Elongation of Secondary Hyphae and Transfer of ^{35}S from Barley to Erysiphe graminis f. sp. hordei during Primary Infection

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ABSTRACT

Over 70% of spores of *Erysiphe graminis* f. sp. *hordei* inoculated onto barley leaves develop secondary hyphae longer than 5 μ by 30 hr after inoculation with compatible parasite/host genotypes. The percentage is reduced with incompatible parasite/host genotypes, the actual percentage being dependent on the particular gene pair that specifies incompatibility.

^{35}S transfer from host to parasite also has been studied with compatible and incompatible parasite/host genotypes. The compatible parasite/host genotypes which had a high efficiency of infection also had high rates of ^{35}S transfer from host to parasite. The incompatible parasite/host genotypes which had low efficiencies of infection also had reduced rates of ^{35}S transfer from host to parasite.

The three parasite/host genotypes in the quadratic check that specify compatibility by the criterion of final infection type show similar kinetics of ^{35}S transfer from host to parasite during primary infection.

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Successful primary infection of barley leaves by the powdery mildew fungus, *Erysiphe graminis* (DC.) Mérat *hordei* Em. Marchal, is judged to occur when haustoria are formed in the epidermal cells of the host and when elongating secondary hyphae, capable of initiating secondary infections, are formed on the leaf surface (3, 4). Over 70% of the spores applied to leaves germinated and resulted in successful infections, provided (i) the environmental conditions were favorable and (ii) the parasite/host genotype was compatible (host-susceptible or pathogen-virulent). The percentage of successful primary infections was reduced, however, with incompatible parasite/host genotypes (3, 4, 5, 9, 10). The percentage of successful infections which develop from the spores applied to leaves is therefore dependent on the particular parasite/host genotype.

The transfer of ^{35}S and ^{32}P from wheat to the fungal structures of *E. graminis* f. sp. *tritici* on the surface of the leaf during primary infection is dependent on the stage of development of the parasite (6) and on the parasite/host genotype (11). The rate of transfer increased after full-sized haustorial bodies were formed and secondary hyphae began to form (6). The rate of ^{35}S transfer with incompatible parasite/host genotypes was lower than with compatible genotypes, but not perfectly consistent with infection efficiency (11).

There are four possible parasite/host genotypes (*Px/Rx, Px/rx, px/Rx,* and *px/rx*) from a single pair of corresponding genes. Only *Px/Rx* specifies incompatibility between host and parasite. *Px/rx, px/Rx,* and *px/rx* all specify compatibility by the criteria of final infection type and morphological development of the parasite during primary infection. With the wheat mildew, the three genotypes, *p1/Pm1, P1/pm1,* and *p1/pm1*, were not identical by the criterion of rate of ^{35}S transfer from host to parasite during primary infection. This is an important observation, because it bears on the question of whether specific interactions occur for parasite/host compatibility or incompatibility.

Our objectives were to (i) determine the effects of environment and parasite/host genotypes on the length of secondary hyphae at various hours after inoculation; (ii) determine the effect of four incompatible parasite/host genotypes on the rates of ^{35}S transfer during primary infection; and (iii) complete the quadratic check (8) for one gene pair using the criteria of final infection type, morphological development of the parasite during primary infection, and ^{35}S transfer from host to parasite during primary infection.

MATERIALS AND METHODS.—Two cultures of *E. graminis* f. sp. *hordei* with genotypes *Pa Pg Pk Pp* (CR3) and *Pa pg Pk Pp* (MSB-70-4) were maintained on barley cultivars Manchuria and Goldfoil, respectively. Environmental conditions for maintenance of cultures and procedures for the production of inocula have been described previously (3, 4, 5). Controlled inoculations made with the rolling method (7) were used for studies of morphological development. Inoculations for studies of ^{35}S transfer were made by dusting conidia onto plants (11).

