

## Association of Mating Type and Virulence in *Helminthosporium maydis*, and Observations on the Origin of the Race T Population in the United States

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### ABSTRACT

Cultures of *Helminthosporium maydis* isolated from diseased corn leaves collected in Iowa, New York, and four Southern states, and from corn seed from Texas and Guatemala in 1971 were tested for mating type and virulence. Race O was found in seed from Guatemala and in 12% of the leaves collected in the South, but in none of the leaves from Iowa or New York. All 50 race T isolates from Iowa and 9 of 10 from New York were mating type A. Mating types A and a occurred in nearly equal frequency among both the race O and race T isolates from

the South. With respect to mating type, the midwestern and northeastern populations of race T remained as they were in 1970. The southern population has changed markedly since 1970, when 80% of the race T isolates were mating type A. The implications of these and other observations which suggest a recent origin of the race T population and counter evidence suggesting a widespread and long term existence of race T in the USA and many other parts of the world are discussed.

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*Additional key words:* southern corn leaf blight.

Although cytoplasmic inheritance of susceptibility to *Helminthosporium maydis* Nisik. & Miyake in corn with Texas cytoplasmic male sterility (T cms) was reported in The Philippines (10, 18) in 1961, it was not observed in the USA until 1969 (6, 15). Smith et al. (17) determined that T cms corn plants were highly susceptible to some isolates, designated race T, but were no more susceptible than corn with normal (N) cytoplasm to other isolates which were designated race O. Collections of *H. maydis* from 1969 in Illinois were race T, whereas collections maintained from 1963 were race O. Scheifele (14) found that race T occurred in 1969 in Iowa, Illinois, Indiana, Ohio, Missouri, Kentucky, Alabama, and Florida. No differences in incidence of *H. maydis* on N and T cms hybrids were detected in surveys of corn

fields in Delaware and Maryland in 1969 and in Pennsylvania as late as 13 August 1970. Ears of corn from 1968, but not the 1964 and 1967 crops, in Iowa contained kernels infected with race T (3). In Florida, race T was found in seed from 1969 but not in seed from 1967 (19).

These observations suggest that race T first appeared in the USA in the Midwest, perhaps in Iowa. Since *H. maydis* is a heterothallic species, Leonard (8) suggested that knowledge of the distribution of mating types, designated A and a, among race T isolates might aid in understanding the origin of race T in the USA. All 25 race T isolates collected in the Midwest in 1970 and tested by Leonard (8) were mating type A. From the South, 33 isolates were mating type A and 8 were a. All 16 North Carolina

isolates from 1968 and 1969 were race O. Nelson et al. (12) found 885 mating type *A* and 30 mating type *a* isolates among race T collections from the Northeast in 1970. Leonard (8) postulated that the original race T population in the USA was recently established in the Midwest, and consisted entirely of isolates of mating type *A*. Genetic recombination with race O in the South may have introduced the mating type *a* allele into the race T population.

In contrast to the observations suggesting a recent origin of race T in the USA, Nelson et al. (13) reported the isolation of race T from diseased leaves collected in North Carolina in 1955 and 1961, in Georgia in 1958, in Tennessee in 1962, in Virginia in 1965, and in Mexico, Argentina, Brazil, Nigeria, Guinea, England, Scotland, and Switzerland from 1960 to 1964. The race T isolates were obtained in 1969 or 1970 from the leaves which had been stored under cool, dry conditions. None of 116 isolates obtained from these and other leaf collections and maintained on artificial media for 4 to 13 years was notably more virulent on T cms than on N cytoplasm corn.

