T-2 Toxin Production by *Fusarium acuminatum* Isolated from Oats and Barley

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Oats grain from South Africa was frequently found to be infested by toxic strains of *Fusarium acuminatum*, as was one barley sample. All 11 toxic strains tested produced T-2 toxin (0.8 to 2,600 mg/kg), and 6 of 11 strains produced diacetoxyscirpenol (0.6 to 8.4 mg/kg). This is the first record of T-2 toxin-producing *Fusarium* isolates from Africa and of the production of large amounts of T-2 toxin at relatively high (25°C) temperatures.

T-2 toxin is produced by isolates of different *Fusarium* species (12) and possibly by *Trichoderma* species (1). There is some doubt as to the number of *Fusarium* species involved due to taxonomic and nomenclatural problems (12), but T-2 toxin production by strains of the following species has been reported (7, 11, 12): *Fusarium acuminatum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. lateritium*, *F. moniliforme*, *F. oxysporum*, *F. poae*, *F. semitectum*, *F. solani*, *F. sporotrichioides*, and *F. tricinctum*. T-2 toxin-producing strains have been reported in Europe (2, 6, 9, 21), North America (3, 18), and Asia (17, 19, 20), but not in Africa, South America, or Australasia.

The optimum temperature for the production of T-2 toxin is comparatively low (8 to 14°C), with yields being much lower or negligible at temperatures of 25°C and above (2, 3, 5, 18).

The object of this study was to determine the dominant fungi occurring in oats grain in the summer rainfall area of South Africa and to evaluate their toxicity so as to determine any possible human health risks.

Twenty-two oats samples were collected at eight localities in the eastern Transvaal and eastern Orange Free State. One barley sample from the Orange Free State was also examined. The dominant fungal species were isolated from the grains by the whole kernel and dilution plate methods (13). Single-conidial isolates of the dominant species were identified and lyophilized. The strains used in toxicity trials and chemical investigations were deposited in the culture collection of the South African Medical Research Council.

*Fusarium* isolates were cultivated on yellow corn kernels in 2-liter glass jars (400 g of maize and 400 ml of tap water per jar) and autoclaved at 121°C for 1 h on each of 2 consecutive days. The corn in each jar was inoculated with a conidial suspension of each isolate and incubated at 25°C in the dark for 21 days. After incubation the culture material was dried at 45°C for 24 h and finely ground in a laboratory mill. The resulting meal was stored at 2°C until it was used in toxicity tests or chemical analysis.

Fungal isolates were evaluated for toxicity in 1-day-old Pekin ducklings (16). T-2 toxin and diacetoxyscirpenol were extracted from dry culture material with methanol-water (1:1). A portion of this extract was transferred to a prepacked Extrelut 20 column (Merck Chemicals), from which the toxins were eluted with dichloromethane. The eluate was dried, redissolved in chloroform-hexane (1:2), and applied to a silica gel column equilibrated with chloroform. The column was washed with benzene followed by benzene-acetone (95:5), after which the toxins were eluted with ether. The dried fraction containing the toxins was dissolved in toluene-acetonitrile and derivatized with N-heptafluorobutyrylimidazole (Pierce Chemical Co.). The derivatized fraction was mixed with 0.1 M phosphate buffer (pH 6.0), and the two phases were allowed to separate. A portion of the organic phase was removed, and the solvent was evaporated under nitrogen. The residue was dissolved in benzene and analyzed by gas chromatography. Chromatographic analysis was performed on a Carlo Erba model 5300 gas chromatograph fitted with a split injector, a fused silica capillary column (SE-30, 25 m by 0.32 mm), and a 63Ni electron capture detector. Quantification was done by comparing peak heights against calibration curves obtained for derivatized standards of T-2 toxin and diacetoxyscirpenol.

