Phytotoxicity of T-2 Toxin Produced by *Fusarium tricinctum*

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ABSTRACT

Fusarium tricinctum isolates which produced T-2 toxin [4, 15-diacetoxy-8-(3-methylbutyryloxy)-12, 13-epoxy-Δ^9-trichothecen-3-ol] caused severe wilt of Early Perfection pea seedlings within 30 hr, and complete necrosis with 72 hr after root immersion in aqueous suspensions prepared from agar cultures. Isolates of *F. tricinctum* and *F. roseum* which did not produce T-2 toxin had no observable effect on pea seedlings.

Crystalline T-2 toxin produced exactly the same symptoms in pea seedlings as T-2 toxin-producing *Fusarium tricinctum* cultures. Root immersion time had a slight effect on subsequent symptom expression. With an immersion time of 20 min, concentrations of T-2 toxin as low as 2.5 ppm could be detected by means of symptom expression alone. Aqueous solutions containing 5.0 ppm of T-2 toxin caused a 40% reduction in average fresh weight and length of pea seedlings 7 days after root immersion for 20 min. T-2 toxin concentrations as low as 0.625 ppm, or 0.0625 mg T-2 toxin in the test solution, caused statistically significant reductions in average fresh weight and length of pea seedlings.

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Additional key word: mycotoxin.

Different isolates of *Fusarium tricinctum* (Cd.) Snyd. & Hans. produce at least three toxic spiro-epoxy derivatives of the trichothecane family. Diacetoxyscirpenol (4, 15-diacetoxy-12, 13-epoxy-Δ^9-trichothece-3-ol) and T-2 toxin [4, 15-diacetoxy-8-(3-methylbutryloxy)-12, 13-epoxy-Δ^9-trichothecen-3-ol] are produced at 8 C, whereas HT-2 toxin [3, 4-dihydroxy-15-acetoxy-8-(3-methylbutyryloxy)-12, 13-epoxy-Δ^9-trichothecene] is produced at 24 C (1, 2, 3, 7). Certain isolates of *F. tricinctum* also produce a toxic butenolide, 4-acetamido-4-hydroxy-2-butenoic acid-γ-lactone (15).

All of these compounds are potent skin irritants and inflammatory agents (1, 2, 3, 11, 12), as are most of the known 12, 13-epoxy-Δ^9-trichothecenes (2, 5, 6, 7, 9, 10). This fact was utilized in the development of a rat skin bioassay for these compounds (1, 2, 7).

Diacetoxyscirpenol is also a potent phytotoxin (5). The production of phytotoxins by *F. tricinctum* was indicated by the work of Bolton & Nuttall (4). They found that root immersion of pea seedlings in sterile, cell-free culture filtrates of *F. poae* (Pk.) Wr. resulted in severe wilting in 24 hr and complete necrosis in 72 hr. According to Snyder & Hansen (13), *F. poae* is a synonym of *F. tricinctum*. These results suggested the possibility of developing a sensitive bioassay for the 12, 13-epoxy-Δ^9-trichothecenes, using a pea wilt test. This possibility was investigated by comparing the effects of T-2 toxin-producing and nonproducing isolates of *F. tricinctum* and of an isolate of *F. roseum* (Lk.) Snyd. & Hans. on pea seedlings, and by determining the phytotoxic properties of crystalline T-2 toxin.

MATERIALS AND METHODS.—*Source and maintenance of isolates.*—Three isolates of *Fusarium tricinctum* and one of *F. roseum* were used in these studies (Table 1). The isolates were maintained as single-conidial cultures on potato-dextrose agar (PDA) slants which were incubated at room temperature for 1 week prior to storage at 4 C.

Preparation of T-2 toxin.—The standard method for production of T-2 toxin in culture utilized ten 500-ml Erlenmeyer flasks containing 100 ml Gregory's medium (8) inoculated with spore suspensions of each of the isolates under study. The inoculated flasks were incubated at 8 C for 14 days, and the contents then macerated in a Waring Blendor and lyophilized. The dry powder was extracted with ethyl acetate, the crude extracts further solvent-purified, derivatized, and analyzed with a gas chromatograph for T-2 toxin content (1). Crystalline T-2 toxin was obtained from isolate T-2 according to the methods described by Bamburg et al. (3).

Pea wilt bioassay.—The ability of *F. tricinctum* and *F. roseum* isolates and of T-2 toxin to cause wilt of pea seedlings was tested by growing Early Perfection peas in the greenhouse under conditions similar to those described by Wells et al. (14). Ten days after planting the seeds in silica sand in stainless steel pans, the seedlings were removed from the pans and the roots washed in tap water and then cut back to a uniform length on a cutting board (14). The roots were then immersed in the test suspensions or solutions for the required period of time, and the seedlings transplanted in Ottawa silica.

