Papilla Formation in Corn Root-Cap Cells and Leaves Inoculated with *Colletotrichum graminicola*

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ABSTRACT

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Inoculation of single cells naturally sloughed from the root caps of corn seedlings may serve as a model system for studying pathogen-induced papilla formation. This approach circumvents the complicating influences of cohort cells in tissues or of chemical treatments used to obtain single cells. Seedlings were grown aseptically, and the outer calyptra cells that sloughed off were collected, washed, and plated on a medium consisting of glucose, inorganic salts, and agar. Conidia of *Colletotrichum graminicola* plated in the same place germinated and formed appressoria on the corn cells. Most appressoria produced penetration pegs, which penetrated directly without papilla formation, penetrated with papilla formation, or induced a papilla without penetration. The same events occurred in disks that were cut from mature leaves, inoculated, and floated on water. In root-cap cell and leaf disk inoculations, the ratio of unpenetrated papillae to penetrations without papillae was greater in dent corn lines than in sweet corn lines. Dent corn leaves formed fewer lesions per square centimeter than did sweet corn leaves inoculated at early tassel stage. When root-cap cells were inoculated, fewer penetrations occurred and more papillae formed in dent corn than in sweet corn. Application of a translational inhibitor, cycloheximide, inhibited papilla formation and enhanced penetration in root-cap cells and in leaf disks. Addition of 1% glucose or sucrose to the medium enhanced papilla formation and decreased penetration in root-cap cells or leaf disks. Thus, the responses of root-cap cells paralleled those of leaves to the regulatory influences of genotype, nutrition, and translational inhibition.

Additional key words: anthracnose, *Zea mays*.

In testing the hypothesis that papilla formation can limit penetration of fungi into grass leaves, Sherwood and Vance (22,24) found that papilla formation was inhibited and penetration was increased when inoculated disks of leaf tissue were floated on solutions of a protein synthesis inhibitor, cycloheximide. In some unreported tests, we noted that the effect was not uniform through the tissue. We reported similar variation in leaf pieces floated on α-amino-oxy-β-phenylpropionic acid solutions, indicating that the inhibitor did not diffuse to all cells equally (23). Experiments using small pieces of leaf supported this possibility. These experiences illustrate the problem of delivering known amounts of compounds to sites of action when studying the regulation of pathogenesis.

The present study began as a search for a system for applying regulatory compounds directly to the cells in which papilla formation was to be induced. Diffusion of compounds through cuticle or from cut edges of tissue seemed unreliable. Gold et al (7) used partly dissected coleoptiles of barley but noted that prior removal of the coleoptile tip changed penetration efficiency in the coleoptile epidermis. This indicated an influence of neighboring cells of the tissue or injury upon events in a given cell. Use of single, uninjured cells would avoid confounding effects of adjacent cells or wounding. Conventional methods for isolating single cells from plant tissue involve enzymatic dissolution of wall components. These conditions undoubtedly affect new wall synthesis (which is necessary for papilla formation) in chemically isolated cells.

The present study explored the use of single, living cells that separate naturally by sloughing off from the surface of root caps. The separated root-cap cells have well-developed, functional walls, cytoplasm, and nuclei. Viability can be maintained for several days in simple media (10). Hawes and Wheeler (12) found that sensitivity of corn root-cap cells to HMT-toxin correlated with susceptibility of corn lines to *Helminthosporium maydis* race T. A similar relation occurred between sensitivity of oat root-cap cells to victorin and reaction of oat lines to *H. victoriae* (9,11).

Colletotrichum graminicola (Ces.) G. W. Wils. was selected to induce papilla formation in corn root-cap cells for several reasons. This pathogen infects both roots and leaves (25–27), making both tissues appropriate for studying pathogenesis. Corn lines differ in frequency of leaf lesions (13), indicating that there may be genetic differences for resistance to penetration. *C. graminicola* induces papilla formation in leaves (19,29), and the pathogen is not noted for producing pathotoxins that kill cells in advance of penetration.

In the first part of this study, I evaluated cultural conditions that support papilla formation and penetration in root-cap cells. In the second part, I compared frequency of penetration and papilla formation in root-cap cells with that in leaf tissue as influenced by genetic and chemical regulation.

