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T-2 Toxin and Diacetoxyscirpenol Metabolism by *Baccharis* spp.†

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Hybrids resulting from crosses between *Baccharis sarothroides* and *B. pilularis* (FS1), *B. sarothroides* (FS2) and *B. megapotamica* (FS3) were tested for their tolerance to trichothecenes as well as their ability to metabolize the toxins. *B. sarothroides* (desert broom) was placed in an aqueous solution containing 500 ppm of T-2 toxin and showed visible signs of toxicity on the twigs at 21 h after exposure but not at 6 h, indicating some resistance. Samples of the twigs harvested 6 and 21 h after treatment contained, respectively, T-2 (0.03 and 2.2 μg/g), HT-2 (0.09 and 7.6 μg/g), and T-2-tetraol (2.1 and 2.6 μg/g). The hybrid FS1 showed no signs of toxicity 6 h after treatment, and its twigs contained T-2 (0.8 μg/g), HT-2 (10.2 μg/g), and T-2-tetraol (10.8 μg/g). The leaves at 6 h contained 0.5 μg of T-2, 1.7 μg of HT-2, 0.01 μg of 3′-hydroxy-HT-2, and 41 μg of T-2-tetraol per g. At 21 h, toxic signs were apparent and the twigs contained T-2 (39 μg/g), HT-2 (62 μg/g), 3′-hydroxy-HT-2 (0.8 μg/g), and T-2-tetraol (22 μg/g). The leaves at 21 h contained 3.6 μg of T-2, 9.4 μg of HT-2, 0.08 μg of 3′-hydroxy-HT-2, and 10.3 μg of T-2-tetraol per g. Cuttings of *B. megapotamica* were placed in a solution of 1,000 ppm T-2 toxin for 72 h and did not show any signs of toxicity. The leaves were harvested at 24, 48, and 72 h after treatment and contained T-2 (225 to 4,099 μg/g), HT-2 (19,000 to 31,000 μg/g), 3′-hydroxy-T-2 (64 to 488 μg/g), and 3′-hydroxy-HT-2 (617 to 825 μg/g). *B. megapotamica* also was tolerant to diacetoxyscirpenol at 1,000 ppm and metabolized it to monoacetoxyscirpenol (149 to 184 μg/g); a trace of scirpentriol was found.

T-2 toxin is a toxic metabolite produced by various species of *Fusarium* which can cause serious toxicological problems in humans as well as farm animals. Residues of the toxin and its metabolites have been found in animal fluids and organs but do not appear to be an important human health problem. The first attempt to elucidate the structures of the catabolic products of T-2 toxin was made during a study of T-2 metabolism in farm animals (8). The toxin, when orally administered to rodents, chickens, and lactating cows, is rapidly metabolized into various products (7, 14, 19). Three major T-2 metabolites (3′-hydroxy-T-2 [TC-1], 3′-hydroxy-HT-2 [TC-3], and T-2-tetraol) were found in urine, blood, and feces of lactating cows administered T-2 toxin (15, 16, 19). These may correspond to several metabolites found in the excreta of chickens and rats (7, 17, 19). Ruminal bacteria and tissue cultures rapidly metabolized T-2 toxin into various products such as HT-2 toxin, T-2 triol, and neosolaniol (11, 13).

In Brazil and in neighboring countries, poisoning of cattle by ingestion of the shrub *Baccharis coridifolia* occurs often and causes mainly gastrointestinal and nervous system disorders. This has been reproduced experimentally by feeding aerial parts of the plants to cows and other animals (4). Careful examination of a large-scale extract of *Baccharis megapotamica* and *B. coridifolia* has shown that these plants contain over 20 closely related macrocyclic trichothecenes (5a, 6).

