# Reduced Fitness Associated with *TOX*1 of *Cochliobolus heterostrophus*

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## ABSTRACT

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*Cochliobolus heterostrophus* strains near-isogenic except for a gene for T-toxin production were examined for differences in fitness on normal cytoplasm maize in the field and greenhouse. A mixture of two strains (one race O, nontoxin-producing, and one race T, toxin-producing) was used to inoculate a field plot of the susceptible hybrid Cornell 281 in Ames, IA, in 1983 and 1984. Different pairs of strains were used each year; lesions were harvested periodically during the season, and the number of lesions yielding race T and race O strains was determined. The frequency of the race T strain decreased significantly compared with the race O strain both years, indicating that the race T strains were less fit than the race O strains. Differences in fitness of near-isogenic strains were also expressed as differences in lesion length. Lesions produced by race T strains were significantly shorter than those produced by race O strains from four backcross generations of the fungus and on three genotypes of maize. We concluded that the toxin gene (*TOX*1) or a gene closely linked to it reduces pathogen fitness on N-cytoplasm maize. Reduced fitness associated with the toxin locus could explain the rapid decrease in frequency of race T after susceptible Texas male sterile cytoplasm maize was replaced with normal cytoplasm maize following the 1970 southern leaf blight epidemic.

*Additional keywords*: *Bipolaris maydis*, stabilizing selection.

*Cochliobolus heterostrophus* (Drechs.) Drechs. (anamorph *Bipolaris maydis* (Nisikado and Miyake) Shoemaker = *Helminthosporium maydis* Nisikado and Miyake = *Drechslera maydis* (Nisikado and Miyake) Subramanian and Jain) is the causal agent of southern leaf blight of maize (1). Two races of *C. heterostrophus*, which differ in alleles at a host-specific toxin locus (*Tox*1) have been distinguished; strains with *TOX*1 (race T) produce T-toxin and cause long, spindle-shaped lesions on leaves of maize with Texas male sterile cytoplasm (T-cms), whereas strains with *tox*1 do not produce T-toxin (race O) and cause smaller, parallel-sided lesions on leaves of T-cms maize. Both races cause small, parallel-sided lesions on leaves of maize with normal (N) cytoplasm (5,20). Race T dominated the *C. heterostrophus* population in the United States in 1970 and caused an epidemic of southern leaf blight on T-cms maize (5). T-cms lines were rapidly replaced by maize with N-cytoplasm after 1970, and the frequency of race T subsequently declined to very low levels (10). The lack of persistence of race T suggests that race T is less fit than race O on N-cytoplasm maize, or more specifically, that the gene for T-toxin production reduces pathogen fitness.

Leonard (9) examined this hypothesis in the greenhouse using near-isogenic strains differing in ability to make T-toxin. He cycled a mixture of race T and race O strains through five asexual generations on N-cytoplasm maize, and found that the frequency of race T in the mixture decreased compared with the frequency of race O. The selection coefficient, 0.12, was not significantly different from zero, however. He also observed that the average lesion length induced by race O was 10% larger than the average lesion length produced by race T, but did not indicate if this difference was significant. Blanco and Nelson (2) observed that race T isolates were less fit than race O isolates in a field study on N-cytoplasm maize. This study did not critically test the effect of the toxin locus on pathogen fitness, however, because a mixture of unrelated isolates was used, and other genes could have affected fitness. These studies suggested that the toxin gene might decrease fitness, and that stabilizing selection was operative.

We have reexamined the hypothesis that the gene for T-toxin production debilitates *C. heterostrophus* on N-cytoplasm maize. Closely related race T and race O strains were tested in the field and the greenhouse for differences in fitness. In 1983 and 1984, T and O strains were used to inoculate a field of susceptible N-cytoplasm maize; changes in race frequency were monitored throughout the season. In 1985, race T and race O strains were examined for differences in lesion length in the field. The same near-isogenic race T and race O strains were tested in the laboratory for differences in spore viability and in the greenhouse for differences in lesion length and infection efficiency.

## MATERIALS AND METHODS

**Strains.** Strains of *C. heterostrophus* (Table 1) were derived from the closely related strains C3 (7) and 380-2-5, kindly donated by O. C. Yoder of Cornell University. The strains used in field and greenhouse studies had a gene for cycloheximide resistance (*Cyh*1R) to distinguish them from naturally occurring field strains. Strains were cultured on complete medium (CM)(7) at room temperature under cool-white fluorescent lights, crossed on Sach's medium (13,14), and stored either on silica gel (15) at 5 C or in 15% glycerol at −70 C.

