ml-a7(LG)* in percentages of ESH with race CR3 of *E. graminis* f. sp. *hordei*.

On all six *Ml* alleles, there was collapse of some parasite units approximately 24–26 hr after inoculation. The percent that collapsed seems to be related to the particular *Ml* allele.

## DISCUSSION

Approximately 30 different genes conferring resistance to *E. graminis* f. sp. *hordei* have been mapped to the *Ml-a* locus on chromosome 5 in barley (2). Thus far, 14 different alleles (or closely linked genes) have been designated *Ml-a* through *Ml-a14* based on differential reactions to many strains of *E. graminis* f. sp. *hordei*. If the six alleles used in this study were true alleles, we would expect that they all would affect the same stage in the ontogeny of the interaction (1). The different levels of infection efficiency may be attributed to modifications of the gene product specified by the different alleles. The results of the infection kinetics are consistent with this hypothesis, though they do not exclude the possibility that the six lines may each have a gene in a unique cistron. For example, lines with *Ml-a6* and *Ml-a13* display identical phenotypes during primary infection by *E. graminis* f. sp. *hordei* (Fig. 2). However, recent data on recombination in this region suggests that *Ml-a6* and *Ml-a13* are in separate cistrons (R. Wise, *unpublished*). The fact that there are three different final infection types suggests that there may be three cistrons, though previous results have suggested that a single host gene can condition different phenotypes with different strains of a pathogen (3).

All six *Ml* alleles affect the formation of ESH. Collapse of some

TABLE 2. Percent elongating secondary hyphae (ESH) of *Erysiphe graminis* f. sp. *hordei* race CR3 on six resistant isolines of barley cultivar Manchuria

| Barley isoline | *Ml-a13* | *Ml-a6* | *Ml-a* | *Ml-a7(Mu)* | *Ml-a10* | *Ml-a7(LG)*[e] |
|---|---|---|---|---|---|---|
| % ESH[a] | 7.5 | 7.8 | 9.7 | 13.2 | 15.7 | 16.1 |
| *P*[b] | | | | | | |
| 0.05[c] | ——— | | | | ——— | |
| 0.01[d] | ——— | | | ——— | ——— | |

[a] Mean percentage of ESH from 12 replications for 24, 26, and 28 hr after inoculation.

[b] Columns connected by the same line are not significantly different from each other at the indicated level of probability according to Tukey's HSD test.

[c] Minimum significant difference = 2.24, $P = 0.05$.

[d] Minimum significant difference = 2.72, $P = 0.01$.

[e] The slope of the regression for lines with *Ml-a7(LG)* was significantly different from the other five from 24 to 28 hr after inoculation as determined by an *f* test of slope ($P<0.001$). The slope of the regression for lines with *Ml-a13, Ml-a6, Ml-a, Ml-a7(Mu)*, and *Ml-a10* was not significantly different from zero ($P<0.05$).

