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Mycotoxin Production by *Fusarium oxysporum* and *Fusarium sporotrichioides* Isolated from *Baccharis* spp. from Brazil†

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***Fusarium oxysporum* isolated from roots of and soil around *Baccharis* species from Brazil produced the trichothecenes T-2 toxin, HT-2 toxin, diacetoxyscirpenol, and 3′-OH T-2 (TC-1), whereas *Fusarium sporotrichioides* from the same source produced T-2 toxin, HT-2 toxin, acetyl T-2, neosolaniol, TC-1, 3′-OH HT-2 (TC-3), *iso*-T-2, T-2 triol, T-2 tetraol, and the nontrichothecenes moniliformin and fusarin C. Several unknown toxins were found but not identified. Not found were macrocyclic trichothecenes, zearalenone, wortmannin, and fusarochromanone (TDP-1).**

In Brazil and neighboring countries, cattle and sheep that graze in pastures harboring shrubs of *Baccharis* species sustain disorders of the gastrointestinal and nervous systems (12). The cause of this toxicity has been postulated as trichothecenes produced by fungi growing in the rhizospheres and roots of *Baccharis* species, with the subsequent translocation of these toxins into the plant roots and aerial plant parts (8). Toxicity has also been attributed to the macrocyclic trichothecenes that are present in the seeds of female plants of *Baccharis* species (6, 7). Isolates of *Fusarium oxysporum* and *Fusarium sporotrichioides* were especially abundant in the rhizosphere of this shrub and were shown to be toxigenic in feeding trials (8). The most toxic (established in feeding trials in rats) of these isolates were tested for their ability to produce known as well as unknown toxins that could explain the toxicity that has been noted. The objective of this study was to determine the toxins that are produced by those isolates of *Fusarium*.

A total of 39 isolates of *F. oxysporum* Schlecht. emend. Snyd. & Hans. and 42 isolates of *F. sporotrichioides* Sherb. found previously in roots and rhizospheres of and soil around *Baccharis* species (8) were grown on autoclaved, long-grain parboiled rice (Uncle Ben's) medium as described by Abbas et al. (2). *Fusarium* species were identified earlier (8) by use of the manual by Nelson et al. (9). Procedures used to isolate these species have been described by Kommedahl et al. (8), and the cultures are stored in the collection at the University of Minnesota, St. Paul.

To assay for trichothecene and nontrichothecene mycotoxins in fungal extracts, the following procedure was used. A total of 20 g of ground fungal culture on rice was wetted with 10 ml of distilled water, extracted with 200 ml of 50% ethyl acetate in acetonitrile 3 times for 1 h each time at room temperature (20 to 24°C), and filtered through Whatman no. 4 filter paper. The filtrates from several extractions were combined and concentrated in a rotary evaporator at 35°C. The residue was dissolved in 60 ml of acetonitrile and partitioned with petroleum ether (boiling point, 60 to 70°C). The acetonitrile portion was condensed in the rotary evaporator, and this residue was dissolved in 10 ml of chloroform-methanol (9:1, vol/vol) and applied to a column (1 by 15 cm) of Florisil packed in the same solvent. To the column, 100 ml of chloroform-methanol (9:1, vol/vol) was added, and the eluate was dried. The residue was redissolved in 2 ml of methanol to prepare it for thin-layer chromatography and analyses as described previously (2). The trichothecene mycotoxins were quantified by gas chromatography by derivatizing the extract with trifluoroacetic acid anhydride (Pierce Chemical Co., Rockford, Ill.) for comparisons with standards and confirmed by using combined gas chromatography-mass spectrometry. Fusarin C was extracted and quantified by using the method described by Gelderblom et al. (4). Moniliformin was extracted and quantified by methods used previously (1, 10, 11).