The production of phytotoxic metabolites by *F. tricinctum* and *F. roseum* was assayed by growing

TABLE 1. Gas chromatograph analyses of culture filtrates for T-2 toxin production by isolates of *Fusarium tricinctum* and *F. roseum*

Species	Isolate no.	Source	T-2 toxin concentration (mg/liter)
F. tricinctum	T-2	Corn, France	103
F. tricinctum	T-2A	Unpigmented mutant of T-2	0
F. tricinctum	223	Corn, Wisconsin	106
F. roseum	353	Corn, Wisconsin	0

single-spore cultures of the isolates listed in Table 1 on potato-dextrose agar in 90-mm petri dishes for 3 weeks at 25 C. A suspension of mycelium and spores was prepared by blending two petri dish cultures of each isolate with 120 ml of distilled water in a Waring Blendor. Two sterile PDA plates treated in the same way served as a control. The roots of 10 pea seedlings were immersed in each test suspension for 15 min, and the seedlings then transplanted.

The phytotoxicity of crystalline T-2 toxin was assayed by dissolving 10 mg toxin in 5 ml absolute ethanol, and adding this solution to 95 ml distilled water under continuous agitation. Dilutions were prepared from this stock solution containing 100 ppm of crystalline T-2 toxin to obtain 100-ml samples of distilled water containing either 10, 1.0, 0.1, or 0.01 ppm of T-2 toxin. Control solutions of distilled water contained 5.0 and 0.5 ml of absolute ethanol. The roots of 10 pea seedlings were immersed in each solution for 10 min, and the seedlings then transplanted.

The effect of root immersion time in aqueous solutions of T-2 toxin on the fresh weight of pea seedlings was determined by preparing dilutions from a stock solution containing 100 ppm of T-2 toxin to obtain 50-ml samples of distilled water containing either 10, 5, 2.5, or 1.0 ppm T-2 toxin. The control solution contained 2.5 ml absolute ethanol. The roots of five pea seedlings were immersed for 20 min and all the seedlings then transplanted. Seven days after immersion of the roots in the test solutions, the seedlings were carefully removed from the Ottawa silica, washed in tap water, and blotted dry, and the fresh weight was determined.

The effect of root immersion in aqueous solutions of T-2 toxin on the fresh weight and length of pea seedlings was determined by preparing dilutions from a stock solution containing 100 ppm of T-2 toxin to obtain 100-ml samples of distilled water containing either 10, 5, 2.5, 1.25, or 0.625 ppm T-2 toxin. A solution of 5 ml absolute ethanol in 95 ml distilled water served as a control. The roots of 18 pea seedlings were immersed in each solution for 20 min, and the seedlings then transplanted. Seven days after immersion of the roots in the test solutions, the fresh weight and length of the seedlings were determined. The length of the seedlings was measured from the point of emergence of the stem from the seed to the terminal node.

RESULTS.–*Phytotoxic metabolites.*–Pea seedlings immersed in suspensions prepared from cultures of *F. tricinctum*, isolates T-2 and 223, which produce T-2 toxin (Table 1), were severely wilted after 30 hr and completely necrotic after 72 hr (Fig. 1). The control seedlings and those treated with the variant T-2A of *F. tricinctum* and isolate 353 of *F. roseum*, which do not produce T-2 toxin (Table 1), appeared completely normal after 72 hr (Fig. 1-A).

Phytotoxicity of T-2 toxin.–All the seedlings immersed in solutions containing either 100 or 10 ppm of T-2 toxin for 10 min showed severe symptoms of wilt after 30 hr. After 48 hr, these seedlings were flaccid and drooping, with white blotches along the midribs of the leaflets. All these seedlings were completely necrotic after 72 hr. None of the controls or the seedlings immersed in solutions containing 1.0 ppm or less of T-2 showed any observable symptoms except some degree of stunting.

Effect of root immersion time on symptom expression and fresh weight of pea seedlings.–Immersion time of the roots in aqueous solutions of T-2 toxin had a slight effect on subsequent symptom expression of pea seedlings. With an immersion time of 10 min, the lowest concentration of toxin which caused wilt of the seedlings after 72 hr was 5 ppm. With an immersion time of 20 min, concentrations of T-2 toxin as low as 2.5 ppm caused wilt of pea seedlings after 72 hr (Fig. 1-B). Concentrations of T-2 toxin as low as 1.0 ppm (or 0.05 mg of T-2 toxin in the test solution) caused statistically significant reductions in the fresh weight of pea seedlings after 7 days, irrespective of the immersion time (Table 2).

