Aggregation of Host Cytoplasm and the Formation of Papillae and Haustoria in Powdery Mildew of Barley

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ABSTRACT

Living host cells in epidermal tissue partially isolated from barley coleoptiles were observed during penetration by *Erysiphe graminis*. The first visible response to the fungus was a well-defined aggregate of host cytoplasm which gathered beneath the appressorial tip of the fungus at 11.2 hours after inoculation. The aggregate contained rapidly-moving cytoplasmic organelles and persisted until 14.7 hours after inoculation. A papilla was produced at the center of many of the aggregates within 12.5-14.1 hours after inoculation. Haustoria were first seen 13.0 hours after inoculation, when aggregates were still present and when papillae were forming. Of a total of 251 appressoria from 10 host-parasite combinations, 94% produced cytoplasmic aggregates, 54% produced papillae, and 67% produced haustoria. Of the appressoria that failed to produce haustoria, 39-100% induced a papilla, depending on the host-parasite combination. Papillae and young haustoria were formed before the specific incompatibility conditioned by single genes in host and parasite was visibly expressed in three host-parasite combinations. The results suggest that the cytoplasmic aggregate has a role in the deposition of the papilla, and that the papilla is a significant component of generalized host resistance to powdery mildew fungi.

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Additional key words: Erysiphe graminis, primary infection of cells.

Cells under attack by powdery mildew fungi frequently produce a papilla on the host wall at the site of attack, probably before the host wall is penetrated (2, 6, 8, 14, 18, 21, 23). Furthermore, large amounts of host cytoplasm have been seen at the site of papilla formation, both in sections fixed for electron microscopy (18, 23) and in living cells in which rapid movement of organelles within the cytoplasmic mass could be seen (3). Although the cytoplasmic aggregate probably has a role in the deposition of the papilla, and the papilla, in turn, may help prevent the fungus from entering the host cell, neither of these possibilities is supported by direct experimental evidence. Whether the papilla has a role in determination of the compatibility conditioned by specific corresponding genes in host and parasite also has not been clear, although the results of Stanbridge et al. (23) indicated that the formation of papillae was independent of such genes. To provide additional information on these relationships, we have timed the development of cytoplasmic aggregates, papillae, and haustoria in living cells of several host-parasite combinations, and have determined how often they occur in relation to one another. The study was done primarily with living tissues from barley coleoptiles mounted in the microculture chambers as used in studies of haustorium development in our laboratory (3, 5, 25).

MATERIALS AND METHODS.—*Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal and *E. graminis* f. sp. *tritici* Em. Marchal were used with *Hordeum vulgare* L. in the ten combinations listed in Table 1. As shown in the table, each combination is designated by host and pathogen in abbreviated form. Based on reactions of first seedling leaves to infection (19), the materials included six fully compatible host-parasite combinations (infection type 4), three highly incompatible combinations (infection type 0), and one partially compatible combination (infection type 2). The barley host lines included Algerian/4*(F14)Man.(R) and Algerian/4*(F14)Man.(S) which differ for genes conditioning reaction to culture CR3 of *E. graminis* f. sp. *hordei* at the *Mla* locus. *E. graminis* f. sp. *tritici* was used with a barley host (Montcalm), not a host species for this fungus.

Primary infection was observed in partially isolated tissues from the inner epidermal surface of barley coleoptiles by use of mounting procedures described elsewhere (5, 25). Coleoptiles and inoculum were grown in 0.6 × 1.2 m aerated plastic film glove bags with 6,800 lux fluorescent light for 16 hours/day (Weston Model 756 illumination meter). Day and night temperatures were 23.0 ± 0.5 C and 20.5 ± 0.5 C, respectively. Some preliminary materials were grown in an Isco Model E-2 plant growth chamber with 10,000-20,000 lux fluorescent light for 12 hours/day at 22.5 × 0.5 C and 21.5 × 0.5 C, day and night temperatures, respectively.

Coleoptiles and inoculum were used 7 days after planting or inoculation. They were harvested near the end of a daily light period, usually within 1 hour. The coleoptiles were split longitudinally, and the inner surface was inoculated with 54-124 mildew spores/mm^2 in a settling tower. These procedures required about 1.5 hours. Then the inoculated pieces were mounted in support wafers, stripped of unwanted tissues, and placed in microchambers. These procedures were as described

elsewhere (5, 25), except that the tissues were mounted immediately after inoculation instead of after 1.5 days. Mounting was completed about 2 hours after inoculation, and the mounted tissues were then incubated in the dark at 90% RH at 18 C, usually until 7.5 hours after inoculation. Thereafter, the mounts were kept on the laboratory bench at 21-23 C until intensive observations were completed 20-22 hours after inoculation. The mounts were again incubated at 18 C, until a final observation at about 36 hours.

