Animal Toxicity of Phytopathogenic Fungi

C. E. MAIN and P. B. HAMILTON

Department of Plant Pathology and Department of Poultry Science, North Carolina State University, Raleigh, North Carolina 27607

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Twelve genera of phytopathogenic fungi comprising 27 species previously reported to produce phytotoxins were tested concurrently for animal and plant toxicity. There appeared to be no direct relationship between plant and animal toxicity.

The discovery that aflatoxin, a metabolite of Aspergillus flavus, caused cancer in mammals stimulated world-wide investigations into the toxic properties of fungi (3, 11). A. flavus is a storage fungus weakly pathogenic to plants. Subsequently, many fungi have been surveyed and found to possess animal toxicity potential. Some of these fungi, such as Cochliobolus carbonum (7), are highly aggressive plant pathogens.

The role of phytotoxic metabolites of plant pathogens in pathogenesis has also received considerable attention during the past ten years (2, 9, 14). Alternaria tenuis, the pathogen causing brown spot of tobacco, produced metabolites shown to be both phytotoxic and toxic to animals (6, 10). Pseudomonas tabaci, the causative organism of the wildfire disease of tobacco, produces a phytotoxin that incites convulsions in mice and rats (12).

A study was undertaken to investigate the possibility of direct relationship between phytotoxic metabolites and their animal toxin potential. A diverse group of plant pathogens known to produce phytotoxins was used. Such a relationship might exist if phytotoxins were able to surmount the physical barriers of the animals.

Selected fungi were obtained from the scientists who had described their phytotoxic properties (Table 1). If the original isolate was not available, a substitute isolate was obtained. The collection consisted of a broad taxonomic spectrum of 12 genera and 27 species. The fungi were grown in synthetic and semisynthetic media and under cultural conditions described in the literature for phytotoxin production. Specific cultural conditions were as described by Brian (2) and Ludwig (9).

A general phytotoxin bioassay using growth inhibition of Chlorella pyrenoidosa (8, 13) was performed on cold, sterilized (Millipore filters, 0.22 μm), crude culture filtrates of each test organism. Uninoculated media processed similarly served as controls. Despite obvious limitations of such a nonspecific assay, it was chosen rather than the varied and laborious phytotoxin assays described in the literature. The animal toxin bioassay consisted of homogenizing mycelium and filtrate of each fungus culture and injecting the brew into mice as previously described (6). Two female Swiss albino mice (DUB/ICR 9, Dublin Animal Laboratories, Dublin, Va.), weighing 12 to 25 g, were injected intraperitoneally with 0.03 ml of homogenate per g of body weight. The innocuousness of this large volume was established by injecting an equal volume of uninoculated medium into control mice. The mice were observed for 72 hr, and mortality was recorded. Chronic toxicity experiments were not attempted.

The phytotoxicity and animal toxicity results are shown in Table 1. In general, the fungi tested were not toxic to mice. The only exception was Penicillium janthinellum which possessed animal toxicity, but was not phytotoxic to Chlorella. Of 27 fungi, 9 were phytotoxic to Chlorella.

The effect of media and cultural conditions on toxin production was investigated. All test fungi were grown in Main's medium (5) enriched with Phytone (Baltimore Biological Laboratory), which represented a common source of plant protein. The cultures were incubated at 25 C in still culture for 3 weeks, and bioassays were performed as described above. A. tenuis (M-1) and Fusarium tricinctum gave positive mouse toxicity but lost their phytotoxic potential. Rhizoctonia solani gave a positive mouse assay, whereas the Chlorella assay was negative. Results with the other fungi were unchanged.

This study indicates that there is no direct
relationship between phytotoxic and animal toxic potential of phytopathogenic fungi as a group. *A. tenuis* (4) and *F. tricinctum* (1) have been reported to produce animal toxins in feedstuffs. Our results support the hypothesis that these two organisms produce animal toxins when provided a plant substrate medium. Apparently, at least nine of the remaining fungi produce phytotoxins but not animal toxins under the conditions of the experiment. It is plausible that phytotoxins and animal toxins have different modes of action. Phytotoxins, as a class, probably pose little hazard to public and animal health, with the few exceptions noted.

