Macrocyclic Trichothecene Mycotoxins in Brazilian Species of *Baccharis*

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ABSTRACT

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The occurrence and distribution of macrocyclic trichothecenes in *Baccharis* spp. plants and the soil in which they were growing were investigated. The six major macrocyclic trichothecenes found were roridins A, D, and E in *B. coridifolia* and baccharinoids B3, B4, and B5 in *B. megapotamica*. Not all plants or plant parts contained trichothecenes, however, and amounts varied from 0 to 480 ppm. Some soils in which *Baccharis* spp. plants were growing also contained trichothecenes. *Myrothecium* spp. known to produce macrocyclic trichothecenes in culture constituted less than 1% of the fungal colonies isolated from soil and rhizospheres. In liquid culture and on rice, only one isolate of *Myrothecium* spp. produced macrocylic trichothecenes. *Baccharis* spp. are probably acquiring fungally produced macrocylic trichothecenes (roridins) and in some cases metabolizing these toxins to baccharinoids. The fungal species responsible is not as yet known, however.

In the screening of plant extracts for antitumor activity, Kupchan et al (15) found that an ethanol extract of the Brazilian shrub *Baccharis megapotamica* Spreng (Asteraceae) showed very high in vivo antileukemic activity. Potent cytotoxic and in vivo active antineoplastic agents called baccharinoids (12,15) that belong to the trichothecene class of terpenoid antibiotics were identified. Although trichothecenes show antitumor properties (4), they are best known as mycotoxins, fungal metabolites that pose a serious hazard to both animal and human health (26). Baccharinoids were present in all of three separate collections of *B. megapotamica* (12), mainly in aerial portions in concentrations of about 0.02% dry weight. The plant material showed no obvious pathogenic or saprophytic fungal activity.

Trichothecenes have been isolated from a variety of common soil fungi, including *Fusarium, Trichothecium, Trichoderma, Myrothecium, Cylindrocarpon*, and *Stachybotrys* (25). Until the finding of their presence in *B. megapotamica*, the trichothecenes had never been found in healthy plants—only in diseased plants (5,19). Trichothecenes (3), including baccharinoids (2), are potent phytotoxins. Since the report of the finding of the baccharinoids in *B. megapotamica*, German workers have reported finding roridins in *B. coridifolia* DC. (6). Roridins differ from baccharinoids in that roridins lack an 8β-hydroxy group in the A-ring (11) (Fig. 1). *B. coridifolia* poses a serious hazard in Brazil because ingestion of the plant by cattle results in illness and death (6), and it has been suggested that the trichothecene mycotoxins are responsible for the toxicity of *B. coridifolia* (6).

The trichothecenes may be divided into two classes: simple (e.g., T-2 toxin and verrucarol) and macrocyclic (e.g., baccharinoids B3, B4, and B5; roridins A, D and E; and verrucarin A) (Fig. 1). It is the macrocyclic trichothecenes that are found in *B. coridifolia* and *B. megapotamica*, but their origin is in doubt. An earlier study (10) showed that *B. megapotamica* grown in Maryland does not yield macrocyclic trichothecenes. Roridin A supplied to these plants was metabolized to baccharinoid B7, a naturally occurring toxin in *B. megapotamica* in its natural habitat (10). These results strongly suggest that the Brazilian *Baccharis* spp. acquire fungal-produced macrocyclic trichothecenes (roridins) and that *B. megapotamica* metabolizes them to the baccharinoids whereas *B. coridifolia* stores them unaltered. This situation is remarkable, as the macrocyclic trichothecenes are very phytotoxic (2), yet *B. megapotamica* and *B. coridifolia*, which contain relatively high concentrations of these toxins, show no apparent phytotoxic symptoms.

In April 1984, an ecological survey was conducted of these *Baccharis* spp. in two districts in Brazil. Herein, we report the results of both chemical and microbiological analyses of plant and soil samples obtained in this survey.