The upper inoculated surface of the mounted tissue was kept dry at all times, whereas the lower surface was kept in contact with 0.01 M $Ca(NO_3)_2$ solution. Observations were confined to a 1.5 mm-diameter hole in an upper support wafer. Serial observations of mounted tissue were made with bright field, dry, objective lenses of ×40-

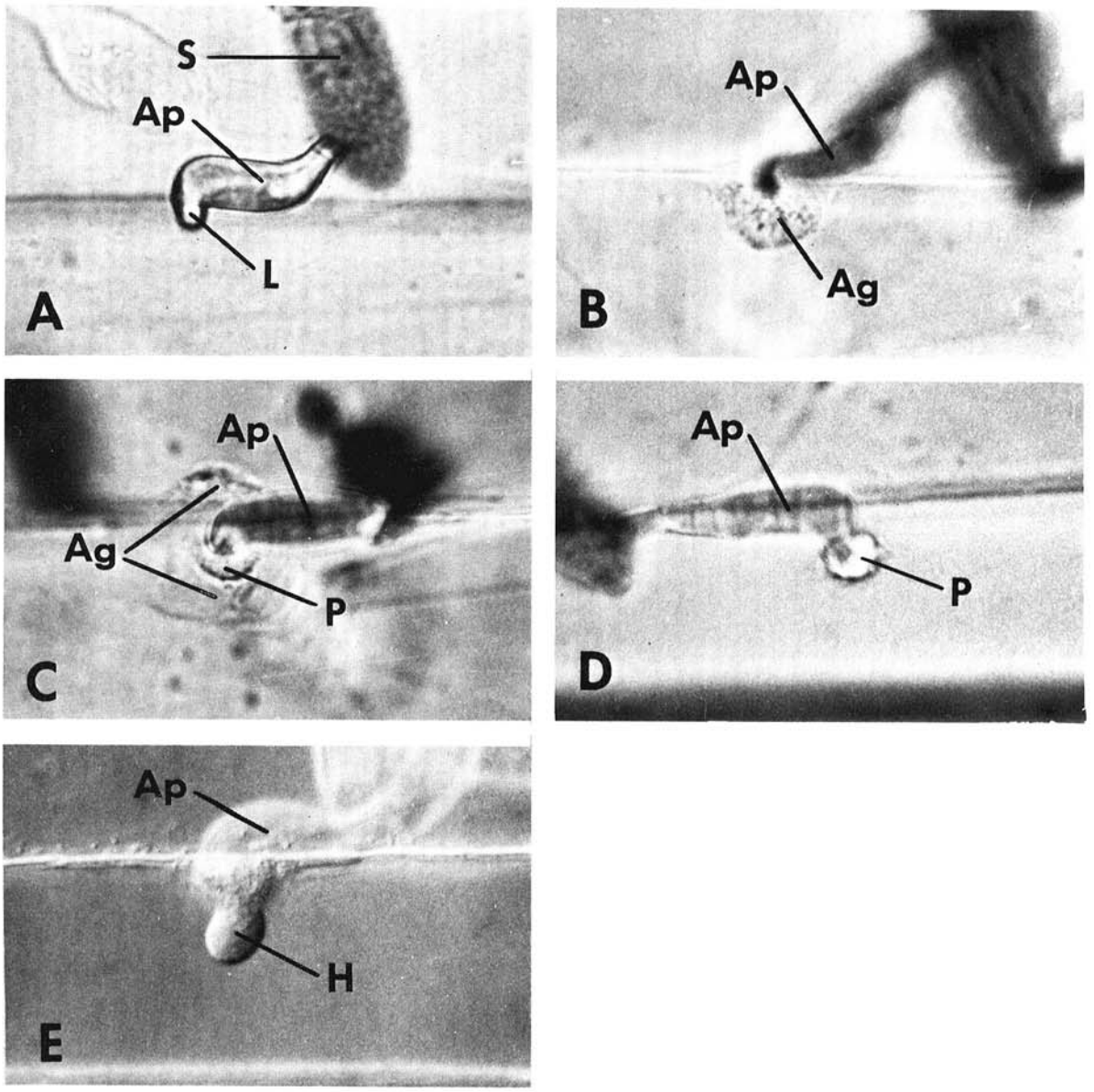

Fig. 1-(A to E). Development of *Erysiphe graminis* f. sp. *hordei* and host responses on epidermal tissues from barley coleoptiles. All specimens were in unfixed, unstained, and living condition. All were race 9 of *Erysiphe graminis* f. sp. *hordei* on Montcalm barley. ×1,100. A-D, bright field; E, differential interference contrast microscopy. **A)** Fully differentiated appressorium (Ap) before visible host response, 11 hours after inoculation. Spore (S); Appressorial lobe (L). **B)** Cytoplasmic aggregate (Ag) in host cell, centered on lobe of appressorium, 11 hours after inoculation. **C)** Papilla (P) developing within a cytoplasmic aggregate, 14 hours after inoculation. An aggregate is present in the cell adjacent to the cell under attack [adapted from (3)]. **D)** A papilla after the cytoplasmic aggregate had dispersed, photographed 64 hours after inoculation. **E)** Haustorium (H) emerging from site of papilla and cytoplasmic aggregate, 14.5 hours after inoculation.

