Genetic Control of Primary Haustorial Development of Erysiphe graminis on Wheat

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ABSTRACT

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Genes conferring resistance in wheat to *Erysiphe graminis* f. sp. *tritici* affect the ontogeny of early interactions between host and parasite. The rate of development of primary haustoria of *E. graminis* f. sp. *tritici* on five near-isogenic lines of wheat containing single genes for reactions to *E. graminis* (MS-1) was determined by direct microscopic observations made after fixation and staining of epidermal strips with aniline blue every 2 hr from 8 through 30 hr after inoculation. Inoculated plants were held under environmental conditions known to favor development of the parasite. With the compatible *Px/pmx* genotype, 87% of the parasite units formed haustoria 35–55 μm long within 30 hr after inoculation. With the incompatible genotypes, *Pla/Pmla, P2a/Pm2a, P3a/Pm3a, and P4a/Pm4a,* the percentages of haustoria 35–55 μm long by 30 hr after inoculation were 15, 66, 18, and 3, respectively. More than 75% of the host cells in the successful infections with *Pla/Pmla, P2a/Pm2a, P3a/Pm3a, and P4a/Pm4a,* in which the haustoria were 35 μm or more in length, were heavily stained, indicating mesophyll collapse and necrogenic protoplasts. These results provide evidence that the different genotypes affect different stages in the host/parasite interactions earlier than previously reported.

Additional key words: host-parasite interactions, powdery mildew, primary infection, *Triticum aestivum*.

A high percentage of spores of *Erysiphe graminis* (DC.) Mérat f. sp. *tritici* Em. Marchal placed on a leaf of wheat will germinate and produce appressoria, haustoria, and elongating secondary hyphae (ESH) if the environmental conditions are appropriate and if the parasite/host genotype specifies compatibility (5).

Genes that confer resistance in wheat to *E. graminis* (the *Pm* genes) are expressed at different stages in the ontogeny of interactions between host and parasite (8). The effects of these genes have been determined by measuring the production of ESH during primary infection, using ESH greater than 10 μm as the criterion of development of a functional relationship between wheat and *E. graminis*. For each ESH greater than 10 μm, a normal haustorium is always present (9). However, no direct studies had been done on the development of haustoria during primary infection in either the compatible or incompatible interactions.

The objectives of this research were (i) to determine the rate of development of haustoria of *E. graminis* on the five near-isogenic lines of wheat containing the *Pm* genes and (ii) to correlate these results with the development of ESH as determined in an earlier study (5).

MATERIALS AND METHODS

Culture MS-1 of *E. graminis* f. sp. *tritici* was cultured on wheat (*Triticum aestivum* 'Little Club'). The environmental conditions under which these stock cultures were maintained have been described (6). Wheat plants were inoculated with *E. graminis* by dusting 5- to 6-day-old plants with conidia from stock cultures. The five near-isogenic wheat lines were designated as *pm* (Chancellor) (CI-1233), *Pm1a* (CI-14114), *Pm2a* (CI-14118), *Pm3a* (CI-14123), and *Pm4a* (CI-14123). The cultivar Chancellor is not known to possess any *Pm* genes and was the recurrent parent in establishing each of the other host lines (1). Each of the *Pm* genes is unique and independent for reactions to *E. graminis* f. sp. *tritici*. Culture MS-1 possesses the *PlaP2aP3aP4a* genes conditioning incompatibility with each corresponding gene *Pmla, Pm2a, Pm3a,* or *Pm4a* in the host (7).

The development of haustorial bodies was measured by direct microscopic observations. Measurements were made every 2 hr from 8 through 30 hr after inoculation. Conidia were applied using "rolling technique" (6) to assure uniform, controlled placement of spores on the abaxial epidermis of the leaf. Every 2 hr after inoculation beginning at hour 8, strips of the abaxial epidermis of the first leaf were removed and fixed in Carnoy's solution for 5 min. Extractable chlorophyll was removed by two or three rinses of 100% methyl alcohol. The epidermal strips were placed in 0.06% aniline blue, which stains fungal material and proptoplasts (3), and incubated at 35 C for 1 hr to intensify the dye in the fungal tissue. The tissue then was transferred to 85% lactic acid for 1 hr and allowed to destain in fresh 85% lactic acid at room temperature for 1 or 2 days. The dark blue-stained haustoria were easily observable with bright-field microscopy.