Five homozygous lines of barley used were designated as follows (1, 2):

$$\frac{mla\ mlg\ mlk\ mlp}{mla\ mlg\ mlk\ mlp}\ (\text{Manchuria or } ml);\quad \frac{Mla\ mlg\ mlk\ mlp}{Mla\ mlg\ mlk\ mlp}\ (Mla);$$

$$\frac{mla\ Mlg\ mlk\ mlp}{mla\ Mlg\ mlk\ mlp}\ (Mlg);\quad \frac{mla\ mlg\ Mlk\ mlp}{mla\ mlg\ Mlk\ mlp}\ (Mlk);$$

$$\text{and } \frac{mla\ mlg\ mlk\ Mlp}{mla\ mlg\ mlk\ Mlp}\ (Mlp).$$

Inoculated plants were maintained under conditions which allow primary infection efficiencies of 80% for *E. graminis* f. sp. *tritici* and 70% for *E. graminis* f. sp. *hordei* (3, 4).

The lengths of secondary hyphae were determined at various hours after inoculation by direct microscopic measurements of all single, isolated parasite units on a 1-cm long section of inoculated leaf. The 1-cm tip portion of the leaf was not used. It is known from previous experiments that parasite units which do not produce haustoria are rarely capable of producing secondary hyphae longer than 5 μ. It is generally considered that secondary hyphae longer than 5 μ indicate successful production of haustoria and the establishment of a compatible functional relationship (4).

The data are presented as per cent of the total number of conidia applied to the plant which attained a particular stage of development at each time of observation. Each experiment was repeated at least 6 times, and ca. 200 parasite units were counted on a single leaf at each observation.

The procedure (11) for determining the rate of ^{35}S transfer from host to parasite was as follows: At various hours after inoculation, 5-day-old barley seedlings were cut at the crown with a razor blade (which had a film of water on the cutting edge) and placed into small vials (25 × 5 mm) which contained about 0.1 ml of a 100-μc/ml solution of $H_2{}^{35}SO_4$ in 0.1 M Na-K-PO_4 buffer (pH 6.9). At the end of 5 hr, each leaf was placed on a paper towel, and an approx 2-cm-long section of the abaxial surface of the leaf was coated with a thin layer of 1.9% parlodion (Mallinckrodt Chemical Works) in 60:40 absolute ether:absolute alcohol solution. The thin film of parlodion dried in about 3 min. The portion of the parasite on the surface of the host was imbedded in the parlodion. The parlodion strip was easily removed from the leaf. Four parlodion strips from four leaves were placed in a scintillation vial and dissolved in 0.4 ml of 60:40 absolute ether:absolute alcohol solution at room temperature for 48 hr. Fifteen ml of scintillation fluid [5 g 2,5-diphenyloxazole and 0.1 g 1,4-bis-2-(5-phenyloxazolyl)benzene in 1 liter toluene] were added to each vial. Radioactivity was determined in a Packard Model 3003 Tri-Carb or Beckman LS-133 Liquid Scintillation spectrometer. Experiments were repeated at least 6 times.

RESULTS.—*Effect of parasite/host genotypes on the length of secondary hyphae.*—The five

TABLE 1. Effect of five different parasite/host genotypes on the percentage of parasite units with secondary hyphae of a given length at stated hour after inoculation

