Toxigenic *Fusarium* Species Found in Roots and Rhizospheres of *Baccharis* Species From Brazil

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Paper 14,574, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 55108.

The authors appreciate the assistance of Gert Hatschbach, botanist with the Prefeitura Municipal de Curitiba, Parana, Brazil, for identification and location of *Baccharis* spp.; and Dr. C.H. Tokarnia, veterinary pathologist, Universidad Federal Fluminense, Rio de Janeiro, Dr. S. S. Barros and Dr. C. S. L. Barros, veterinarians at the University in Santa Maria, RS, Brazil, for background information. Thanks are expressed to Patricia McClellan Burnes for laboratory assistance at Minnesota.

Work was supported in part by funds from U. S. Army Contract DMAD 17-82-C-2240 with the University of Maryland (B. B. Jarvis, Department of Chemistry).

Accepted for publication 3 October 1986 (submitted for electronic processing).

ABSTRACT

Kommedahl, T., Abbas, H. K., Mirocha, C. J., Bean, G. A., Jarvis, B. B., and Guo, M. 1987. Toxigenic *Fusarium* species found in roots and rhizospheres of *Baccharis* species from Brazil. Phytopathology 77:584-588.

Fusarium species were more abundant in the rhizosphere than in roots or soil of *Baccharis* species collected in southern Brazil. *Fusarium sporotrichioides* occurred more abundantly in roots and rhizospheres than in soil surrounding roots. More *Fusarim* species were isolated from *B. coridifolia* than from four other *Baccharis* species. Thirty-nine isolates of *F. oxysporum* and 42 of *F. sporotrichioides* from roots, rhizospheres, and soil of *Baccharis* species, grown on rice substrate and fed to rats in a 1:1 mixture with rat ration, caused below-normal weight gains, and 18 of the isolates of the two *Fusarium* species were lethal to rats in a 5-day test. T-2 toxin was produced by three isolates of *F. sporotrichioides*.

Baccharis comprises about 350 species and is found in the Americas from Massachusetts to Tierro del Fuego. Members of this genus are shrubs especially common on the grassland highlands of South America (campos) where they are toxic to livestock. Species in North America, however, have not been reported toxic to livestock. Jarvis et al (9) found that roots of *B. megapotamica* Spreng. can absorb and translocate trichothecenes to shoots, and the mycotoxins are chemically altered in the plant to structures analogous to those found in plants growing in their native habitat. Plants grown from seed in non-Brazilian soil are not toxic to animals and do not contain trichothecenes. Thus they (9) postulated that North American soils lack the trichothecene-producing soil fungi that serve as a source of the mycotoxin.

Baccharis coridifolia DC. is a common and important plant poisonous to cattle and sheep in southern Brazil and neighboring countries (3,20). This species is two to four times more toxic during flowering and seeding stages (March) than in the sprouting stage (October-November) in Brazil. Other species of *Baccharis* occur in Brazil and include *B. megapotamica*, *B. myriocephala* Baker (= *B. pseudomyriocephala* Teodoro), *B. semiserrata* DC., and *B. uncinella* DC.

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The toxins that could account for the poisoning of livestock were thought to be trichothecenes, produced by isolates of *Cephalosporium*, *Fusarium*, *Myrothecium*, and *Stachybotrys* species (5,16,21). Trichothecenes are toxic to livestock, humans, bacteria, fungi, viruses, and insects (7).

The class of trichothecenes reported in these Brazilian species of *Baccharis* (5,10,12) is the macrocyclic trichothecenes (8,19) that have been reported to be produced by four genera of fungi: *Myrothecium* (7), *Stachybotrys* (7), *Verticimonosporium* (15), and *Cylindrocarpon* (14). However, only one instance each of such toxin production has been reported for the latter two genera. Of interest is whether these *Baccharis* species may also contain the simple trichothecenes, such as T-2 toxin, which in general are far more common in nature than are the macrocyclic trichothecenes. These simple trichothecenes are produced by a number of common soil fungi, the most notable of which are *Fusarium* species.

Fungi have been the only reported source of these compounds except for the Brazilian shrubs, *B. coridifolia* and *B. megapotamica* (2,3,9–11). The trichothecenes in *B. megapotamica* are called baccharinoids. There is some question whether these trichothecenes are produced by the plant or whether fungi in soil, in rhizospheres, or in roots produce the mycotoxin, which is then absorbed and translocated in the plant (3). Thus, our objectives were to assay for *Fusarium* species in the roots and rhizospheres of *Baccharis* species and in nearby soil, ascertain toxicity to laboratory rats from rice culture of *Fusarium* species isolated, and determine if *Fusarium* species produce trichothecenes in culture.