The data are presented as percent of the total number of conidia applied that had produced haustoria of various lengths at different times after inoculation. The experiments were repeated on 4 days. When different near-isogenic lines were compared, all lines were tested on the same day.

RESULTS

At 8 hr after inoculation with the compatible parasite/host genotype, *Px/pmx* (Chancellor), all the parasite units applied had attempted penetration or had formed haustoria 5 μm or less in length (Table 1, and Fig. 1). Formation of appendages on the haustorial bodies was evident 18 hr after inoculation in all the compatible and incompatible interactions. With this compatible parasite/host genotype, by 30 hr after inoculation, the percentages of parasite units with haustoria of a given length were: 0%, 0–5 μm; 1%, 5–15 μm; 1%, 15–25 μm; 8%, 25–35 μm; 43%, 35–55 μm; and 44%, 45–55 μm. There were five collapsed ectoparasitic structures by 30 hr after inoculation, and there were no distorted haustoria in the host cell. The meshophyll cells showed no sign of necrosis and

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TABLE 1. Effect of genotype of host and pathogen on development of haustoria during primary infection

Genotypes (parasite/host)	Time after inoculation (hr)	Parasite units with haustoria of a given length (μm)						Haustoria		
		0-5 (%)	5-15 (%)	15-25 (%)	25-35 (%)	35-45 (%)	45-55 (%)	Distorted (%)	Collapsed (%)	Total observed (no.)
Px/pmx										
	8	100								304
	10	99	1							267
	12	90	10							388
	14	22	73	3					2	256
	16	21	56	25					2	221
	18	13	25	57	4				1	228
	20	8	9	57	27					239
	22	4	5	27	63	2				232
	24	7	5	19	50	20				235
	26	5	3	14	50	26	2			189
	28	5	4	8	18	59	6			185
	30		1	1	8	43	44			209
P1a/Pm1a										
	8	100								299
	10	100								298
	12	91	8						2	314
	14	69	21	2				5	3	221
	16	38	25	33				2	2	231
	18	50	8	29	4			6	3	219
	20	29	7	28	24			7	5	249
	22	25	10	24	11	2		24	5	236
	24	46	11	14	8	4		19	2	250
	26	34	3	6	11	4		42		252
	28	41	4	6	12	8	1	33		217
	30	33	3	8	19	8	7	26		265
P2a/Pm2a										
	8	100								322
	10	98							1	313
	12	92	8						1	411
	14	53	35	7				1	4	230
	16	35	18	47					2	260
	18	18	9	63	8			2	1	234
	20	17	6	34	44					222
	22	23	4	16	51	5				241
	24	16	4	14	32	31		2	2	239
	26	24	2	3	41	33	2			239
	28	21	2	5	9	42	18	2	1	221
	30	19		3	7	35	31	4		198
P3a/Pm3a										
	8	100								277
	10	98	1							277
	12	91	9							317
	14	23	69	4				3	2	223
	16	47	23	30						261
	18	26	15	50	7				1	216
	20	36	5	34	22				3	221
	22	27	4	30	22			13	4	284
	24	23	1	12	37	9		16	1	268
	26	23	3	10	19	6		32		244
	28	34	2	4	15	8	2	34	2	200
	30	35	2	6	12	11	7	29		251
P4a/Pm4a										
	8	100								346
	10	98	2							224
	12	90	10							372
	14	52	38	8	1				2	233
	16	58	20	21					2	205
	18	51	7	30	8			4	2	271
	20	70	4	9	5			6	5	230
	22	44	6	20	10			11	8	291
	24	46	2	11	15	4		14	9	295
	26	64	3	4	3	1		16	8	231
	28	49	2	4	3	3	1	38		198
	30	60	2	4	3	2	1	18	10	199

none of the host cells was stained with the dye.