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**LITERATURE CITED**

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**Table 1. Plant and animal bioassay results of 27 phytopathogenic fungi selected on the basis of previously reported potential to produce phytotoxic metabolites**

| Organism | Isolate no. | Source | Host plant(s) | Bioassay results* | Mouse assay |
|----------|-------------|--------|---------------|------------------|------------|
| *Alternaria citri* (Ell. & Pier.) | b | N. D. Fulton | Citrus | - b | - c |
| *A. solani* (Ell. & Mart.) Jones & Grout | U-105 | G. B. Lucas | Tomato, potato | + | - |
| *A. tenuis* (Nees)* | M-1 (R-396) | C. E. Main | Tobacco | + | - |
| *A. tenuis* (Nees) | 3048 F | N. D. Fulton | Cucumber | - | - |
| *Ceratocystis fagacearum* (Bretz.) Hunt | A | A. Kelman | Oak | - | - |
| *C. fimbriata* (Ell. & Halst.) Elliott | S1 | J. Kue | Sweet potato | - | - |
| *C. ulmi* (Buisman) | w20 | A. Kelman | Elm | - | - |
| *Endothia parasitica* (Murr.) Anderson & Anderson | | H. L. Barnett | Chestnut | + | - |
| *Fusarium lateritium* Nees. | | N. N. Winstead | Ornamentals | - | - |
| *F. oxysporum* f. conglutinans (wr.) Snyder & Hansen | N-8 | N. N. Winstead | Cabbage | - | - |
| *F. oxysporum* f. lycopersici (Sacc.) Snyder & Hansen | N-3 | N. N. Winstead | Tomato | + | - |
| *F. oxysporum* f. nivorum (E. F. Sm.) Snyder & Hansen | Cg-8 | N. N. Winstead | Watermelon | + | - |
| *F. roseum* f. cerealis (Cke.) Snyder & Hansen | FSCM-1 | O. H. Calvert | Small grain | + | - |
| *F. tricinctum* (Cda.) Snyder & Hansen | T-1 | E. B. Smalley | Corn | + | - |
| *Helminthosporium sativum* (Pam. King & Bakke) | 100 | R. R. Nelson | Wheat | + | - |
| *H. victoriae* (Meeham & Murphy) | 7002 I | R. R. Nelson | Oats | + | - |
| *Nectria cinnabarina* (Tode & Fr.) | A-25 | G. B. Ouelette | Peach | - | - |
| *Penicillium fumiculosum* (Thom) | A-27 | R. J. Stipes | Peanut | - | - |
| *P. janthinellum* (Biourge) | A-27 | R. J. Stipes | Peanut | - | + |
| *Periconia circinata* (Mang.) Sacc. | P-2A | R. P. Scheffer | Sorghum | - | - |
| *Phytophthora infestans* (Mont.) de Bary | 149 | Z. A. Barker | Potatoes | - | - |
| *P. parasitica* var. nicotiana (Breda de Haan) Tucker | 1156 | J. L. Apple | Tobacco | - | - |
| *Rhizoctonia solani* (Kuehn) | 282 | R. L. Sherwood | Cotton | - | - |
| *R. leguminicola* | 242 | R. L. Sherwood | Red clover | - | - |
| *Sclerotinia homeocarpa* (Bennett) | T-72 | R. M. Endo | Turf grass | + | - |
| *S. sclerotiorum* (Lib.) de Bary | 1177 | R. G. Grogan | Potato | - | - |
| *Stereum purpureum* (Per. & Fr.) Fr. | FP71382-SP | F. F. Lombard | Peach | - | - |

* Bioassay results of cultures grown under conditions described in the literature for production of phytoxins.
* Plus indicates presence of inhibition zone around assay disc on agar plates seeded with *Chlorella pyrenoidosa*.
* Plus indicates mortality of at least one of two mice injected intraperitoneally with 0.03 ml of homogenized culture per g of body weight.
* *Alternaria tenuis* (Nees.); *A. longipes* (Ell. & Ev.) Mason.
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