×63 magnification with maximum numerical apertures of 0.75. For high resolution bright-field, phase-contrast, or interference-contrast microscopy, tissues were removed from the mounts and placed in dilute surfactant (0.1% Tween 20) under a cover slip. Such tissues were discarded after 1-2 hours.

The tissues had 30-81 germinated spores per mm^2 as 42-66% of applied spores germinated. A high percentage of the germinated spores produced appressoria, 84% in one typical experiment with Han-9, but the percentage of appressoria was not recorded in most experiments.

RESULTS.—*The visible events.*—The germinating spore usually produced an appressorium with a lobe on one side of the appressorial apex by 9 hours after inoculation (Fig. 1-A). These appressoria were generally similar among all host-parasite combinations, except that Mont-trit had longer apical lobes than others, and lobes that were slightly narrower at the tip than at the base. With all host-parasite combinations, a second lobe often formed midway along the side of the appressorium if the host had not been penetrated at the first lobe. The second lobe developed 15-36 hours after inoculation, long after any events at the primary lobe had been completed. The second lobe [similar to those described by Benada (1)] occurred most frequently with Han-9 and Mont-trit, the two host-parasite combinations with the lowest rates of haustorium formation as will be shown later.

The host cell usually did not respond to the presence of the lobed appressorium until about 11 hours after inoculation, when cytoplasm suddenly aggregated around the point of attack beneath the appressorial lobe (Fig. 1-B). The aggregate consisted of a seething mass of

TABLE 1. The host-parasite combinations used in the present study, and the infection types produced by each on first leaves

Designation for combination	Barley host: Cultivar or other designation	Barley host: C.I. Number	Mildew parasite: *E. graminis* f. sp.	Mildew parasite: Race	Mildew parasite: Culture	Infection type on 1st leaves
Man-9	Manchuria	2330	*hordei*	9	Wild[a]	4
Han-9	Hanna	906	*hordei*	9	Wild	4
Mont-9	Montcalm	7149	*hordei*	9	Wild	4
Mont-3	Montcalm	7149	*hordei*	3	CR3	4
Atlas-3	Atlas	4118	*hordei*	3	CR3	4
AlgS-3	Algerian/4*(F14)Man.(S)[b]	16138	*hordei*	3	CR3	4
AlgR-3	Algerian/4*(F14)Man.(R)[b]	16137	*hordei*	3	CR3	0
GoldR-3	Goldfoil/4*(F14)Man.(R)[c]	16139	*hordei*	3	CR3	0
PsakR-3	Psaknon/4*(F14)Man.(R)[c]	16145	*hordei*	3	CR3	2
Mont-trit	Montcalm	7149	*tritici*	Unknown	Wild[d]	0

[a]Found in a growth chamber, probably from field sources.

[b]Algerian/4*(F14)Man.(S) and Algerian/4*(F14)Man.(R) comprise a near-isogenic pair of lines which differ at the *Mla* locus for genes that condition reaction to culture CR3 of *E. graminis* f. sp. *hordei* (20).

[c]The resistant member of a pair of near-isogenic lines which differ in genes that condition reaction to culture CR3 of *E. graminis* f. sp. *hordei* (20). The susceptible member of the pair was not studied.

[d]Collected from the field, St. Paul, Minn., 1972.

TABLE 2. Production of aggregates, papillae, and haustoria in barley tissues under attack by *Erysiphe graminis*

Host-parasite combination[a]	Number of appressoria	Percentage of appressoria that produced the indicated structure: Aggregates	Papillae	Haustoria
Compatible:				
Man-9	27	93	70	67
Han-9	28	71	46	39
Mont-9	27	96	70	63
Mont-3	24	96	46	83
Atlas-3	24	100	58	58
AlgS-3	24	96	63	67
	Total 154	Avg 92	59	63
Incompatible:				
AlgR-3	26	100	46	81
GoldR-3	24	100	67	83
PsakR-3	23	96	30	78
Mont-trit	24	92	46	46
	Total 97	Avg 97	47	72
All:				
	Total 251	Avg 94	54	67

[a]See Table 1 for full descriptions.

rapidly moving cytoplasmic organelles and had well-defined, though shifting, outer boundaries. The aggregate was 10-30 μm in diameter, and was connected to cytoplasmic streams which appeared to enter and leave the aggregate. After 1-2 hours, the aggregate shrank to two-thirds to one-half its original diameter. The outer boundaries of the aggregate became more sharply defined and more stable in position. Occasionally, an aggregate developed in an adjacent cell as well as in the one under attack (Fig. 1-C), if the appressorial lobe was close to the wall between the two cells. Later the aggregate dispersed completely or left only a few organelles in the region that it once occupied. Sometimes the aggregate reformed one or more times for short periods, only to disperse again. The nucleus of the host sometimes came to the aggregate and remained there, but usually stayed in a distant part of the host cell.