MATERIALS AND METHODS

Production of inoculum. An isolate of *C. graminicola* from corn (*Zea mays* L.) was provided by Herman L. Warren, USDA, Lafayette, IN. Cultures were maintained on oatmeal agar (Difco Lab, Detroit, MI) in 10-cm-diameter petri dishes at 22 C with 12 hr of fluorescent light daily. Conidia from 15- to 20-day-old cultures were washed by suspending and centrifuging them twice in sterile water to remove the spore matrix (3,17) and were suspended in sterile water at 3×10^5 conidia per milliliter for inoculations.

Inoculation of corn leaves. The four sweet corn lines used, and their sources, were (i) Early Sunglow Hybrid and (ii) Golden Cross Bantam, Burpee Co., Warminster, PA 18974, and (iii) inbred IA5125 sugary and (iv) inbred IA453 sugary, Charles Boyer, Pennsylvania State University. The four dent corn lines were (i) 38X, Doebler's Hybrids Inc., Jersey Shore, PA 17740 and (ii) (Pa91 × B73) × T250, (iii) (Pa762 × H84) × T252, and (iv) (H96

× Pa887P) × B84, M. W. Johnson, Pennsylvania State University.

Plants were grown singly in commercial peat moss:vermiculite mix in 15-cm-diameter clay pots in a greenhouse at 25–33 C. Liquid fertilizer solution (20-20-20) was added weekly. Plants were inoculated in the early tassel stage 32–41 days after seeding. Inoculum suspension was sprayed on both surfaces of each leaf to runoff. Plants were incubated for 48 hr at 24 ± 2 C in a walk-in moist chamber (14) and returned to the greenhouse.

Various numbers of corn lines were inoculated in four tests. Each test involved a separate planting date and two plants per line. Five to 7 days after inoculation, 20-cm^2 sampling areas were outlined with ink in the center third of leaves five, six, and seven (from the bottom) of each plant. The number of lesions per square centimeter was determined in the sampling areas.

Inoculations were also conducted on 7-mm-diameter disks cut from mature leaves and floated on water in petri dishes as described earlier (24).

Production of corn root-cap cells. Root-cap cell cultures were prepared by aseptic techniques. Corn seeds (not treated with fungicide) were surface disinfested in lots of 100 seeds by immersion for 9 min in freshly prepared sodium hypochlorite:70% ethanol 1:1 (v/v) with frequent stirring. The disinfestant was decanted and the seeds were rinsed five times with sterile water. The seeds were soaked for 2 hr in sterile water, blotted dry on sterile filter paper, and plated with the embryo downward on 1% water agar. Ten seeds were placed in each 10-cm-diameter petri plate. Plates were kept in plastic bags in a dark incubator at 28 C for 48 hr. Contaminated seedlings were discarded.

The tip 1–2 mm of about 50 primary roots and the adhering clusters of root-cap cells were excised and placed in 4 ml of sterile water in a 12-ml tapered centrifuge tube. The contents were agitated gently in a vortex mixer for 30 sec. After 30 min, the contents were mixed again and suspended cells were pipetted to another tube. The cells were washed three times with sterile water to remove polysaccharide slime (8). Each washing consisted of suspending the cells in 4 ml of water, vortex-mixing gently for 10 sec, centrifuging at low speed (about 100 *g*) for 3 min, and discarding the supernatant. The final pellet was suspended in 2 ml of water (about 5,000 cells per milliliter) and used immediately. Most cells were completely separated from others, but a few remained in groups of 2–10 cells.

Inoculation of root-cap cells. Root-cap cells were inoculated on agar media in 10-cm-diameter petri plates. The standard medium contained 10 g of dextrose, 10 g of agar, and Pfeffer's salts mixture in 1,000 ml of water. Pfeffer's salts consisted of 0.8 g of $Ca(NO_3)_2$, 0.2 g of KNO_3, 0.2 g of $MgSO_4$, 0.2 g of KH_2PO_4, 0.2 g of KCl, and a trace of $FeCl_3$ (16). One-tenth milliliter of conidial suspension (ca. 3,000 conidia) was spotted on a 12-mm-diameter area of the agar surface. Four inoculum spots were made per plate. The plates were left uncovered in the flowing air of a transfer hood until free moisture evaporated (30–60 min). Then 0.1 ml of corn root-cap cell suspension (ca. 500 cells) was overlaid on each inoculum spot, and free moisture was evaporated as above. Lids were replaced and plates were incubated in plastic bags in dark incubators for 24–48 hr. The colonies were stained with cotton blue-lactophenol. The stained agar slabs were cut out, mounted on microscope slides with coverslips, and examined with a bright-field microscope at ×500.