Effect of T-2 toxin concentration on fresh weight and length of pea seedlings.–Pea seedlings immersed in aqueous solutions containing less than 1.0 ppm of

TABLE 2. Effect of T-2 toxin concentration and root immersion time on fresh weight of pea seedlings

Concentration of T-2 toxin (ppm)	Fresh weight of peak seedlings (g)[a] Immersion time (min) 10	20
0	1.3727	1.4039
1	1.1585[b]	1.1788[c]
2.5	1.1855[c]	1.0318[b]
5	1.1608	0.9813[b]
10	1.0113[b]	0.8425[b]
100	0.8585[b]	0.9397[b]

[a] Each value represents the average weight of five Early Perfection pea seedlings 7 days after immersion of the roots in the test solutions.

[b] Significantly different from the control ($P < .01$).

[c] Significantly different from the control ($P < .05$).

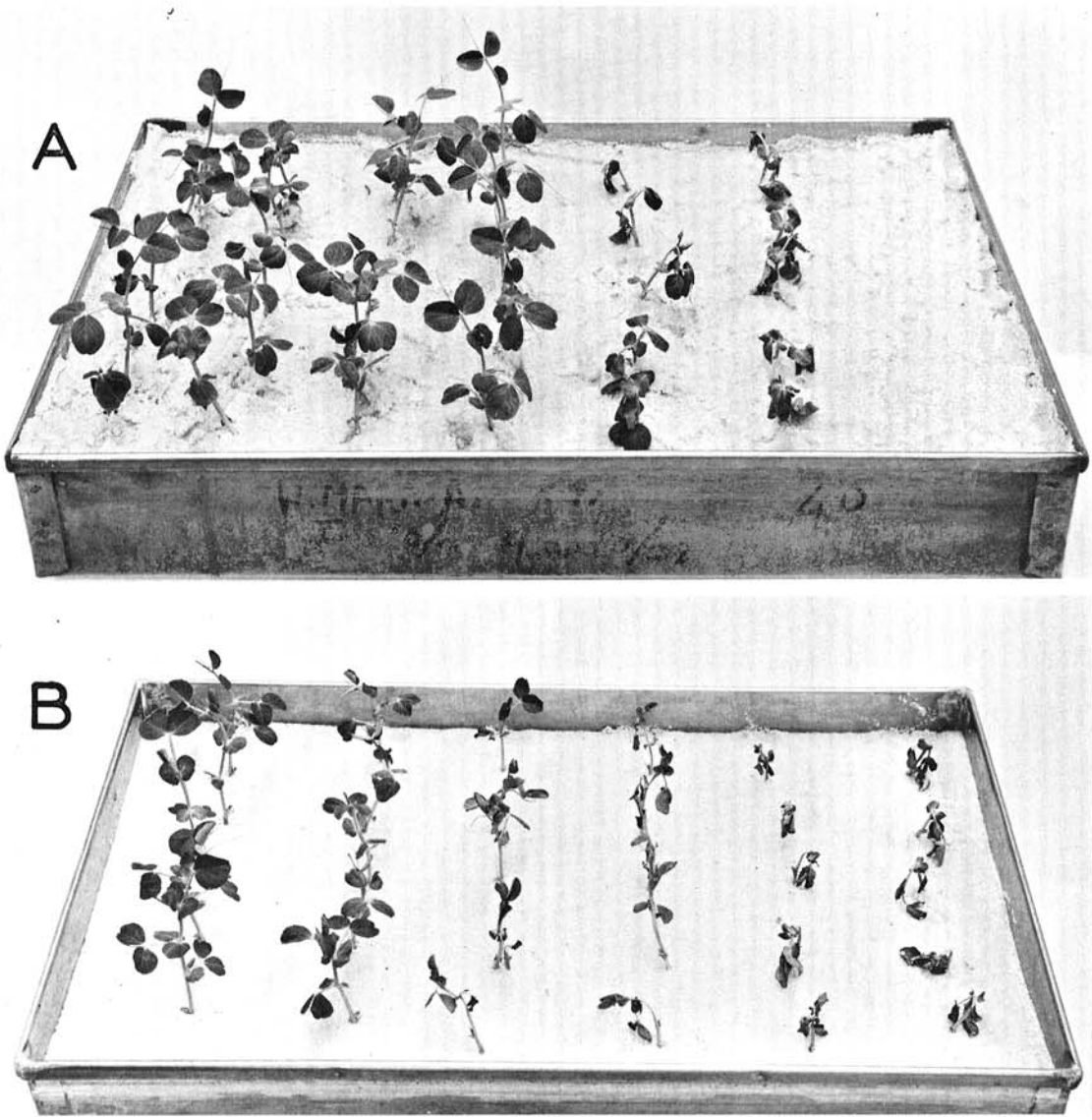

Fig. 1. A) Early Perfection pea seedlings 72 hr after immersion of the roots in aqueous suspensions prepared from agar cultures of *Fusarium tricinctum* isolates. (Left two rows) Control. (Central two rows) Mutant T-2A. (Right two rows) Isolate T-2. **B)** Early Perfection pea seedlings 72 hr after immersion of the roots in aqueous solutions of T-2 toxin for 20 min. Rows of five seedlings from left to right: Control, 1.0, 2.5, 5.0, 10.0, and 100.0 ppm of T-2 toxin.