MATERIALS AND METHODS

Sources of plant and soil samples. The two areas in Brazil where samples were collected were Curitiba and Santa Maria, which is about 320 km south of the city of Curitiba and near the border with Uruguay. *B. megapotamica* grows in a large stand (site 1) about 30 km southeast of Curitiba. Three collections of this plant from the same location had been made previously, including a large-scale collection of about 7,725 kg (dry weight) of plant material in 1978 (12). Inspection of site 1 revealed other species of *Baccharis*, including *B. unicinella* DC., *B. semiserrata* Steudel, *B. myriocephala* DC., and *B. camporum* DC. In addition, *B. megapotamica* was in flower (March), and separate collections of

both male and female plants were made.

Along a road running west of Curitiba, three separate collections of *B. coridifolia* (sites 2, 3, and 4) were made. Site 2 was about 35 km west of Curitiba, site 3 was another 2 km west, and site 4 was about 20 km west of site 3. Site 5 was a pasture near Santa Maria and contained numerous plants of *B. coridifolia* that caused cattle intoxications (6). In addition to plant material, soils from around the plant roots (rhizosphere) and soils from nearby areas where no *Baccharis* spp. plants were growing were collected.

These samples were obtained by inserting an Oakfield soil sampler at about a 45-degree angle under the plant so as to collect roots and adjoining soil. The samples were put into plastic bags and taken to the laboratory (in Minnesota), where roots were separated from soil by means of a No. 5 sieve with a 4-mm mesh; the separated soil and roots were stored in plastic bags at 5 C until assayed for fungi within a few days. Roots from each host and location were cut aseptically into fragments 2 cm long and introduced into 125-ml Erlenmeyer flasks each containing 25 ml of autoclaved 0.12% water agar. The flasks were placed on a rotary shaker for 10 min to separate rhizosphere fungi from the roots. One milliliter of this rhizosphere suspension was dispensed by pipette onto either of two agar media: 1) pentachloronitrobenzene agar (PCNB) (14,20) supplemented with aureomycin or 2) peptone rose-bengal agar (RB). The dispensed suspension was distributed evenly over the agar surface by means of a bent sterilized glass rod. The Warcup soil-plate method (27) was used to isolate fungi from soil, in which a soil sample weighing approximately 0.5 g was allowed to air-dry, then crushed into a fine powder. *Myrothecium* was isolated more frequently on PCNB than on RB.

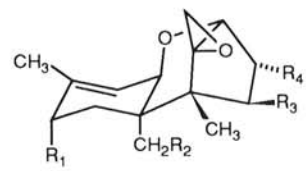
All work with media, soils, and fungi was done in a laminar-flow biological safety cabinet. Petri dishes of cultures were incubated at approximately 24 C under fluorescent and black (ultraviolet) lamps at 5,300 lx for 12 hr/day to foster fungus sporulation. For each field sample, 12 rhizosphere dishes and 12 soil dishes (each with six PCNB and six RB) were prepared.

Analysis of plant samples and soil. Dried plant material (5 g) was crushed and covered with 50 ml of 90% methanol/water. After 12 hr, the methanol extract was drained and another 50 ml of 90% aqueous methanol was added. After 12 hr, the extract was drained and the two fractions were combined and washed with two 50-ml portions of hexane. The methanol extract was treated with 75 ml of 1.5% ferric gel solution at pH 4–4.5 obtained by diluting 10 ml of a 15% solution of ferric chloride hexahydrate to 100 ml with distilled water and adjusting the pH with 1 N sodium hydroxide. The resulting mixture was filtered. The precipitate was washed with about 25 ml of 50% aqueous methanol. The filtrate was evaporated to 50 ml and extracted with two 25-ml portions of dichloromethane. The dichloromethane fractions were combined, dried over sodium sulfate, concentrated, and passed through a silica gel column (5 × 0.3 cm, 230–400 mesh). The column was washed with 3 ml of dichloromethane (discarded), and the trichothecenes were eluted with 10% methanol in dichloromethane. The solvent was removed under a stream of dry nitrogen gas, and the resulting gummy material was dissolved in 1.0 ml of dichloromethane. Quantitative analysis was performed by injecting 10 μl of these solutions into a chromatograph. Five-gram samples of air-dried soil samples were treated in the same manner except that ferric gel was not used.