The first 50 ± 5 separated root-cap cells found with attached appressoria were classified into four groups: no papilla or penetration, penetration only, papilla only, and papilla with completed penetration. Three or four replicate colonies were classified to give a total of 150–200 cells classified per treatment.

For estimates of viability, corn cell suspensions were plated on fungus-free areas of the plates and examined at the beginning and end of incubation using the fluorescein diacetate vital staining method of Widholm (28).

Statistical analyses were done with the Statistical Analysis System (20).

RESULTS

Microscopic observations. Germ tubes and hyphae of *C. graminicola* did not show orientation toward root-cap cells, and contact between hyphae and root-cap cells appeared random. About 30–60% of the contacts induced no visible reaction by the corn cell. In the remainder of contacts, the fungus formed an appressorium. Nearly all appressoria on root-cap cells were single celled, only slightly wider than the hypha, and unpigmented. Occasionally they were larger, globose, and brown. Papillae formed beneath many appressoria; they varied from minute, hemispheric appositions 2–3 μm thick to elongate protuberances 10 μm long surrounding a thin penetration peg. Most penetrations from appressoria involved passage by a peg through unthickened host wall; some were accompanied by a small papilla. Hyphae exited from the host cells through a narrow pore in the host wall after little proliferation inside the cell. Appressoria, papillae, and penetrations occurred at any location on the surface. There was no obvious difference between unpigmented and pigmented appressoria in ability to initiate penetration or papilla formation in root-cap cells.

Cytoplasts of attacked corn cells showed a range of integrity from no apparent vacuolization or plasmolysis, to extensive vacuolization and plasmolysis, to collapse into a dense aggregate. About 60–90% of cytoplasts associated with papilla formation

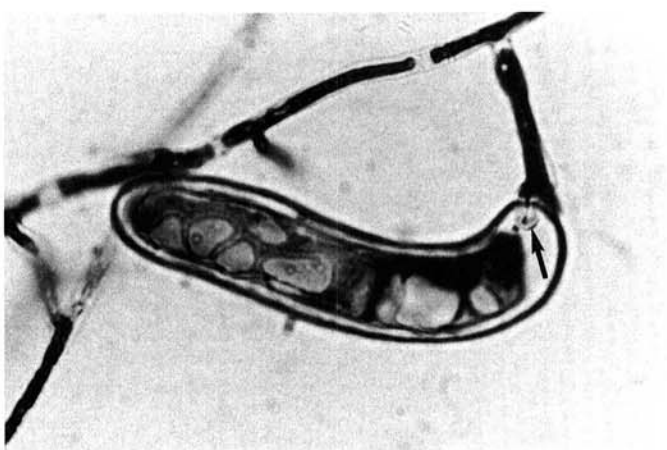

Fig. 1. Corn root-cap cell showing papilla formation without penetration in response to *Colletotrichum graminicola*. Appressorium (upper right) was only slightly enlarged. Hemispheric papilla (arrow) was formed inside corn cell wall beneath appressorium and encased thin (darkly stained) penetration peg. At time of sampling, 27 hr after inoculation, host cytoplast adjacent to papilla was drawn away from host wall and stained darkly with cotton blue. (ca. ×950)

