

Survey of Tall-Fescue Pasture: Correlation of Toxicity of *Fusarium* Isolates to Known Toxins

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Several aspects of fescue foot in cattle suggest that this disease is caused by fungi growing on fescue grass. Certain fungi isolated from winter pasture yield toxins when grown on synthetic medium. Most of these toxin producers belong to the genus *Fusarium*. All but 1 of the 21 toxic and 7 questionably toxic *Fusarium* isolates produce either 4-acetamido-4-hydroxy-2-butenic acid γ -lactone, or 4 β ,15-diacetoxy-8 α -(3-methylbutyryloxy)-12,13-epoxytrichothec-9-en-3 α -ol, or both.

Tall fescue (*Festuca arundinacea* Schreb.) pasture, often used as a winter forage, occasionally causes a disease known as fescue foot in grazing cattle. The sporadic, seasonal, and regional occurrence of this disease suggests that it is caused by a fungus or fungi growing on the grass (4). Keyl et al. (2) isolated *Fusarium tricinctum* NRRL 3249, which was toxic in both a rabbit skin and mouse assay. When cultured in the laboratory on natural or synthetic media, this fungus produced at least three toxins: 4-acetamido-4-hydroxy-2-butenic acid γ -lactone (I), 4 β ,15-diacetoxy-8 α -(3-methylbutyryloxy)-12,13-epoxytrichothec-9-en-3 α -ol (II), and a third not yet identified (3).

In 1967, grass was collected from a fescue pasture in Missouri where 11 out of a herd of 100 cattle were severely affected with fescue foot. Samples were taken for mycological examination from six different areas within this pasture, plus three other samples from nearby fields. One of the three was orchard grass (*Dactylis glomerata* L.), intended as a control, and the other two were tall fescue samples from a farm having a history of being toxic each year. Since there were no cattle on this second fescue pasture, we do not know if the grass was toxic at the time of sampling. The 200 fungal isolates obtained from these nine grass samples represented 19 genera, the most abundant being *Fusarium*, *Epicoccum*, *Cladosporium*, and *Alternaria* (4).

Of these 200 isolates previously tested, culture extracts of 25 were toxic to mice; 23 of the toxic extracts were from isolates of the genus *Fusarium*. Another 29 of the fungi were questionably toxic, and 9 of these were from the genus *Fu-*

sarium. Three of the toxic *Fusarium* strains were isolated from orchard grass (4).

The present study was undertaken to see whether toxins I and II accounted for toxicity of the *Fusarium* isolates and to confirm their toxicity to the mouse.

MATERIALS AND METHODS

Toxins I and II were detected qualitatively by thin-layer chromatography (TLC) of fungal extracts. Ethyl acetate extracts of Sabouraud's agar cultures were spotted on Silica Gel G plates and developed, by Honegger's sandwich method (1), with ethyl acetate-toluene (3:1). After the plates were air-dried, they were sprayed with concentrated sulfuric acid and heated at 125 C. Toxin I has an R_F of about 0.2 and forms a tan to brown trailing spot. Toxin II has an R_F of about 0.6 and forms an oval spot that varies in color from green to dark grey. The detection limits of pure compounds I and II are of the order of 10 μ g.

The 32 *Fusarium* strains, reported as either toxic or questionably toxic (4), were regrown at 15 C on Sabouraud's agar from lyophilized cultures prepared shortly after isolation. *F. tricinctum* NRRL 3249 and NRRL 3299 (strain T-2 given us by E. B. Smalley, University of Wisconsin), which usually produce toxins I and II, were also included. Extracts of the cultures were made by steeping the diced agar in ethyl acetate for several days. The extracts were concentrated, and samples were evaporated to dryness under reduced pressure. The residues were suspended in Ringer's solution, and each suspension was assayed in two male white mice (25 \pm 5 g). Each mouse received a single intraperitoneal injection equivalent to extract from 0.1 of a petri plate (about 2.5 ml of agar). The criterion of toxicity was death of both mice within 4 days. The toxicity was recorded as questionable if only one of the two mice died or both mice became sick during this time.