Genotypes (parasite/host)	Hr after inoculation	% of parasite units with secondary hyphae of a given length						Functional secondary hyphae (%) (>5-μ length)
		0	1-5 μ	6-10 μ	11-15 μ	16-20 μ	>20 μ	
Pa Pg Pk Pp / *mla mlg mlk mlp*	16	67	25	8	0	0	0	8
	18	45	37	18	0	0	0	18
	20	33	42	25	0	0	0	25
	22	17	53	22	7	1	0	30
	24	14	43	30	8	4	1	53
	26	11	31	44	6	5	3	58
	28	7	16	38	14	6	19	77
	30	6	17	33	5	9	30	77
Pa Pg Pk Pp / *Mla mlg mlk mlp*	16	76	24	0	0	0	0	0
	18	60	33	7	0	0	0	7
	20	59	34	7	0	0	0	7
	22	53	40	7	0	0	0	7
	24	42	50	8	1	0	0	9
	26	38	53	6	3	0	0	9
	28	34	52	11	1	2	0	14
	30	30	53	11	1	3	2	17
Pa Pg Pk Pp / *mla Mlg mlk mlp*	16	78	19	3	0	0	0	3
	18	50	40	9	1	0	0	10
	20	37	49	13	1	0	0	14
	22	39	45	11	4	1	0	16
	24	34	40	22	3	1	0	26
	26	31	44	20	4	1	0	25
	28	34	39	23	2	2	0	27
	30	21	52	19	2	1	5	27
Pa Pg Pk Pp / *mla mlg Mlk mlp*	16	69	26	5	0	0	0	5
	18	56	36	8	0	0	0	8
	20	47	41	12	0	0	0	12
	22	41	47	11	1	0	0	12
	24	36	49	12	2	1	0	15
	26	29	48	19	2	1	0	23
	28	25	47	22	2	3	1	28
	30	26	49	13	4	1	7	28
Pa Pg Pk Pp / *mla mlg mlk Mlp*	16	67	29	4	0	0	0	4
	18	49	41	11	0	0	0	10
	20	49	41	10	0	0	0	10
	22	39	46	14	1	0	0	15
	24	30	48	16	4	2	0	22
	26	25	53	15	5	2	0	22
	28	22	44	13	3	8	10	34
	30	18	44	17	5	5	11	38

near-isogenic host lines were inoculated with culture CR3 and subjected to environmental conditions required for optimum infection efficiency (3, 4, 5), and the length of secondary hyphae at various hours after inoculation was measured (Table 1). With the compatible parasite/host genotype (Genotype 1), 67% of the parasite units had formed no secondary hyphal initials by 16 hr after inoculation; 25% had formed secondary hyphae shorter than 5 μ in length; and 8% had formed secondary hyphae which were longer than 5 μ. By 30 hr after inoculation, only 6% had not produced secondary hyphae and 77% had secondary hyphae longer than 5 μ. The distribution of lengths of secondary hyphae is presented graphically in Fig. 1-A.

Genotype 2 (Table 1) is an incompatible parasite/host genotype on the basis of the *Pa/Mla* gene pair. At 30 hr after inoculation, only 17% of the parasite units had secondary hyphae longer than 5 μ. More than half of the parasite units produced secondary hyphae of 5 μ or less in length. Almost a third did not produce secondary hyphae at all. The distribution of lengths of secondary hyphae is presented graphically in Fig. 1-B.

Genotypes 3, 4, and 5 in Table 1 are incompatible genotypes on the basis of gene pairs *Pg/Mlg*, *Pk/Mlk*, and *Pp/Mlp*, respectively. All three gene pairs reduce the per cent of parasite units with secondary hyphae longer than 5 μ. The distributions of the lengths of

secondary hyphae for any given hour after inoculation are similar.

Effect of light period on elongation of secondary hyphae.—The kinetics of the formation of elongating secondary hyphae is influenced by light (3, 4). The sequence of light conditions for the highest efficiency of infection and the most synchronous development of the parasite was designated as standard conditions: darkness for the 1st hr after inoculation, low light the 2nd through 6th hr, darkness the 7th through 20th hr, and low light after the 20th hr (3, 4, 5, 9). These conditions were established for *E. graminis* f. sp. *tritici* on wheat (3, 4), and were later found to be acceptable though less satisfactory for *E. graminis* f. sp. *hordei* on barley (5). The data for the compatible parasite/host genotype in Table 1 and Fig. 1-A reveal that, with the standard conditions, there are basically two types of elongating secondary hyphae; namely, secondary hyphae 6-10 μ in length and secondary hyphae 20 μ or greater in length.