Isolation of roridins A and E from plant material. An additional 20 g of dried plant material (plant 13) was extracted in the manner described above and combined with the previous extract of the 5-g sample from plant 13 before analysis by high-performance liquid chromatography (HPLC). The combined extract was subjected to flash chromatography (23) over 10 g of silica gel packed in dichloromethane with 5% 2-propanol in dichloromethane as eluate. The fraction shown by thin-layer chromatography (TLC) to contain roridins A and E was subjected to preparative TLC (1-mm plate, 2% methanol in dichloromethane) on the centrifugally accelerated Chromatotron (Harrison Research, Palo Alto, CA). The fraction containing roridin E was recrystallized from acetone/hexane to give 10 mg of roridin E. The fraction containing roridin A was recrystallized from acetone/ether to give 4 mg of roridin A. The identities of these compounds were confirmed by comparing the chromatographic behavior (TLC and HPLC) and proton nuclear magnetic resonance (^{1}H-NMR) spectra of authentic samples of roridins A and E.

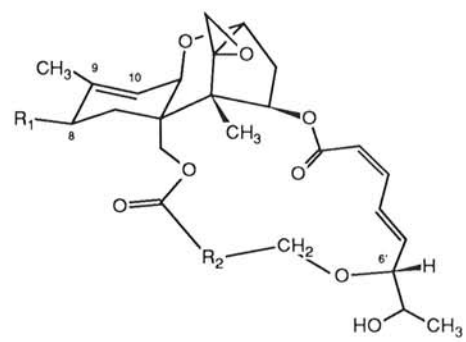
Trichothecene production in liquid culture. Twenty-two isolates of *Myrothecium* spp. (21 of *M. verrucaria* (Alb. & Schw.) Ditm. ex Fr. and one of *M. roridum* Tode ex Fr.) and 14 isolates of *Cylindrocarpon destructans* (Zins.) Scholten were grown on lima bean agar (Difco Laboratories, Detroit, MI) for 7 days at 22–26 C. These species were tested because they produce macrocyclic trichothecenes (9), although there is only one report of such toxin production by a *Cylindrocarpon* sp. (18).

Spores of *Myrothecium* or *Cylindrocarpon* were introduced into two different liquid media and onto sterilized rice substrate (17). A two-stage fermentation procedure was used for the liquid cultures. The inoculum was first grown out in a seed medium (corn steep liquor/glucose), then transferred either to a potato broth medium or to a sucrose/glycerol medium (11), which is particularly effective for producing macrocyclic trichothecenes (11). The ability to produce trichothecenes was evaluated in a small-scale fermentation that involved a two-stage process. Blocks of lima bean agar bearing spores and mycelia were introduced into a 125-ml Erlenmeyer flask containing 25 ml of medium (15.6 g of glucose, 10 ml/L of corn steep liquor) that had been sterilized at



T-2 Toxin: $R_1 = (CH_3)_2CH_2COO$; $R_2 = R_3 = OAc$; $R_4 = OH$

Verrucarol: $R_1 = R_4 = H$; $R_2 = R_3 = OH$



Roridin A: $R_1 = H$; $R_2 = CHOHCH_3CH_2$

Roridin D: $R_1 = H$; $R_2 = CH(O)CCH_3CH_2$

Roridin E: $R_1 = H$; $R_2 = CH{=}CCH_3CH_2$

Baccharinoid B3: $R_1 = OH$; $R_2 = CHOHCHCH_3CH_2$

Baccharinoid B4: $R_1 = OH$; $R_2 = CH(O)CCH_3CHOH$

Baccharinoid B5: 9,10-epoxide; $R_1 = H$; $R_2 = CH(O)CCH_3CHOH$

Verrucarin A: C6' = C=O; $R_1 = H$; $R_2 = CHOHCHCH_3CH_2$

Fig. 1. Structures of trichothecene mycotoxins.

