Ear-Rotting Potential of Helminthosporium maydis Race T in Corn

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ABSTRACT

The ear-rotting potential of *Helminthosporium maydis* race T was determined by inoculating ears of two separate strains of the 'Pioneer 3306' hybrid corn. One strain had male-sterile cytoplasm and the other, normal male-fertile cytoplasm. Inoculum-laden toothpicks were inserted in the base, center, and tip of the ear. Inoculations were made 10, 20, 30, and 40 days after flowering and rated 28 days after inoculation.

Ears from plants with T-cytoplasm had more extensive rotting than those from normal cytoplasm plants. Ears inoculated at the center had the greatest amount of rot. Rotting was highest when inoculations were made at 10 and 20 days after flowering, and lower at 30 and 40 days. Kernels next to the lesion were infected, but were not visibly damaged.

Certain tissues of corn ears with the T-cytoplasm were much more susceptible to infection than others. Susceptibility decreased with age of the tissues. Since *H. maydis* race T was shown to be seed-transmitted, the possibility exists that the fungus may be disseminated in infected kernels that are not visibly damaged.

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Additional key words: Zea mays, southern corn leaf blight, T-cytoplasm.

During the 1970 epiphytotic of *Helminthosporium maydis* Nisik. & Miyake (*Cochliobolus heterostrophus* Drechsl.), the infection types were strikingly different for strains of corn, *Zea mays* L., having the Texas male-sterile cytoplasm (cms-T) than for those having the normal male-fertile cytoplasm (cmf-N). The importance of the *H. maydis* - corn cytoplasm associations has been reported (1, 7, 12, 13). These investigations resulted in the identification and designation of a new race, *H. maydis* race T (11). The new race extensively damaged plants with cms-T cytoplasm, whereas the original or old race, designated as *H. maydis* race O (11), produced a low-type infection on plants with either cms-T or cmf-N. Race T not only infects cms-T corn in the interveinal leaf tissues, as does race O, but also is potentially able to invade veinal tissues of leaves, sheaths, ear shanks, husks, and kernels of primary ears, and completely destroys secondary ear shoots. Environmental factors, especially temperature, failed to limit the new race T to the southern corn-growing areas in the United States, as had been the case with race O. Thus, the fungus spread widely into the cornbelt states in 1970 (9). Since lesions caused by race T on the husks of ears sometimes result in kernel infections, a study was undertaken to determine the conditioning factors associated with this type of infection. Some of the factors studied included: (i) location of the lesion on the ear, and (ii) the stage of ear development at the time of the infection.

MATERIALS AND METHODS.–Mature ears from plants containing cms-T cytoplasm, infected with *H. maydis* race T, and with husk lesions that had penetrated to the kernels, were obtained from fields near Columbia, Missouri, in 1970. The infected ears were sampled at four sites relative to the lesion area located near the center of the ear. The four sampling sites were: (i) the lesion area, which included all visible affected kernels; (ii) the lesion-margin area, a concentric band (several kernels wide) adjoining the lesion area; (iii) a second concentric band of kernels adjoining the lesion margin area; and (iv) a third band, also several kernels wide, outside the second band. Kernels from the four areas were plated on potato-dextrose agar (PDA) to establish the presence or absence of infection by race T. Kernels from these four areas were also planted in the greenhouse to determine the effect of seed infection on seedling emergence. Kernels taken from the various areas on the ear were observed for damage due to infection by *H. maydis*, and the seedlings that emerged were observed for blighting. Leaf samples from these seedlings were incubated in moist chambers to induce sporulation of *H. maydis*.

A field experiment was conducted in 1971 to measure the ear-rotting potential of *H. maydis* race T. Two strains of the corn hybrid, 'Pioneer 3306', one with cytoplasm cms-T and the other with cmf-N, were used. The treatments, plus controls, were arranged in a completely randomized block consisting of four inoculation dates, beginning 10 days after flowering and continuing at 10-day intervals to 40 days after flowering, and three inoculation sites. The intervals coincided approximately with stages of ear development from blister to beginning dent (6). The three inoculation sites were the tip, middle, and base of the ear. Ears were inoculated by inserting

