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Formation of Trichothecenes by *Fusarium solani* var. *coeruleum* and *Fusarium sambucinum* in Potatoes

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Fusarium solani var. *coeruleum* can form deoxynivalenol in potato tubers and in liquid medium, although concentrations observed in the rot were highly variable; acetyldeoxynivalenol and HT-2 toxin were detected in 1 to 3 tubers only (of 57). Trichothecenes were also detected in a very few (3 of 20) cultures of *Fusarium sambucinum* in potato tubers.

Fusarium solani var. *coeruleum* (Sacc.) Booth and *Fusarium sambucinum* Fuckel f.6 Wollenweber (*F. sulphureum* Schlecht) are the two most common fungi causing dry rot of potatoes in Europe and North America (1). Both species have been shown to produce one or more type A trichothecenes, such as diacetoxyscirpenol, but not type B (8-keto) trichothecenes (5, 7, 10) (Fig. 1). No previous attempts have been made to examine cultures of these fungi in potatoes for trichothecene mycotoxins.

Potato tubers were washed with distilled water, flame sterilized, and punctured with either a nail punch (20 by 2 mm) or a cork borer (6 mm in diameter up to a depth of 20 mm). *F. solani* var. *coeruleum* or *F. sambucinum* were originally isolated from potatoes, maintained in potato tubers, and then subcultured onto potato dextrose agar (PDA) plates. After incubation for 10 to 14 days at 23°C, conidia were harvested and used to inoculate potato tubers within 30 to 60 min by immersing the tubers in a spore suspension (10^3 spores per ml of distilled water) for 30 s. The *Fusarium* species were identified at the Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario. Inoculated tubers were stored at either 4°C (for up to 75 days plus 4 days at ambient temperature after day 60) or 15°C (18 to 63 days). Rot, together with ca. 0.5 cm of surrounding potato tissue, was excised and analyzed for trichothecenes after blending with acetonitrile and cleanup of the extract on an alumina-charcoal column (2); the column used in this method was washed with 25 ml of acetonitrile-water (84:16) to elute the trichothecenes. Screening for eight trichothecenes—deoxynivalenol (DON), 3-acetyldeoxynivalenol (ADON), nivalenol (NIV), fusarenone X, diacetoxyscirpenol, neosolaniol, T-2 toxin (T-2), and HT-2 toxin (HT-2)—was carried out by temperature-programmed gas-liquid chromatography (GLC) on a column (183 cm by 2 mm [inner diameter]) of 3% OV-3 with electron capture (EC) detection, after derivatization with heptafluorobutyrylimidazole, (8, 9). In addition, or alternatively, underivatized trichothecenes, with the exception of NIV and the inclusion of monoacetoxyscirpenol, were screened by a newly developed capillary GLC-chemical ionization tandem mass spectrometric (MS/MS) technique (SCIEX TAGA 6000 triple quadrupole MS/MS system) (P.-Y.Lau, T. Sakuma, and P. M. Scott, manuscript in preparation). GLC conditions used in this system were as follows: column (15 m by 0.32 mm [inner diameter]) of DB-5 fused silica (J & W Scientific) held at 140°C for 30 s and then programmed to 250°C at 20°C/min and held there for 2 min; injector temperature, 200°C, splitless mode; nitrogen carrier gas at a flow rate of 7 ml/min; retention times in the range of 4 to 7 min. The first mass analyzer selected the following molecular ions of interest; *m/z* 296, 324, 338, 366, 354, 382, 318, 466, and 424 for DON, monoacetoxyscirpenol, 3- or 15-ADON, diacetoxyscirpenol, fusarenone X, neosolaniol, zearalenone, T-2, and HT-2, respectively; these ions were then fragmented in the second quadrupole by collision with argon (2.2×10^{14} atoms per cm^2), producing daughter ions at *m/z* 248, 264, 248, 153, 153, 122, 250, 121, and 86, respectively, that were analyzed by the third mass analyzer. Determination and confirmation of trichothecenes was carried out, after derivatization, by capillary GLC-MS selected ion monitoring [MS(SIM)] (3, 9), which was necessary because of interferences in potato rot observed by GLC-EC, particularly near the retention time of DON and at the retention time of NIV. In general, good agreement was obtained between the two MS techniques, and in 48 potato tubers analyzed by both methods, only three false-positives for DON were observed with the MS/MS system. Overall method recovery after spiking fresh potatoes (30 g) with 2 μg of trichothecene per g was in the range of 69 to 109% for diacetoxyscirpenol, T-2, HT-2, DON, ADON, and fusarenone X and 20% for NIV as determined by GLC-EC. To study trichothecene formation by the same strain of *F. solani* var. *coeruleum* in liquid medium, a solution of 1% glucose, 0.1% peptone, and 0.1% yeast extract (12) was inoculated with 3×10^5 to 5×10^5 spores per ml and incubated at 23°C for up to 21 days. The strain had previously been maintained on a PDA slant for 11 weeks at 4°C. A 7- to 10-ml sample of liquid culture medium was removed under sterile conditions at intervals, filtered, and evaporated at 50°C with a rotary evaporator; the residue was then dissolved in an equal volume of acetonitrile-water (84:16), passed through the alumina-charcoal column, and analyzed by GLC-EC and GLC-MS(SIM).