121 C for 15 min. After 2 days with shaking (100 rpm), 5 ml of medium was transferred to 50 ml (in a 250-ml Erlenmeyer flask) of two different production media (300 g of potato extract [Difco] per liter and 1 g of $NH_4H_2PO_4$, 3 g of K_2HPO_4, 5 g of NaCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 40 g of sucrose, and 8 ml of glycerol per liter) that had been sterilized at 121 C for 15 min. The production media were incubated at 25 C for 7 days at an agitation speed of 150 rpm.

Trichothecene production on rice. Each isolate of *Myrothecium* or *Cylindrocarpon* was grown in 1-L Erlenmeyer flasks on 200 g of autoclaved converted parboiled rice. Deionized water (120 ml) was added to the rice in each flask, and the flasks were autoclaved at 121 C for 1 hr on two consecutive days. The rice was inoculated with blocks from lima bean agar, and the flasks were shaken by hand daily for 1 wk. Rice cultures were incubated at 22–26 C for 4 wk.

Extraction of cultures and analysis of trichothecenes. Liquid cultures were filtered through Whatman No. 1 filter paper, and the filtrates were extracted three times with 25-ml portions of ethyl acetate. The mycelia remaining from filtration were soaked in methanol overnight and filtered. The methanol filtrate was concentrated until only an aqueous phase remained, which was extracted with 25 ml of ethyl acetate three times. The ethyl acetate extracts were pooled, concentrated, and analyzed for trichothecenes by TLC.

Rice cultures were extracted three times with 500 ml of methanol by agitating the flasks in a sonicator. The methanol extracts were combined and concentrated to give an aqueous phase that was defatted three times with hexane, then extracted three times with 300 ml of ethyl acetate. The ethyl acetate extracts were combined, concentrated to dryness, dissolved in a small amount of dichloromethane, and analyzed for trichothecenes by TLC.

Isolation of trichothecenes from *Myrothecium* and *Cylindrocarpon*. A culture of *M. verrucaria* isolate MV 2575 grown on rice substrate (200 g) for 4 wk was extracted with methanol (vide supra). The methanol extract was concentrated, extracted with dichloromethane, dried with sodium sulfate, and concentrated to give 0.6 g of a dark brown gum. This material was subjected to flash chromatography on 80 g of silica gel. Elution with dichloromethane containing increasing amounts of methanol (0–5%) gave a series of fractions, one of which (eluted with 2% methanol) upon crystallization from dichloromethane/hexane gave 3 mg of verrucarin A. The identity of this verrucarin A was verified by comparing its chromatographic (TLC and HPLC) and spectrometric (^{1}H-NMR) properties with those of an authentic sample of verrucarin A (13). TLC analysis of the ethyl acetate extract of rice cultures of the *Cylindrocarpon* isolates was also done. The ethyl acetate extract was further purified by preparative TLC on the Chromatotron (2% methanol in dichloromethane), followed by ^{1}H-NMR analysis of the resulting crystalline compounds, which, when the TLC plates were sprayed with 4-(*p*-nitrobenzyl)pyridine reagent (24), gave colors suggestive of trichothecenes.

General physical and chemical methods. The ^{1}H-NMR spectra were determined in deuteriochloroform ($CDCl_3$) on an IBM SY-200 MHz spectrometer, with tetramethylsilane as an internal standard. Flash chromatography (23) was carried out on silica gel 60 (230–400 mesh), and TLC was done on silica gel plates (0.25 mm thick). The Chromatotron was used for preparative TLC. Visualization of trichothecenes was effected with 4-(*p*-nitrobenzyl)pyridine and tetraethylenepentamine spray (24), preceded by irradiation with a short-wavelength UV light.

Normal-phase HPLC was carried out on a Gilson model 302 gradient HPLC equipped with a LDC UV-III monitor (254-nm wavelength) and Shimadzu Chromatopac C-R3A integrator. The solvent in pump A was 70% hexane in dichloromethane, and the solvent in pump B was 50% dichloromethane in 2-propanol. The column employed was a 5-μ Spherisorb amino column (250 × 4.6 mm) with a flow rate of 1 ml/min for the following gradient (time, % B): 0, 5; 10, 30; 14, 55. Under these conditions, the following retention times were observed: roridin E, 6.4 min; roridin D, 6.9 min; roridin A, 9.7 min; B5, 13.5 min; B3, 17.1 min; and B4, 17.8 min.

