Histological Reactions and Effects on Position of Epidermal Nuclei in Susceptible and Resistant Corn Inoculated with Helminthosporium maydis Race T

M. R. Contreras and C. W. Boothroyd

Graduate Student and Professor, respectively, Department of Plant Pathology, Cornell University, Ithaca, N.Y. 14853. Based on a M.S. thesis submitted by the senior author to Cornell University.

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ABSTRACT

The first detectable manifestation of differential host response in susceptible (W64A^{tcms} and M017^{tcms}) and resistant (W64A and M017) corn seedlings inoculated with *Helminthosporium maydis* (*Cochliobolus heterostrophus*) race T was an effect on the position of epidermal nuclei in the incompatible reaction. The nuclei in epidermal cells surrounding the penetration site were positioned next to the radial walls most proximal to the penetrated cell. This event occurred simultaneously with penetration and formation of a primary hypha 6-9 hours after inoculation. Results of inoculations with *H. carbonum* (a nonpathogen of these inbreds) and *H. victoriae* (a nonpathogen of corn) suggest that the phenomenon may be associated with incompatible corn-fungus interactions. Hyphal growth of the pathogen in resistant corn was arrested about 12 hours after inoculation, and had apparently ceased by 18-24 hours. The pathogen colonized susceptible tissue readily, with changes in the morphology of the infected tissue occurring later than in the incompatible reaction. It is suggested that differential disease reaction is determined very early after penetration.

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Orillo (8) and Jennings and Ullstrup (6) have given accounts of the histological interactions of *Helminthosporium maydis* Nisikado & Miyake (*Cochliobolus heterostrophus* Drechsler) with susceptible and resistant corn. Only brief reports have been given, however, of the histological aspects of *H. maydis* race T affecting corn tissue (2, 3). The objective of the present investigation was to obtain, by means of light microscopy, information on the histological events that occur during the initial interactions between *H. maydis* race T and compatible and incompatible lines of dent corn (*Zea mays* L.). The earliest observable event after penetration was the apparent migration of epidermal nuclei toward the penetration site in incompatible interactions. The authors earlier reported this observation in abstract form (4).

Mechanical injury or infection of a plant cell has been reported to affect the position of the nucleus in the affected cell and, in some cases, the position of nuclei in neighboring cells as well. Benda (1) illustrated photographically the approach of the nucleus toward a wound site in an epidermal cell of *Nicotiana langsdorfii*. Tomiyama (11) observed migration of epidermal nuclei towards infection sites in resistant potato leaf tissue inoculated with *Phytophthora infestans* (Mont.) de Bary. More recently, Somasekhara and Pappelis (10) indicated that nuclei in epidermal cells of onion bulbs became oriented toward wound sites, and more markedly so in the direction of infection sites in tissue inoculated with *Botrytis allii* Munn. Pappelis et al., (9) have since reported the phenomenon in more detail in onion epidermal cells wounded or inoculated with *B. allii* Munn. and *Aspergillus niger* van Tiegh.

MATERIALS AND METHODS.—Seedlings of corn inbreds W64A and M017 in normal or Texas cytoplasmic male sterile (tcms) lines were inoculated at the four- to five-leaf stage with *Helminthosporium maydis* race T. The inoculum source was a 2-week-old culture of an isolate (T6) grown on PDA under fluorescent lamps (14 hours of light/day) and at 26 C. Isolate T6 is virulent on corn with Texas male-sterile cytoplasm, whereas it does not affect corn with normal cytoplasm. A suspension of conidia in water was applied to the foliage with a De Vilbiss atomizer. The plants were enclosed in plastic bags immediately after inoculation and placed in a large chamber at 27 C. The same procedure was followed in tests with *H. carbonum* Ullstrup race 2 and *H. victoriae* M. & M.

Leaf tissue for whole mounts was fixed in a mixture containing equal parts of glacial acetic acid and 95% ethyl alcohol, cleared in lactophenol, and stained with 0.1% acid fuchsin in lactophenol. Cross sections of leaf tissue (24 to 36 μm thick) were made with a Hooker (Lab line) fresh-tissue microtome (5) and stained with 0.1% acid fuchsin or cotton blue in lactophenol. All tissues were mounted in 50% glycerin for observation.