MATERIALS AND METHODS

Soil samples and assay procedure. Eighty-nine samples of soil were collected in three geographical locations in southern Brazil (Table 1). Samples were collected in the field by inserting an Oakfield soil sampler at about a 45° angle under the plant so as to collect roots as well as the soil around them. These samples were put into plastic bags and taken back to the laboratory, and the roots were separated from the soil by screening with a No. 5 sieve having 4-mm-mesh screen. The separated soils were stored in plastic bags at 5 C until assayed, usually within a few days. The roots were cut aseptically into 2-cm-long fragments. These fragments were placed into three groups: diameters 1.5 mm or wider, 1.0–1.5 mm, and less than 1.0 mm. All work with soil, roots, and rhizospheres was done in a laminar flow biological safety cabinet.

To assay roots, four root fragments were placed in an 125-ml Erlenmeyer flask containing 25 ml of autoclaved 0.12% water agar. The flask was placed on a rotary shaker for 10 min, then each one of the four roots was placed in a petri dish containing an agar medium—two roots on pentachloronitrobenzene agar (PCNB) (17) supplemented with aureomycin, and two roots on peptone-dextrose rose-bengal agar (RB). The root fragments were not surface disinfected.

In the rhizosphere study, the 0.12% water agar suspension used during the preparation of the root fragments served as a source of the rhizosphere organisms. Using a sterilized pipette, 1 ml of this suspension was dispensed onto each of two media: PCNB and RB. The dispensed suspension was distributed over the agar surface by using a bent sterilized glass rod.

The Warcup soil-plate method (22) was used to isolate fungi from soil. An air-dried soil sample, approximately 0.5 g, was crushed to a fine powder. A flattened, sterilized nichrome needle was used to transfer a microsample of powdered soil into the bottom of a sterile petri dish. Approximately 10 ml of melted and cooled (to 45 C) agar was added and the dish was then swirled to attempt uniform distribution of soil particles. Both PCNB and RB agar media were used. However, the results are based almost entirely on counts made on PCNB.

Petri dishes containing roots, rhizosphere soil, and field soil were incubated at approximately 24 C under fluorescent and black (ultraviolet) lamps (5,300 lx) for 12 hr/day to foster growth and sporulation of fungi. After 7–10 days, fungal colonies were counted and transferred to acidified noncommercial potato-dextrose agar (PDA). *Fusarium* species were identified by using the manual of Nelson et al (18).

TABLE 1. Soil and root samples taken from *Baccharis* species at different locations in southern Brazil in March 1984

Baccharis spp.	Samples (no.)	Location	Elevation
B. coridifolia	8	Palmeira[a]	850 m
	11	Pulgas de Sima[b]	900 m
	11	Curitiba area[c]	1,000 m
	4	Santa Maria[d]	1,000 m
B. megapotamica	15[e]	Curitiba	800 m
	10[f]		950 m
B. myriocephala	3	Curitiba[g]	800 m
B. semiserrata	2	Curitiba[g]	800 m
B. uncinella	2	Curitiba[g]	800 m
None present	11	Curitiba[h]	800 m

[a] Location is 10 km west of Palmeira, which is west of Curitiba.
[b] Location between Palmeira and Curitiba.
[c] West of Curitiba on mountain slope, but adjacent to pasture.
[d] Two locations near Santa Maria on the Vista Alegre Ranch, Julio de Castilhos County (63 km from two of Julio de Castilhos, in the area known as Igresjinha dos Quevedos). Soil pH here was 5.6.
[e] One sample from pistillate plant; others from staminate plants.
[f] Mountain slope.
[g] Samples taken in the vicinity of plants of *B. megapotamica*, wet swamp, near Curitiba.
[h] Soil samples taken from pasture near plants of *B. megapotamica* but in which no *Baccharis* species were present.

From each field sample, 12 rhizosphere dishes (six PCNB, six RB), 12 soil dishes (six PCNB, six RB), and eight roots 2 cm long (four PCNB, four RB) were prepared; however, *Fusarium* grew mainly in the PCNB medium.