Reversed-phase chromatography was carried out on a 5-μ C8 Supelco column (250 × 4.6 mm) at a flow rate of 1.2 ml/min on an Altex model 332 gradient HPLC. The solvent system was methanol/water using the following gradient (time, % methanol): 0, 50; 10, 50; 14, 80. Under these conditions, the following retention times were observed: B5, 6.0 min; B4, 6.4 min; B3, 8.0 min; roridin A, 10.0 min; roridin D, 11.5 min; and roridin E, 13 min. This chromatographic system was equipped with a LKB model 2140 Rapid Spectral Diode Array Detector interfaced with an IBM XT-PC microcomputer that recorded and plotted the UV spectra (200–300 nm) of all peaks observed on the chromatograms. Only those chromatographic peaks that gave UV spectra consistent with the assigned macrocyclic trichothecene were accepted for quantitative analysis on normal-phase HPLC.

Standard concentration curves were generated by injecting known amounts of authentic samples of roridins A, D, and E (13) and baccharinoids B3, B4, and B5 (12) into the chromatograph (normal-phase HPLC) and measuring the areas under the peaks with the Shimadzu C-R3A integrator.

RESULTS

Analysis of macrocyclic trichothecenes from collected samples of *Baccharis*. Table 1 gives the plant sample number, collection description, and chemical analyses of the plant material. The six major macrocyclic trichothecenes found were roridins A, D, and E (in *B. coridifolia*) and baccharinoids B3, B4, and B5 (in *B. megapotamica*). Under the HPLC conditions used, baccharinoid B3 and its diastereomer, baccharinoid B7 (12), were inseparable, and so the values given for baccharinoid B3 include any contribution made by baccharinoid B7. Other trichothecene congeners, such as the miotoxins, which are closely related in structure to the roridins (7), may have been present in *B. coridifolia*, but only in small quantities. Some *B. megapotamica* extracts contained other baccharinoids (12), but in concentrations considerably below those of baccharinoids B3 (B7), B4, and B5. Identities of the peaks in the HPLC chromatograms were

TABLE 1. Concentrations of macrocyclic trichothecenes[a] found in *Baccharis* spp. in Brazil

Plant sample no.	Location[b]	Specie/sample	Concentration[c] of: RA	RD	RE	B3	B4	B5
1	Site 3	*B. coridifolia*/entire plant[d]	60	25	90	0	0	0
2	Site 1	*B. unicinella*/entire plant	0	0	0	0	0	0
3	Site 5	*B. coridifolia*/entire plant	25	55	0	0	0	0
4	Site 1	*B. megapotamica*/seed heads	0	0	0	55	65	55
5	Site 1	*B. semiserrata*/entire plant	0	0	0	0	0	0
6	Site 5	*B. coridifolia*/seed heads	25	30	25	0	0	0
7	Site 5	*B. coridifolia*/lower portion of sample 6	0	0	0	0	0	0
8	Site 1	*B. megapotamica*/staminate heads	0	0	0	0	0	0
9	Site 2	*B. coridifolia*/leaves	0	0	0	0	0	0
10	Site 1	*B. megapotamica*/pistillate heads	0	0	0	30	60	55
11	Site 4	*B. coridifolia*/leaves	0	0	0	0	0	0
12	Site 1	*B. myriocephala*/entire plant	0	0	0	0	0	0
13	Site 2	*B. coridifolia*/flower	300	55	480	0	0	60
14	Site 4	*B. coridifolia*/seed heads	0	0	0	0	0	0
15	Site 2	*B. coridifolia*/seed heads	130	0	90	0	0	20
16	Site 1	*B. camporum*/entire plant	0	0	0	0	0	0

[a] RA = roridin A; RD = roridin D; RE = roridin E; B3 = baccharinoid B3; B4 = baccharinoid B4; B5 = baccharinoid B5.

