Elongation of Secondary Hyphae of Erysiphe graminis f. sp. tritici on Wheat with Compatible and Incompatible Parasite/Host Genotypes

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ABSTRACT

The lengths of secondary hyphae of *Erysiphe graminis* f. sp. *tritici* 20-30 h after inoculation varies with the genotype of host and parasite. With a compatible parasite/host genotype (*Px/pmx*), or the incompatible genotype (*P2/Pm2*) that does not affect primary infection, secondary hyphae elongate at about 2-3 μm/h. Secondary hyphae that become longer than 10 μm with the incompatible genotype, *P3a/Pm3a*, continue to elongate the first 26 h at the same rate as hyphae with the compatible genotype. Secondary hyphae that begin to elongate on the incompatible genotypes, *P1/Pm1* and *P4/Pm4*, cease elongation at about 26 and 22 h after inoculation, respectively.

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A high percentage of spores of *Erysiphe graminis* (DC.) Merat f. sp. *tritici* em. Marchal on a wheat leaf will germinate, produce appressoria, haustoria, and elongating secondary hyphae if given the appropriate environmental conditions, and if the parasite/host genotype specifies compatibility (6, 11). With incompatible genotypes, the same percentage of spores germinate and produce appressoria but few of them produce secondary hyphae that are longer than 10 μm (11). Almost all parasite units (i.e., those derived from one spore) produce secondary hyphae, but only parasite units that have haustoria appear to be able to produce secondary hyphae longer than 10 μm (7, 11). We have not observed secondary hyphae shorter than approximately 10 μm to produce secondary appressoria and haustoria. Therefore, those elongating hyphae which cease growth before reaching 10 μm in length are considered to be nonfunctional.

Several incompatible parasite/host genotypes affect the percentage of parasite units that produce elongating secondary hyphae (11). A few elongating secondary hyphae are formed with all genotypes, but the percentage that develops is dependent on the particular parasite/host genotype (11).

The objectives of the research reported herein were: (i) to collect quantitative data on the kinetics of elongation of secondary hyphae as a function of time after inoculation; and (ii) to determine whether the secondary hyphae that begin to elongate with incompatible genotypes develop similarly to secondary hyphae with compatible genotypes.

MATERIALS AND METHODS.—*Erysiphe graminis* f. sp. *tritici*, genotype *P1 P2 P3a P4* (5, 12) (MS-1) was maintained on wheat, *Triticum aestivum* ('Little Club'). Environmental conditions for maintenance of cultures, procedures for the production of inocula, and procedures for obtaining high infection efficiency and synchronous development of the parasite were described previously (6, 7, 8). Controlled inoculations on the abaxial leaf surface were made by the rolling method (9).

The five homozygous lines of wheat used were designated as follows (1, 2):

$$\frac{pm1\ pm2\ pm3\ pm4}{pm1\ pm2\ pm3\ pm4} = (pmx \text{ or 'Chancellor'})$$

$$\frac{Pm1\ pm2\ pm3\ pm4}{Pm1\ pm2\ pm3\ pm4} = Pm1$$

$$\frac{pm1\ Pm2\ pm3\ pm4}{pm1\ Pm2\ pm3\ pm4} = Pm2$$

$$\frac{pm1\ pm2\ Pm3a\ pm4}{pm1\ pm2\ Pm3a\ pm4} = Pm3a$$

$$\frac{pm1\ pm2\ pm3\ Pm4}{pm1\ pm2\ pm3\ Pm4} = Pm4$$

Each of the four loci (*Pm1, Pm2, Pm3a,* and *Pm4*) are known to condition different reactions to powdery mildew. The host genotype is abbreviated in this study as *Pm1* rather than as a diploid *Pm1 Pm1*, since the wheat lines used are homozygous for these genes. Parasite/host combinations are referred to by the corresponding genotypes which specify compatibility or incompatibility of the relationship. The compatible parasite/host genotype, *Px/pmx*, was the standard to which all other genotypes were compared. The incompatible genotypes were *P1/Pm1, P2/Pm2, P3a/Pm3a,* and *P4/Pm4*. The *P1/Pm1* genotype specifies incompatibility. The *Pm1* gene in the host recognizes only the *P1* gene in the pathogen, even though the pathogen possessed many other *P* genes.