**MATERIALS AND METHODS.**—Collections of *H. maydis*-infected corn leaves in 1971 were obtained from C. M. Martinson, Department of Botany and Plant Pathology, Iowa State University, Ames; C. W. Boothroyd, Department of Plant Pathology, Cornell University, Ithaca, N. Y.; and M. C. Futrell, Department of Plant Pathology and Weed Science, Mississippi State University, State College. Isolates from infected seed were obtained from R. A. Fredericksen, Department of Plant Science, Texas A&M University, College Station, and from N. A. Lapp, North Carolina Department of Agriculture, Raleigh. The leaf samples were air-dried and kept in closed packets until isolations were attempted. Isolates of *H. maydis* were obtained by incubation of samples in moist chambers for 1 or 2 days and selection of conidia from the conidiophores that formed. In a few cases in which the disease reaction of the sample was of a resistant type, more than 2 days' incubation was required.

Inoculum for virulence tests of each isolate was

prepared by a scraping of conidia and mycelial fragments from a 7-day-old culture on potato-dextrose agar (1% dextrose) in a 9-cm petri dish and suspension of the inoculum in ca. 50 ml water. The inoculum suspension was sprayed on B 37 and B 37 T cms corn plants in the 4- to 5-leaf stage with a modified hand sprayer attached to a pump which delivered air at 5 to 10 psi. The plants were grown in an air-conditioned greenhouse at 20 to 24 C, inoculated, incubated in a moist chamber 20 to 24 hr at 20 C, and returned to the greenhouse. Isolates which induced lesions on B 37 T cms which were more than twice as large as those on B 37 plants, or isolates which induced at least twice as much blight on B 37 T cms as on B 37 plants, were designated race T. Inoculations were repeated for isolates classified as race O to confirm the race designation.

Mating type was determined by a pairing of unknown isolates with highly fertile isolates of mating types *A* and *a*. Mycelial plugs of known and unknown isolates were placed at opposite sides of corn leaf discs on modified Sachs agar (5) in petri dishes which were kept in the dark at 22 C. Perithecia formed within 1 week in the area where mycelia of compatible isolates intermingled on the leaf discs. Ascospores were present in perithecia after 3 weeks.

**RESULTS.**—As in 1970, isolates of race T predominated in the Northeast and Midwest (Table 1). All isolates from Iowa and nearly all from New York were mating type *A*, whereas mating types *A* and *a* occurred with nearly equal frequencies among race T and race O isolates collected in the South in 1971. In 1 year, the percentages of mating types *A* and *a* in the South has shifted from 80% *A* and 20% *a* in 1970 to 47% *A* and 53% *a* in 1971.

**DISCUSSION.**—Because the sexual stage of *H. maydis* has been observed rarely in the field (11), it is apparent that random genetic recombination alone could not account for more than a very small part of the change in the Southern race T population from 1970 to 1971. The mating type *a* individuals of race T must have been favored by natural selection in the South. It is more likely that this selection was due to some gene(s) linked to the mating type locus than to

TABLE 1. Mating type and pathogenic race designation of isolates of *Helminthosporium maydis* collected in 1971

Origin of isolates	Number of isolates of designated race and mating type			
	Race T mating type <i>A</i>	Race T mating type <i>a</i>	Race O mating type <i>A</i>	Race O mating type <i>a</i>
Iowa	50	0	0	0
New York	9	1	0	0
Kentucky	1	1	3	0
Tennessee	2	0	0	0
Mississippi	4	6	2	10
Texas	1 <sup>a</sup>	0	0	0
North Carolina	107	123	10	10
Guatemala	0	0	0	2 <sup>a</sup>

<sup>a</sup> Isolated from diseased seed.

the mating type alleles themselves, because neither mating type allele is favored over the other in the established race O populations (8, 11). Selection due to gene(s) linked to the mating type locus might be expected if race T first occurred in the Midwest and consisted entirely of mating type A. The race O population in the South would be well adapted to the climatic conditions there, so race T isolates in the South which resulted from crosses of Midwestern race T, mating type A isolates with race O, mating type *a* isolates would tend to combine the climatic adaptation of the race O isolates with the virulence of race T. Progeny with the mating type *a* allele and the genes linked to it would tend to have more genes conditioning adaptation in the South than progeny with the A allele and the genes linked to it.