[b] Site 1 = along dirt road about 30 km southeast of Curitiba; site 2 = along highway 277 about 35 km west of Curitiba; site 3 = along highway 277 about 37 km west of Curitiba; site 4 = along highway 277 about 57 km west of Curitiba; site 5 = pasture near Santa Maria about 320 km south of Curitiba.

[c] Parts per million of dried plant material ± 10%.

[d] Leaves, stem, flower, and seeds.

confirmed by conducting the HPLC analysis under two conditions. Quantitative assessments of peaks as well as of retention times were made on an amino column under normal-phase conditions and retention times were confirmed by reversed-phase analysis on a C8 column. In addition, all peaks were analyzed on a diode array detector for homogeneity, and the UV spectra of the peaks obtained in this manner matched the UV spectra of the relevant macrocyclic trichothecene. Furthermore, 25 g of plant sample 13, which was particularly rich in roridins A and E, was extracted and fractionated to give 4 mg of roridin A and 10 mg of roridin E, whose structures were confirmed by comparing the physical and spectral properties with those of authentic samples (13).

Analyses of the plant material (Table 1) showed a number of interesting results. At site 1, only *B. megapotamica* contained the toxins; the other *Baccharis* spp. (plant samples 2, 5, 12, and 16) growing among plants of *B. megapotamica* contained no detectable amounts. Furthermore, the pistillate heads of *B. megapotamica* (plant sample 10) contained high concentrations of baccharinoids B3, B4, and B5, whereas the staminate heads (plant sample 8) contained none. Also, the concentration of these toxins differed markedly according to the plant part analyzed. At site 2, with *B. coridifolia* plants, roridins were not detected in the leaves of plant sample 9 but were found in amounts of several hundred parts per million in the flower of plant sample 13 and the seed heads of plant sample 15. The same was true of *B. coridifolia* plants collected at site 5, several hundred kilometers to the south near Santa Maria; roridins were detected in appreciable amounts in the seed heads of plant sample 6 and all of plant sample 3 but were not found in the lower leaves of plant sample 7 (the lower portion of plant sample 6). Plant sample 6 contained roridins A, D, and E, but plant sample 3 contained only roridins A and D.

The three collection sites for *B. coridifolia* near Curitiba (sites 2, 3, and 4) were along highway 277 west of the city. Plant samples from sites 2 and 3 were gathered from a lowland pasture and contained large concentrations of roridins in the upper plant. Plants, including seed heads, collected from site 4 (plant samples 11 and 14), however, were devoid of these toxins. Relative to sites 2 and 3, site 4 was elevated, and few *B. coridifolia* plants were found growing there. The presence of baccharinoid B5 in plant samples 13 and 15 is questionable. Large-scale collections of *B. coridifolia* have not shown this compound (6,7), and the peak in the chromatogram assigned to B5 may be an unidentified macrocyclic trichothecene such as a miotoxin (7).

Analysis of soil samples for macrocyclic trichothecenes. A large number of soil samples were collected from sites 1 through 5 and analyzed for microflora (vide infra). After examination for soil fungi, the samples were extracted and analyzed for macrocyclic trichothecenes. Because these samples contained very few interfering contaminates, our analytical method could easily detect and quantify these toxins at the concentration of 50 ppb and above. Soils located near roridin-containing plants of *B. coridifolia* (sites 2, 3, and 5) had 100–2,000 ppb of roridins. The soil near a *B. coridifolia* plant at site 4 contained none of the macrocyclic trichothecenes. The same was true of soils collected near plants of *B. unicinella* and *B. semiserrata*. However, of three soil samples obtained near the *B. myriocephala* plant, two contained 300–500 ppb of roridin A and one contained 450 ppb of roridin E; no baccharinoids were detected. Soil samples from around *B. megapotamica* plants contained 150–2,500 ppb of roridins A and E and 50–1,500 ppb of baccharinoids B4 and B5. These amounts are lower limits for the concentrations of these toxins in soils. We found that only about one-half of the amount of these toxins could be recovered from soils spiked with 1 ppm of roridin A, indicating that trichothecenes may be irreversibly bound to the soil. Because the soil samples were not chemically analyzed for nearly a year after being collected, appreciable losses of these toxins may have occurred.