The timing and length of the light periods were found to be critical for the formation of elongating secondary hyphae of *E. graminis* f. sp. *tritici* on wheat (3), and the possibility that different conditions would be favorable for *E. graminis* f. sp. *hordei* was examined. The second light periods were begun at 16, 18, 20, and 22 hr after inoculation. Only slight effects on the distribution of lengths of secondary hyphae were observed when the second light periods were begun after 18 or 22 hr after inoculation. The results of having light periods of 1-5 and 16 hr after inoculation are presented in Fig. 1-C,

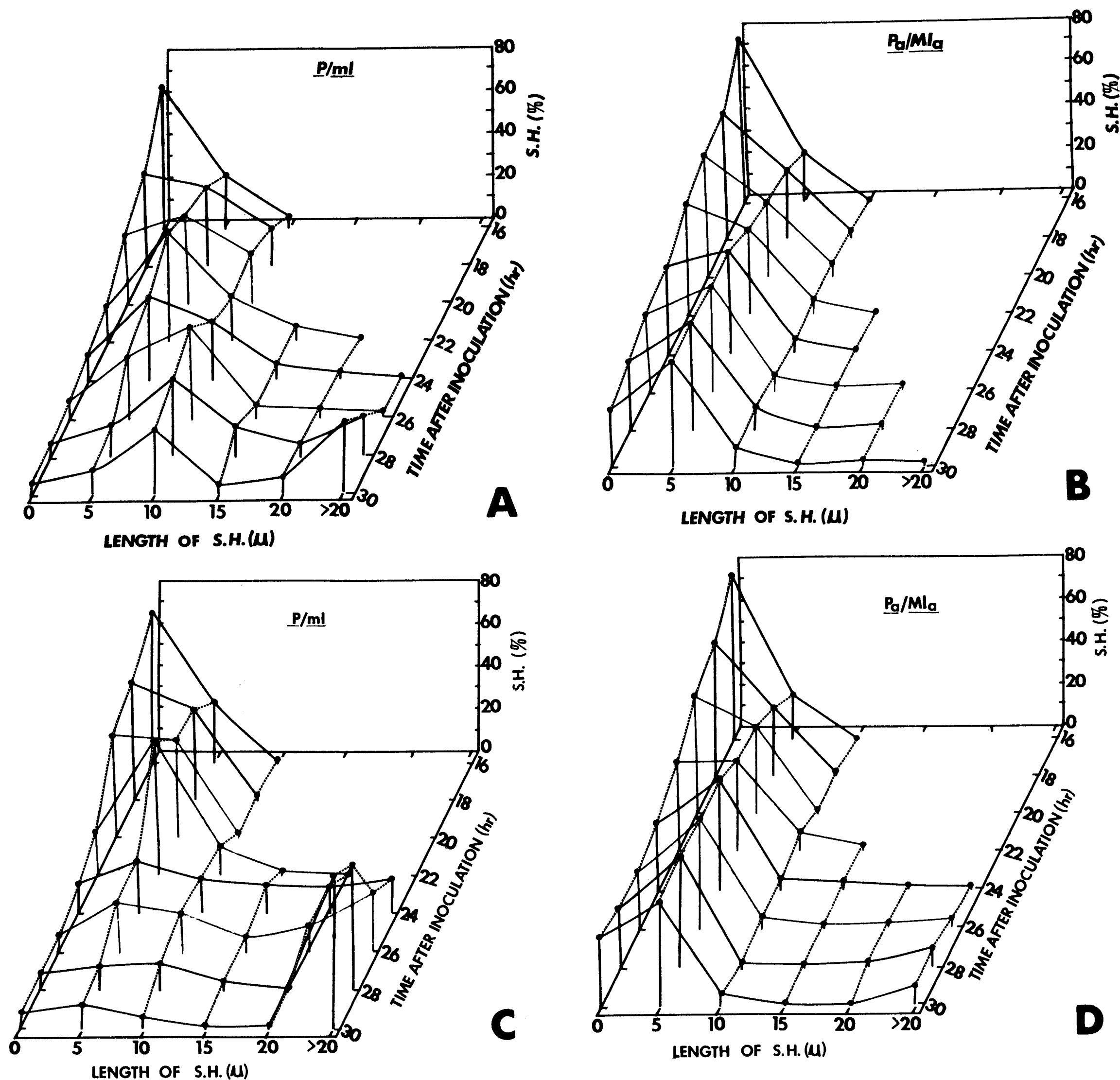
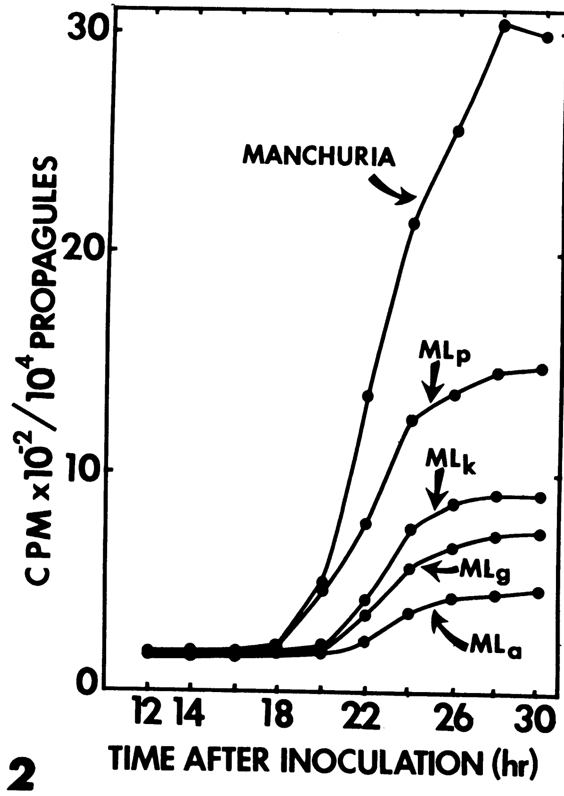