With the incompatible parasite/host genotype, *P1a/Pm1a* (Fig. 2), fewer parasite units had large haustoria when compared with genotype *Px/pmx*. Additionally, 26% of the parasite units that were applied had distorted haustoria, evidenced by the tendency of the haustoria to lose their normal shape. Host cells containing distorted haustoria also were heavily stained by the dye. By 30 hr after inoculation, approximately 95% of the infected cells were heavily stained with the dye. Collapse of the ectoparasitic portion of the parasite was greatest approximately 20–22 hr after inoculation.

With the incompatible parasite/host genotype, *P2a/Pm2a*, (Table 1, Fig. 3), 19% of the parasite units formed hausstoria 5 μm or less in length and 66% had haustorial bodies 35–55 μm in length by 30 hr after inoculation. Only 4% of the haustoria were distorted and

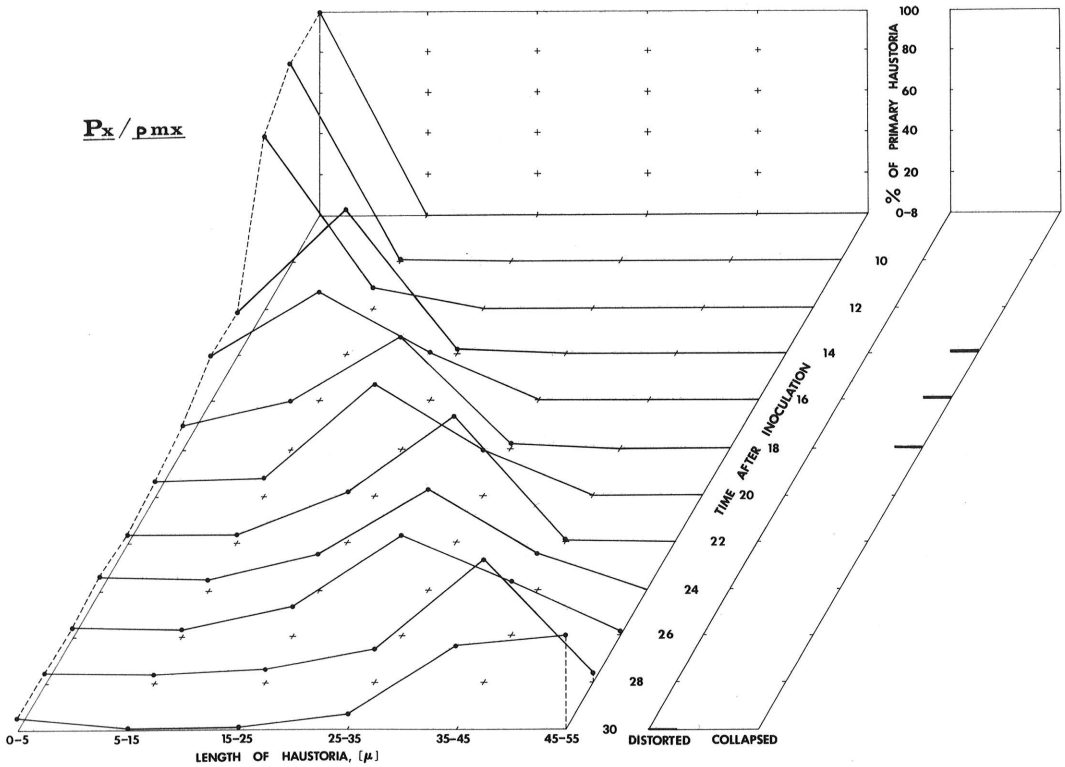

Fig. 1. Effect of parasite/host genotype *Px/pmx* on development of primary haustoria of Erysiphe graminis f. sp. *tritici* on wheat leaves.