Isolation of fungi from soil and rhizosphere of *Baccharis* plants. *M. roridum* was isolated from two locations in southern Brazil: at Curitiba (950 m elevation) from the rhizosphere of *B. megapotamica* plants and at Pulgas de Sima (900 m elevation) from the rhizosphere of *B. coridifolia* plants. *M. verrucaria* was isolated near Curitiba (1,000 m elevation) from both the soil and the rhizosphere of soil around roots of *B. coridifolia* plants. Although present in the rhizosphere and soil, *Myrothecium* spp. did not constitute more than 1% of the fungal colonies isolated by means of the two agar media. Faster growing fungi such as *Fusarium* spp. (14) may have masked the presence of *Myrothecium*. Subsequent assays indicated the presence of *Myrothecium* in the same soil and rhizosphere samples, but their numbers were low relative to those of *Fusarium* spp.

Analysis of fungal cultures for macrocyclic trichothecenes. The *Cylindrocarpon* isolates did not grow well in liquid culture and did not appear to produce appreciable quantities of secondary metabolites. Analyses of the extracts of these cultures were negative for the presence of trichothecenes. On rice, two of the 14 isolates of *Cylindrocarpon* showed evidence of potential toxin production in that TLC analyses of their extracts showed three spots that gave a positive reaction to the 4-(*p*-nitrobenzyl)pyridine spray reagent (24), and these spots could be visualized on the plates with UV light. Several milligrams of each of these compounds were isolated, and the compounds showed cytotoxicity against L-1210 leukemic cells (ED_{50} values of 1.1, 0.49, and 0.0078 μg/ml). However, ^{1}H-NMR spectral analysis made it clear that none of these compounds was a trichothecene.

Initially, none of the liquid or rice cultures of the Brazilian isolates of *Myrothecium* appeared to produce trichothecenes in the laboratory. We have examined many isolates of *M. verrucaria* and *M. roridum* from around the world, and until now we had never found any of these fungi that did not produce trichothecenes. In some trials, production was low, especially if the isolate did not sporulate well (11), and we occasionally observed production only in rice cultures. Several of the Brazilian isolates of *Myrothecium* sporulated well, but we observed production of a macrocyclic trichothecene (verrucarin A) by only one of these isolates, MV 2757, from the rhizosphere of a *B. coridifolia* plant from site 2.

DISCUSSION

The results of this study support the earlier suggestions (10) that *Baccharis* spp. acquire fungal-produced macrocyclic trichothecenes (roridins) and that *B. megapotamica* plants metabolize the roridins to baccharinoids, whereas *B. coridifolia* plants store the toxins unaltered. The fact that *B. coridifolia* plants collected from site 4 contained no roridins while those collected from nearby sites 2 and 3 did contain appreciable quantities of these toxins argues against these compounds being of purely plant origin. There is a possibility, however, that this result reflects a variation within the plant species to produce these compounds.

Although this study strongly supports the thesis that certain *Baccharis* spp. are acquiring the mycotoxins from fungi, it is unclear as to which species of fungi are involved and by what mechanism these compounds are transferred to the plants. The fact that only one of the Brazilian isolates of *Myrothecium* produced trichothecenes in the laboratory, and then only in low yield, is surprising. The method used (TLC) to analyze the liquid and rice cultures of the *Myrothecium* spp. can readily identify isolates that are good producers of macrocyclic trichothecenes (11). Although TLC analyses of the extracts of the Brazilian cultures of *Myrothecium* gave no clear indication of the presence of macrocylic trichothecenes, several isolates (MV 2736, MV 2753, MV 2757, MV 2759, MV 2763, and MR 210) were grown on rice and fed to rats. Only isolate MV 2757 was toxic to the animals (C. J. Mirocha, *personal communication*). Purification of the extract of MV 2757 grown on rice gave verrucarin A, but in low yield. These results make it clear that the *Myrothecium* spp. isolated from the soils and rhizospheres associated with these Brazilian *Baccharis* spp. are at best weak producers of macrocylic trichothecenes, at least in the laboratory. Trichothecene production by *Myrothecium* when the fungus is in contact with plants is a possibility. In their native habitat, *B. megapotamica* and *B. coridifolia* may release chemicals into the soil that trigger toxin production by *Myrothecium*.