Organelles were densely packed in the young aggregate, making observations by light microscopy difficult. The organelles were generally less than 1 μm in diameter and were predominantly spherical at first, but elongate bodies became visible later when the organelles became less abundant. Vesicles 0.5 - 2.0 μm long sometimes were present at the margin of older aggregates and seemd to be oriented along the margin in a radial pattern centered on the point of attack. Similar bodies were found by McKeen and Rimmer (18) in electron micrographs. One to two hours after the cytoplasm first aggregated, unidentified stationary structures were often seen at the center of the moving mass of cytoplasm beneath the edge of the appressorial lobe. These consisted of one or more empty vesicles about 1 μm in diameter, or a crescent-shaped line concentric with one side of the appressorial lobe.

A papilla often developed within the aggregate of cytoplasm (Fig. 1-C). The papilla had a rough outer margin (as viewed from above). It was difficult to see at first because the dense aggregate of cytoplasm blocked the view. Later the papilla enlarged, was seen more readily (Fig. 1-D), and usually had a diameter of 4-9 μm. Some unusually large papillae, 10-15 μm in diameter, developed with Mont-trit.

Young haustoria developed at about the same time that papillae did and were sometimes indistinguishable from them at first, but the smooth margin of the young haustorium was usually distinct from the rough surface of the papilla. The haustorium elongated rapidly until it extended beyond the margin of the cytoplasmic aggregate (Fig. 1-E), and later became branched in the digitate form of *E. graminis*. In AlgR-3 and GoldR-3, incompatibility prevented normal haustorial development as will be described later.

By 36 hours after inoculation, a hypha had grown from 50% or more of the appressoria that had produced normal haustoria. Hyphae never developed if the haustorium was absent except for one appressorium that produced a short hyphalike lobe [in agreement with findings of Ellingboe and coworkers (9, 15)]. Hyphal growth was highly variable with both compatible and incompatible host-parasite combinations, probably as a result of overcrowding among colonies, as was found by Sullivan (24) for the infection densities used here.

The infection peg was not visible in microculture mounts, and no study was made to determine when the peg formed in relation to aggregate formation or to other events.

Frequency of events.—To monitor events during fungal attack, approximately 24 germlings with well-differentiated appressoria (test appressoria) were selected at 7.5 - 8.5 hours after inoculation for each of the ten host-parasite combinations listed in Table 1. The selections usually were made before signs of host response were visible. The test appressoria and underlying host cells were then observed at about 45-minute intervals from 8.5 to about 21 hours after inoculation, with a final

TABLE 3. Combinations of aggregates (agg), papillae (pap), and haustoria (haust) in barley tissues under attack by *Erysiphe graminis*

Host-parasite combination[a]	Number of appressoria	Percentage of appressoria that produced the indicated combination of structures					
		None	Agg only	Agg-pap	Agg-haust	Agg-pap-haust	Other
Compatible:							
Man-9	27	7	11	15	11	56	0
Han-9	28	29	4	29	21	18	0
Mont-9	27	4	11	22	15	48	0
Mont-3	24	4	0	13	50	33	0
Atlas-3	24	0	8	33	33	25	0
AlgS-3	24	4	8	25	29	38	0
	Total 154	Avg 8	7	23	26	36	0
Incompatible:							
AlgR-3	26	0	9	12	46	35	0
GoldR-3	24	0	0	17	33	50	0
Psak[?]-3	23	0	4	13	61	17	4[b]
Mont-trit	24	8	25	21	21	25	0
	Total 97	Avg 2	9	16	40	32	1
All:							
	Total 251	Avg 5.6	8.0	20.0	32.0	34.5	0.4

[a] See Table 1 for full descriptions.
[b] Represents one specimen which produced a haustorium alone.

observation at 36 hours. In these materials, aggregates were usually well defined, although short-lived ones may have been missed occasionally. Papillae and haustoria were difficult to see when first formed in an aggregate, but they were confirmed after the aggregate dispersed. Only permanent structures were scored as papillae; ephemeral or trace deposits were not.