Toxicity of fungal isolates to laboratory rats. To test each fungus isolate for toxicity to rats, the fungus was grown on a rice substrate, using a method of Eugenio et al (4), modified by Abbas et al (1), in which 200 g of long-grain parboiled rice in 120 ml of distilled water was allowed to stand for 1 hr in a 1-L flask, shaken, cotton stoppered, and autoclaved for 1 hr at 121 C. The flasks were cooled for 24 hr at room temperature (22–25 C) and then reautoclaved, shaken, and inoculated with isolates of *Fusarium* to be tested. The cultures in flasks were incubated again at room temperature and shaken daily for 10 min for the first few days to enable the hyphae to penetrate the rice substrate thoroughly. The cultures were then incubated for 2 wk at 25–27 C, followed by 2 wk at 10 C. The mass of fungus-invaded rice was broken up, transferred to a screen-bottomed tray, and air-dried in a ventilated hood. The moldy rice was ground to the consistency of flour by using a coffee grinder in a fume hood. This material was then mixed 1:1 with a ground (to a flour) complete rat chow (Ralston-Purina), and fed to 20-day-old virgin female Sprague-Dawley rats (Bio-Lab Corp., St. Paul, MN) housed in individual cages. The animals and feed were weighed at the beginning and at the end of the experiment. Three rats were used for each fungal isolate and the controls, which were fed only rat chow mixed 1:1 with noninfested rice. Rats were observed daily for 5 days, and symptoms and death were recorded. Surviving rats were killed by cervical dislocation and examined for pathological changes in various tissues.

Isolation of mycotoxin from fungal cultures. Samples (8–18 g each) of the dried moldy rice cultures of *F. sporotrichioides* were treated with 50 ml of 90% aqueous methanol in a sonicator (Branson Cleaning Equipment Co., Shelton, CT) for 30 min. The solvent was decanted and the extraction repeated with 50 ml of 90% methanol. The aqueous methanol extracts were combined and filtered by gravity through Whatman No. 1 filter paper, and the solid material on the filter paper was washed with 10 ml of methanol. The filtrate was washed with hexane (twice, 30 ml each) in a 250-ml separatory funnel. The hexane layer was discarded. The

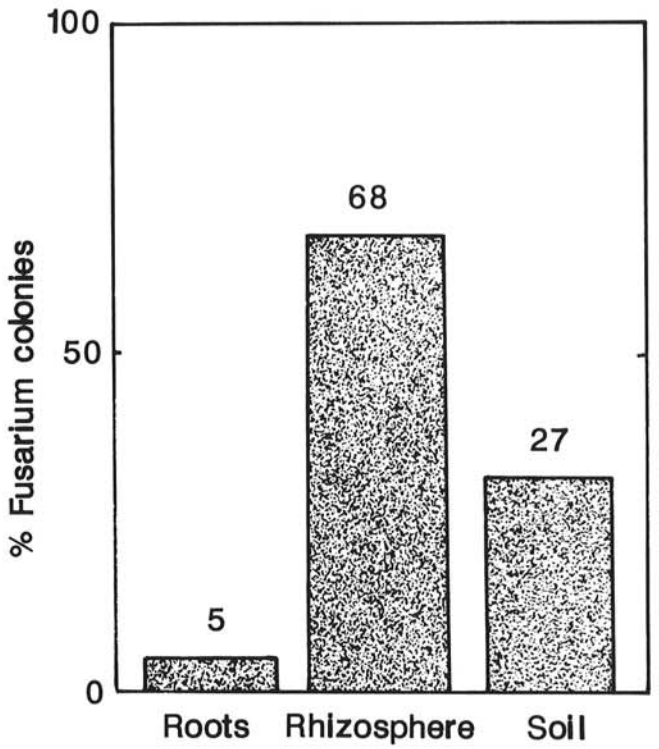

Fig. 1. Percentage of colony-forming units of *Fusarium* in roots, rhizosphere, and soil of *Baccharis* species, from Brazil based on 6583 colonies.

aqueous methanol solution was slowly added to a solution of 100 ml of ferric gel solution. Ferric gel solution was prepared by adding 100 ml of distilled water to 10 ml of 15% ferric chloride ($FeCl_3 \cdot 6H_2O$) and the pH of the ferric chloride solution was adjusted to about 4–4.5 by adding 1 N NaOH solution. The mixed solution was allowed to stand for 5 min and was then filtered through Whatman No. 1 filter paper. The precipitate was washed with 50 ml of 50% methanol. The filtrate and wash were combined and concentrated in vacuo to remove methanol. The resulting water solution (about 40 ml) was extracted with dichloromethane (three times, 25 ml each). The organic extracts were combined, dried over sodium sulfate, and the drying agent was removed. The dichloromethane extract was concentrated in vacuo and the resulting material was transferred to a 1-dram vial with a small amount of dichloromethane. The solution in the vial was dried with a stream of dry nitrogen.