The position of nuclei in epidermal cells was estimated by the following procedures. Randomly selected leaf

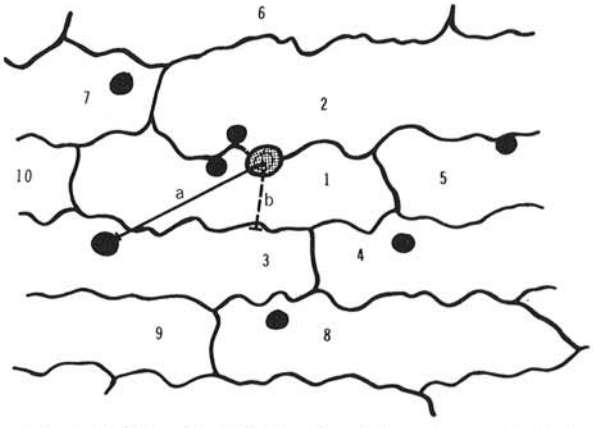

Fig. 1. Position of nuclei (dots) in epidermal corn cells (1-10) in relation to an appressorium of *Helminthosporium maydis* race T. a = distance from appressorium to nucleus, b = distance from appressorium to a point in the wall of the same cell closest to the appressorium. A smaller value for the difference between the two measurements indicates closer proximity of the nucleus to the appressorium.

TABLE 1. Mean[a] values in μm indicative of the effect of penetration by *Helminthosporium maydis* race T on the position of nuclei in 10 epidermal cells surrounding different sites in resistant (M017) and susceptible (W64A^{tcms}) corn. A smaller[b] value indicates greater proximity of nuclei to the appressorium or the penetrated epidermal cell

	Inbred M017		Inbred W64A^{tcms}
Site	Before penetration[c]	After penetration	after penetration
1	22.4	0.3	25.5
2	26.1	0.6	29.4
3	20.5	0.6	30.9
4	68.6	1.2	39.2
5	20.6	16.7	23.1
6	17.2	9.8	24.8
7	34.0	27.4	24.0
8	25.8	0.4	20.6
9	18.1	2.1	21.2
10	29.9	1.0	23.9
= X	28.32 ± 4.77 A	6.01 ± 2.92 B	26.26 ± 1.84 A

[a]Means obtained from differences between two straight-line measurements, one going from the center of the appressorium to the nucleus of an epidermal cell, and the other from the center of the appressorium to that point in the wall of the same cell closest to the appressorium. Each mean is composed of 10 such differences.

[b]Means with standard errors followed by the same letter do not differ statistically, $P = 0.05$, according to Duncan's multiple range test.

[c]Measurements taken at sites where a very young appressorium had developed, but no signs of penetration were evident.

surface regions, each bearing a germinating conidium, were sketched free-hand 6-12 hours after inoculation. The epidermal cells of the region were numbered 1 through 10, the smaller numbers being those of the epidermal cells closest to an appressorium; increasingly larger numbers corresponded to cells farther away. Straight-line measurements (in μm) were made for each of the cells from (i) the center of the appressorium to the nucleus of a given cell and from (ii) the appressorium to a point in the wall of the same cell that was closer to the appressorium (Fig. 1). The difference between the two measurements gave an indication of the relative position of the nucleus in the cell with respect to the site of penetration; i.e., a smaller value indicated that the nucleus was closer to the appressorium or penetration site.

RESULTS.—Prepenetration and penetration activities of *H. maydis* race T were similar in both susceptible and resistant tissue. Germination was mostly bipolar and was noticeable as early as 1 hour after inoculation. Appressoria usually formed at the depression between two adjoining epidermal cells. Penetration was always associated with appressoria and took place 3-9 hours after inoculation. The penetration peg swelled upon gaining ingress into an epidermal cell, and then gave rise to a primary hypha.

Reaction of tissue compatible to Helminthosporium maydis race T.—Secondary hyphae grew rapidly in susceptible tissue of W64A^{tcms} and were observed in adjacent epidermal cells some 300 μm from the site of penetration, deep in the mesophyll, and near the lower epidermis of the leaf within 12 hours of inoculation. Profuse ramification had occurred 18 hours after inoculation; hyphae generally spread intercellularly, but they were also observed inside of mesophyll cells. Changes in host cell morphology were apparent after 18 hours with the formation of small granular bodies associated with advancing hyphae and a slight brown discoloration in tissue more proximal to the site of penetration. Collapse of the tissue occurred 24 hours after inoculation. Lesions in W64A^{tcms} first were visible macroscopically 12-18 hours after inoculation. Lesion development began as chlorotic specks which by about 48 hours expanded to lesions 8-13 mm in length, each with a conspicuous necrotic center (3-5 mm in length). By 96 hours, most of the lesion tissue was necrotic; lesions were