Aggregates were induced by 92-100% of test appressoria in all host-parasite combinations except Han-9, which had 71% (Table 2). Papillae were induced by 30-70% of test appressoria; haustoria were produced by 39-83%. The frequency of these structures was generally the same in both compatible and incompatible host-parasite combinations (Table 2).

The combinations of aggregate (agg), papilla (pap), and haustorium (haust) that occurred with each host-parasite combination are shown in Table 3. The absence of all three structures was uncommon except for Han-9, and aggregates were induced by most test appressoria as noted earlier. Furthermore, only 0-11% of test appressoria produced an aggregate without a papilla or a haustorium, except for Mont-trit in which 25% of test appressoria induced aggregates alone. Papillae and haustoria always occurred in association with an aggregate, except for only one test appressorium (in PsakR-3). Papillae were present in many instances in which haustoria failed to form, and papillae were also frequently present when haustoria were formed. Thus papillae without haustoria (agg-pap) were produced by 20.0% of test appressoria, haustoria without papillae (agg-haust) by 32.0%, and haustoria with papillae (agg-pap-haust) by 34.5%. The percentages for these combinations of structures were similar in both compatible and incompatible host-parasite combinations.

Timing of events.—The times that aggregates, papillae, and haustoria were first seen were recorded for eight host-parasite combinations (Tables 4 and 5). The times for papillae and haustoria were recorded when a structure was first seen to be forming beneath the appressorial lobe, although it usually could not be identified with certainty until a later observation. Also recorded were the times that papillae became well defined (when a continuous structure was visible along the entire margin of the

TABLE 4. Timing of events during primary infection by *Erysiphe graminis* in relation to formation of the cytoplasmic aggregate (agg) by the host

Host-parasite combination[c]	Hours in designated interval[a,b]				
		From agg to:			
	Inoculation to agg	Papilla		Haustorium	Agg dispersed
		1st sign	Well defined		
Mont-9	10.8 ± 0.4 x	1.0 ± 0.5 x	1.9 ± 0.7 x	2.3 ± 0.5 x	5.1 ± 1.3 x
Mont-3	11.1 ± 0.5 x	1.7 ± 0.8 x	3.1 ± 1.2 xy	1.6 ± 0.4 x	3.2 ± 0.6 xy
Atlas-3	11.1 ± 0.4 x	1.2 ± 0.5 x	2.4 ± 0.4 x	2.0 ± 0.6 x	4.9 ± 0.9 x
AlgS-3	11.1 ± 0.4 x	1.5 ± 0.5 x	3.9 ± 1.3 xy	1.8 ± 0.4 x	2.5 ± 0.5 y
AlgR-3	11.2 ± 0.3 x	1.7 ± 0.7 x	2.9 ± 0.8 xy	1.7 ± 0.4 x	2.6 ± 0.6 y
GoldR-3	11.1 ± 0.4 x	1.4 ± 0.8 x	3.7 ± 0.8 y	1.8 ± 0.8 x	3.2 ± 0.9 xy
PsakR-3	10.8 ± 0.3 x	1.2 ± 1.2 x	2.8 ± 1.1 xy	1.5 ± 0.4 x	2.5 ± 1.0 y
Mont-trit	12.4 ± 0.5 y	1.3 ± 0.5 x	2.1 ± 0.7 x	1.9 ± 0.7 x	3.7 ± 1.3 xy
Avg[d]	11.2 ± 0.1	1.3 ± 0.2	2.8 ± 0.3	1.8 ± 0.2	3.5 ± 0.3

[a] Mean $\pm t_{.05s_{\bar{x}}}$, after Snedecor (22). Means within a column differ significantly from other means in the column only if they are not followed by the same letter, as determined from the confidence intervals shown.

[b] For the number of specimens in a category, add the appropriate combinations of structures of Table 3; e.g., for the total number of specimens in the category "from agg to papilla" in Table 4, add numbers of specimens shown for agg-pap- and agg-pap-haust in Table 3.

[c] See Table 1 for full descriptions.

[d] Averages are weighted for differences in numbers among host-parasite combinations.

TABLE 5. Timing of events during primary infection by specimens of *Erysiphe graminis* grouped according to the combination of aggregate (agg), papilla (pap), and haustorium (haust) that was present. Averages are for the eight host-parasite combinations of Table 4.