Each sample was dissolved in 1 ml of dichloromethane and spotted onto 0.25-mm F-254 thin-layer chromatography (TLC) plates (E. Merck, Darmstadt, West Germany) along with T-2 toxin and roridins A and E standards. The plates were developed with 5% methanol in dichloromethane and visualized with short wavelength UV light, which yields dark spots for the macrocyclic trichothecenes (e.g., roridins A and E), followed by spraying the plates with a solution of sulfuric acid/vanillin/ethanol (20/1/3). When these plates are heated with a hot-air gun, the trichothecene spots turn a blue-gray.

TABLE 2. Percentage of colony-forming units (cfu) of 11 *Fusarium* species in the roots, rhizospheres, and soil of *Baccharis* species in the field

Fusarium	Roots[a] (%)	Rhizosphere[b] (%)	Soil[c] (%)
acuminatum Ell. & Ev. *sensu* Gordon	6	5	1
avenaceum (Fr.) Sacc.	0	0	<1
equiseti (Corda) Sacc. *sensu* Gordon	5	3	<1
merismoides Corda	0	<1	0
oxysporum Schlect. emend. Snyd. & Hans.	46	55	82
poae (Peck) Wollenw.	<1	<1	0
sambucinum Fuckel	1	<1	1
semitectum Berk. & Rav.	0	<1	0
solani (Mart.) Appel & Wollenw. emend. Snyd. & Hans.	3	1	17
sporotrichioides Sherb.	39	35	<1
tricinctum (Corda) Sacc.	0	1	0

[a] Total cfu from roots = 292.
[b] Total cfu from rhizosphere = 4,509.
[c] Total cfu from soil = 1,782.

TABLE 3. Toxicity to rats fed a complete ration mixed 1:1 with a rice culture of isolates of *Fusarium oxysporum* from roots and rhizospheres of *Baccharis* spp. or from soil near roots

Source of *Fusarium*	Isolate no. (Host)[a]	Avg. wt. feed eaten (g)	Avg. wt. change (g)[b]	Congestion and tissue hemorrhage[c]	Dead rats (no.)
Roots	59 m	3.33 ± 1.20	−14.33 ± 0.33	+B	3
	557 c	29.00 ± 2.08	2.33 ± 1.33	−	0
	745 c	6.33 + 3.33	−16.00 ± 0.00	+B	3
	Control	45.33 ± 2.33	27.00 ± 0.57	−	0
Rhizosphere	26 m	5.66 ± 5.81	−6.00 ± 1.73	−	0
	30 m	19.33 ± 1.20	3.00 ± 2.00	−	0
	42 m	27.00 ± 3.51	6.00 ± 1.00	−	0
	93 m	29.66 ± 1.76	4.33 ± 2.84	−	0
	108 m	21.00 ± 1.73	5.00 ± 2.08	−	0
	375 m	4.00 ± 4.72	−12.66 ± 1.33	+B	2
	503 m	17.00 ± 5.68	4.00 ± 1.53	−	0
	527 c	39.33 ± 0.33	16.66 ± 1.45	−	0
	552 c	8.66 ± 4.05	−5.66 ± 1.45	+B,IN	0
	598 c	1.00 ± 0.00	−14.33 ± 0.33	+B	3
	617 c	12.00 ± 2.88	−7.00 ± 4.04	−	0
	724 c	28.00 ± 2.08	5.33 ± 0.88	−	0
	962 c	29.66 ± 4.48	−9.66 ± 1.45	−	1
	983 c	12.00 ± 6.65	−6.66 ± 2.90	−	0
	984 c	13.33 ± 6.69	−11.66 ± 1.45	+B	3
	Control	37.87 ± 1.31	22.87 ± 1.63	−	0
Soil	1,412 c	25.00 ± 3.21	4.00 ± 2.30	−	0
	1,437 c	29.00 ± 3.60	6.66 ± 3.52	−	0
	1,458 c	30.66 ± 1.85	7.00 ± 2.33	−	0
	1,473 c	28.00 ± 3.60	5.00 ± 2.64	−	0
	1,581 my	7.00 ± 1.00	−11.00 ± 1.52	−	0
	1,712 c	32.66 ± 0.66	12.66 ± 1.20	−	0
	1,912 0	26.33 ± 1.85	4.00 ± 1.52	−	0
	1,917 0	29.00 ± 3.21	6.66 ± 1.76	−	0
	1,930 0	13.33 ± 1.20	−5.66 ± 1.85	−	0
	1,972 0	3.00 ± 1.15	−14.00 ± 0.00	+B	3
	2,008 0	32.66 ± 1.20	13.33 ± 5.04	−	0
	2,010 0	18.00 ± 3.05	3.66 ± 1.20	−	0
	2,156 c	12.00 ± 5.56	−7.00 ± 4.58	+B	1
	2,167 c	25.33 ± 1.33	10.66 ± 1.66	−	0
	2,232 c	13.33 ± 4.50	−6.00 ± 4.04	+IN	0
	2,307 c	9.33 ± 0.66	−14.00 ± 0.00	+B,IN	3
	2,358 0	23.33 ± 4.33	4.33 ± 1.85	−	0
	2,374 0	7.66 ± 1.96	−6.00 ± 4.04	−	1
	2,379 0	2.33 ± 1.33	−13.00 ± 0.57	+B	3
	2,711 c	15.66 ± 2.60	−5.00 ± 4.50	−	0
	2,714 c	10.66 ± 3.17	−15.66 ± 0.33	+B	3
	Control	43.33 ± 1.20	23.66 ± 2.40	−	0