A corresponding gene pair that conditions incompatibility is expressed in the presence of any number of other corresponding gene pairs that specify compatibility (13). Therefore, we designate only the part of the parasite/host genotypes that specifies incompatibility.

The lengths of secondary hyphae were determined at various times after inoculation by direct microscopic measurements of all single, isolated parasite units on leaf sections (1 cm long). The 1-cm tip portion of the leaf was not used.

The data are presented as the percentage of the total number of conidia applied to the leaf having secondary hyphae according to hyphal length and time after inoculation. New leaf sections were used for each observation. From 100-150 parasite units were counted

TABLE 1. The percentages of secondary hyphae of given lengths at various times (h) after inoculation with different parasite/host genotypes

Genotypes (parasite/host)	Time after inoculation (h)	% of parasite units with secondary hyphae of a given length					Functional secondary hyphae (%)
		0-5 μm	6-10 μm	11-15 μm	16-20 μm	>20 μm	(>10 μm in length)
Px/pmx	20	68	16	16	1	0	17
	22	56	26	17	2	0	19
	24	17	33	38	12	0	50
	26	14	20	35	21	10	66
	30	16	12	25	11	37	73
P1/Pm1	20	83	10	6	0	0	6
	22	74	19	7	1	0	8
	24	66	18	14	1	0	15
	26	56	25	14	4	1	19
	30	63	23	10	2	2	14
P2/Pm2	20	67	21	10	1	0	11
	22	54	28	16	3	0	19
	24	18	28	41	11	2	54
	26	14	14	30	27	14	71
	30	12	11	14	10	53	77
P3a/Pm3a	20	77	14	8	1	0	9
	22	71	19	8	1	0	9
	24	50	31	16	2	0	18
	26	45	24	16	10	4	30
	30	47	23	17	6	6	29
P4/Pm4	20	85	11	4	0	0	4
	22	79	14	6	1	0	7
	24	77	17	4	1	0	5
	26	76	17	5	2	0	7
	30	75	18	5	1	0	6

on a single leaf for each observation. Results are the averages of at least five experiments repeated on different days.

RESULTS.—At 20 h after inoculation with the genotype *Px/pmx*, 68% of the parasite units had either no secondary hyphae or secondary hyphae 5 μm or less in length, 16% were 6 to 10 μm, 16% were 11 to 15 μm, 1% were 16 to 20 μm, and no parasite units had secondary hyphae longer than 20 μm (Table 1, Fig. 1). By 24 h after inoculation, more parasite units (38%) had secondary hyphae 11 to 15 μm long than in any of the other classes of lengths. Thirty-three percent were 6 to 10 μm long. By 30 h after inoculation 37 percent secondary hyphae were greater than 20 μm long. The distribution of lengths of secondary hyphae as a function of time after inoculation as presented in Fig. 1-A shows that secondary hyphae elongation occurred from 20 h through 30 h after inoculation.

The *P1/Pm1* genotype was shown to affect infection efficiency (11, 12); and in this experiment, 15 to 20% of the percentage of secondary hyphae were longer than 10 μm (Table 1, Fig. 1-B). Of those secondary hyphae that grew longer than 10 μm, only a few elongated to more than 15 μm. There was no apparent change in the distribution of secondary hyphae from 24 to 30 h after inoculation.

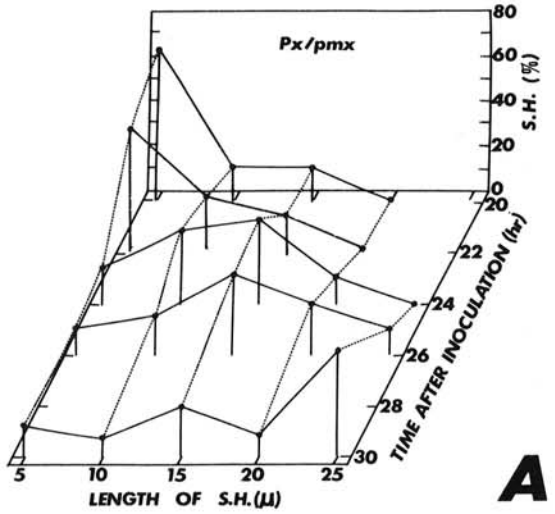
In none of the previous experiments (11) has it been possible to demonstrate an effect of the *P2/Pm2* genotype during primary infection. Most of the parasite units (77%) eventually produced secondary hyphae longer than 10 μm, and most secondary hyphae became much longer than 10 μm (Table 1, Fig. 1-C).