Since potato tubers can be stored at different temperatures depending upon the intended use (6), we studied growth of the *Fusarium* strains and trichothecene formation in tubers stored at both 4 and 15°C. Trichothecenes were found in rot from 4 of 17 tubers after storage for up to 71 days at 4°C (Table 1). A second batch of potato tubers was inoculated by a different technique (cork borer) for making the inoculum holes and incubated at 15°C. The results presented in Table 2

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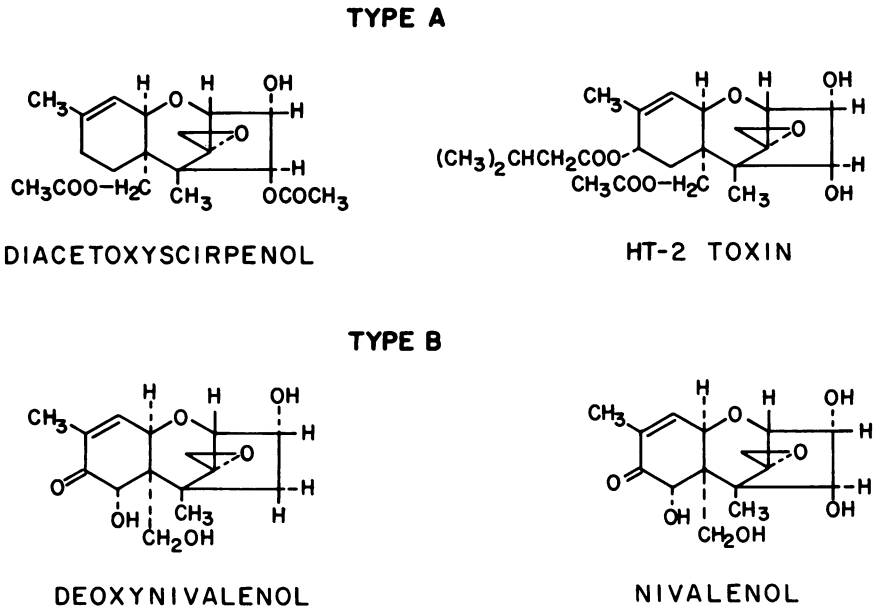

FIG. 1. Structures of some trichothecene mycotoxins.

demonstrate formation of DON (also ADON and HT-2) by *F. solani* var. *coeruleum* in potato tubers. Very large variations in DON production (from undetected to 23.8 μg/g) were observed among tubers of the same variety, inoculated with the same amount of inoculum and stored for the same time period (18 days). DON concentration was not proportional to fungal growth, and longer storage times resulted in lower incidence of DON, suggesting toxin destruction. Growth of *F. sambucinum* was very weak, and DON (0.011 μg/g) was detected in only 1 of 12 tuber samples analyzed after 32 to 63 days storage, generally at 15°C (7 days at 23°C were included in the storage period of three tubers). Therefore, for further sudy we concentrated on *F. solani* var. *coeruleum*.