[a] c = *B. coridifolia*, m = *B. megapotamica*, my = *B. myriocephala*, and 0 = no host (soil only); Control = rats fed 1:1 mixture ration and sterile rice.
[b] Average of three rats per isolate.
[c] + = Definite toxic effect; B = bladder hemorrhage; IN = intestinal hemorrhage; − = no toxic effect.

RESULTS

***Fusarium* in roots, rhizospheres, and soil.** *Fusarium* species were more abundant in the rhizosphere than in soil or roots, comprising 68% of the colony-forming units (cfu) of *Fusarium* species in the rhizosphere (Fig. 1). Of the *Fusarium* species isolated from roots, rhizospheres, and soil, *F. oxysporum* was the most abundant (Table 2). *F. sporotrichioides* was isolated frequently from roots and rhizospheres but not from soil. Nine other species of *Fusarium* were infrequently isolated from the three habitats. More colonies of *Fusarium* were isolated from rhizospheres of *B. coridifolia* (953 cfu per gram of soil) than from *B. megapotamica* (106 cfu/g), *B. unicinella* (63 cfu/g), *B. semiserrata* (24 cfu/g), or *B. myriocephala* (17 cfu/g). In soil in which *Baccharis* species were absent (Table 1), 190 cfu per gram of soil was isolated.

Toxicity of *Fusarium oxysporum*. Twenty-one of the isolates of *F. oxysporum* were obtained from *B. coridifolia*, eight from *B. megapotamica*, one from *B. myriocephala*, and seven from soil where *Baccharis* spp. were growing (Table 3). Substances that were lethal to rats were found in two of three isolates from roots, four of 15 isolates from rhizospheres, and six of 21 isolates from soil. Rice cultures of 20 of the 39 isolates of *F. oxysporum* incorporated into the ration caused rats to make less than normal weight gains during the 5-day test period and 12 isolates caused congestion and tissue hemorrhage in the bladder or the intestines. Thirty-eight of the 39 isolates tested caused decreased food consumption. Toxic substances were produced by *Fusarium* in soils from all three *Baccharis* species based on weight changes and death of rats, but there was no relation of *Baccharis* species and occurrence of toxins. Also, toxins were produced by isolates of *F. oxysporum* from nonrhizosphere soils (Table 3).