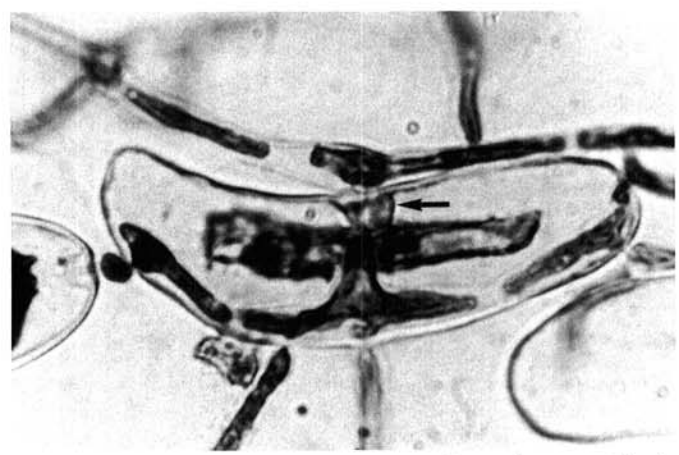

Fig. 2. Corn root-cap cell penetrated by *Colletotrichum graminicola*. Slightly enlarged intercalary appressorial cell (top center) subtended penetration peg and surrounding papilla (arrow). By time of sampling at 27 hr after inoculation, host cytoplast was collapsed and stained darkly and hyphae had exited host cell from at least five points (right, bottom, left). One hypha had induced papilla formation without penetration in cell to left. (ca. ×950)

were intact or slightly plasmolyzed at the point of the papilla (Fig. 1). About 70% of cells that were penetrated had extensive cytoplasmic vacuolization or clumping and appeared dead (Fig. 2).

Effect of temperature and medium. Because penetration attempts on leaves lead to papilla formation, or penetration, or both, or neither, it was desirable in the root-cap cell study to establish standard incubation conditions that would support all four alternatives. Cultivar Early Sunglow was used to test the effects of temperature, glucose concentration, and salts concentration.

In the tests of temperature, inoculated root-cap cells were incubated on 1% sucrose, 1% agar medium at 20, 24, 28, and 32 C for 27 hr (test 1) or 40 hr (test 2). In test 1, there was no significant effect of temperature. Ranges in the four classes were (i) no penetration or papillae—10–17%; (ii) penetration only—25–28%; (iii) papilla only—50–57%; and (iv) penetration and papilla—6–9%. In test 2, papilla formation was more frequent at 28 than at 20 C (Table 1). Host-cell viability declined greatly at 32 C.

Glucose concentration was tested using Pfeffer's salts, 1% agar, and 0.1, 0.3, 1, 3, and 5 g of glucose/100 ml of medium. Incubation was 40 hr at 28 C. One percent glucose supported significantly more papilla formation (45%) and less penetration (44%) than the other media (Table 2). A second test gave similar results.

Inorganic salt concentration was tested using 1% glucose and 1% agar with Pfeffer's salts at 0, 0.5, 1, 2, and 4×. Incubation was at 24 C for 26 hr. Two tests gave the same results. Penetration, without papillae, was least frequent on 1× media (Table 3). Papilla formation was most frequent on the 0.5 and 1× and least on 0 and 4× media. Noninoculated cells retained greater viability on 0.5 and 1× media.

The standard culture conditions adopted were 1% glucose, Pfeffer's salts, and incubation at 24 C. Within the ranges tested, these conditions supported the highest ratio of papillae per penetration and the lowest frequency of appressorial sites that had no papilla or penetration.

Effect of genotype on penetration and papilla formation. When leaves of plants at early tassel stage were inoculated, significantly more lesions per square centimeter were formed on sweet corn lines than on dent corn lines (Table 4). The distinction was absolute in tests 1, 2, and 4. In test 3, the most lesions were formed on Golden Bantam sweet corn; IA453 sweet corn and B84 dent corn were intermediate, and the fewest lesions were formed on 38X and T250 dent corn.

Leaf disk inoculation was used to study penetrations and papillae in Early Sunglow sweet corn and B84 and 38X dent corn leaves. Appressoria were globose and brown. Most appressoria (42–69%) did not subtend papillae or penetration. Penetration was most frequent in Early Sunglow (Table 5). Papilla formation without penetration was more frequent in the dent corn lines (17–39%) than in Early Sunglow (2–3%).

Root-cap cells were used in five inoculations of two to four corn lines per test. In tests 1, 2, 3, and 5, the sweet corn lines formed significantly more penetrations and fewer papillae than the dent corn lines (Table 6). In test 4, Golden Bantam and Early Sunglow sweet corn were similar to B84 dent corn, and all formed more penetrations and fewer papillae than T250 dent corn.