There are data that strongly implicate the macrocyclic trichothecenes as the causative agents of an induced toxicosis by *B. coridifolia* in cattle (6). Furthermore, these studies make it clear that the toxicity of *B. coridifolia* depends on the growth stage of the plant, i.e., that plants at the end of the growth season and in flower are the most toxic. This suggests that *Baccharis* may trigger the fungal production and acquisition of roridins only late in its growing season in Brazil (March and April). The behavior of these trichothecene-containing *Baccharis* spp. is similiar to that of plants infected with endophytes (22). Myrothecium has never been reported to be endophytic, although some species are parasitic, even pathogenic (16).

That the toxins become concentrated in the flowers and seed heads of *Baccharis* is interesting and perhaps serves some ecological purpose. Related to this is the finding that the ansa macrolide maytansine, which also appears to be a fungal-produced antibiotic, is accumulated in the seeds of a West Indian tree, *Maytenus rothiana* (21).

The presence of the trichothecene mycotoxins in the soil samples taken from the rhizospheres of plants is important. Clearly, these toxins have an appreciable lifetime in the soil, and their presence could influence the soil ecology. The pathway for introduction of trichothecenes into the soil is unknown. They may arise by microbial activity in the soil or by exudation through the roots of the plants. Another possibility is decomposing plant residue. In any event, because the baccharinoids were found in soils associated with *B. megapotamica* plants, it appears that some of the toxins in the soil may have come from the plant itself.

The plant material extracts were analyzed by gas liquid chromatography/mass spectrometry for the nonmacrocyclic trichothecenes: T-2 toxin, T-2 tetrol, HT-2, diacetoxyscripenol, nivalenol, and deoxynivalenol (C. J. Mirocha, *personal communication*). Only the *B. semiserrata* plant was positive, and only 1 ppm of T-2 toxin was detected. This would indicate that *B. megapotamica* and *B. coridifolia* do not establish a relationship with fungal producers of the simple trichothecenes (e.g., *Fusarium*).

The trichothecene mycotoxins are the most potent small-molecule protein-synthesis inhibitors known in eukaryotic systems (1) and are also among the most potent of the plant-growth inhibitors (2). Clearly, there is something special about *Baccharis* spp. in that these toxins have no measurable deleterious effect when absorbed by these plants. This latter statement also holds for the simple trichothecenes because when *B. megapotamica* was fed T-2 toxin in a manner similar to that described for roridin A (10), no adverse effect was detected. This immunity to the trichothecenes is not general for the *Baccharis* genus, since a species in Maryland, *B. halimifolia*, is sensitive to the phytotoxic trichothecenes at levels nonphytotoxic to *B. megapotamica* (B. B. Jarvis and G. A. Bean, *unpublished*). The origin of the insensitivity of *B. megapotamica* and *B. coridifolia* to the trichothecenes is obscure. The protein-synthesis inhibitory effect of the trichothecenes is brought about by the binding of the compounds to the 60S subunit of the ribosomes. *M. verrucaria* is unaffected by T-2 toxin because this mycotoxin is unable to bind to the ribosomes of *M. verrucaria* (8). *B. coridifolia* and *B. megapotamica* may also possess this unusual property. An alternate explanation could be that these plants sequester the toxins in structures such as the vacuoles or some other subcellular unit. However, the fact that *B. megapotamica* metabolizes the roridins to baccharinoids strongly suggests that these compounds do enter the cells of this plant, but why they do not elicit a toxic response is unknown.

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