Fig. 1. Length of secondary hyphae of *Erysiphe graminis* f. sp. *hordei* at various hours after inoculation with compatible (A, C) or incompatible (B, D) parasite/host genotypes and two light regimes. **A)** Compatible parasite/host genotype (*P/ml*) with light treatment 1-6 and 20- hr after inoculation. **B)** Incompatible parasite/host genotype (*Pa/Mla*) with light treatment as in A. **C)** Compatible parasite/host genotype (*P/ml*) with light treatment 1-5 and 16- hr after inoculation. **D)** Incompatible parasite/host genotype (*Pa/Mla*) with light treatment as in C.



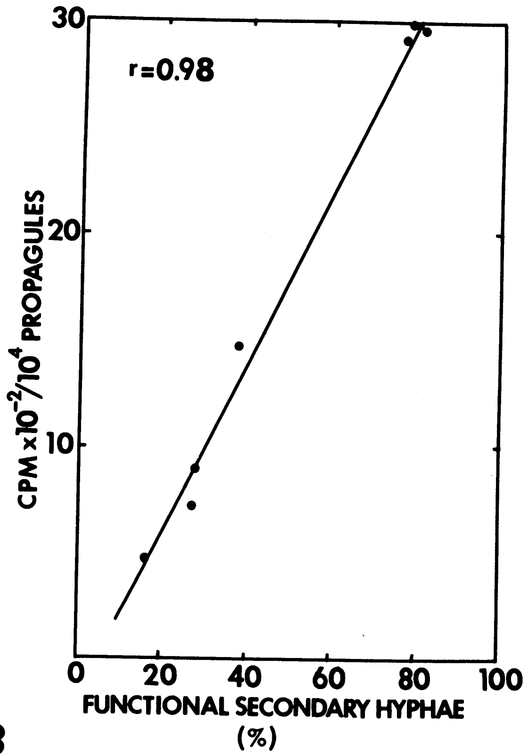


Fig. 2-3. 2) Rates of transfer of ^{35}S from barley leaves to the hyphae of *Erysiphe graminis* f. sp. *hordei* CR3 (*Pa Pg Pk Pp*), which is on the surface of the leaves with barley cultivar Manchuria and host lines with *Mla, Mlg, Mlk,* or *Mlp.* 3) The relationship between rates of ^{35}S transfer from barley to the hyphae of *Erysiphe graminis* f. sp. *hordei* which is on the surface of the leaves and formation of functional secondary hyphae with compatible or incompatible parasite/host genotypes.