Effect of sugar and cycloheximide on penetration and papilla

TABLE 1. Percentage of Early Sunglow corn root-cap cells showing penetration or papilla formation after inoculation with *Colletotrichum graminicola* and incubation at various temperatures[w]

Class	20 C	24 C	28 C	32 C
Penetration only[x]	52 a[y]	51 a	51 a	56 a
Papilla only[x]	30 b	36 ab	43 a	37 ab
Penetration and papilla[x]	2 a	3 a	2 a	2 a
Viability[z]	89	83	75	41

[w] Incubation was for 40 hr at 24 C on 1% sucrose, 1% agar medium.
[x] About 50 root-cap cells with appressoria were classified in each of four replicates.
[y] Values in rows not sharing a common letter are significantly different in Duncan's multiple comparisons test at alpha = 0.05.
[z] Two replicates of 100 noninoculated cells at 27 hr were done; viability was 96% at the start.

TABLE 2. Percentage of Early Sunglow corn root-cap cells showing penetration or papilla formation after inoculation with *Colletotrichum graminicola* and incubation with various glucose concentrations[w]

Class	0.1%	0.3%	1%	3%	5%
Penetration only[x]	64 b[y]	59 b	44 c	66 b	76 a
Papilla only[x]	27 bc	35 b	45 a	24 c	12 d
Penetration and papilla[x]	1 a	1 a	1 a	1 a	1 a
Viability[z]	46 c	43 c	52 bc	56 ab	61 a

[w] Incubation was for 40 hr at 28 C on media with indicated percentage of glucose and with Pfeffer's salts and 1% agar.
[x] About 50 root-cap cells with appressoria were classified in each of four replicates.
[y] Values in rows not sharing a common letter are significantly different in Duncan's multiple comparisons test at alpha = 0.05.
[z] Three replicates of 100 noninoculated cells at 40 hr were done; viability was 96% at the start.

TABLE 3. Percentage of Early Sunglow corn root-cap cells showing penetration or papilla formation after inoculation with *Colletotrichum graminicola* and incubation with various Pfeffer's salts concentrations[w]

Class	0×	0.5×	1×	2×	4×
Penetration only[x]	33 a[y]	19 bc	13 c	19 bc	29 ab
Papilla only[x]	40 b	68 a	65 a	44 b	35 b
Penetration and papilla[x]	11 a	3 bc	2 c	7 b	4 bc
Viability[z]	74 bc	87 a	80 ab	71 c	68 c

[w] Incubation was for 26 hr at 24 C on media with 1% glucose and 1% agar.
[x] About 50 root-cap cells with appressoria were classified in each of four replicates.
[y] Values in rows not sharing a common letter are significantly different in Duncan's multiple comparisons test at alpha = 0.05.
[z] Three replicates of 100 noninoculated cells at 26 hr were done; viability was 98% at the start.

TABLE 4. Number of lesions per square centimeter on corn leaves inoculated at the early tassel stage with *Colletotrichum graminicola*[x]

Corn line	Test 1	Test 2	Test 3	Test 4
Sweet corn				
IA5125	6.2 a[y]	14.5 a	...[z]	10.7 a
IA453	4.6 a	12.5 a	4.9 b	...
Golden Bantam	4.7 a	12.2 a	7.2 a	...
Early Sunglow	...	...	...	7.0 b
Dent corn				
T250	1.2 b	1.7 b	0.9 c	...
T252	0.4 b	0.4 b	2.9 bc	...
B84	0.6 b	2.9 b	3.8 b	...
38X	0.4 b	...	1.0 c	3.2 c

[x] The basic sample consisted of 20-cm^2 area in center third of each of leaves five, six, and seven on two plants per entry per test.
[y] Values in columns not sharing a common letter are significantly different in Duncan's multiple comparisons test at alpha = 0.05.
[z] Not tested.

TABLE 5. Penetration and papilla formation in corn leaf samples floated on 1% glucose solution after inoculation with *Colletotrichum graminicola*

	Percentage of cells					
	Test 1			Test 2		
Class[y]	Early Sunglow	B84	38X	Early Sunglow	B84	38X
Penetration only	28 a[z]	18 b	5 c	19 a	12 ab	6 b
Papilla only	3 b	17 a	20 a	2 b	28 a	39 a
Penetration and papilla	6 b	16 a	6 b	2 b	18 a	2 b

[y] About 50 epidermal cells with appressoria were classified in each of four replicates.
[z] Values in rows in tests not sharing a common letter are significantly different in Duncan's multiple comparisons test at alpha = 0.05.