Combination of structures[b]	Hours in designated interval[a]				
		From agg to:			
	Inoculation to agg	Papilla		Haustorium	Agg dispersed
		1st sign	Well defined		
Agg only	11.7 ± 0.6 x	...	...	...	2.2 ± 0.9 x
Agg-pap	11.3 ± 0.4 x	1.0 ± 0.3 x	2.9 ± 0.7 x	...	5.3 ± 1.0 y
Agg-haust	11.1 ± 0.2 x	...	...	1.6 ± 0.2 x	2.6 ± 0.4 x
Agg-pap-haust	11.2 ± 0.3 x	1.4 ± 0.3 x	2.9 ± 0.3 x	2.1 ± 0.3 y	3.7 ± 0.5 z

[a] Mean $\pm t_{.05s_{\bar{x}}}$, after Snedecor (22). Means within a column differ significantly from other means in the column only if they are not followed by the same letter, as determined from the confidence intervals shown.

[b] There were 32-67 specimens per combination, except that there were 15-16 for "Agg only."

appressorial lobe) and the times that aggregates dispersed (when only trace amounts of particles remained in the region formerly occupied by the aggregate).

The overall sequence of events during infection is indicated by the average times for each event at the bottom of Table 4. Aggregates formed 11.2 hours after inoculation. Expressed as time after formation of the aggregate, papillae were seen first at 1.3 hours, haustoria at 1.8 hours, and well defined papillae at 2.8 hours. The aggregates dispersed at 3.5 hours. Thus papillae and haustoria were first seen within 0.5 hour of one another, and papillae were still developing when haustoria were first seen. Papillae and haustoria both developed while aggregates were present.

Variation in the timing of events was generally small among the eight host-parasite combinations. The average time of aggregate formation for any given host-parasite combination was within 0.4 hour of the average for all (11.2 hours after inoculation) except for Mont-trit in which aggregates were first seen at 12.4 hours. (The data are insufficient to attribute this delay to host or parasite since neither *E. graminis* f. sp. *tritici* nor *E. graminis* f. sp. *hordei* was tested on wheat.) The times between aggregate formation and the first sign of the papilla and the haustorium were remarkedly uniform among all host-parasite combinations, including Mont-trit (Table 4). Each type of event occurred in any single host-parasite combination within 0.5 hour of the average for all, and differences among them were not significant. Similarly, the papilla became well-defined in each combination within 1 hour of the average for all, although the data show that the papilla became well defined significantly later in GoldR-3 than in Mont-9, Atlas-3, or Mont-trit. Likewise, the aggregate dispersed in each combination within 1.6 hours of the average for all, but significantly later in Mont-9 and Atlas-3 than in AlgS-3, AlgR-3, or PsakR-3 (Table 4).

In Table 5 the timing of events is listed for groups of appressoria that produced given combinations of structures. The haustorium formed earlier in the agg-haust group than in the agg-pap-haust group, which suggests that early formation of the haustorium tended to reduce formation of the papilla. The data also show that aggregates dispersed later if papillae were formed than if they were not.

Incompatibility.—Host cells in AlgR-3 collapsed 16-21 hours after inoculation. Cytoplasmic streaming stopped at 15-18 hours, and host cells lost turgor 1-3 hours later as the host cytoplasm clumped and haustoria degenerated. About half the test appressoria also degenerated, whereas the others remained healthy in appearance, including some attached to degenerate haustoria. Host cells that were not under attack by the fungus also collapsed, especially in parts of the tissue that had unusually heavy inoculum loads. The cells definitely collapsed after the aggregate had dispersed and well after any papillae and haustoria had been produced, although the haustoria were not fully grown at the time of host cell death. In AlgS-3, with host near-isogenic to the host of AlgR-3, the host cells remained alive through 35-36 hours after inoculation, and hyphae grew out from about half of the test appressoria that had produced haustoria (as in other compatible host-parasite combinations).

In GoldR-3, incompatibility was expressed by formation of malformed and small haustoria as their growth was retarded in the period 16-20 hours after inoculation. The central body was more nearly spherical than usual, and the haustorial lobes were small and unusually globular. Host cells and haustoria remained alive, and some appressoria produced hyphae by 35 hours. In PsakR-3, a slight retardation in growth of haustoria was noted by 20 hours after inoculation, but about 50% of the test appressoria with haustoria produced hyphae by 35 hours. Some host cells in PsakR-3 collapsed, but collapse was unrelated to the amount or location of appressoria and probably resulted from injury during mounting instead of in response to the pathogen.

With barley coleoptile tissues under attack by *E. graminis* f. sp. *tritici* (Mont-trit), no signs of incompatibility were seen, an unexpected result. The cells of the host remained alive in all cases, and hyphae developed as they did in fully compatibile host-parasite combinations, so that small colonies of mildew hyphae were present at 72 hours. Thus, for reasons unknown, incompatibility was not expressed between the wheat pathogen and the nonhost test tissue from barley coleoptiles.