Baccharis coridifolia and *B. megapotamica* are tolerant of the trichothecenes. *B. coridifolia* stores verrucarins as well as roridins in the seeds of the female plant. However, the whole plant can metabolize the toxins to various derivatives; e.g., *B. megapotamica* can hydroxylate the A ring of the macrocyclic trichothecenes (5). Based on this, we decided to test other *Baccharis* species, such as those found in the desert of Arizona as well as *B. megapotomica*, for their ability to tolerate and metabolize the nonmacrocyclic trichothecenes (T-2 and diacetoxyscirpenol [DAS]).

MATERIALS AND METHODS

Species and their sources. Samples of two different types of *Baccharis* plants were collected from the desert region of Tucson, Arizona, in spring 1983 and 1984. The first samples were branches and leaves of the hybrid species which resulted from a cross between *B. sarothroides* (desert broom) and *B. pilularis*, which was given the code number FS1. The second sample consisted of branches of *B. sarothroides*, which was given the code number FS2. All samples were collected fresh directly from the wild shrubs and kept in plastic bags with water (refrigerated) to remain fresh until the time of experiments (within 5 days). Another sample of *B. megapotomica*, which was given the code number FS3, was grown in the greenhouse.

Reference standards. T-2 toxin was produced and purified in this laboratory by the method described in detail before (3). HT-2 toxin, T-2-tetraol, and DAS were purchased in analytically pure form from Sigma Chemical Co., St. Louis, Mo. 3′-Hydroxy-T-2 (TC-1) and 3′-hydroxy-HT-2 (TC-3) were the gifts of T. Yoshizawa, Department of Food Science, Faculty of Agriculture, Kagawa University, Kagawa-Kun, Japan.

Uptake of T-2 toxin by *Baccharis* species. Ten cuttings between 25 and 30 cm in length of *Baccharis* species FS1 and FS2 were placed in a 125-ml Erlenmeyer flask containing 500 ppm T-2 toxin in 15% ethanol (100-ml solution). Cuttings of

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TABLE 1. Metabolism of T-2 toxin by *Baccharis* species when placed in a solution containing 500 ppm T-2

Sample[a]	Sampling time (h)	Toxin concn (ppb)[b]					Symptoms or signs[c]
		T-2	HT-2	TC-1	TC-3	T-2-tetraol	
Control							
1	6	0	0	0	0	0	–
2	21	0	0	0	0	0	–
FS1							
Leaves	6	493	1,761	0	9.6	40,923	+NL
	21	3,633	9,443	0	6.7	10,301	+NW
Twigs	6	771	10,183	0	0	10,811	–
	21	39,161	62,289	0	76.9	22,431	+NB
FS2 twigs	6	31	86	0	0	2,097	–
	21	2,182	7,597	0	0	2,628	+NB

[a] Controls 1 and 2 included cuttings (twigs with leaves on them and twigs only) of the non-toxin-treated *Baccharis* species. FS1 stands for hybrids resulting from a cross of *B. sarothroides* with *B. pilularis*. FS2 stands for *B. sarothroides*.
[b] Detection and quantitation of T-2 metabolites were done by GC/MS based on external standards.
[c] –, No toxic effects; +, toxic effects; NL, necrosis of lower leaves; NW, necrosis of whole plants; NB, necrosis and black color.

B. megapotomica were placed in solutions of either 1,000 ppm T-2 or 1,000 ppm DAS. A layer of cotton was wrapped around the stems of the cuttings at the junction of the neck of the flask in order to anchor the plants. Aluminum foil was placed over the cotton in order to prevent evaporation. The control group consisted of 10 cuttings of the same samples placed in a 125-ml Erlenmeyer flask containing aqueous 15% ethanol. All flasks were kept under room conditions (22 to 25°C) and observed frequently for any morphological changes and symptoms. After 6 and 21 h, the cuttings were removed and their stems were cut 2 cm above the point of contact with the surface of the solution to avoid direct contamination of the sample with the toxin solution. The cuttings were then allowed to air dry in a hood for 72 h. The leaves were removed and kept separate from the small twigs. The volume of solution transpired was recorded.