D. A much larger percentage of the secondary hyphae were longer than 20 μ by 28-30 hr with the latter light treatment (Fig. 1-C). At 22 hr after inoculation, only 15% of the secondary hyphae were more than 6 μ long in contrast to 80% after 28 hr. Most of the latter were more than 20 μ long. The altered light treatment stimulated 70% of the applied parasite units to produce long secondary hyphae by 30 hr after inoculation. Only 10% of the secondary hyphae were longer than 6 μ but shorter than 20 μ in length. Few secondary hyphae 11 to 20 μ in length were observed (Fig. 1-C). These data suggest that once secondary hyphae begin to elongate, they rapidly elongate to lengths greater than 20 μ.

The length of the light period did not affect the per cent of elongating secondary hyphae with the incompatible parasite/host genotype *Pa/Mla*. The altered light treatment, however, did affect the percentage of elongating secondary hyphae that attained a length greater than 20 μ. (Fig. 1-B, D).

Transfer of ^{35}S with compatible and incompatible parasite/host genotypes.—The rate of transfer of ^{35}S from host to parasite at different times after inoculation is presented in Fig. 2. The kinetics of ^{35}S transfer from Manchuria to culture CR3 is very similar to transfer observed with a compatible genotype of wheat mildew (11). The rate of ^{35}S transfer with the five different genotypes was similar up to 18 hr after inoculation. Differences in the rate of transfer were noted beginning 20 hr after inoculation. The rates of transfer were dependent on the genes involved, and were correlated with infection efficiency (Fig. 3)

Quadratic check.—The four possible genotypes involving one corresponding gene pair with two alleles in the parasite and two alleles in the host is called the quadratic check (8, 10). The quadratic check was established for the gene pair *Pg/Mlg* with two lines of barley, one homozygous *Mlg Mlg* and one homozygous *mlg mlg* (both have recessive *ml* genes at other loci) and two cultures of the *E. graminis* f. sp. *hordei*, CR3 and MSB-70-4, which differ in that CR3 has *Pg* and MSB-70-4 has *pg*. The quadratic check for this pair of corresponding genes is presented in Fig. 4. Only one genotype, *Pg/Mlg*, gave an infection type distinctive from the other three genotypes 7 days after inoculation, and is listed as - (minus) to imply an incompatible parasite/host relationship. Indistinguishable infection types were obtained with the other three genotypes *Pg/mlg, pg/Mlg,* and *pg/mlg*, and they are listed as + (plus) to indicate a compatible parasite/host relationship. Only with *Pg/Mlg* did we observe a reduced primary infection

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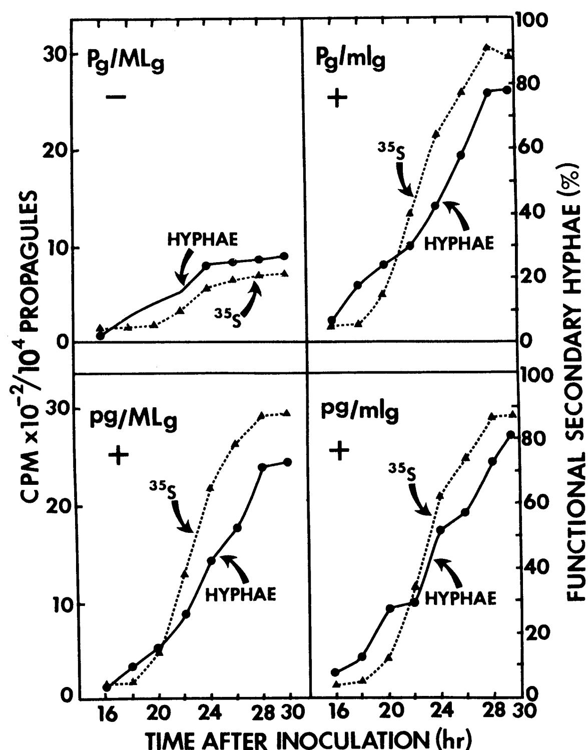

Fig. 4. Quadratic check with two highly isogenic lines of barley containing either *Mlg Mlg* or *mlg mlg*, and 2 races of *Erysiphe graminis* f. sp. *hordei* containing either *Pg* or *pg*. + = compatible parasite/host relationship; − = incompatible parasite/host relationship; Hyphae = per cent of functional secondary hyphae; and ^{35}S = ^{35}S in the parasite on the surface of the leaf.

efficiency and a reduced rate of ^{35}S transfer from host to parasite during primary infection. The three genotypes *Pg/mlg, pg/Mlg,* and *pg/mlg* were experimentally indistinguishable by the criteria of kinetics of formation of elongating secondary hyphae and the rate of ^{35}S transfer from host to parasite.