Cultures identified as *F. acuminatum* Ell. & Ev. (14) were among the fungi most often isolated from oats from mainly lower grades of oats from the eight localities. *F. acuminatum* was isolated from 100% of the grains in three oats samples, whereas the incidence ranged from 5 to 40% for the remain-

<table>
<thead>
<tr>
<th>Locality</th>
<th>Cultivar</th>
<th>Grade</th>
<th>Strain no.</th>
<th>DAS* (mg/kg)</th>
<th>T-2 toxin (mg/kg)</th>
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<tr>
<td>Amersfoort</td>
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<td>II</td>
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<td>MRC 3827</td>
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<td>MRC 3821</td>
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<td>III</td>
<td>MRC 3822</td>
<td>1.6</td>
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<td>Clipper (barley)</td>
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<td>MRC 3937</td>
<td>1.3</td>
<td>45.0</td>
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</table>

* Corresponding author.

* DAS, Diacetoxyscirpenol.

* ND, Not detected.
ing 19 samples. Ten of these isolates of *F. acuminatum* from oats and one from barley grain from the same area were tested for toxicity. The macroconidia of these strains, obtained from oats and barley collected over a large geographical area, were similar to those described for *F. acuminatum* (14). However, they all differed from typical representatives of *F. acuminatum* in culture characteristics and in the rapid production of abundant microconidia, sporodochia, and chlamydospores. The taxonomy of these isolates is being further investigated.

In duckling trials, the 11 isolates were all highly toxic and caused the death of four of four ducklings within 4 days. In skin tests on rabbits, ethyl acetate extracts of 6 of these 11 isolates were dermotoxic.

The corn culture material of these 11 isolates was analyzed for the trichothecenes T-2 toxin and diacetoxyscirpenol. All of the strains produced T-2 toxin, and 6 of the 11 strains produced diacetoxyscirpenol at 25°C (Table 1). The culture material of two strains (MRC 3933 and MRC 3936) yielded relatively high amounts of T-2 toxin. The yield of 2,600 mg/kg from MRC 3936 at 25°C is among the highest yields of T-2 toxin ever obtained and is much higher than those previously reported at temperatures above 20°C. This high yield of T-2 toxin was verified by repeated analysis of independent cultures.

The diacetoxyscirpenol yields of all the producing strains were low (0.6 to 8.4 mg/kg) compared with the amounts described in previous reports (12). Seven strains produced both T-2 toxin and diacetoxyscirpenol, and MRC 3936 was the highest producer of both trichothecenes (Table 1).

To verify the identity of the T-2 toxin detected in these cultures, the compound was isolated and purified from a culture of strain MRC 3936 and subjected to mass spectral analysis. The extraction and purification was performed in the same way as for gas chromatographic analysis except on an eight times larger scale, and the fraction containing the T-2 toxin was further purified by two consecutive preparative thin-layer chromatography steps on preparative silica thin-layer chromatography plates. The mass spectrum (Fig. 1) of this isolated fraction (70 eV, probe temperature 130°C) showed excellent agreement with that reported for T-2 toxin (6, 15).

The production of unspecified levels of T-2 toxin by *F. acuminatum* has been reported for one isolate from fescue

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**FIG. 1.** Mass spectra of (A) the toxin isolated from *F. acuminatum* MRC 3936 and (B) standard T-2 toxin as previously reported (15).
hay in the United States (4), one isolate from oats in Germany (9), and 13 of 14 isolates from wheat and barley in Japan (8). The levels of T-2 toxin produced by three of four isolates of F. acuminatum from wheat and barley in Japan were fairly low, 8.35 to 37.15 mg/kg (19).

One isolate each of F. sulphureum (10) and F. heterosporum (7), which have been reported to produce T-2 toxin, were subsequently identified as F. acuminatum (12). The former isolate produced 0.38 to 37.5 mg/kg at 15°C and 1.5 mg/kg at 25°C (10). The other one produced unspecified amounts of T-2 toxin at 27 to 30°C (7).

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LITERATURE CITED