Extraction and purification of T-2 metabolites. Dried leaves and twigs were ground in a mortar and pestle and extracted twice in succession with 100 ml of acetonitrile each time. The combined extracts were filtered through Whatman filter paper number 4 and defatted with petroleum ether (60 to 70°C bp) two times with 100 ml each time. The acetonitrile layer was then evaporated to dryness on a rotary evaporator at 45°C. The residue was redissolved in methanol-H_2O (50: 20, vol/vol). Upon addition of water, the extract became yellow and milky, containing brown oily droplets. This suspension was placed on a rotary evaporator to drive off the methanol. The remaining suspension was then added to an XAD-2 column (14 by 2.5 cm) as described in detail before (19). The column was washed with 100 ml of distilled water, which was discarded. Then 10 ml of 90% methanol was added to the column to elute the toxins, which were collected in a 250-ml round-bottomed flask. The eluate was evaporated to dryness on a rotory evaporator at 45°C. The residue was dissolved in 4 ml of methanol, and portions of this solution were analyzed by thin-layer chromatography as described in detail before (1) and gas chromatography/mass spectroscopy (GC/MS) with both selected ion monitoring and total ion chromatographic methods.

Determination of T-2 metabolites by GC/MS. Determination and confirmation of T-2 metabolites were performed on a Hewlett Packard 5987B GC/MS. Samples and standards were derivatized to their corresponding trifluoroacetate (TFA) esters prior to analysis by GC/MS. TFA esters were prepared by adding 200 μl of trifluoroacetic acid anhydride (Pierce Chemical Co., Rockford, Ill.) to dry samples in a half-dram glass screw cap vial. Excess TFA anhydride was driven off with nitrogen. The vial was rotated for adequate mixing for injection of the sample into the GC/MS. One microliter was taken from the derivative TFA esters for injection and delivered onto a DB-5 capillary column (0.25 mm by 30 m; J and W Scientific, Rancho Cordova, Calif.). Column head pressure was 12 lb/in^2 with an exit flow of 1 to 2 ml. The oven temperature program was 80 to 300°C at 20°C/min. A splitless injection procedure was used with a delay time of 0.5 min. The MS was set in the positive chemical ionization mode for detection of positive ions. A source pressure of 0.4 mm Hg methane and a temperature of 150°C were chosen as optimum conditions. The instrument was tuned with perfluorotributylamine. Determination of toxins in samples was based on external standards for known T-2 toxin metabolites and molecular weight for unknown related T-2 compounds.

RESULTS

Symptoms. Control groups did not exhibit any toxic signs on the leaves and twigs (Table 1). Symptoms of necrosis on the hybrid (FS1) did not appear until 4 to 6 h after treatment but were clearly visible at 6 h, after which they progressed up the stems. After 12 h, the whole leaves showed necrosis that started from the margins of the leaves toward the center of the blade and then turned black. Such symptoms were difficult to see on the samples of FS2 because they did not have leaves, but the stalks turned black within 21 h. The results are listed in Table 1. When *B. megapotamica* was treated with T-2 toxin and DAS at 1,000 ppm, the leaves and twigs did not show symptoms or signs of necrosis during the 72-h experiment.

Metabolites in *Baccharis* species. The control groups of each species did not produce any trichothecenes. The twigs and leaves (analyzed separately) of the *B. sarothroides*-*B. pilularis* hybrid (FS1) at 6 and 21 h after exposure contained T-2, HT-2, 3′-hydroxy-HT-2, and T-2-tetraol in significant amounts (Table 1). The twigs of *B. sarothroides* (FS2) at 6 and 21 h also contained T-2, HT-2, and T-2-tetraol but did not contain 3′-hydroxy-HT-2 as in the hybrid (Table 1).

Cuttings of *B. megapotamica* placed in aqueous solutions of T-2 toxin and DAS (both at 1,000 ppm) were not toxic to the plants and were metabolized quickly. Thus, within 24 h, T-2 toxin was converted to HT-2 toxin (31,209 μg/g) and TC-1 and TC-3 (488 and 617 μg/g, respectively) (Table 2). The same metabolites were found at 48 and 72 h as were found at 24 h except for T-2-tetraol, which appeared at 48 h.