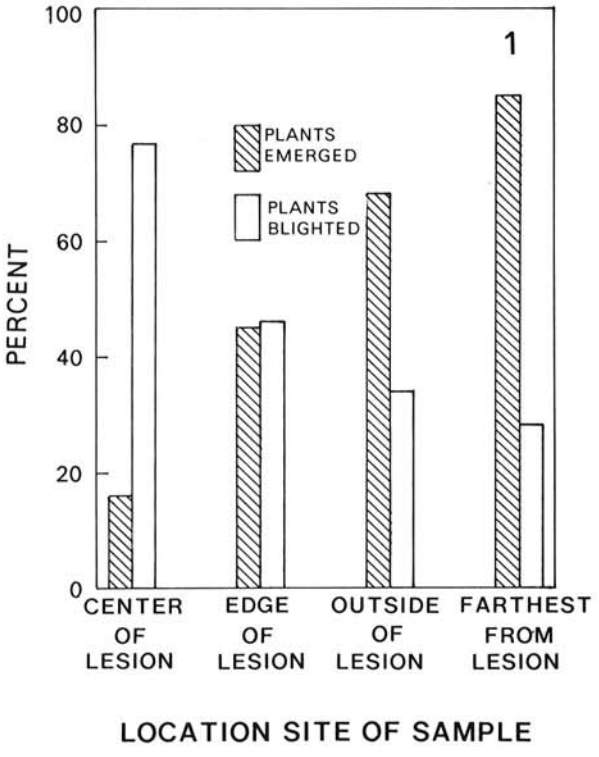

Fig. 1. Relation of plants emerged and plants blighted in greenhouse plantings from seed sampled from four sites relative to *Helminthosporium maydis* race T lesion area located near the center of the ear.

toothpicks infested with the fungus into the cob through the husks. The controls were treated similarly, but sterile toothpicks were used. The toothpicks were clipped off at the husk surface to prevent a wick effect and dehydration of corn tissues. Each ear was rated for rot 28 days after inoculation. To determine the amount of spread by the fungus, two single rows of kernels from each of three ears from each treatment were plated on PDA. The rows sampled were taken through the point of inoculation lengthwise of the ear (average of 20 kernels per sample) and around the ear (average of 16 kernels per sample). Samples included both the obviously affected kernels and the apparently sound ones.

RESULTS.–Only a small percentage of the kernels sampled from the center of naturally occurring lesions produced seedlings. However, the percent of seedling emergence increased with distance from the infection center. For example, seedling emergence was 16% for kernels taken at the lesion center, but increased to 85% for kernels taken farthest from the lesion. Many seedlings that emerged subsequently became blighted (Fig. 1), but the percent of blighting also became progressively less with distance from the lesion center. Of the 84 seedlings that emerged from kernels sampled at the center of the lesion, almost 80% were blighted, whereas of the 227 plants that emerged from kernels sampled farthest away from the infection site, only 28% were blighted. That blighting of plants was due to *H. maydis*, was substantiated by sporulation of the fungus on the seedling leaves. Remnant seed were plated on PDA, and a pure culture of *H. maydis* was obtained from nearly every affected kernel. Occasionally, *Fusarium moniliformae* Sheldon was found with *H. maydis*.

The stage of ear development at the time of inoculation was critical, as was the type of cytoplasm of the plant (Fig. 2). Damage was most severe for ears on the cms-T plants inoculated earliest (10 days) and inoculated in the middle part of the ear; the lesions in the central area of the ear extended through the cob tissues to envelop the kernels on the far side of the inoculation site. None of the inoculations of ears on the cmf N corn plants resulted in disease or infections significantly different from the controls. Disease damage to the kernels was significantly different from that of the controls only when ears having cms T cytoplasm were inoculated in the middle sites 10 and 20 days after flowering (Fig. 3).

Isolations from kernels taken from rows around the ear and lengthwise through the lesions, revealed that the race T fungus infected kernels in both the horizontal and longitudinal directions at all three inoculation sites (Table 1). Pathogen recoveries averaged 49 percent for kernels sampled around the ear, and 58 percent for the longitudinal samples. Recovery of *H. maydis* was greatest from the inoculations made at both the 10th and 20th day after flowering. At the 30th day, recovery was somewhat lower but still three to six times greater than for the inoculations made at 40 days, or for the controls. Pathogen recovery from the tip, middle, and base of the ear were 50, 51, and 40 percent, respectively, indicating that the fungus was able to establish infections more or less equally at each of the three inoculation sites (Table 1).