**Field experiments.** Race T and race O near-isogenic strains were examined for differences in fitness in the field during 1983 and 1984. Each year, a field of susceptible, normal-cytoplasm maize (Cornell 281) was inoculated with a mixture containing one strain of each race. The strains used in 1983 (K22-R-48 and K22-R-55) were siblings from the fifth backcross of *TOX*1 to C3 (*tox*1). The strains used in 1984 (K36-P2-4-2 and K36-P4-1-3) were siblings from the ninth backcross of *TOX*1 to C3 (*tox*1); the four additional backcrosses increased average isogenicity. Mating type was used as a marker to distinguish 1983 inoculum from 1984 inoculum. Lesions were collected throughout the season so that changes in race frequency could be determined.

Cornell 281 (W182BN × (A632 × A634))(Halsey Farms, Trumansburg, NY) was sown with 0.76-m row spacing at 28,000 plants per hectare on 0.3 ha of a Spillville loam at Hinds Farm,

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Ames, IA, on 29 April 1983 and 9 May 1984. Debris was plowed under before planting each year. Fertilizer (80 kg/ha of N, 70 kg/ha of $P_2O_5$, 100 kg/ha of $K_2O$) was incorporated at planting.

Inoculum for the 1983 and 1984 experiments was prepared by growing the race T and race O strains separately in flasks containing 50 or 100 ml of modified Richard's medium broth (150 ml of V-8 juice, 10 g of $KNO_3$, 5 g of $KH_2PO_4$, 0.02 g of $FeCl_3 \cdot 6H_2O$, 2.5 g of $MgSO_4$, 50 g of sucrose per liter of distilled water). In 1983, fungus plus liquid (13 days growth) from 600 ml of broth was fragmented briefly in a blender and mixed with 4.5 kg of sandblasting sand. In 1984, fungus only (9 days growth) from 800 ml of broth was fragmented and mixed with 6 kg of sandblasting sand. The sand-fungus mixtures were dried on newspaper, then tested for viability on CM amended with 100 $\mu$g/ml of streptomycin sulfate. The race T and race O inocula were mixed in equal proportion based on the proportion of sand grains bearing viable inoculum.

Plants were inoculated 6 July 1983 (68 days after planting) and 24 June 1984 (45 days after planting) by sprinkling 0.1–0.8 g of inoculum into each whorl. The plot was irrigated every two to three evenings, or more frequently in dry weather, for 10 min using overhead sprinklers.

At least 900 lesions, with no more than one lesion per plant, were collected every 3 wk (1983) or every 2 wk (1984). Leaf pieces with lesions were stored dry at 5 C (1983) or at 22–25 C (1984) until isolations were made (usually 2–9 wk). Lesions were surface sterilized for 30–60 sec in 0.5% sodium hypochlorite solution, rinsed in sterile water, and placed in a sterile moist chamber. A single conidium was isolated from each sporulating lesion. Colonies were stored on CM at 2–5 C until phenotypes were determined (usually 1–4 wk).

All isolates were tested for resistance to cycloheximide on CM supplemented with 40 $\mu$g/ml of cycloheximide. In 1983, all cycloheximide-resistant isolates and 122 cycloheximide-sensitive isolates were tested for T-toxin production. Mating type was determined for over 200 isolates. In 1984, all isolates were assayed for mating type and toxin production as well as cycloheximide resistance. Mating type was determined by noting the presence or absence of pseudothecia when isolates were paired with mating type 1 and mating type 2 tester strains. Petri plates (9 cm) containing 20 ml of Sach's medium were flooded with 3 ml of a spore suspension (250 conidia per milliliter) of the tester strain and covered with a single layer (0.2 g per plate) of sterile, naturally senescent maize leaf pieces that had been sieved through a 5-mm-mesh screen and retained on a 0.6-mm-mesh screen. The plates were allowed to dry and inoculated 24 hr later with mycelial cubes 1.5 mm on a side; 10 isolates were tested per plate. The plates were incubated 7 days in the dark in an open plastic bag.