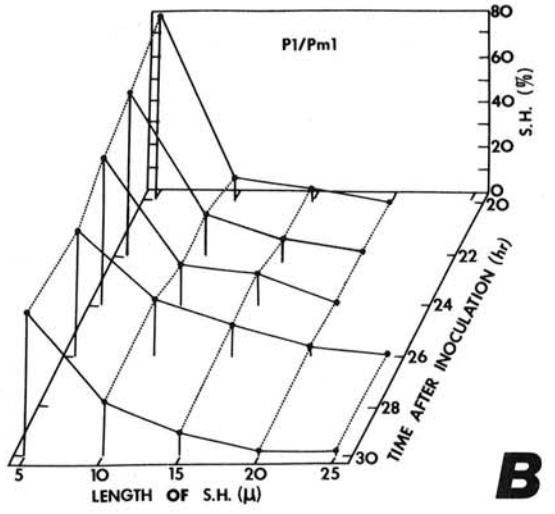
The *P3a/Pm3a* genotype reduced the number of secondary hyphae that attained a length greater than 10 μm to approximately 30 percent (Table 1, Fig. 1-D). The secondary hyphae on some of the parasite units became more than 20 μm long.

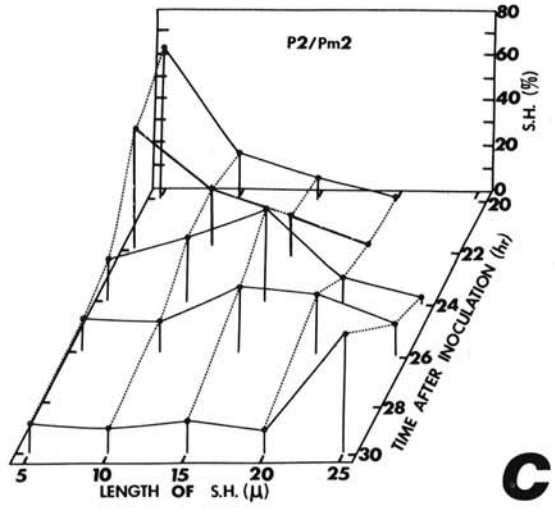
The *P4/Pm4* genotype reduced secondary hyphae that got longer than 10 μm to approximately 5 percent (Table 1, Fig. 1-E). Few secondary hyphae were more than 15 μm long and none was longer than 20 μm. The maximum percentage of secondary hyphae longer than 10 μm was reached by 22 h after inoculation.

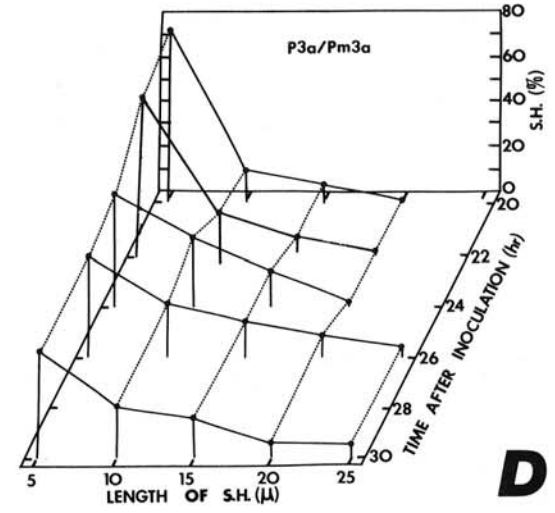
Statistical treatment of the data, summarized in Table 1, show that there are no differences between genotypes *Px/pmx* and *P2/Pm2* in the percent of secondary hyphae longer than 10 μm. Each of the three genotypes *P1/Pm1*, *P3a/Pm3a*, and *P4/Pm4* is different from the other four genotypes based on the percent of secondary hyphae longer than 10 μm. Analyses of the profiles presented in Fig. 1 show no significant differences between *Px/Pmx* and *P2/Pm2*, *P1/Pm1* and *P4/Pm4*, and *P1/Pm1* and *P3a/Pm3a*. All other comparisons are significantly different with $(P) \leq 0.05$.