TABLE 2. Metabolism of T-2 toxin and DAS by leaves of *B. megapotamica* when placed in a solution containing 1,000 ppm toxin

Toxin	Toxin concn (ppm)[a] at:		
	24 h	48 h	72 h
T-2	4,099	+	225
HT-2	31,209	+	19,630
TC-1	488	+	64.2
TC-3	617	+	825
T-2-tetraol	–	+	+
DAS	285	40	118
MAS	184	125	149
Scirpenetriol	+	+	+
Control[b]	–	–	–

[a] Symbols: –, No detectable metabolite; +, metabolite detectable but not quantitated.
[b] *B. megapotamica* branches placed in free toxin in aqueous solution (15% ethanol).

DAS was also quickly metabolized within 24 h into monoacetoxyscirpenol (MAS) (184 μg/g) and scirpenetriol (not quantitated).

DISCUSSION

The metabolites of T-2 toxin are important and of interest to researchers because of their potential occurrence as residues in edible portions of farm animals; moreover, their identification is important to establish their importance in human health. All animal systems reported to date metabolize T-2 into HT-2, 3'-hydroxy-T-2 toxin (TC-1), and 3'-hydroxy-HT-2 toxin (TC-3) as well as T-2-tetraol, which suggests that the same enzyme systems are operative within these biological systems. This theme has been well studied in cows, monkeys, guinea pigs, rats, mice, microorganisms, and tissue culture (9, 10, 18). However, little has been published on the metabolism of T-2 in plants such as *Baccharis* spp. (8). Preliminary studies conducted in our laboratory demonstrated that the three major T-2 metabolites found in *B. sarothroides* were HT-2, TC-1, TC-3, and/or T-2-tetraol and that the test plants had some resistance of these toxins. This study was prompted because of the lack of toxicity noted in *Baccharis megapotomica* treated with the macrocyclic trichothecenes, as reported by Jarvis (5).

Our results indicate that *Baccharis* species are relatively tolerant of T-2 toxin, as evidenced by the fact that a high concentration (500 ppm) of T-2 toxin in solution did not kill the plants outright but rather caused a slight necrosis in the leaves and stems up to 6 h; serious necrosis did not occur until 21 h after treatment. The treatment of the plants is severe because they are exposed to the solution of T-2 toxin constantly, as opposed to animals, which are given a single dose and then left to metabolize the toxin. Of greater importance is the fact that the plants were able to metabolize the toxin into HT-2, T-2-tetraol, and TC-3. This metabolism is similar to that found in cows dosed with T-2 toxin (2), chickens (12), and rats (18). This information is of basic importance in that it demonstrates that similar enzyme systems capable of reduction and hydration reactions are found in both animals and plants, thereby supporting the principle of chemical similarity in diverse biological systems. Of practical importance is the discovery that we can use plants to synthesize T-2 derivatives that are difficult or costly to obtain in appreciable yield from animal systems.

B. sarothroides exhibited greater tolerance to T-2 toxin than the *B. sarothroides-B. pilularis* hybrid. Further investigation is necessary to determine whether greater tolerance or resistance is exhibited during senescence, i.e., seed formation. Recent information (5) indicates that the macrocyclic trichothecenes produced by *B. coridipholia* produce their toxins in the seeds and only on female plants.

The hydroxylation products (TC-1 and TC-3) were prominent among the metabolites of T-2 produced by *B. megapotamica*. It appears that hydroxylation is a detoxification mechanism in certain plants as well as animals, as all of the above are appreciably less toxic than the parent T-2 toxin. On the other hand, *B. megapotamica* deacetylated DAS at the C-4 position and formed the hydroxyl derivative called MAS. The latter is not less toxic, as expected, but rather is more toxic in animals and perhaps in plants as well. No data on the phytotoxicity of MAS exist. However, hydroxylation of DAS at the C-4 and C-15 positions results in definite loss of toxicity by formation of scirpenetriol.

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