Toxin-producing ability was determined by examining lesion types formed on two to four seedlings each of W64A-T (T-cms) and W64A-N (normal cytoplasm) in the greenhouse (22–28 C). The whorls of seedlings, which were 7–15 days old and had expanded second leaves (16), were filled with a Tween 20 solution (one drop per 125 ml). Conidia and hyphae of the fungus were scraped from an agar colony with a sterile toothpick to form a dense ball (diameter = 2–3 mm), then distributed among the seedlings by dipping the toothpick into the whorls. The amount of inoculum deposited on each seedling was not uniform. Controls were race O and race T laboratory strains. The plants were held at 100% relative humidity at 23–26 C for 14–18 hr, then placed in the greenhouse. Symptoms were observed 4–6 days after inoculation. Race T was easily distinguished from race O by its production of bright chlorotic streaks and tan to gray runner lesions on W64A-T and small, tan lesions on W64A-N. Race O caused small tan lesions on both W64A-N and W64A-T. Isolates yielding ambiguous symptoms were retested.

Changes in frequency of race T and race O cycloheximide-resistant isolates were examined using a regression test for a linear trend in proportions (18). Confidence intervals were calculated using binomial tables (19).

The race T and race O near-isogenic strains used in the 1983 and 1984 experiments were also examined for differences in relative fitness by measuring lesion length in the field in 1985. Cornell 281 was planted on 3 May 1985 as described for the 1983 and 1984 experiments. Strains K22-R-48, K22-R-55, K36-P2-4-2, and K36-P4-1-3 were grown 12 days on ground-corn-leaf agar (GCLA), a medium that yields spores with high viability (21). Conidia were suspended in 0.05% water agar at 2–5 C, filtered through three layers of cheesecloth, and stored on ice until use. Fully expanded, healthy leaves were inoculated using a Chromist sprayer (Gelman Sciences, Inc., Ann Arbor, MI), then covered with a plastic bag at least 14 hr. On 16 June 1985, 43 days after planting, each isolate was used to inoculate one leaf on each of nine plants; 5 ml of a 1,000 spores per milliliter suspension were used per leaf. On 21 August 1985, 110 days after planting, each isolate was used to inoculate one leaf on each of 24 plants; 2–10 ml of a 6,000 spores per milliliter suspension were used per leaf. Lesion lengths in the middle halves of the leaves were measured 2 (August trial) or 3 (June trial) wk after inoculation using a dissecting microscope with transmitted light and a Field Finder slide (Teledyne Gurley, Troy, NY) with 0.1-mm divisions. Significant differences between the mean lesion lengths for each isolate within a trial were detected using analysis of variance with leaves as observations, followed by an LSD multiple comparison test.

**Greenhouse and laboratory experiments.** The relative fitness of race T and race O near-isolines was also examined by measuring components of fitness, including spore viability, infection efficiency, and lesion length. Germination experiments compared K22-R-48 with K22-R-55 and K36-P2-4-2 with K36-P4-1-3 for differences in spore viability. A conidial suspension made from cultures grown 14 days on GCLA was spread on water agar at a density of 200–500 spores per square centimeter. Conidia (170–400 per isolate) were examined 200 min later at 35× for germ tube formation. Each comparison was repeated twice.

Inoculum for lesion length experiments was prepared using the procedure for 1985 field inoculum from 13–15-day-old cultures grown on GCLA. Two or four isolates from each backcross generation were compared three times. For each isolate, 7–10 plants in the five-leaf stage (one per 10-cm pot) were placed in an inoculation chamber and the fourth leaves (taped flat horizontally) were inoculated simultaneously with 6,000 spores in 24 ml of water agar using a Chromist sprayer. Plants were incubated in a dew chamber at 22 C for 24 hr, then placed in the greenhouse. Lengths of lesions in the middle 16 cm of the fourth leaf and separated by at least 5 mm from any other lesion were measured 6 days after inoculation using a dissecting microscope and a Field Finder slide.

TABLE 1. Genotypes of *Cochliobolus heterostrophus* used in field and greenhouse experiments

| Cross | Backcrosses[a] | Strain | Genotype[b] |
|---|---|---|---|
| K22 | 5 | K22-R-48 | *Cyh*1R *MAT*1-1 *TOX*1 |
| | | K22-R-55 | *Cyh*1R *MAT*1-1 *tox*1 |
| K36 | 9 | K36-P2-4-2 | *Cyh*1R *MAT*1-2 *TOX*1 |
| | | K36-P4-1-3 | *Cyh*1R *MAT*1-2 *tox*1 |
| K38 | 10 | K38-R-12 | *Cyh*1R *MAT*1-2 *TOX*1 |
| | | K38-R-15 | *Cyh*1R *MAT*1-2 *TOX*1 |
| | | K38-R-32 | *Cyh*1R *MAT*1-2 *tox*1 |
| | | K38-R-33 | *Cyh*1R *MAT*1-2 *tox*1 |
| K39 | 11 | K39-R-1 | *Cyh*1R *MAT*1-1 *tox*1 |
| | | K39-R-10 | *Cyh*1R *MAT*1-1 *TOX*1 |
| | | K39-R-14 | *Cyh*1R *MAT*1-1 *tox*1 |
| | | K39-R-25 | *Cyh*1R *MAT*1-1 *TOX*1 |