TABLE 2. Mean[a] values in μm indicative of the effect of penetration of three *Helminthosporium* spp. on the position of nuclei in 10 epidermal cells surrounding different penetration sites in resistant (M017) and susceptible (W64A^{tcms}) corn. A smaller[b] value indicates greater proximity of nuclei to the penetrated epidermal cell

	Inbred M017			Inbred W64A^{tcms}		
Site	*H. carbonum*	*H. victoriae*	*H. maydis* race T	*H. carbonum*	*H. victoriae*	*H. maydis* race T
1	6.35	3.04	7.36	9.65	3.30	18.03
2	3.81	6.60	2.03	1.27	5.08	12.44
3	0.50	1.27	1.01	0.00	12.44	21.59
4	0.76	9.90	5.58	2.54	11.93	26.67
5	3.30	2.28	8.12	6.03	6.35	11.17
6	0.76	12.19	8.12	7.62	6.85	18.49
7	6.09	6.33	0.50	15.44	19.05	7.11
8	1.01	0.25	2.54	9.63	27.43	20.06
9	4.31	2.28	2.28	4.06	28.44	31.75
10	5.08	1.27	3.30	4.31	13.46	17.78
= X	3.19 ± 0.72 A	4.44 ± 1.26 A	4.08 ± 0.93 A	6.06 ± 1.48 A	13.43 ± 2.83 B	18.51 ± 2.56 C

[a]Means obtained from differences between two straight-line measurements, one going from the center of the appressorium to the nucleus of an epidermal cell and the other from the center of the appressorium to that point in the wall of the same cell closest to the appressorium. Each mean is composed of 10 such differences.

[b]Means with standard errors followed by the same letter do not differ statistically, $P = 0.05$, according to Duncan's multiple range test.

elongate (about 20 mm in length), and had increased in width occupying two to three adjacent interveinal spaces (4-5 mm). After 6 days, tissues surrounding the lesions became chlorotic and extended from the lesion towards the apex of the leaf; many leaves were desiccated The overall reaction of W64A^{tcms} was one of high susceptibility.

The histological events in susceptible M017^{tcms} corn followed a pattern similar to that described for W64A^{tcms}. Hyphae, however, did not spread as rapidly as in the latter. Secondary hyphae ramified in the penetrated cell, crossed to two or three neighboring epidermal cells, and had advanced downwards to the upper mesophyll region in 12 hours. Cells of the mesophyll located directly below penetrated epidermal cells showed some granulation of cytoplasm and a yellow discoloration 12-18 hours after inoculation. Cell collapse was seen in the mesophyll directly below the penetration site and in surrounding tissue in 18-24 hours. This collapsed center was brown and it was surrounded by a yellow discoloration 100-200 μm in width which exhibited granulation. Lesion expansion continued in similar fashion; i.e., a yellow discolored area associated with advancing hyphae at the lesion borders and surrounding a brown collapsed center. The incipient lesions on M017^{tcms} first were visible macroscopically as small discolored areas 18 hours after inoculation; by 48 hours they became conspicuous chlorotic lesions, about 2-4 mm in length, with no visible necrosis. The lesions expanded slowly and a light brown center developed in the middle of the chlorotic lesion by 96 hours. By 6 days, each lesion consisted of an oval shaped, brownish center (2-3 mm in length) surrounded by a chlorotic halo (3-4 mm in width). Little or no leaf killing was seen. The overall reaction of M017^{tcms} was one of moderate susceptibility.

Reaction of tissue incompatible to Helminthosporium maydis race T.—Secondary hyphae in tissue with normal cytoplasm (W64A and M017) were limited to the epidermal cell penetrated first, or to a few (two to four) adjacent cells. Nuclei of epidermal cells adjacent to the site of penetration moved, in their respective cells, to a point closer to the penetrated cell 6-9 hours after inoculation. This effect on the position of epidermal nuclei may have occurred during or a few hours after penetration. No attempt was made to determine the time of occurrence with more precision. Six to 12 hours after inoculation, a few of the cells (four to six) of the mesophyll directly below the penetrated epidermal cell showed granules and, in some cases, brown discoloration of the tissue was evident in a radius 30-40 μm from the penetration site. Arrest of hyphal growth was apparent 12 hours after inoculation, at about the same time that discoloration of the tissue was apparent. Eighteen to 24 hours after inoculation, hyphal growth apparently had ceased. Cells in close proximity to hyphae were in an advanced stage of collapse; other cells in the area appeared normal. Twenty-four hours after inoculation, minute chlorotic areas were visible macroscopically in both W64A and M017. These areas became small, round-to-elongate necrotic lesions, about 1-3 mm in length, in 48-72 hours. Lesions on W64A were slightly larger than those on M017. The lesions were limited to single interveinal spaces and the damage caused to the plants was minimal. The overall reaction of both inbreds was one of high resistance.

