Lack of Toxicity of *Helminthosporium maydis*-Invaded Corn and Culture Filtrates to Chicks and Mice

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Six isolates of *Helminthosporium maydis*, obtained from southern leaf blight-damaged corn, were grown separately on autoclaved corn and fed to chicks and mice to evaluate their toxicogenicity. Two-, three-, and four-week-old culture filtrates from three pathogenic isolates grown separately on modified Fries medium were also evaluated for toxicogenicity. None of the invaded corn samples or culture filtrates affected the weights of chicks or mice when compared to controls. Postmortem examinations did not reveal significant gross lesions. *H. maydis* was not toxigenic under our experimental conditions.

In view of a recent report on mycotoxin production on corn by *H. maydis* (A. Ciegler et al., Bacteriol. Proc., p. 6, 1971), however, we wanted to assess the toxicity of autoclaved corn invaded by pure cultures of known pathogenic ("race T") isolates of *H. maydis* and of culture filtrates to chicks and mice.

The isolates of *H. maydis* used in this study were isolated from blight-damaged corn in 1970, three from leaves and three from grain. One of the isolates from grain was obtained from E. S. Luttrell (University of Georgia, Athens). The isolates were tested for pathogenicity by using the method described by Smith et al. for single spore isolates (8) and for pathotoxin production by using the root inhibition method described by Luke and Wheeler (7) on two corn hybrids, Coker 52 [Texas male-sterile cytoplasm (Tms)] and Coker 71 (normal cytoplasm).

Test diets, each containing 60% autoclaved corn heavily invaded by a single isolate of *H. maydis*, were prepared as previously described (3, 4). Each diet was fed ad libitum for 2 weeks to a group of 10 1-day-old Babcock B-300 cockerels and for 3 weeks to a group of three randomly bred male white mice (minimum age 30 days). Control chicks received a commercial chick starter diet, and control mice received a commercial laboratory diet.

Each pathogenic ("race T") isolate also was grown separately on modified Fries medium similarly to the method described by Luke and Wheeler for the production of victorin, the pathotoxin produced by *H. victoriae* (7). Culture filtrates were collected from each of these isolates.
after 2, 3, and 4 weeks of incubation. One milliliter of each culture filtrate was given orally, via a plastic tube attached to a tuberculin syringe, to each of three 1-day-old chicks, and 1 ml of each filtrate was injected intraperitoneally into each of three 25-g male white mice. The chicks were then fed a commercial chick starter diet for 1 week, and the mice were fed a commercial laboratory diet for 3 weeks, both ad libitum. Control chicks and mice each received 1 ml of modified Fries medium. Water was provided ad libitum for all groups in both the feeding and filtrate tests.

Initial and weekly body weights and daily observations were recorded for each group of chicks and mice. All chicks and mice were killed at the end of the test periods and examined for gross lesions. Tissues were collected in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for future examinations by microscope.

By the classification scheme of Hooker et al. (6), the six isolates were tentatively classified as follows: two produced a susceptible reaction on both hybrids and were designated as "race O"; three produced a susceptible reaction on Coker 52 (Tms) and were designated as "race T"; and one produced a resistant reaction on both hybrids and was given no race designation. Only the "race T" isolates produced a positive pathotoxin reaction. Fifty-fold dilutions of "race T" culture filtrates reduced the growth of Coker 52 (Tms) roots more than 50%.

Growth suppression of 10% or more, as compared to controls, was not recorded for any of the groups of chicks or mice fed H. maydis-invaded corn. One mortality was recorded in one group of chicks on the second day of the test. The cause of death was omphalitis and was thought to be unrelated to the invaded corn.

Growth suppression was not recorded for any of the groups of chicks or mice given H. maydis culture filtrates; however, growth stimulation of 10% or more, as compared to controls, was recorded for three of the nine groups of chicks. No mortalities were recorded during the culture filtrate test period.

Postmortem examinations of the chicks and mice at the end of the test periods did not reveal lesions associated with the test diets or culture filtrates. One chick fed fungus-invaded corn had severe fibrinopurpurative epicarditis and localized peritonitis in the umbilical region. These lesions were characteristic of a bacterial infection and were thought to be unrelated to the test.

Our data do not support the findings of Ciegler et al. (Bacteriol. Proc., p. 6, 1971) that H. maydis produces a mycotoxin on corn. They gave no details on the race of H. maydis used or on the extraction procedures and concentration factors involved in obtaining their solvent extracts before intraperitoneal injection into mice, rats, and a pig. It is possible that their experimental conditions preclude analogy to situations involving naturally infected corn.

Under our experimental conditions, H. maydis-invaded corn and culture filtrates were not toxic to chicks or mice. If H. maydis does produce a toxin which affects animals, a much higher concentration of toxin should occur in corn which is inoculated with a pure culture and incubated in naturally infected corn. Our data add support to the accumulating evidence that H. maydis, per se, is not toxic to animals.

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