[a] Number of backcrosses to C3 (*Cyh*1S *MAT*1-2 *tox*1). The donor parent of the first cross was 380-2-5 (*Cyh*1 R *MAT*1-1 *TOX*1). The donor parent of each subsequent cross was a *Cyh*1R *MAT*1-1 *TOX*1 offspring of the previous cross.

[b] Alleles at the *Cyh*1 locus are *Cyh*1R for cycloheximide resistance and *Cyh*1S for sensitivity; alleles at the *MAT*1 locus are *MAT*1-1 (previously *MAT*A) and *MAT*1-2 (previously *mat*a) for opposite mating types; alleles at the *Tox*1 locus are *TOX*1 for T-toxin production and *tox*1 for lack of T-toxin production.

Isolates tested for lesion length differences on Cornell 281 included K22-R-48 and K22-R-55, K36-P2-4-2 and K36-P4-1-3, and eight progeny (Table 1) from two additional backcross generations: K38 and K39. In addition, K36-P2-4-2 and K36-P4-1-3 were tested for differences in lesion length on the inbreds W64A-N and B37-N. Significant differences between the mean lesion lengths for each isolate were detected with a two-way analysis of variance with trials as blocks and leaves as observations, followed by an LSD multiple comparison test.

Infection efficiency experiments compared K22-R-48 with K22-R-55 and K36-P2-4-2 with K36-P4-1-3 on Cornell 281, and used the same protocol as lesion length experiments, except that 34,000 spores were applied in 48 ml of water agar. Five plates of 2% water agar were placed in the inoculation chamber between the leaves so that the average number of germinable spores applied per square centimeter could be determined. Lesions between 7 and 17 cm from the tip of the fourth leaf were counted 4–7 days after inoculation. Infection efficiency on each leaf was determined by dividing the lesions counted per square centimeter by the average number of viable spores applied per square centimeter. The mean infection efficiencies for each isolate were compared using a Student's *t* test.

TABLE 2. Frequencies of cycloheximide-resistant (laboratory) and cycloheximide-sensitive (naturally occurring) *Cochliobolus heterostrophus* isolates from a field plot of N-cytoplasm maize (Cornell 281) in Ames, IA

| | 1983[a] | | 1984 | |
|---|---|---|---|---|
| Genotype | Percent | Total | Percent | Total |
| *Cyh*1R *TOX*1 *MAT*1-1 | 35 | | 0 | |
| *Cyh*1R *TOX*1 *MAT*1-2 | 0 | | 19 | |
| *Cyh*1R *tox*1 *MAT*1-1 | 65 | | 0 | |
| *Cyh*1R *tox*1 *MAT*1-2 | 0 | | 81 | |
| | | 927 | | 1,589 |
| *Cyh*1S *TOX*1 *MAT*1-1 | 0 | | 0 | |
| *Cyh*1S *TOX*1 *MAT*1-2 | 0 | | 0 | |
| *Cyh*1S *tox*1 *MAT*1-1 | 32 | | 64 | |
| *Cyh*1S *tox*1 *MAT*1-2 | 68 | | 36 | |
| | | 575 | | 653 |

[a] All 1983 cycloheximide-resistant isolates were tested for toxin production. Of these, 89 were tested for mating type. Mating type and toxin production were determined for 122 of the 575 cycloheximide-sensitive isolates. All 1984 isolates were tested for cycloheximide resistance, mating type, and toxin production.

## RESULTS

The number of cycloheximide-resistant (CyhR) and cycloheximide-sensitive (CyhS) isolates obtained from the 1983 and 1984 field experiments is shown in Table 2. All CyhR isolates from the 1983 sample were mating type 1 and all CyhR isolates from 1984 were mating type 2, suggesting no carryover of inoculum in the field from 1983 to 1984. These results also suggest that the frequency of cycloheximide resistance in the natural population was low, because both mating types were present in the CyhS population, yet no CyhR isolate of the noninoculated mating type was detected. Cycloheximide resistance was therefore used to distinguish laboratory strains from field strains. The 129 CyhS isolates tested in 1983 and all 1984 CyhS isolates were race O.