To confirm the formation of trichothecenes by *F. solani* var. *coeruleum*, production of DON in liquid medium was demonstrated. In the medium used in this study, shaking the culture in the dark was found to be essential for toxin production, which was associated with spore (microconidia) formation and nonproduction of red-brown pigment. Maximum concentration of DON was 2.0 μg/ml [by GLC-EC; 1.5 μg/ml by GLC-MS(SIM)] at 10 days, with a drop in concentration thereafter. The experiment was repeated with a single spore isolate from an original PDA plate, but maximum production of DON was six to eight times less, which may be due to maintenance of the culture on PDA (one more subculturing was carried out). Similarly, reinoculation of a subculture of *F. solani* var. *coeruleum* in potatoes (variety Sebago) resulted in no trichothecene formation until after 25 and 32 days at 15°C, when 0.19 and 0.03 μg of DON per g, respectively, was detected in rots from two tubers, and growth of the fungus in all samples (15 tubers) was weak. Other investigators have reported loss of toxicity as well as culture variation by maintenance of *Fusarium* strains on PDA (4, 11).

The present data indicate for the first time that *F. solani* var. *coeruleum* forms a type B trichothecene (DON) in potato tubers and in liquid medium, albeit in very variable amounts. Further studies are needed on a number of freshly isolated strains of this species as well as on *F. sambucinum*, for which indications of trichothecene formation were also obtained. Our results suggest that trichothecenes may occur

TABLE 1. Trichothecenes in positive samples[a] of potato tubers inoculated with *F. solani* var. *coeruleum* or *F. sambucinum* and stored primarily at 4°C

Potato variety	*Fusarium* species	Fungal growth	Storage conditions	Trichothecenes detected in rot (μg/g)[b]
Shepody	*F. solani* var. *coeruleum*	Fair[c]	60 days at 4°C (+ 4 days at 23°C)	HT-2 (0.24)
Shepody	*F. sambucinum*	Good[d]	60 days at 4°C (+ 4 days at 23°C)	DON (0.017), NIV (0.22)
Shepody	*F. sambucinum*	Good	67 days at 4°C (+ 4 days at 23°C)	NIV (0.046), HT-2 (0.33)
Russett Burbank	*F. sambucinum*	Fair	71 days at 4°C (+ 4 days at 23°C)	DON (0.14)

[a] Total number of tuber samples, representing eight varieties, that were analyzed was 17 (9 were inoculated with *F. solani* var. *coeruleum* and 8 with *F. sambucinum*).
[b] Determinations by GLC-MS(SIM).
[c] About 0.5 by 2.5 cm.
[d] About 0.9 by 2.8 cm.

TABLE 2. Trichothecenes in three potato varieties inoculated with *F. solani* var. *coeruleum* and stored at 15°C for 18 days[a]

Potato variety	Tuber no.	Dimensions of fungal growth (cm)	Trichothecenes detected in rot (μg/g)[b]
Shepody	1	0.85 by 2.7	DON (0.005), HT-2 (0.29)
	2	1.1 by 2.8	DON (0.091)
	3	0.6 by 2.6	DON (0.010)
Russett Burbank	1	1.3 by 3.0	DON (0.020)
	2	2.5 by 3.5	ND[c]
	3	0.85 by 2.5	DON (0.033)
Sebago	1	1.3 by 3.0	DON (23.8), ADON (1.6), HT-2 (1.4)
	2	1.1 by 2.8	DON (0.018)
	3	1.8 by 3.5	ND

[a] Of 24 other tubers stored for 25 to 39 days with fungal growth dimensions of 0.8 to 2.75 by 2 to 5 cm, only two contained DON (0.010 and 0.016 μg/g).

[b] Determinations by GLC-MS(SIM).

[c] ND, Not detected by GLC-EC or GLC-MS/MS (limit, 0.003 to 0.004 μg/g) or both.

naturally in potatoes infected with *Fusarium* dry rot and which could be fed to livestock.

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