TABLE 6. Penetration and papilla formation in root-cap cells of corn lines inoculated with *Colletotrichum graminicola*[y]

	Percentage of cells												
	Test 1		Test 2		Test 3			Test 4				Test 5	
Class	Golden Bantam	38×	IA5125	B84	Golden Bantam	B84	38×	Golden Bantam	Early Sunglow	B84	T250	IA453	T252
Penetration only	37 *[z]	5	38 *	14	55 a	37 b	21 c	52 a	47 a	48 a	33 b	45 *	15
Papilla only	46 *	90	38 *	59	36 c	51 b	68 a	37 b	34 b	42 b	59 a	34 *	73
Penetration and papilla	1	1	3	2	1 a	1 a	2 a	3 a	3 a	1 a	2 a	2	1

[y] About 50 root-cap cells with appressoria were classified in each of four replicates per entry per test, using 1% glucose, Pfeffer's salts, and 1% agar for 27 hr at 24 C.

[z] Asterisk indicates that the difference between lines within a test was significant at $P = 0.05$ in a pairwise t test. Values in row of a test not sharing a common letter were significantly different in Duncan's multiple comparisons test at alpha = 0.05.

TABLE 7. Percentage of Early Sunglow corn leaf epidermal cells showing penetration and papilla formation after inoculation with *Colletotrichum graminicola* and incubation on aqueous solutions[x]

Class[y]	Water	Sucrose	Cycloheximide	Cycloheximide + sucrose
Penetration only	34 b[z]	18 c	51 a	55 a
Papilla only	8 b	17 a	0 c	0 c
Penetration and papilla	9 a	10 a	0 b	0 b

[x] Leaf disks (10 mm diam) were floated on distilled water with or without 1% sucrose or 12.5 ppm cycloheximide at 25 C for 72 hr.

[y] About 50 epidermal cells with appressoria were classified in each of four replicates.

[z] Values in rows not sharing a common letter are significantly different in Duncan's multiple comparisons test at alpha = 0.05.

TABLE 8. Percentage of Early Sunglow corn root-cap cells showing penetration and papilla formation after inoculation with *Colletotrichum graminicola* and incubation with cycloheximide[w]

Class	0 ppm	5 ppm	15 ppm
Penetration only[x]	20 b[y]	36 ab	51 a
Papilla only[x]	61 a	31 b	14 b
Penetration and papilla[x]	2 a	4 a	1 a
Viability[z]	98	91	93

[w] Incubation was for 26 hr on 1% glucose, Pfeffer's salts, 1% agar medium.

[x] About 50 root-cap cells with appressoria were classified in each of four replicates.

[y] Values in rows not sharing a common letter are significantly different in Duncan's multiple comparisons test at alpha = 0.05.

[z] Two replicates of 100 noninoculated cells at 26 hr were done; viability was 99% at the start.

formation. Early Sunglow leaf disks were inoculated and floated on distilled water or on 1% sucrose solution. The disks on 1% sucrose had a higher frequency of papilla formation and lower frequency of penetration than disks on water (Table 7). When water or 1% sucrose solution was supplemented with cycloheximide (12.5 $mg \cdot L^{-1}$), papilla formation was totally inhibited and penetration was increased significantly (Table 7).

Early Sunglow root-cap cells were inoculated onto 1% glucose, Pfeffer's salts agar medium. Drops of water containing cycloheximide at 0, 5, or 15 $mg \cdot L^{-1}$ were placed onto inoculated cells and evaporated. The experiment was conducted twice with the same results; one test is shown in Table 8. Cycloheximide at 15 $mg \cdot L^{-1}$ significantly increased penetration and reduced papilla frequency. Viability of noninoculated cells remained high in the cycloheximide treatments.

DISCUSSION

Papilla formation is an induced response involving synthesis and deposition of wall materials (1,21,24). The discovery that fungal challenge can induce papilla formation in unattached corn root-cap cells provides evidence that single, isolated cells are capable of an induced response to a pathogen resembling that of intact tissues. Although Hawes and Wheeler (11,12) reported that sensitivity of root-cap cell suspensions to pathotoxins was correlated with whole plant reaction, their studies did not distinguish whether the toxin/host-cell interactions involved induced or constitutive components.

The results indicate that papilla formation responses in root-cap cells may be effective in reducing penetration. In general, treatments that resulted in an increase in frequency of papilla formation gave a corresponding decrease in frequency of penetration. The correlative evidence is similar to that reported for leaves (23).