The relative frequency of the race T strain decreased significantly ($P < 0.0001$) during the growing season in 1983 (Fig. 1). In 1984, frequencies of the two strains 2 wk after inoculation were significantly different from 50% (Fig. 2), possibly because of inaccurate determination of inoculum viability before mixing. However, the number of CyhR isolates in each of the first three lesion harvests was large (350–600 lesions), especially compared with the last two harvests (100–135 lesions), enabling detection of a small but significant overall decline in the relative frequency of the race T strain ($P = 0.03$). Disease severity was low (fewer than 10 lesions per leaf) both years; changes in strain frequency were therefore probably not caused by differences in competitive ability.

The mean lengths of lesions produced by race T strains were significantly shorter than the mean lengths of lesions produced by race O strains from the same backcross generation in the greenhouse (Table 3). Differences were observed among progeny of four backcross generations and on three genotypes of maize. Significant differences in lesion length between near-isogenic race T and O strains were also observed in the 1985 field experiment (Table 4), although lesions produced on mature plants in field conditions were smaller than those observed on young plants in the greenhouse.

The germinability on agar of the conidia produced by the K22 and K36 pairs of strains was very high, averaging $99.0 \pm 0.3\%$ over all trials. No difference in germinability between race T spores and race O spores was detected. In infection efficiency tests, no consistent significant differences between race T and race O strains were detected, nor were any trends observed. The smallest difference in infection efficiency that could have been detected was 18%.

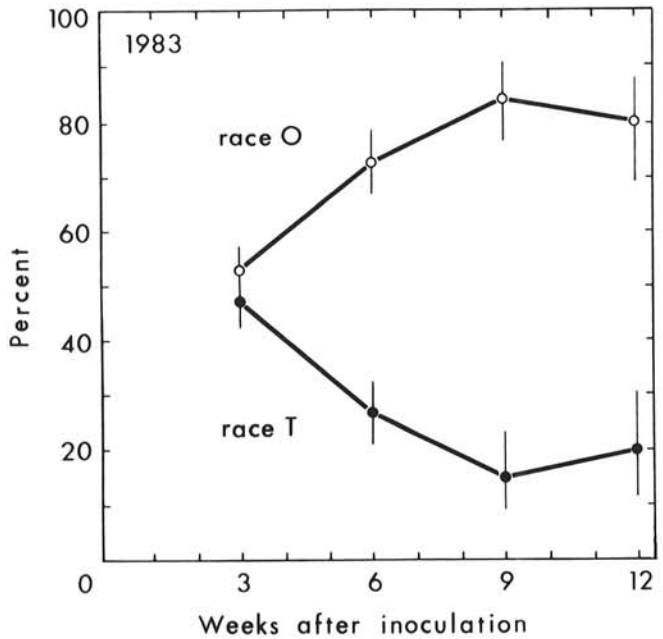


**Fig. 1.** Changes in frequency of *C. heterostrophus* strains (race O = K22-R-55, race T = K22-R-48) on N-cytoplasm maize (Cornell 281) in a field at Ames, IA, in 1983. Vertical lines indicate 95% confidence intervals.