Fukuki & Aragaki (4) showed that isolates of race O in Hawaii sporulated better at high temperatures, whereas isolates of race T sporulated better at moderate temperatures. Hooker et al. (7) suggested that race T may have a lower temperature optimum than race O, which tends to be limited to the warmer part of the USA. It is significant that these observations were made in areas where there is little apparent diversity in the race T population. In the southern USA, the differences in adaptation of race T and race O may soon disappear if they have not already.

Many observations suggest a recent origin of the race T populations in the USA. The association of T cms cytoplasm with susceptibility to *H. maydis* was not observed before 1969 (6, 15), even though corn breeders and pathologists were aware of such an association in The Philippines in 1961 (10, 18). Race T has not been found in corn seed harvested before 1968 (3, 19). The absence of mating type *a* in the race T population in the Midwest (8), where race T was first detected, suggests an imbalance in a very young population rather than a stable condition of a long established population. The rapid increase in the frequency of the mating type *a* allele in the race T population of the South clearly shows that the mating type *a* allele is not necessarily deleterious to race T, and that the preponderance of mating type A in 1970 did not represent a stable condition. The absence of race T type virulence among 116 isolates maintained on artificial media for 4 to 13 years (13) might be regarded as evidence that these isolates were race O when they were first isolated, rather than that some of them were race T when first isolated but later lost the ability to induce blight in T cms corn plants.

Evidence that race T did occur widely in the USA before 1965 is based on the isolation of race T from diseased leaf collections which had been stored for 4 to 13 years (13). Isolates were obtained by incubation of pieces of the stored leaves on moistened filter paper in petri dishes to allow the fungus to grow and sporulate on the leaves. Leonard (9) has shown that this method is subject to considerable hazard of contamination from airborne conidia of *Helminthosporium* spp., especially if the contaminating species is the same as, or morphologi-

cally similar to, the species expected from the leaves. Conidia of *H. maydis* deposited upon incubated leaves can germinate and produce sporulating colonies within 2 days (9). This is well within the length of time that might be required for the fungus within the stored leaf to resume growth and sporulate.

Craig (2) presented evidence that a corn cultivar widely grown in Nigeria for several years and highly susceptible to race T appeared resistant to *H. maydis* before 1970. He expressed doubt that race T occurred in Nigeria before 1970 even though Nelson et al. (13) isolated race T from five leaf samples collected there in 1963.

The apparent discrepancies between observations pointing to a recent origin of race T in the USA and the isolation of race T from stored leaf collections suggest two possible explanations. The race T isolates from the stored leaves may have arisen as contaminants, in which case they should be almost exclusively of mating type A. Nelson et al. (13) stated that mating types A and *a* occurred with nearly equal frequency among the isolates, but did not state specifically whether the mating type determinations referred to were made with the isolates obtained when the leaves were collected or with those obtained after storage of the leaf samples. The second possible explanation is that there may be two types of race T: one which has been widespread throughout the world since at least 1955, but which lacked the ability to cause noticeable damage in T cms corn in the field; and a second, more damaging type which has occurred only recently in the USA but has existed for a longer period of time in The Philippines. In either case, the midwestern USA seems an unlikely place for the appearance of a new, virulent race of *H. maydis* unless it was introduced into that area from another part of the world. Mutation from race O or from a weak race T to a virulent race T would be much more likely in the South, where *H. maydis* has long been common, rather than in the Midwest, where *H. maydis* was rarely found before 1969.

If race T did not arise in the Midwest, it is likely that it was introduced into that area in infected seed. The ability of race T to develop and sporulate on seedlings from infection within the seed has been demonstrated by Boothroyd (1). The threat of seed transmission has prompted the New Zealand Department of Agriculture to place an embargo on the importation of seed and other raw corn products from the USA (16). The apparent jump of race T from the Midwest to Florida and Alabama and the sudden appearance of race T in Hawaii and Nigeria seem to justify this precaution, and may serve as examples of the manner in which race T first appeared in the midwestern USA.

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