Events in primary leaves.—To learn if the events observed in coleoptile test tissues also occurred in leaf tissues, primary leaves of Mont-3, Atlas-3, AlgS-3, AlgR-3, and Mont-trit were examined in dry mounts or in temporary wet mounts as follows: The leaves were detached, infiltrated with water by repeated application and withdrawal of a vacuum, placed on water on a microscope slide, covered with microscope immersion oil (to make wax deposits on the leaf surface invisible), and then covered with a cover slip. After about 1 hour the mounted leaves were discarded and hence were not used for serial observations. Leaves in the wet mounts were poorer optically than were epidermal tissues of coleoptiles, but cytoplasmic streaming, papillae, and haustoria usually could be seen in the leaf epidermis.

Aggregates of cytoplasm were seen frequently in leaves examined 11-16 hours after inoculation, and haustoria were seen beneath about one-third of the observed appressoria at 24-28 hours. Papillae were usually present if haustoria were not. Thus the cytoplasmic aggregate, the papilla, and the haustorium in leaves were generally similar to the same structures in coleoptile tissue. However, the leaves differed from coleoptiles in several ways: (i) Haustoria were not found in combination with papillae beneath any one appressorial lobe, indicating that papillae did not continue to be formed after the haustorium had entered the leaf cell, and suggesting that papillae, when formed, prevented haustorium formation more effectively in leaves than they had in coleoptiles; (ii) About two-thirds of the appressoria located on subsidiary cells and on other cells near stomates, produced haustoria in those cells, but the appressoria located on epidermal cells at a distance from stomates (cells separated from guard or subsidiary cells by at least two intervening cells) produced virtually no haustoria. Hirata (10) and Lin and Edwards (14) likewise have reported that cells near stomates were more susceptible to mildew than cells distant from them. The cells distant from stomates usually had well-differentiated papillae at the site of

attack and the appressoria thereon had secondary and tertiary lobes, indicating that the fungus had tried repeatedly to enter the host. The response of coleoptile tissues, which lack stomates, had been generally more uniform; (iii) The incompatibility in AlgR-3 was expressed differently in leaves than it had been in coleoptiles in that only about half of the leaf cells in which a haustorium was produced collapsed, as had all such cells in coleoptiles. The remaining half stayed alive, and the appressoria associated with these living host cells later produced a hypha. As in coleoptiles, however, papillae appeared to be independent of specific incompatibility because they were produced at about the same frequency in both AlgR-3 and AlgS-3 (of 50 appressoria observed in each, two-thirds induced papillae); (iv) With *E. graminis* f. sp. *tritici* on barley (Mont-trit), papillae formed more frequently in leaves than they had in coleoptiles, as 80-90% of appressoria induced papillae in leaves, and appressoria rarely produced haustoria. Most appressoria produced secondary lobes, indicating that the fungus had failed to enter the host cell at the primary lobe. Thus leaves of barley were more resistant to *E. graminis* f. sp. *tritici* than they were to *E. graminis* f. sp. *hordei* (as expected). This contrasted with coleoptiles of barley which had been equally susceptible to both. The papilla appeared to be an important factor in the barley leaf's high degree of resistance to the wheat mildew fungus.

DISCUSSION.—Our results show that host cytoplasm consistently aggregates at the site of attack by *E. graminis* before a papilla or a haustorium becomes visible within the host cell. Because evidence by electron microscopy indicates that the papilla can be formed before the host wall is penetrated by the fungus (8, 18), it follows that the cytoplasm also aggregates before the wall is penetrated. However, the closeness in time between the appearance of the aggregate and the haustorium (within 2 hours) suggests that the fungus may have started to penetrate before the aggregate developed. The fungus probably penetrates the host wall with the help of wall-degrading enzymes (2, 4, 18), and these (or their products) may have induced the aggregate. Evidence for a chemical inducer is provided by the occasional occurrence of an aggregate in the cell adjacent to the one being attacked, as noted elsewhere (3).

The cytoplasmic aggregate probably has a role in the deposition of the papilla. The large mass of material comprising the papilla was deposited within a 1.5-hour period (from the first sign to a well-defined structure, Table 4). These materials were rapidly transported and assembled while the aggregate was present, and rapidly moving cytoplasm within the aggregate undoubtedly was involved in the deposition processes. Furthermore, the aggregate persisted longer when papillae were produced than when they were not (Table 5). On the other hand, aggregates often did not produce a papilla, and no difference in configuration or movement was detected between aggregates that produced papillae and those that did not. We need to understand more completely at the ultrastructural level how the papilla is assembled so we can clarify the relations between the papilla and the aggregate. It should be noted that cytoplasm sometimes aggregated at sites of attack by *Phytophthora infestans* in both compatible and incompatible host-parasite combinations although papillae were not produced (11).