Of 39 isolates of *F. oxysporum*, 1 (isolate 598) produced 600 μg of T-2 toxin per g and <1 μg of HT-2 toxin, diacetoxyscirpenol, and 3′-OH T-2 toxin (TC-1) per g. Of 42 isolates of *F. sporotrichioides*, 1 isolate (isolate 1266) produced 3 μg of T-2 toxin per g and 10 μg of HT-2 toxin per g; 3 isolates (isolates 689, 1230, and 1235) produced acetyl T-2 toxin (1 to 2 μg/g), T-2 toxin (250 to 600 μg/g), neosolaniol (8 to 10 μg/g), HT-2 toxin (30 to 80 μg/g), T-2 triol (1 to 2 μg/g), T-2 tetraol (1 to 2 μg/g), and TC-1, 3′-OH HT-2 toxin (TC-3), and *iso*-T-2 toxins (<1 μg/g) (Table 1).

Of 42 isolates of *F. sporotrichioides*, 11 isolates produced fusarochromanone (TDP-1), wortmannin, or zearalenone. Of 42 isolates of *F. sporotrichioides*, 11 isolates (isolates 218, 599, 804, 837, 888, 938, 945, 951, 978A, 1019, and 1235) produced the mutagenic mycotoxin fusarin C in the range 7.5 to 67.2 μg/g (Table 1). None of the 42 isolates of *F. sporotrichioides* produced macrocyclic trichothecenes. None of the 39 isolates of *F. oxysporum* or 42 isolates of *F. sporotrichioides* produced fusarochromanone (TDP-1), wortmannin, or zearalenone. A total of 7 of 39 isolates of *F. oxysporum* and 3 of 42 isolates of *F. sporotrichioides* produced toxins that were not identified by studies of toxicity in rats (8). The chemical identity of trichothecenes produced by *F. oxysporum* and *F. sporotrichioides* was confirmed by derivatizing the extract with trifluoroacetic acid anhydride and analyzing the product by combination gas chromatography-mass spectrometry in the positive ionization mode with methane at 66.7 Pa (0.5 torr) of source pressure. Moniliformin and fusarin C were confirmed by UV spectroscopy (1).

F. oxysporum has been reported to produce trichothecenes, as determined by thin-layer chromatographic analysis (3, 5) but not by gas chromatography-mass spectrometry,

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TABLE 1. Toxins produced by *F. oxysporum* and *F. sporotrichioides* and the number of isolates of each species grown on a rice medium

Toxin	No. of *F. oxysporum*[a]	Amt (μg/g) of toxin produced	No. of *F. sporotrichioides*[a]	Amt (μg/g) of toxin produced
Acetyl T-2	0	0	3	1–2
T-2 toxin	1	600	4	30–600
HT-2 toxin	1	<1	4	30–80
Diacetoxyscirpenol	1	<1	0	0
3′-OH T-2 toxin	1	<1	3	<1
Neosolaniol	0	0	3	8–10
T-2 triol	0	0	3	1–2
T-2 tetraol	0	0	3	1–2
3′-OH HT-2 toxin	0	0	3	<1
iso-T-2 toxin	0	0	3	<1
Macrocyclic trichothecenes	ND[b]	ND	0	0
Moniliformin	16	25–2,550	13	19–1,750
Fusarin C	0	0	11	8–68
Zearalenone	0	0	0	0
Wortmannin	0	0	0	0
Fusarochromanone	0	0	0	0
Unknown[c]	7		3	

[a] Values represent a range among the isolates: 39 of *F. oxysporum* and 42 of *F. sporotrichioides*.
[b] ND, Not determined.
[c] Unknown indicates that pathological effects and death were noted in rats fed a diet of rice medium containing fungi in which the toxin was not one of those identified (8).

raising doubt as to whether they are produced. We found that only 1 of 38 isolates of *F. oxysporum* produced copious amounts of T-2 toxin and trace amounts of other trichothecenes. Our results with *F. sporotrichioides* were similar to those reported previously (2, 13). Many isolates of *F. sporotrichioides* produced the mutagenic mycotoxin fusarin C, which is the first report of fusarin C associated with *F. sporotrichioides* isolated from *Baccharis* species in Brazil.

The discovery that strains of *F. oxysporum* and *F. sporotrichioides* inhabit the rhizospheres of *Baccharis* species (8) and can produce trichothecenes, fusarin C, moniliformin, and some unidentified toxins indicates that livestock grazing in pastures infested with *Baccharis* species are exposed to these mycotoxins; however, we did not establish that these toxins account for the intoxications reported in livestock.

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