Toxicity of *F. sporotrichioides*. Thirty-four of the isolates of *F. sporotrichioides* were obtained from *B. coridifolia*, six from *B. megapotamica*, and only one isolate from soil (Table 4). Toxins lethal to rats were found in four of 15 isolates from roots, one of 26 isolates from rhizospheres, and none in the single isolate from soil. Five isolates in the ration each caused congestion and tissue hemorrhage in the bladder, intestines, and stomach. All of the lethal isolates came from roots of *B. coridifolia*. Rice cultures of *F. sporotrichoides* in the ration from roots, rhizospheres, and soil of *Baccharis* species decreased feed consumption and daily weight gains (Table 4). All of the 41 isolates of both *Fusarium* species that

TABLE 4. Toxicity to rats fed a complete ration mixed 1:1 with a rice substrate supporting growth of *Fusarium sporotrichioides* isolated from roots and rhizospheres of *Baccharis* spp. or from soil near roots

Source of *Fusarium*	Isolate no. (Host)[a]	Avg. wt. feed eaten (g)[b]	Avg. wt. change (g)	Congestion and tissue hemorrhage[c]	Dead rats (no.)
Roots	119 c	6.33 ± 1.20	−6.66 ± 1.20	−	1
	689 c	4.33 ± 3.33	−16.33 ± 1.45	−	3
	803 c	27.00 ± 2.30	12.00 ± 0.57	−	0
	804 c	15.66 ± 0.66	−1.00 ± 0.57	−	0
	913 c	16.33 ± 1.76	−8.33 ± 0.33	−	0
	942 c	23.00 ± 1.52	12.33 ± 1.20	−	0
	951 c	10.66 ± 2.18	−10.33 ± 0.88	−	0
	1,017 m	15.33 ± 2.60	−2.66 ± 0.33	−	0
	1,072 m	22.33 ± 1.66	3.66 ± 0.88	−	0
	1,076 m	16.00 ± 2.08	−2.66 ± 0.66	−	0
	1,097 m	28.66 ± 2.02	4.00 ± 2.08	−	0
	1,230 c	2.66 ± 0.66	−17.33 ± 0.88	+IN	2
	1,235 c	3.33 ± 0.33	−13.00 ± 0.57	−	2
	1,238 c	6.66 ± 3.28	−11.00 ± 1.00	+B	0
	1,240 c	10.33 ± 3.84	−15.33 ± 1.20	−	3
	Control	45.33 ± 2.33	27.00 ± 0.57	−	0
Rhizosphere	146 c	28.33 ± 1.76	16.66 ± 1.20	−	0
	218 c	26.66 ± 1.45	12.66 ± 2.60	−	0
	427 m	10.33 ± 5.54	−1.00 ± 0.57	−	0
	547 c	19.33 ± 1.20	−1.33 ± 0.33	−	0
	549 c	21.33 ± 1.45	6.66 ± 0.88	−	0
	599 c	8.00 ± 2.00	−1.66 ± 0.33	−	0
	600 c	15.00 ± 4.35	2.66 ± 1.66	−	0
	632 c	8.00 ± 5.00	−1.00 ± 0.57	−	0
	709 c	12.66 ± 3.52	−7.00 ± 4.16	−	0
	716 c	22.00 ± 2.08	13.33 ± 1.85	−	0
	759 c	22.66 ± 3.17	4.00 ± 1.52	−	0
	814 c	31.00 ± 2.64	18.66 ± 1.45	−	0
	830 c	20.00 ± 3.46	2.33 ± 0.33	−	0
	837 c	17.00 ± 0.57	7.33 ± 2.66	−	0
	872 c	22.66 ± 2.72	4.66 ± 1.45	−	0
	888 c	11.66 ± 0.88	−3.66 ± 1.45	−	0
	895 c	18.33 ± 4.33	5.00 ± 1.73	−	0
	923 c	13.33 ± 3.38	−2.33 ± 0.88	−	0
	934 c	10.00 ± 5.19	−10.66 ± 0.66	−	0
	938 c	16.66 ± 2.72	−7.00 ± 0.57	−	0
	954 c	18.00 ± 1.52	8.00 ± 1.15	+IN	0
	978 c	11.00 ± 0.57	3.33 ± 0.66	−	0
	1,008 m	24.00 ± 1.15	16.66 ± 1.33	−	0
	1,019 m	11.00 ± 2.64	−4.00 ± 1.73	−	0
	1,259 c	9.66 ± 2.33	−11.33 ± 0.33	+IN	0
	1,266 c	6.00 ± 7.00	−13.66 ± 1.20	+S	1
	Control	37.87 ± 1.31	22.87 ± 1.63	−	0
Soil	1,515 0	33.33 ± 2.02	16.33 ± 0.33	−	0
	Control	43.33 ± 1.20	23.66 ± 2.40	−	0