Effects on the position of epidermal nuclei.—Observations of whole mounts of W64A and M017 tissue indicated that *H. maydis* race T affected the position of nuclei in epidermal cells adjacent to the site of penetration. This effect was extensive, involving as many as 30 epidermal cells, and was dependent upon penetration by the fungus into an epidermal cell as shown in Table 1. No precise observations were made to determine if the fungus had any minor effects on position of nuclei prior to penetration; i.e., during germination or formation of appressoria. The effect on the position of nuclei, first detected 6-9 hours after inoculation, was the earliest detectable manifestation of differential host reaction to the pathogen. There was no apparent effect on the position of nuclei in susceptible cells, except in the cells adjacent to the site of penetration. By 6-9 hours after inoculation, nuclei of these cells often appeared to have migrated toward the penetration site; this effect, however, was always limited to two or three cells and was never as extensive as in incompatible tissue.

Inoculation of leaves of W64A^{tcms} or M017 (the lines most susceptible and most resistant, respectively, to *H. maydis* race T) with *H. carbonum* race 2 (a nonpathogen of these inbreds) resulted in the same effect on the position of nuclei. *H. victoriae* (nonpathogenic to corn) caused the phenomenon in leaves of M017, but results with W64A^{tcms} were not conclusive. Mean values for position of nuclei are given in Table 2.

DISCUSSION.—Interaction between *H. maydis* race T and susceptible and resistant tissues appear similar to those described for other diseases. There were no differences in prepenetration and penetration activities of the fungus on susceptible and resistant tissues. The first detectable difference was the extensive effect on the position of nuclei in resistant but not in susceptible tissue at 6-9 hours after inoculation. A brown discoloration developed in both susceptible and resistant tissues, with marked differences in the development time required in both cases. Brown discoloration was observed first in resistant tissue 9-12 hours after inoculation; whereas, it was not observed until 18-24 hours after inoculation in susceptible tissue. Arrest of hyphal growth in resistant tissue occurred at about the same time that discoloration of the tissue was apparent; i.e., about 12 hours after inoculation.

It is evident from our observations that in resistant tissue epidermal cells removed from the penetration site respond rapidly to penetration of *H. maydis* race T into the leaf. The position of epidermal nuclei close to the radial walls proximal to the epidermal cell penetrated first is indicative of this response. This phenomenon can be elicited in a corn line resistant to *H. maydis* race T by species of *Helminthosporium* other than *H. maydis*. The effect on the position of nuclei, furthermore, is not unique to a corn line resistant to *H. maydis* race T; it can also be elicited in corn tissue susceptible to *H. maydis* by at least one other species of *Helminthosporium*. It appears from our observations that the phenomenon may be associated with incompatible corn tissue-fungus interactions. White et al. (13) observed that the breakdown of the tonoplast occurred within 6 hours after inoculation of susceptible corn leaf tissue with *H. maydis* race T, and that this was

followed by the dispersal of cytoplasm from the periphery of the cell into the vacuolar space. In our studies, movement of epidermal nuclei towards the site of penetration suggests a type of defense mechanism, followed by a resistant reaction; conversely, lack of nuclear movement suggests an early adverse effect of the pathogen on plant cell integrity and, therefore, subsequent susceptibility. It is conceivable then that the phenomenon involving the position of nuclei in epidermal cells near cells penetrated by *H. maydis* race T is a manifestation of plant disease response.

Formation of granules and brown discoloration are events commonly associated with reaction of disease resistance (12). Müller (7) pointed out that in diseases involving necrotrophic fungi, the spread of the pathogen often appears to depend on a balance between the pathogenic activity of the fungus and the speed of counteraction by the host. In this study, a brown discoloration developed in both susceptible and resistant tissues, with marked differences in the time it took to develop in both cases; i.e., 6-9 hours longer in susceptible W64A^{tcms} than in the resistant W64A. Growth of secondary hyphae in resistant tissue was arrested very early in lesion development; i.e., within 12 hours of inoculation, and probably before brown discoloration was noticeable. It is suggested that host response conducive to differential disease reaction occurs very early in the interaction, probably within 12 hours of penetration.

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