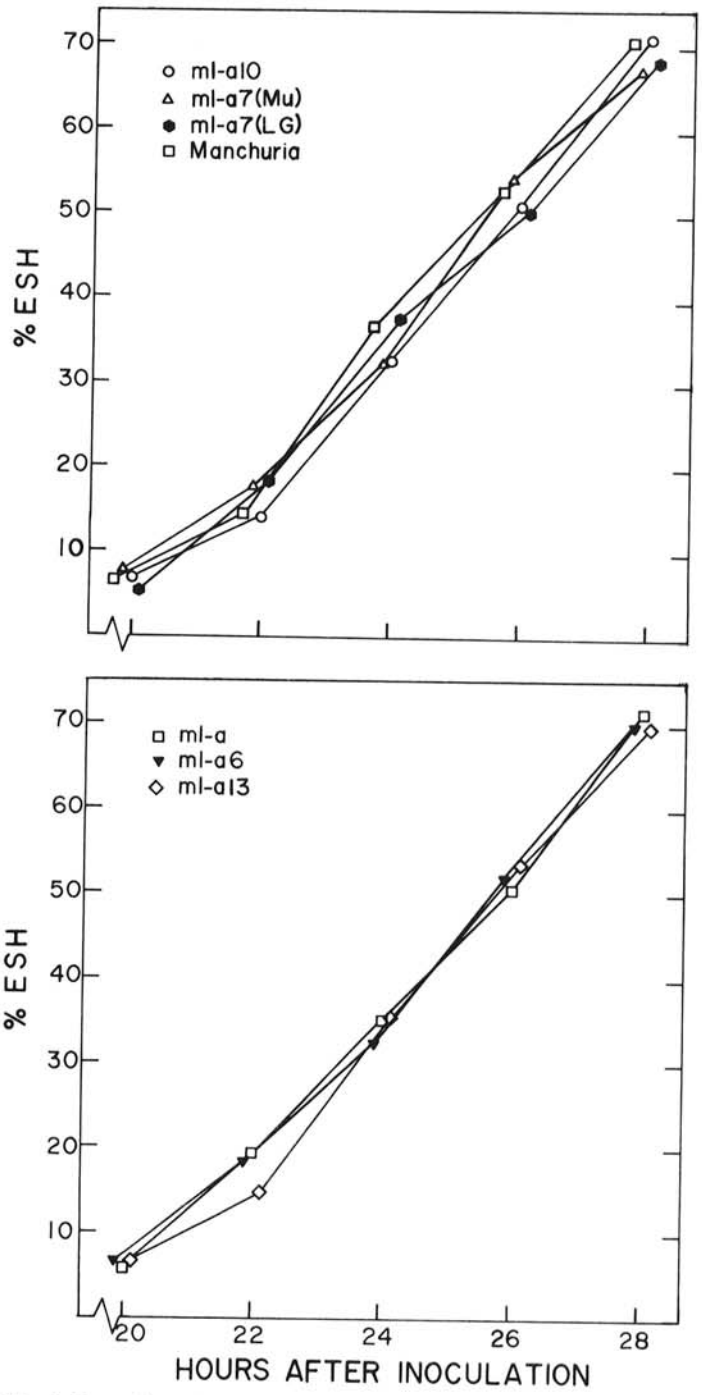


**Fig. 1.** Formation of elongating secondary hyphae (ESH) on susceptible isolines of barley by *E. graminis* f. sp. *hordei* race CR3.

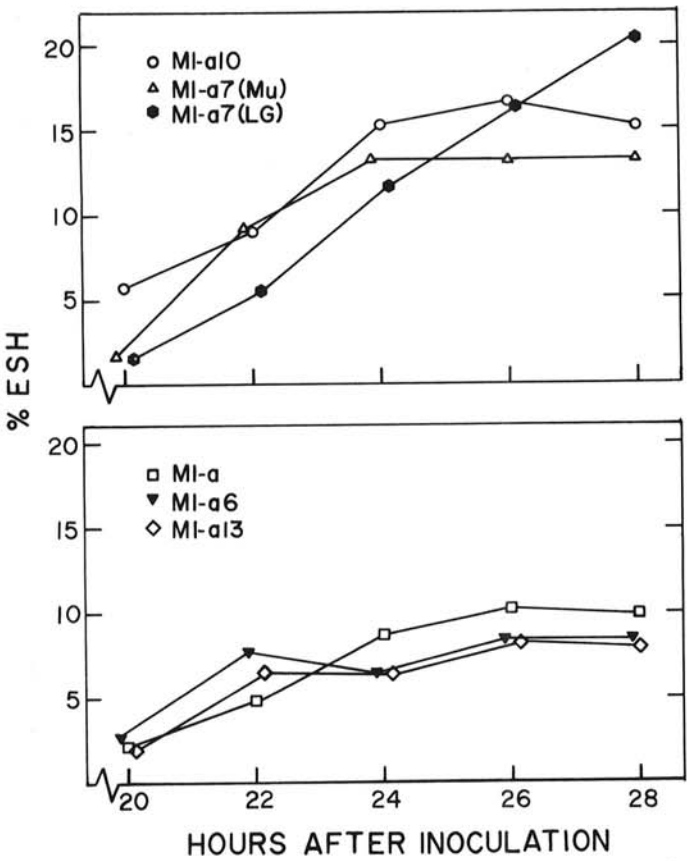


**Fig. 2.** Formation of elongating secondary hyphae (ESH) by *E. graminis* f. sp. *hordei* race CR3 on near isogenic barley lines that possess different genes at the *Ml-a* locus. The standard error for 24, 26, and 28 hr after inoculation is 0.91. The slope of *Ml-a7(LG)* was significantly different from the other five from 24 to 28 hr after inoculation as determined by an *f* test of slope ($P < 0.001$).