DISCUSSION.—The development of elongating secondary hyphae has been used extensively as an indication of the establishment of a compatible, functional relationship between host and parasite during primary infection of wheat and barley by *Erysiphe graminis* (3, 4, 5, 9). The basis for this argument is the observation that a large per cent of the parasite units can form short secondary hyphae (generally less than 2 μ in length, almost always less than 5 μ) even when haustoria are not formed in the host cell, but very rarely does a secondary hypha become longer than about 5 μ in the absence of a haustorium. The data presented here show that genotypes which allow a high efficiency of infection also allow for a high percentage of secondary hyphae longer than 5 μ (Table 1). Genotypes which allow a low infection efficiency allow for a low percentage of secondary hyphae longer than 5 μ (Fig. 1, Table 1).

The bimodal distribution of lengths of secondary hyphae longer than 5 μ at 30 hr after incubation under standard conditions suggests that the parasite population is not synchronous (Table 1, Fig. 1-A). When the light treatments were 1-5 and 16- hr after inoculation (rather than the standard conditions developed for wheat mildew), a major portion of the parasite units produced secondary hyphae longer than 20 μ by 30 hr after inoculation (Fig. 1-C). The parasite population was quite synchronous in its development during primary infection, but the environmental regime needed for synchronous development of *E. graminis* f. sp. *hordei* was slightly different than was needed for *E. graminis* f. sp. *tritici* (3, 4, 9).

Secondary hyphae presumably do not have to obtain any particular length before initiating secondary infections. Secondary hyphae 6-10 μ long may be just as successful in initiating secondary appressoria and secondary infections as secondary hyphae 20 μ long. What is considered important by the development of the altered light treatments is that a major portion of the population of parasite units was of sufficient physiological uniformity to be capable of being stimulated to produce long secondary hyphae over a limited period of time.

The rate of ^{35}S transfer from host to parasite with compatible genotypes of the barley mildew was similar to rates observed with compatible genotypes of the wheat mildew. By calculating transfer rate on a per parasite unit basis, a high correlation between infection efficiency and rate of ^{35}S transfer is evident (Fig. 3). The transfer efficiency per parasite unit with secondary hyphae longer than 6 μ with incompatible genotypes may be less at 30 hr after inoculation than with compatible genotypes (Fig. 2, 3). A more detailed analysis is necessary to determine whether the parasite units that do form secondary hyphae with incompatible genotypes are less efficient at any given hour after inoculation than compatible genotypes. No morphologically observable effects of the incompatible genotypes were observed with the parasite units which do produce elongating secondary hyphae as was observed with the wheat mildew. With wheat mildew, *P4/Pm4* and *P1/Pm1* genotypes cause parasite units with elongating secondary hyphae to collapse at ca. 21-22 and 26-28 hr after inoculation, respectively.

The quadratic check helps to identify the uniqueness of a particular interaction. The simplest hypothesis of the data presented in Fig. 4 is that the only unique interaction occurs with the genotype *Pg/Mlg*. The three genotypes for high infection type, +, were indistinguishable on the basis of formation of elongating secondary hyphae and rates of ^{35}S transfer from host to parasite. When the quadratic check was completed for the corresponding gene pair *P1/Pm1* affecting wheat mildew, the genotype *p1/Pm1* was different from *P1/pm1* and *p1/pm1* by the criterion of rate of ^{35}S transfer. Whether the pattern with the *Pg/Mlg* pair affecting barley mildew or the *P1/Pm1* pair affecting wheat mildew is the more universal phenomenon must await completion of the quadratic check for many corresponding gene

pairs and several host lines and parasite cultures for single gene pairs.

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