[a] c = *B. coridifolia*, m = *B. megapotamica*, 0 = no host (soil only), Control = rats fed 1:1 mixture of ration and sterile rice.
[b] Average of three rats per isolate.
[c] + = Definite toxic effect; B = bladder hemorrhage; IN = intestinal hemorrhage; S = stomach hemorrhage; − = no toxic effect.

caused weight loss were associated with the small thymus and small spleen (Tables 3 and 4). TLC analysis of the methanol extract of the rice cultures of *F. sporotrichioides* were all negative for the macrocyclic trichothecenes. However, three isolates (689, 1,230, and 1,235) did produce T-2 toxin (500–1,200 ppm), and these same isolates also proved toxic to rats (Table 4). Chemical analysis of plant tissue of the samples of *B. coridifolia* and *B. megapotamica* collected in this study were negative for the presence of simple trichothecenes (e.g., T-2 toxin), whereas most of these samples contained several hundred parts per million of the macrocyclic trichothecenes (B. B. Jarvis et al, *unpublished*).

DISCUSSION

That *Fusarium* species, especially *F. sporotrichioides*, were more abundant and more frequently isolated from the rhizosphere and roots of *Baccharis* species than from nearby soil indicates that these fungi occupied a habitat in which mycotoxins could have been produced and be taken up by plants. Furthermore all 41 cultures of *F. sporotrichioides* and all 39 cultures of *F. oxysporum* that were isolated from roots and rhizospheres of *Baccharis* species proved toxic but not necessarily lethal to rats in our 5-day test. Twelve of the isolates of *F. oxysporum* and six of the isolates from *F. sporotrichioides* were lethal. *F. oxysporum*, however, was more abundant than *F. sporotrichioides* in soil—a common observation generally. Isolates of *F. sporotrichioides* from roots and rhizospheres of *B. coridifolia* were lethal to rats, whereas isolates from *B. megapotamica* were not. On the other hand, there were isolates of *F. oxysporum* from roots, rhizospheres of both species of *Baccharis*, and from soil that were lethal to rats. Where rats survived they failed to make normal weight gains during the trial period when fed rations of infected rice. Thus, it was apparent that one or more mycotoxins were produced by *Fusarium* species that were toxic or lethal to rats, but it was not established that such toxins were produced by these fungi in the roots or rhizospheres of plants of *Baccharis* species.

F. sporotrichioides (13) and *F. oxysporum* (6) have been reported among the list of *Fusarium* species that produce mycotoxins (13) and the cultures of both species that we isolated were also toxigenic, based on rat-feeding trials with *Fusarium*-infected rice, but not by chemical analysis of the products of metabolic activity in roots, rhizosphere, or soil. Not every isolate of each species of *Fusarium* growing in rice cultures was toxic to rats, which could be expected in that isolates of species in the rhizosphere comprise a population of genotypes that undoubtedly differ in a variety of characteristics, including the production of different kinds and amounts of mycotoxins.

Simple trichothecenes (T-2) as well as some unidentified toxins were found in infected rice cultures; however, macrocyclic trichothecenes were not produced by these *Fusarium* species, indicating that the macrocyclic trichothecenes such as baccharinoids and roridins, extracted from plants of *B. coridifolia* (11), were not produced by our isolates of *Fusarium* species. We postulate that *Myrothecium* species could have produced the macrocyclic trichothecenes previously identified in *B. coridifolia*. Some colonies of *Myrothecium* appeared on the isolation medium selective for *Fusarium* species but their role was not determined at this time.

Several questions are still unresolved: whether livestock poisoning is attributable to simple or macrocyclic trichothecenes, or both; whether species of *Fusarium* or other fungi produce such toxins at the root surface; and that these toxins produced on roots can be taken up and translocated in the roots to the shoots of *Baccharis* plants. Jarvis et al (9) had demonstrated previously that trichothecenes applied to roots were readily taken up and translocated to shoots.

Roots are not likely to be colonized uniformly by fungi; moreover, the proportion of *Fusarium* to other genera of microorganisms may vary with the specific population of other organisms and with the microenvironment. This can have an unpredictable influence on mycotoxin production in or on roots and account for variation in results obtained.

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