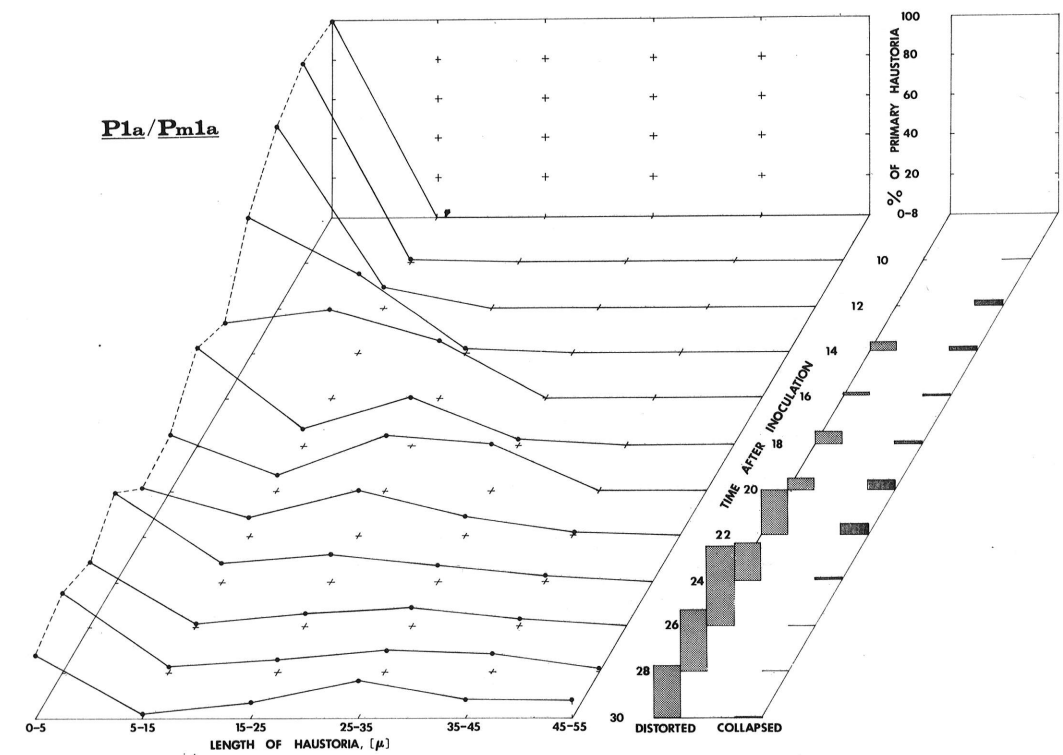

Fig. 2. Effect of parasite/host genotype *P1a/Pm1a* on development of primary haustoria of *Erysiphe graminis* f. sp. *tritici* on wheat leaves.

relatively few host cells were stained by the dye. The results with *P2a/Pm2a* differ only slightly from the results with *Px/pmx*.

The percentages of haustoria of a given length with genotype *P3a/Pm3a* are shown in Table 1 and Fig. 4. The highest percentage of parasite units with haustoria was reached by 28 hr after inoculation and almost all infected host cells were stained by the aniline blue.

The *P4a/Pm4a* genotype (Fig. 5) showed that few parasite units had haustoria longer than 5 μm by 30 hr after inoculation. Approximately 6% of the parasite units had haustoria 35 μm or longer at this time; 18% of the haustoria were distorted, and 10% had collapse of the ectoparasitic portion. Essentially all infected host cells stained with the dye. As early as 14 hr after inoculation, groups of mesophyll cells had collapsed beneath the penetrated cell, and