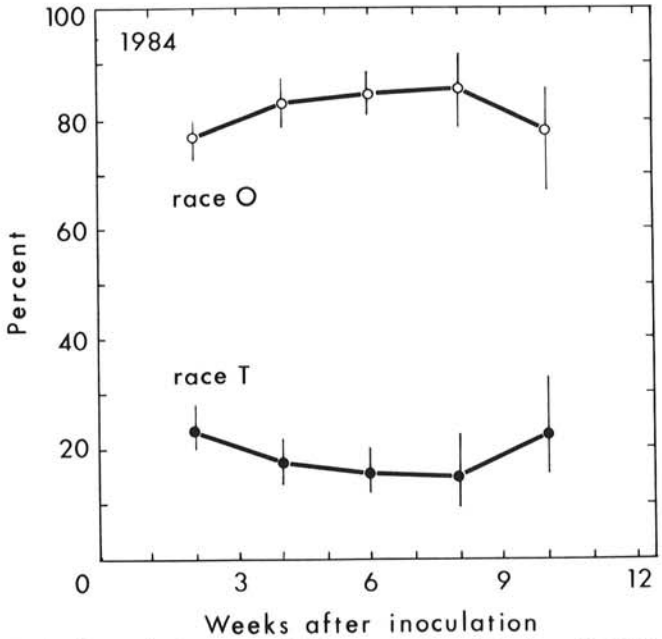


**Fig. 2.** Changes in frequency of *C. heterostrophus* strains (race O = K36-P4-1-3, race T = K36-P2-4-2) on N-cytoplasm maize (Cornell 281) in a field at Ames, IA, in 1984. Vertical lines indicate 95% confidence intervals.

## DISCUSSION

Our data strongly suggest that either the *TOX*1 gene for production of T-toxin or a gene closely linked to it reduces the fitness of race T strains of *C. heterostrophus* on normal cytoplasm maize. Race T decreased significantly in frequency compared with near-isogenic race O strains on N-cytoplasm maize (Cornell 281) in two years of field experiments. Comparisons of lesion lengths produced on N-cytoplasm maize in the greenhouse and the field verified an association of toxin production with reduced fitness. The results suggest that the changes in race frequency observed in the field may have resulted from differences in lesion length. Jenns et al (6) found a highly significant correlation between number of spores produced per lesion and lesion length of race O isolates on N-cytoplasm maize. Thus, relative lesion length could have epidemiological significance through its association with the amount of secondary inoculum produced. No difference in infection efficiency or spore viability was detected between race T and O strains in the greenhouse and the laboratory. However, this does not preclude any effect of spore viability or infection efficiency in the field.

Leonard (9) also observed that the mean lesion length of race T strains was shorter than that of near-isogenic race O strains. The larger reduction (an average of 38% on B37 compared with 10% on B37 in Leonard's study) that we observed between our near-isolines could be the result of different genetic backgrounds of the near-isogenic strains, different environments, or differences in experimental technique.

Debilitation associated with T-toxin production might not be characteristic of the average race T population, as only a single source of the *TOX*1 allele was examined, and an atypical gene affecting fitness may be linked to this particular *TOX*1 allele. However, because our results are similar to those obtained by Leonard (9) using a different strain of race T, and because race T seems to be less fit than race O in the natural population, we consider our results applicable to the natural race T population. Because *TOX*1 is an unnecessary virulence gene when *C. heterostrophus* infects N-cytoplasm maize, the reduced fitness of race T strains is consistent with Vanderplank's stabilizing selection hypothesis (22). Our results, however, do not necessarily imply that similar phenomena will be found for other virulence genes or other pathogens.

The difference in fitness between the near-isolines was probably caused by the toxin locus or a closely linked gene. We examined progeny from four backcross generations; progeny from the final generation had been backcrossed 11 times. Based on 11 backcrosses and unrelated initial parents, an average of 0.05% of the genome unlinked to the toxin locus would differ in race T and race O strains, and gene differences linked to the toxin locus would, on the average, be within a region no more than 9 centimorgans on either side of the toxin locus (18 centimorgans total)(11). The parents of our backcrosses, however, are very closely related, and the size of the nonisogenic region linked to the toxin locus is probably smaller than 18 centimorgans.

If the presence of the toxin gene reduces pathogen fitness on N-cytoplasm maize, the cause might be reduced metabolic efficiency. Lim and Hooker (12) demonstrated that T-toxin is produced by race T on N-cytoplasm maize. Tegtmeier et al (20) determined that the amount of toxin produced in culture represents about 2% of the dry weight of race T strains closely related to our strains. T-toxin production is probably a substantial drain of cell resources into the production of an unnecessary metabolite and could reduce pathogen efficiency. It does not seem to affect growth in culture, however, because no differences in growth rate have been found between near-isogenic race T and race O strains (7). Alternatively, an interaction between T-toxin and N-cytoplasm maize may subtly inhibit disease expression; this possibility has not been investigated (3).

If the reduction in pathogen fitness is caused instead by a gene closely linked to the toxin locus rather than the toxin locus itself, this gene could still affect race changes in the field. There is evidence that *C. heterostrophus* undergoes little genetic recombination in the field (4,8). A gene might, therefore, remain linked with the toxin locus for a considerable time and affect race frequency even if it is not a virulence gene (23).