In all treatments, there was a relatively low frequency of papillae that did not prevent penetration. I did not determine whether the papillae that were accompanied by penetration were formed before or after penetration was completed. Penetrations accompanied by papillae were observed in leaves as well as root-cap cells. Politis and Wheeler (19) gave ultrastructural evidence that penetration of corn leaves by *C. graminicola* can be successful where leaf papillae are formed. Root-cap cell papillae associated with penetrations were usually small and poorly stained, suggesting that ineffectiveness of resistance to penetration might result from a delayed or weak papilla response. Aist and Israel (2) and Gold et al (7) showed that timing of papilla initiation relative to penetration peg initiation can be critical to success of penetration of barley by *Erysiphe*.

The discussion below indicates that the ratio of papillae without penetration to penetrations without papillae responded to genetic and chemical regulation. The direction of change in root-cap cells paralleled the direction of change induced in leaf tissue by the same factors. This indicated that the *C. graminicola*/corn root-cap cell system may reflect the potential of leaf tissue to form papillae.

Corn lines differed significantly in papillae per penetration in root-cap cells (Table 6), indicating that the response of root-cap cells is genetically determined. Sweet corn lines had more penetrations and fewer papillae than dent corn lines in both root-cap cell (Table 6) and leaf sample (Table 5) inoculations. In inoculations of whole plants (Table 4), sweet corn lines formed more lesions per square centimeter than dent corn lines. If we assume that lesion frequency is related to penetration frequency, it appears that whole leaves of sweet corn, like leaf disks and root-cap cells, had a higher frequency of penetration than those of dent corn.

Most studies of susceptibility of corn lines to anthracnose (5,6,18) have not measured resistance to penetration or have not distinguished between resistance to penetration and resistance to lesion enlargement. Jenns and Leonard (13) showed significant differences among inbred lines of corn for lesion frequency and for lesion length and found that the two traits were not always related. Resistance to penetration (lesion frequency) in leaves is strongly influenced by plant age, leaf position (15), and illuminance (13). Penetration should be considered separately from lesion enlargement in studies of resistance.

Papilla formation responded to regulation by exogenously supplied chemicals. The translational inhibitor cycloheximide inhibited papilla formation and increased penetration in root-cap cells (Table 8) and leaf tissue samples (Table 7). Inhibition in root-cap cells appeared dosage-dependent. The findings were consistent with previous reports on the effect of cycloheximide in leaves (22,24). Compared with sugar-free control media, media with 1% glucose or sucrose enhanced papilla formation and

decreased penetration in root-cap cells (Table 2) and leaf samples (Table 7). Whether the sugar enhanced plant systems, fungal systems, or both requires further study. It is noteworthy that even in the absence of an added energy source, apparently some root-cap cells had sufficient reserves to form effective papillae.

The study was not intended to establish whether host-cell death preceded penetration, but it did indicate that the matter is complex. Most of the penetrated cells had collapsed cytoplasts and appeared dead. In the Pfeffer's salts experiment (Table 3), the percentage of penetrated cells was similar to that of noninoculated control cells that were naturally dead (fluorescein diacetate negative) at the time of sampling. This finding suggests that penetration occurred principally in weak or dead cells. However, evidence from other tests suggests that penetration did not occur only in dead cells. In the cycloheximide tests (Table 8) at 26 hr, up to 51% of cells were penetrated, but only about 10% of noninoculated control cells on cycloheximide were dead. With 5% glucose medium (Table 2), the percentage of penetrated cells exceeded the percentage of viable control cells. Thus, at least some cell death may have been the result of penetration.

The single root-cap cell inoculation system may offer advantages over tissue systems. Regulatory compounds can be delivered to the cell in known amount and form. Events in living cells can be viewed by light microscopy, using techniques similar to those described (2,4,7) for partly dissected coleoptiles. Confounding effects of cohort cells of tissues would be avoided. One-on-one confrontations of host and pathogen cells can be manipulated in a gnotobiotic environment. The cells are of one type only; however, they vary in size and longevity. The present study shed no new insight on the question whether variation in penetration within a population of cells was due to variation in host or pathogen or both.

Ultimately, each step in the sequence of events leading to pathogenesis and resistance must be examined in the context of natural host and pathogen associations. Validating tests with tissues should be conducted whenever extending the results obtained from single cells.

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