DISCUSSION.–The results from natural field inoculations demonstrated the severe ear-rotting potential of *H. maydis* race T for cms-T plants. This was evident by poor seed germination and seedling survival. Artificial inoculations with this same race T resulted in little or no rotting of ears of cmf-N plants of the same genotype. Since we used the same hybrid but different cytoplasms, we have assumed they were nearly the same genetically.

It is difficult to explain why the kernels in the center part of the ear supported better growth of the race T fungus than the kernels that developed slightly later at either the tip or the base of the ear. Possibly certain chemical differences associated with kernels from either the base, center, or tip position of the ear may account for the differential fungal growth observed. Fergason et al. (4) found a significant linear decrease in amylose content from the base to the tip of the ear. Jellum (8) reported the effects of kernel position on the ear for oil composition among some inbred lines, whereas for others he found no effects. No reports are available on the effect of kernel position on amino acids. The current evidence of kernel-position effect for some chemical components

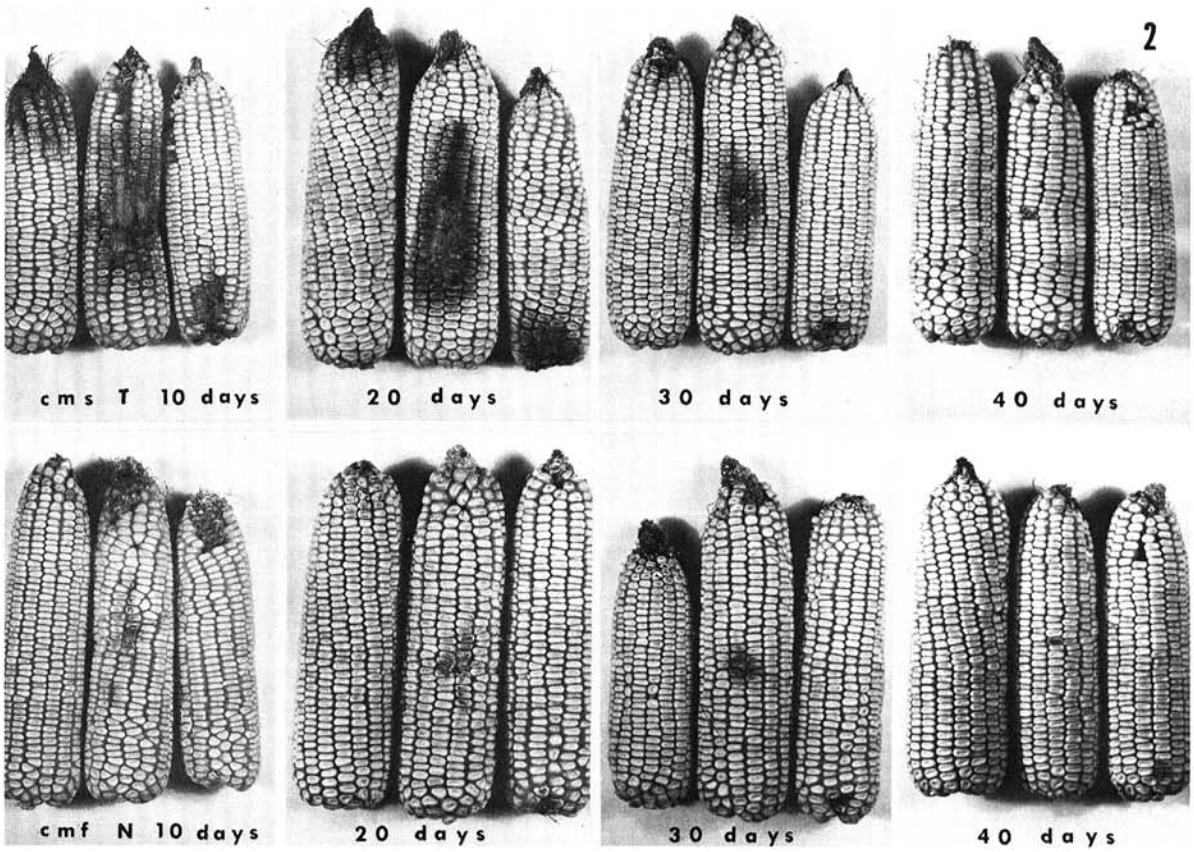

Fig. 2. Texas cytoplasmic male-sterile corn (cms T upper row), and normal cytoplasmic male-fertile corn (cmf N lower row) inoculated with *Helminthosporium maydis* race T-infested toothpicks inserted at the tip, middle, or base of 'Pioneer 3306' ears 10, 20, 30, or 40 days after flowering (left to right) and photographed 28 days after inoculation.

suggests that further studies are needed to determine whether chemical differences account for greater fungal growth on kernels from the center position of the ear.