DISCUSSION.—The data suggest that it is possible to observe, with time, the distribution of a parasite population having either no, or predominantly very short, secondary hyphae to a distribution of a parasite population having predominantly secondary hyphae greater than 10 μm. The data with the compatible genotype *Px/pmx* (Fig. 1-A), suggest that, once the formation of secondary hyphae is initiated, secondary hyphae elongate at the rate of about 2.5 μm/h. Similar









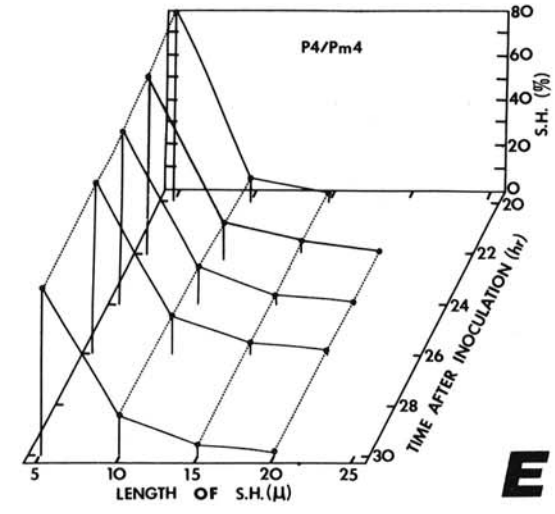


Fig. 1-(A to E). Effect of five different parasite/host genotypes on the length of secondary hyphae (S.H.) of *Erysiphe graminis* f. sp. *tritici* at various times (h) after inoculation. **A)** genotype *Px/pmx;* **B)** genotype *P1/Pm1;* **C)** genotype *P2/Pm2;* **D)** genotype *P3a/Pm3a;* and **E)** genotype *P4/Pm4*. Those secondary hyphae grouped in class 25 refer to all secondary hyphae greater than 20 μm long.

results were obtained with the incompatible genotype *P2/Pm2* which has not been shown to affect primary infection (Fig. 1-C). This is a much slower rate of elongation of secondary hyphae than that observed with *E. graminis* f. sp. *hordei* (4).

Three genotypes have been shown to affect the percentage of secondary hyphae that attain a length >10 μm (11) (Fig. 1-B, 1-D, 1-E). *P4/Pm4* not only causes a reduction in the percentage of parasite units that produce haustoria and elongating secondary hyphae, but it also causes a collapse of most parasite units that produce haustoria and a discoloration of the host cell adjacent to the haustorium (10). The collapse of the parasite units that have produced haustoria was observed at about 21-22 h after inoculation (10). Our data suggest that there is no detectable increase in the lengths of secondary hyphae subsequent to 22 h after inoculation (Table 1, Fig. 1-D).

P1/Pm1 also affects the percentage of parasite units that produce haustoria and are capable of producing secondary hyphae longer than 10 μm (10). More than half of the parasite units that produce haustoria collapsed about 26-28 h after inoculation. Our data suggest that cessation of elongation of secondary hyphae longer than 10 μm occurred by 26 h after inoculation, or earlier.

P3a/Pm3a also affects the percentage of parasite units that produce haustoria and elongating secondary hyphae (10, 11). Our data suggest that approximately 30% of the parasite units that produce haustoria also produce secondary hyphae that are longer than 20 μm. The relative proportions of secondary hyphae in the 11 to 15, 16 to 20, and >20 μm length ranges are approximately the same as with the compatible genotype *Px/pmx* through 26 h after inoculation. The parasite units which are successful in producing haustoria have secondary hyphae which appear to elongate to the same extent as with a compatible genotype the first 26 h after inoculation.

We interpret these results to provide additional supporting evidence that the different genotypes affect different stages in the ontogeny of interactions between host and parasite (3).

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