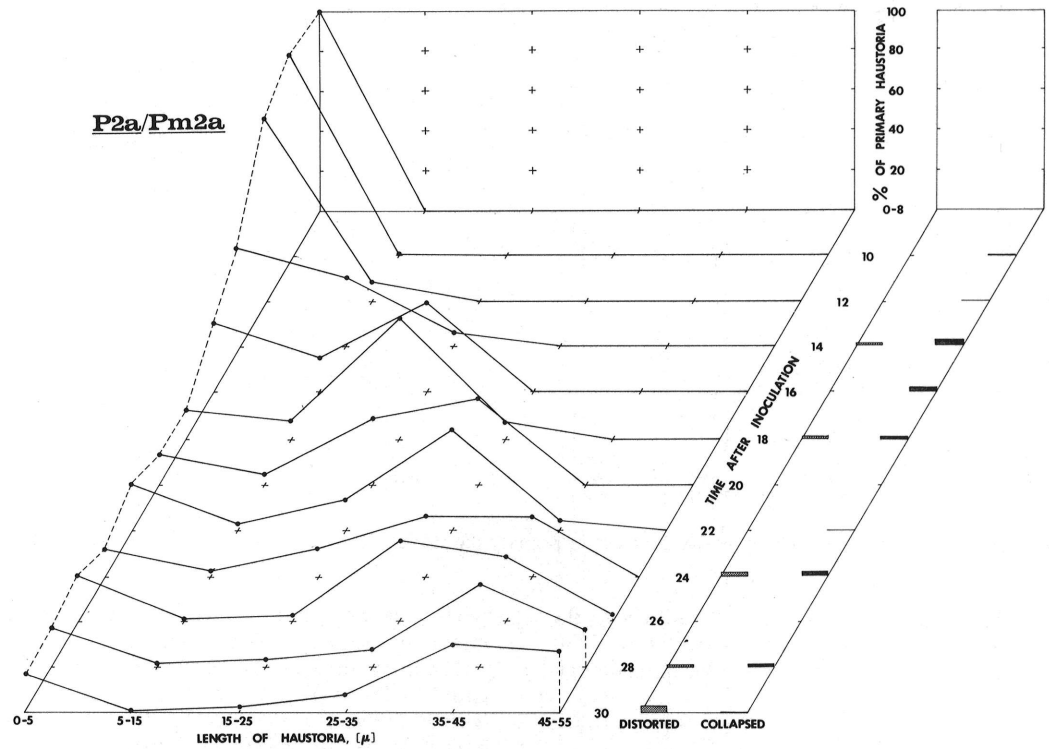

Fig. 3. Effect of parasite/host genotype *P2a/Pm2a* on development of primary haustoria of *Erysiphe graminis* f. sp. *tritici* on wheat leaves.