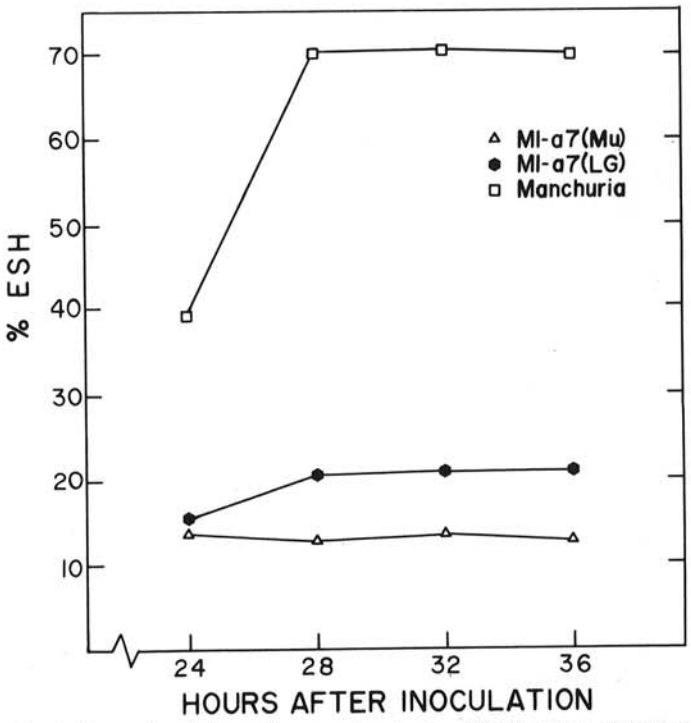


**Fig. 3.** Formation of elongating secondary hyphae (ESH) on isogenic barley lines *Ml-a7(Mu)* and *Ml-a7(LG)*. The standard error for 28, 32, and 36 hr after inoculation is 0.78.

parasite units occurs at approximately the same time. Based on the pattern of development of ESH, there appears to be four groups of genes based on how they affect primary infection (Table 2). A significant difference in the percent ESH was found between the three groups of genes: (*Ml-a, Ml-a6, Ml-a13*), *Ml-a7(Mu)*, and [*Ml-a10, Ml-a7(LG)*]. In addition, a significant difference was found in the rate of development of ESH between *Ml-a7(LG)* and the other five. The different levels of compatibility may be attributed to the interaction of each specific host/parasite gene pair since each of the host lines with recessive *ml* genes had kinetics indistinguishable from Manchuria. It was interesting to find a significant difference between the degree of compatibility of *Ml-a7(Mu)* and *Ml-a7(LG)* with *E. graminis* f. sp. *hordei*. Moseman and Jørgensen (7) suggested that these two genes may be identical, based on the final infection type determined by using differential races of *E. graminis* f. sp. *hordei*. In addition, tests with about 65 different isolates of *E. graminis* f. sp. *hordei* from Europe, North America, Japan, and Israel have shown that alleles in 'Long Glumes' and 'Multan' are susceptible only to cultures virulent on *Ml-a7* (J. H. Jørgensen, *personal communication*). Although final infection type is satisfactory for differentiating gross qualitative differences, it is not very useful for determining when incompatibility is expressed during the early stages of disease development.

The primary infection kinetics of *E. graminis* is a quantitative method of determining the degree of compatibility in the interaction between host and parasite. By using this method, we have demonstrated a repeatable difference in compatibility in the isolines *Ml-a7(Mu)* and *Ml-a7(LG)* with race CR3 of *E. graminis* f. sp. *hordei*. The specificity of the interaction between host and parasite is presumably determined by the product of the host gene and the product of the parasite gene and hence their respective nucleotide sequences. A different specificity most likely reflects a different nucleotide sequence. Recent experiments at Risø National Laboratory have tentatively revealed an additional resistance gene in the Multan isoline with a complementary avirulence gene in CR3 (J. H. Jørgensen, *personal communication*). If the primary resistance gene in isolines *Ml-a7(Mu)* and *Ml-a7(LG)* is *Ml-a7*, then the lower infection efficiency observed on *Ml-a7(Mu)* compared to *Ml-a7(LG)* may be due to a synergistic effect of the gene at the *Ml-a* locus and a second gene in *Ml-a7(Mu)* due to their interaction with (possibly) two complementary avirulence genes in CR3. Alternatively, the difference observed between *Ml-a7(Mu)* and *Ml-a7(LG)* may represent two different alleles at the *Ml-a* gene complex. To our knowledge, these two genes have not been differentiated by recombination or by different races of *E. graminis* f. sp. *hordei* (2,7). Based on the evidence presented here, we feel that a distinction is warranted and suggest the gene from cultivar Long Glumes at the *Ml-a* locus tentatively be designated *Ml-a15*.

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