T-2 toxin showed no visible symptoms except a slight stunting. Seven days after root immersion, the average fresh weight and length of these seedlings were, however, significantly ($P<.01$) less than those of the controls (Table 3). Immersion of the roots of pea seedlings for 20 min in an aqueous solution containing 5.0 ppm of T-2 toxin resulted in a 40% reduction in average fresh weight as well as in length after 7 days (Fig. 2).

DISCUSSION.–Only the isolates of *F. tricinctum* which produce T-2 toxin caused wilting of pea seedlings within 30 hr and complete necrosis within 72 hr. The mutant T-2A and the isolate of *F. roseum*, which do not produce the toxin, had no observable effect on pea seedlings. These results presented good presumptive evidence that T-2 toxin is phytotoxic and causes severe wilt and necrosis of peas. The subsequent experiments showed that crystalline T-2 toxin could produce exactly the same symptoms in peas as the macerated cultures of toxin-producing isolates of *F. tricinctum*. These symptoms included severe wilt within 30 hr, stunting, vein clearing, white blotches along the midribs of the leaflets, brown, sunken lesions on the leaflets, and complete necrosis within 72 hr.

Concentrations of T-2 toxin as low as 2.5 ppm could be detected by means of symptom expression alone, whereas concentrations as low as 0.625 ppm (or 0.0625 mg T-2 toxin in the test solution) caused

TABLE 3. Effect of T-2 toxin concentration on fresh weight and length of pea seedlings

T-2 toxin concentration (ppm)	Average weight[a] (g)	Average length[a] (cm)
0.000	1.4899	13.93
0.625	1.2573[b]	12.73[b]
1.250	1.2343[b]	12.18[b]
2.500	1.2131[b]	9.56[b]
5.000	0.8981[b]	8.42[b]
10.000	0.8593[b]	7.05[b]

[a] Average weight and length of 18 Early Perfection pea seedlings determined 7 days after immersion of the roots in each toxin-containing solution for 20 min.
[b] Significantly different from the control ($P < .01$).

highly significant reductions in the fresh weight and length of pea seedlings.

The dosage response curves between T-2 toxin concentration, fresh weight reduction, and length reduction were not linear. Nevertheless, the fact that the test solutions containing less than 0.1 mg of T-2 toxin caused highly significant reductions in fresh weight and length of pea seedlings suggests that the pea wilt test definitely has some potential as a bioassay for T-2 toxin. It would be most interesting to extend these studies to other 12, 13-epoxy-Δ^9-trichothecenes like trichodermin, trichothecin, crotocin, trichodermol, verrucarol, and diacetoxyscirpenol (2) to determine whether the pea wilt test is specific for T-2 toxin or whether other members of the trichothecane family, or other toxic metabolites of *Fusarium* spp., behave in a similar way.

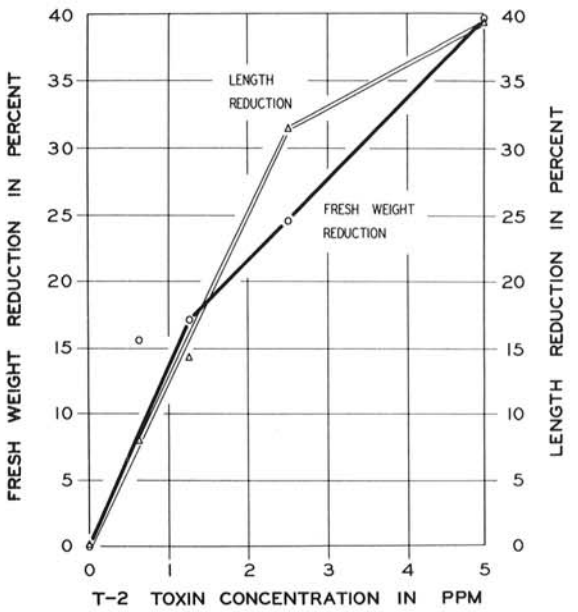

Fig 2. Reduction in fresh weight and length of pea seedlings caused by immersion of the roots in aqueous solutions of T-2 toxin for 20 min.

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