Smith (21) observed fixed specimens of *Erysiphe* spp. in several hosts and concluded that papillae were formed by host cells as an impediment to the intruding fungus and implied that the outcome depended on the relative speed of host and parasite in their respective activities. The results here are consistent with Smith's interpretation in that: (i) the haustorium and the papilla were definitely formed at about the same time; (ii) the outcome varied among individual specimens within a given host-parasite combination; and (iii) the haustoria without papillae were formed earlier than those with papillae (Table 5). This last point suggests that the haustorium, if formed early enough, may somehow turn off deposition of the papilla.

The fact that haustoria and papillae were formed at about the same time suggests that the 45-minute intervals we used between observations might have been too long for a critical comparison of the timing between the two events. Recent observations by time-lapse photography have indicated that most of the growth in diameter of papillae occurs in only 15-20 minutes (Bushnell, *unpublished*). Nevertheless, our present data from coleoptile tissue indicates that the early stages of papilla formation had been completed by the time the haustorium had enlarged enough within cells to be visible from above, and that deposition of the papilla continued until 0.8 hour after the haustorium first became visible (see agg-pap-haust, Table 5). In leaves on the other hand, the papilla response seemed to be under a more decisive on-off control, as papillae were seen only when haustoria did not form, and papilla deposition was not seen around necks after haustoria were produced. We did not see evidence in leaves that papillae were deposited ahead of the developing fungus and then penetrated as Edwards and Allen (8) concluded from an electron microscope study.

Development by germlings of the mildew fungus stopped at four points in the course of infection after appressoria were produced in our study with coleoptile tissues: (i) Some germlings failed to elicit any host response or show other sign of attempted penetration, possibly because the fungus did not try to penetrate the cuticle and wall, or because it did not penetrate far enough to induce a response. This absence of a response was rare except for Han-9 (Table 3). (ii) Some germlings induced an aggregate of host cytoplasm, but the aggregate later dispersed without sign of papilla or haustorium. In these cases the fungus probably penetrated farther than in the first case, but we speculate that, at most, it only partially penetrated the host wall. (iii) Some germlings induced papilla formation and then failed to produce a haustorium. Here fungus development was probably greater than in the two preceding cases, but we did not determine the extent of host wall penetration. In these cases, the papilla or chemical factors associated with it apparently prevented haustorium formation as has been discussed by others (6, 7, 8, 14). These possibilities occurred with a significant percentage of the fungus population in all the host-parasite combinations we studied. The highest percentage (80-90%) occurred with the wheat mildew fungus on nonhost leaves of barley, in line with observations by others that papillae often are formed in inappropriate host species under attack by powdery mildew fungi (6) or by *Olpidium viciae* (13). (iv) Development of germlings in specifically incompatible

host-parasite combinations was retarded only after young haustoria had been produced in the host cells. Incompatibility was expressed either by hypersensitive death of host cells (with the *Mla* gene) or by retarded fungus development (with the *Mlp* and *Mlg* genes), and the incompatibility clearly did not involve the cytoplasmic aggregate or the papilla.

Only a part of the fungus population induced hypersensitive death of host cells with the *Mla* gene in leaves, whereas another part developed haustoria and hyphae. Similar differences in development among individual germlings on leaves has been reported for the *Mla* and other genes by several workers (9, 12, 14, 15, 16, 17). Ellingboe and coworkers (9, 15, 16) exhaustively studied the development of appressoria and hyphae (which they termed elongating secondary hyphae), but they usually did not observe papillae, haustoria, or cytoplasm inside host cells and therefore did not clearly relate incompatibility to hypersensitivity or to other events and structures inside of host cells. Our results with leaves show that a part of the parasite population with *Mla* failed to produce haustoria because of the nonspecific action of papillae, another part specifically induced rapid hypersensitive death of host cells after a young haustorium had been produced, and a third part produced the beginnings of a mildew colony. In contrast, host cell death immediately after a young haustorium is formed has not been observed in most studies of incompatibility conditioned by the *Mla* and other genes in leaves or coleoptiles of barley (9, 12, 14, 15, 16, 17). In some cases the methods of observation were probably unsuited for observation of cell death, but in others the hypersensitive death of cells may not have occurred because of unsuitable environmental factors or low infection densities.

Our results for the papilla are generally in agreement with the findings of Stanbridge et al. (23). They showed that a small part of the parasite population stopped growth without papilla formation (0.6%), a large part with papilla formation (50-80%), and that the incompatibility conditioned by specific genes (*Mla, Mla6,* or *Mla6* and *Mlg* combined) was expressed as a halt in fungus growth and hypersensitive death of host cells only after a haustorium had been produced in host cells. Both studies emphasize that a high percentage of germlings fail to enter the host when a papilla is produced, regardless of the host-parasite combination or the presence of specific incompatibility. Although we do not know how the papilla excludes the fungus, it apparently is an important component of generalized host resistance to powdery mildew fungi.

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