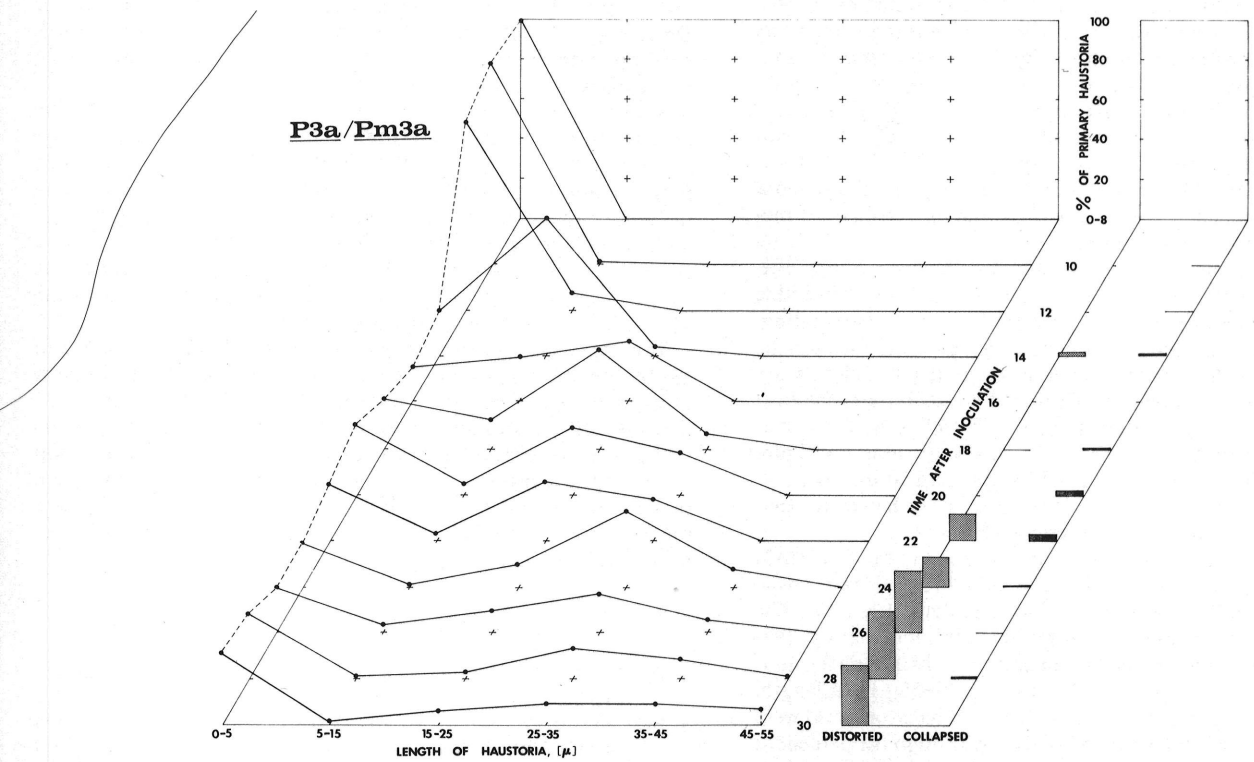

Fig. 4. Effect of parasite/host genotype *P3a/Pm3a* on development of primary haustoria of *Erysiphe graminis* f. sp. *tritici* on wheat leaves.

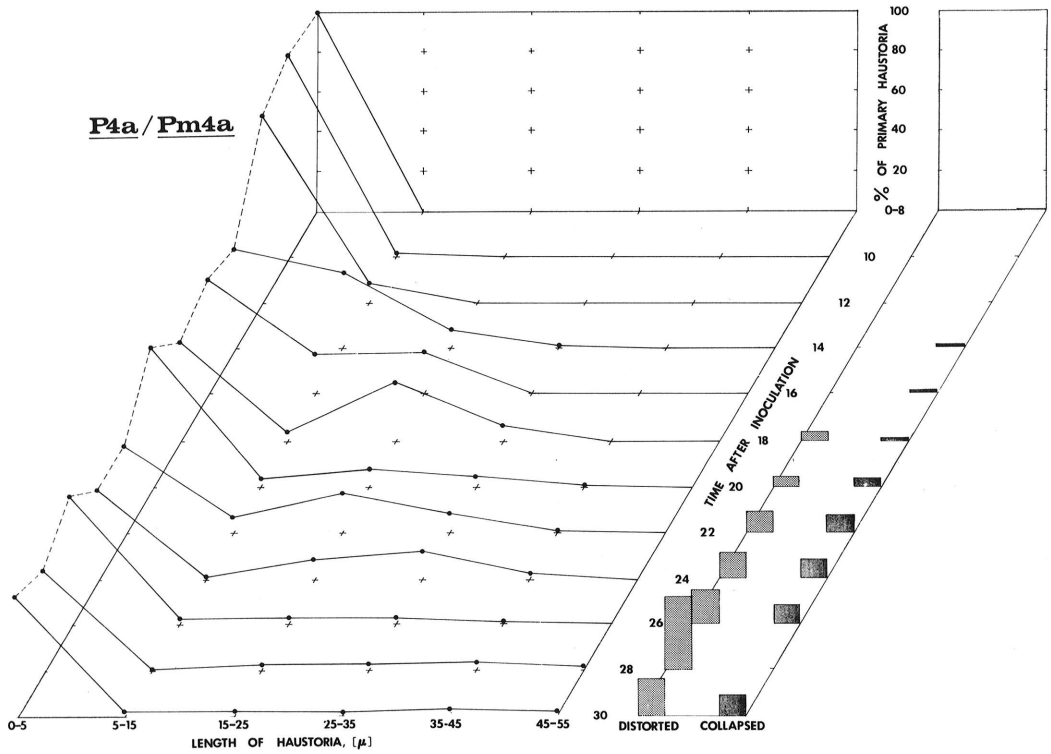

Fig. 5. Effect of parasite/host genotype *P4a/Pm4a* on development of primary haustoria of *Erysiphe graminis* f. sp. *tritici* on wheat leaves.

usually the highest percentage of collapsed cells adjoining the infected cells was observed by 28 hr after inoculation. This phenomenon did not occur with the other compatible or incompatible interactions.