Our results suggest that the decrease in frequency of race T in the field after T-cms maize was replaced in 1971 and 1972 with N-cytoplasm maize could have been caused, in whole or in part, by *TOX*1 or a closely linked gene. We observed that the fitness of race T was reduced on the two public inbreds in most widespread use in 1970, W64A and B37 (24), as well as on Cornell 281. Thus, the expression of differences in fitness was not dependent on host genotype. Although it dominated the pathogen population in 1970, race T has been detected rarely in the field since 1975 (10,17). Our study supports previous suggestions that race T failed to persist in the field because it was less fit than race O on normal cytoplasm maize and that reduced fitness is associated with the toxin gene (2,9). The reduced fitness of race T implies that it will remain a

TABLE 3. Mean length of lesions produced on N-cytoplasm corn in the greenhouse by near-isogenic *Cochliobolus heterostrophus* strains differing in a gene for T-toxin production

| Variety | Cross | Trial | Lesion length (mm) *tox*1[w] | *TOX*1 | Decrease[x] (%) |
|---|---|---|---|---|---|
| Cornell 281 | K22 | 1 | 5.0 | 3.6 | |
| | | 2 | 5.5 | 4.0 | |
| | | 3 | 6.0 | 4.1 | |
| | | Mean | 5.5 a[y] | 3.9 b | 29 |
| | K36 | 4 | 6.1 | 3.9 | |
| | | 5 | 6.1 | 4.2 | |
| | | 6 | 8.8 | 6.6 | |
| | | Mean | 7.1 a | 5.0 b | 30 |
| | K38[z] | 7 | 6.5 6.5 | 4.6 6.1 | |
| | | 8 | 7.1 8.5 | 6.3 6.7 | |
| | | 9 | 7.4 8.1 | 6.6 7.5 | |
| | | Mean | 7.1 ab 7.8 a | 6.0 c 6.9 b | 13 |
| | K39[z] | 10 | 9.6 8.2 | 7.5 7.1 | |
| | | 11 | 10.3 9.3 | 7.6 9.1 | |
| | | 12 | 7.6 7.3 | 6.2 5.5 | |
| | | Mean | 9.0 a 8.2 b | 7.0 c 7.1 c | 18 |
| W64A | K36 | 13 | 6.6 | 4.0 | |
| | | 14 | 7.3 | 4.9 | |
| | | 15 | 7.0 | 4.5 | |
| | | Mean | 7.1 a | 4.5 b | 37 |
| B37 | K36 | 16 | 5.3 | 3.3 | |
| | | 17 | 5.4 | 3.1 | |
| | | 18 | 5.3 | 3.4 | |
| | | Mean | 5.3 a | 3.3 b | 38 |

[w] *TOX*1 (race T) strains produce T-toxin, *tox*1 (race O) strains do not produce T-toxin.
[x] Difference between *tox*1 and *TOX*1 means divided by *tox*1 mean.
[y] Means in the same line followed by the same letter are not significantly different at $P = 0.05$ using a protected least significant difference test.
[z] Two *TOX*1 progeny and two *tox*1 progeny from this cross were compared.

TABLE 4. Mean length of lesions produced by near-isogenic *Cochliobolus heterostrophus* strains differing in a gene for T-toxin production on N-cytoplasm maize (Cornell 281) in a field at Ames, IA

| Inoculation date | Lesion length (mm) K22 *tox*1 | K22 *TOX*1 | K36 *tox*1 | K36 *TOX*1 | Decrease[y] (%) |
|---|---|---|---|---|---|
| 16 June 1985 | 1.3 a[z] | 0.8 b | 1.2 a | 0.9 b | 32 |
| 21 August 1985 | 1.7 a | 1.3 b | 1.6 ab | 1.4 b | 18 |
| Mean | 1.5 | 1.1 | 1.4 | 1.2 | 20 |

[y] Difference between average of *tox*1 and *TOX*1 means divided by average *tox*1 mean.
[z] Means in the same line followed by the same letter are not significantly different at $P = 0.05$ using a protected least significant difference test.

minor fraction of the pathogen population unless its pathogenic fitness is improved, or toxin production provides a selective advantage (as on T-cytoplasm maize). Occasional small-scale plantings of T-cms maize would probably not increase the frequency of race T in the general pathogen population. If the frequency of race T did increase due to large-scale plantings or repeated plantings in the same location of T-cms maize, plantings of N-cytoplasm maize would likely soon reduce race T to a small fraction of the pathogen population.

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