The major damage to the ear was to the center part rather than the tip or base when the fungus was introduced at the 10th and 20th day after flowering. These observations confirm the idea that each part of the ear (i.e., the tip, center, and base) can react differently to ear-rotting pathogens. For example, *Diplodia maydis* (Berk.) Sacc. usually is confined to the base of the ear, whereas *Gibberella roseum* (Lk.) Snyd. & Hans. is usually confined to the tip of the ear. Our results indicate that the center part of the ear is more severely damaged than the tip or the base when inoculated with *H. maydis* race T. Presumably this would also be true as a result of natural infection.

The ability of the fungus to invade adjoining tissue from the inoculation site both longitudinally and around the ear was of special interest. Often kernels that appeared healthy were identified as being infected with race T of *H. maydis*. Since both Boothroyd's (2) and our experiments showed that *H. maydis* can be seed-transmitted, infected kernels without visible damage could have been a means of

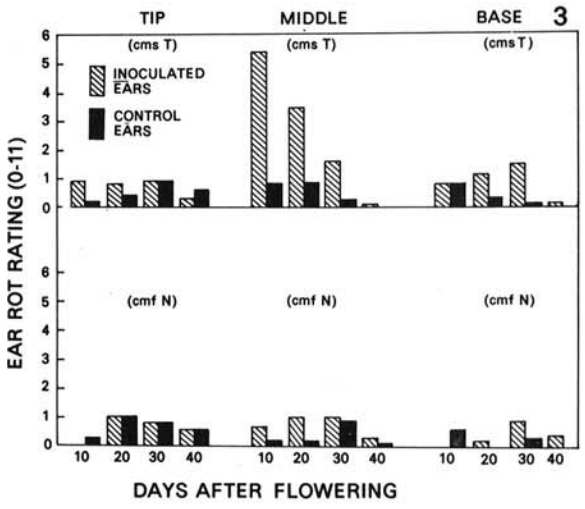

Fig. 3. Comparative ear-rot ratings of 'Pioneer 3306' corn ears in two cytoplasms, inoculated 10, 20, 30, and 40 days after flowering at the tip, middle, and base of the ear and rated 28 days after inoculation with *Helminthosporium maydis* race T (0 = no disease, 11 = 100% of ear visibly infected).

TABLE 1. Comparative movement of *Helminthosporium maydis* race T infections from the site of inoculations made at four intervals after flowering as measured by percentage pathogen recovery from kernels sampled around the ear and lengthwise through the inoculated site at the tip, middle, or base of the ear[a]

Inoculation site on the ear	Position kernels sampled	Time of inoculation at days after flowering				Sterile control	Position mean	Site mean
		10	20	30	40	40		
Tip	Row around ear through lesion	73	92	76	14	24	56	50
	Row lengthwise through lesion	70	73	57	9	17	45	
Middle	Row around ear through lesion	86	62	63	24	7	49	51
	Row lengthwise above lesion	83	88	79	23	19	56	
	below lesion	69	71	71	21	14	48	
Base	Row around ear through lesion	48	59	42	26	0	34	40
	Row lengthwise through lesion	72	75	46	27	5	45	
Mean		72	74	61	20	11		

[a] Nine hundred kernels of 'Pioneer 3306' cms T out of 1,891 sampled were infected.

distributing the fungus over wide areas, as was observed in 1970.

One might speculate how a fungus such as *H. maydis* race T differs genetically from *H. maydis* race O to give such a pronounced differential response on a specific host tissue. Nelson & Kline (10) showed conclusively the genetic complexity for pathogenicity in *C. heterostrophus*. Even with only a very few isolates of the species and strains of *Helminthosporium* that were used to test the reaction of nine gramineous hosts, a minimum of 13 genes were identified. Of these, one or two perhaps are involved in the SCLB syndrome. The combination of genes in both the host and the parasite interact to give a high-infection type which is the reverse of what Daly (3) suggests when an obligate parasite is involved. Most *H. maydis* race T cultures – cms-T strains (1, 7), and even some *H. maydis* – cmf-N strains (5) produce a high-infection type association.

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