DISCUSSION

The data with the compatible genotype *Px/pmx* suggest that both development of haustoria and the ectoparasitic portion of the parasite are reasonably synchronized (Fig. 1). With the compatible *Px/pmx* genotype, penetration by the parasite units is detectable as early as 8–10 hr after inoculation. By 18 hr after inoculation, appendages are present on the primary haustorial body and relatively few of the ectoparasitic structures have collapsed. The fact that the haustoria were not distorted and the host cells were not stained with aniline blue indicates that the infected cell is not greatly affected. By 30 hr after inoculation, 85% of the conidia applied had produced primary haustoria and established a functional relationship with the host (9).

The *P1a/Pm1a* parasite/host genotype affects infection efficiency (2), development of the haustoria, and uptake of aniline blue by host cells during primary infection. By 30 hr after inoculation, 33% of the parasite units had produced no haustoria or infected the host cell and formed haustoria 5 μm or less in length. Twenty-six percent of the parasite units had distorted haustoria and the host cells in which they were located stained with aniline blue. Normal haustoria were formed by 19% of the parasite units, a figure comparable to the formation of ESH longer than 10 μm by 26 hr after inoculation (9). Apparently, therefore, only haustoria that develop normally support the production of ESH.

Published studies have not demonstrated an effect of the *P2a/Pm2a* gene interaction during primary infection (4). Most of the parasite units, 77%, eventually produced ESH greater than 10 μm (9). The development of haustoria show that by 30 hr after inoculation, 19% of the parasite units had formed no haustoria or haustoria 5 μm or less in length, while only 66% had haustoria 35–55 μm in length. Four percent of the parasite units had distorted haustoria and were in host cells that were stained with the dye. Although the percent of ESH does not show an effect of *P2a/Pm2a* in primary infection, a study of development of haustoria indicates that *P2a/Pm2a* genotype significantly differs from the parasite/host genotype *Px/pmx* interaction during primary infection.

The interactions of *P3a/Pm3a* were similar to those of *P1a/Pm1a* and *P4a/Pm4a* (Fig. 2, 4, and 5). The percentage of parasite units with *P3a/Pm3a* is not in complete agreement with the percent of parasite units that have formed ESH more than 10 μm long. Thirty-three percent of the parasite units support growth of ESH greater than 10 μm by 26 hr after inoculation (5, 7). For the data with *P3a/Pm3a* to be in agreement with both parameters, it was necessary to include 25–35 μm haustoria and those 35 μm or longer. Only 18% of the infected host cells with the incompatible *P3a/Pm3a* genotype were normal 30 hr after inoculation. Apparently, smaller haustoria were supporting the development of ESH.

Reduction in the percentage of the parasite units that produced primary haustoria and discoloration of the host cells adjacent to the infected cell were evident with *P4a/Pm4a* (7). Sixty percent of the parasite units either failed to penetrate the host cells or penetrated the host cells and stopped growing. Eighteen percent of the parasite units were distorted in appearance. Rudimentary haustoria and stained cells were observed. Of the total number of parasite units applied, 10% showed collapse of the ectoparasitic portion and only 3% had haustoria 35–55 μm in length.

The data also show that development of haustoria in the incompatible interactions is not an all-or-none phenomenon. In some cases, the host cells were penetrated and development of the parasite unit was rapidly halted. In other infected cells, the parasite unit appeared to develop normally for a few hours; then development stopped, leaving a rudimentary haustorium. The uptake of the aniline blue by the infected host cell in these interactions led to the assumption that a large percentage of the infected cells were measurably affected.

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