RESULTS AND DISCUSSION

The results of the mouse assay (Table 1) agreed well with the previous assay (4). Of the 32 *Fusarium* strains retested, 22 gave the same results. Of the 10 assays which differed, six were changes from toxic to questionably toxic or vice versa, and two were changes from questionably toxic to nontoxic. Only two samples changed from toxic to nontoxic. These changes may reflect the heterokaryotic nature of the strains.

The ethyl acetate extracts were examined by

TLC for toxins I and II (Table 1). Sometimes infrared spectroscopy was used to confirm the presence or absence of toxin I (4). In all of the toxic cultures toxin I or toxin II, or both, were present. In those cultures which were nontoxic, neither of these two compounds could be detected. In one questionably toxic culture, number 14, neither toxin I nor II could be detected. The three toxic *Fusarium* isolates that came from orchard grass, 5, 17, and 18, contained toxins I and II just as did the isolates from tall fescue. Detection of toxins I and II does not rule out the presence of other

TABLE 1. *Mycotoxins, toxicity, and pigmentation in Fusarium isolates*

Culture no. ^a	Color of agar	Yield ^b	Toxicity	Toxin I ^c	Toxin II ^c
Control	None	3.4	Nontoxic	No ^d	No
NRRL 3249	Brown	32.0	Questionably toxic	No ^d	Yes
NRRL 3299	Yellow, red spots	48.0	Toxic	Yes ^d	Yes
1	Yellow	26.8	Toxic	Tr ^d	Yes
2	Yellow	17.6	Toxic	No ^d	Yes
3	Yellow	30.2	Toxic	Yes ^d	Yes
4	Yellow	34.5	Toxic	Yes	Yes
5	Yellow, red spots (+)*	22.8	Questionably toxic	Tr ^d	Yes
6	Yellow, red spots (+)	34.0	Toxic	No ^d	Yes
7	Tan, red spots (+)	37.6	Nontoxic	No ^d	?
8	Tan, red spots (++)	19.1	Toxic	No	Yes
9	Yellow, red spots (+++)	53.5	Toxic	Yes	Yes
10	Yellow, red spots (+++)	51.1	Toxic	Yes	Yes
11	Yellow, red spots (+++)	44.2	Toxic	Yes	Yes
12	Yellow, red spots (+++)	13.9	Toxic	No ^d	Yes
13	Red orange	29.1	Toxic	Yes ^d	Yes
14	Reddish brown	27.9	Questionably toxic	No	No
15	Reddish brown	33.5	Toxic	Yes	?
16	Reddish brown	26.8	Toxic	Yes	Yes
17	Reddish brown	41.8	Toxic	Yes	Yes
18	Reddish brown	36.7	Toxic	Yes ^d	Yes
19	Reddish brown	18.2	Toxic	No ^d	Yes
20	Dark, reddish brown	40.4	Toxic	Yes ^d	Yes
21	Dark, reddish brown	31.4	Toxic	Tr ^d	Yes
22	Dark, reddish brown	38.7	Toxic	Yes ^d	Yes
23	Yellow-grey	10.6	Nontoxic	No	No
24	Purple	31.9	Nontoxic	No	No
25	Purple	48.4	Questionably toxic	Yes ^d	No
26	Purple	48.4	Toxic	Yes ^d	No
27	Purple	37.9	Questionably toxic	Yes ^d	No
28	Purple	32.3	Questionably toxic	Yes ^d	No
29	Purple	41.9	Questionably toxic	Yes ^d	No
30	Purple	45.0	Toxic	Yes ^d	No
31	Purple	22.0	Nontoxic	No	No
32	Purple	40.8	Questionably toxic	Yes ^d	No

^a NRRL numbers refer to permanent cultures of the Agricultural Research Service Culture Collection; the *Fusarium* species numbers 1-32 refer to this publication only.

^b Yield of ethyl acetate extractives per agar plate (about 25 ml of agar).

^c Thin-layer chromatography evidence.

^d Infrared evidence.

* Red spots increase from (+) to (+++).

toxins in any or all of the toxic or questionably toxic strains.

All except the first three entries in Table 1 are arranged in order of increasing color density of the pigments which diffuse into agar. The data seem to indicate that pigmentation and toxicity per se are not related; neither is pigmentation nor yield of extractable material. However, pigmentation may be related to which toxin is produced. None of the dark purple cultures produced any of toxin II, although most produced toxin I.

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