Incidence and Toxigenicity of Seedborne Fusarium Species from Annual Medicago Species in South Africa

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ABSTRACT


Six Fusarium species were isolated from seed of annual Medicago species (M. littoralis and M. truncatula) in a field grazed by sheep near Swellendam, South Africa. F. acuminatum was the predominant fungus isolated from seeds collected during summer and F. reticulatum from seeds collected during winter. F. avenaceum, F. equiseti, F. graminearum Gr. I and F. sambucinum occurred less frequently. Cultures on autoclaved corn of five single-conidial isolates of each of the six Fusarium species were tested for toxicity in 2-day-old ducklings. All Fusarium isolates with the exception of four isolates of F. equiseti caused 100% mortality. Cultures on corn of one isolate of each species were dosed to sheep by means of a stomach tube; F. acuminatum, F. avenaceum, and F. reticulatum were lethal. Single doses of 5 g/kg of culture material of F. acuminatum and F. reticulatum caused death within 2 and 18 hr, respectively, whereas culture material of F. avenaceum administered twice at 5 g/kg caused death within 48 hr. Moniliformin was the most abundant of the five mycotoxins analyzed in culture material of the three species lethal to sheep. A single dose of 10 mg/kg of crystalline moniliformin resulted in death of a sheep within 18 hr. These results indicate that toxigenic Fusarium species associated with medics present a potential hazard to grazing animals.

Annual Medicago species (medics) are important pasture legumes in the winter rainfall region of the Cape Province of South Africa. During the dry summer months (October to March) sheep on some farms survive exclusively on medic seedpods. Several workers have isolated Fusarium species from medic seed in Australia (5,6,10,15). Lamprecht et al. (7) reported that Fusarium species were commonly associated with medic crowns and roots in the Cape Province, but no information is available on the occurrence of Fusarium species on medic seed in South Africa. The potential danger of toxigenic Fusarium species associated with medic seeds to grazing animals has not been assessed (9). This paper reports on the incidence and toxigenicity of Fusarium species associated with medic seed in South Africa.

MATERIALS AND METHODS

Incidence of Fusarium species. Medic seedpods were collected in a medic field grazed by sheep near Swellendam, Cape Province,
during the summer (March) and winter (July) of 1984. The field had been planted to *Medicago littoralis* 'Rhode Harbinger' and *M. truncatula* Gaertn. 'Jemalong' and 'Hannaford'. Seeds were separated from seedpods and 242 seeds collected in summer and 400 collected in winter were surface-disinfested for 5 min in 1% sodium hypochlorite, plated on 2% malt extract agar containing 0.02% novocrostomycin, and incubated at room temperature (20–25°C) for 5 days. All cultures of *Fusarium* species that grew from the seeds were started from single conidia and grown on potato-dextrose agar and carnation-leaf agar (3) containing 0.02% novocrostomycin in divided petri dishes. Cultures were incubated at 20°C under a light bank with one 40 W cool-white fluorescent and one 40 W black light (Philips TL 40W/80 RS F40HL.B) tube suspended 25 cm above the cultures. *Fusarium* cultures were identified according to Nelson et al. (11). Single-conidial cultures of representative *Fusarium* isolates were preserved by lyophilization and deposited in the culture collection of the South African Medical Research Council (MRC).

**Toxicity tests.** Suspensions of lyophilized conidia of five isolates of each *Fusarium* species were used to inoculate autoclaved, moistened yellow corn kernels (400 g of corn and 400 ml of distilled water in 2-L glass fruit jars). Cultures were incubated at 25°C for 21 days, dried at 50°C, milled, mixed with commercial chicken feed (50%, by weight), and fed ad libitum to groups of four 1-day-old Pekin ducklings for 14 days (12). Culture material toxic to ducklings of one isolate of each *Fusarium* species was dosed to a sheep by means of a stomach tube. The dosage rate was 5 g of culture material per kilogram of body weight per day. A single dose of 10 mg/kg of crystalline moniliformin dissolved in water was administered to a sheep by means of a stomach tube. Moniliformin was isolated as the sodium salt from corn cultures of *F. graminearum* (Wollenw. & Reinking) Nelson, Toussoun & Marasas strain MRC 115 by a modification of the procedure of Steyn et al. (13). The crystalline product containing one molecule of crystalline water was 98% pure. A control sheep was dosed with uninoculated, autoclaved corn meal. All the sheep were 6 to 10-mo-old and weighed 29–36 kg.

**Chemical analyses.** Culture material of *Fusarium* isolates dosed to sheep were analyzed chemically for *Fusarium* mycotoxins. Moniliformin was determined by the high-performance liquid chromatography (HPLC) procedures described by Thiel et al. (14). Zearealenone was extracted and the extracts purified by the standard procedure of the Association of Official Analytical Chemists (4). Quantification of zearealenone was done by HPLC using a fluorescence detector and confirmation by thin-layer chromatography. The trichotheccenes diacetoxyscirpenol, deoxynivalenol, and T-2 toxin were determined by capillary gas chromatography and electron capture detection.

**RESULTS**

**Incidence of seedborne *Fusarium* species.** Six *Fusarium* species were isolated from *Medicago* (M. *littoralis* and *M. truncatula*) seeds (Table 1). The predominant species isolated were *F. acuminatum* Ell. & Ev. sensu Gordon (22.3%) from seeds collected in summer, and *F. reticulatum* Mont. (6.5%) from seeds collected in winter. *F. avenaceum* (Fr.) Sacc., *F. equiseti* (Corda) Sacc., *F. graminearum* Schwabe Group 1, and *F. sambucinum* Fuckel occurred less frequently.

**Toxicity.** All five isolates of five out of the six *Fusarium* species tested caused 100% mortality in ducklings. In the case of *F. equiseti*, only one isolate was toxic.

One isolate of each *Fusarium* species toxic to ducklings was also tested for toxicity to sheep (Table 2). Cultures of *F. acuminatum*, *F. avenaceum*, and *F. reticulatum* were lethal to sheep. Single doses of 5 g/kg of culture material of *F. acuminatum* and *F. reticulatum* caused death within 2 and 18 hr, respectively. Culture material of *F. avenaceum* administered twice at 5 g/kg caused death within 48 hr. The isolates of *F. equiseti*, *F. graminearum* Group 1, and *F. sambucinum* that were highly toxic to ducklings, caused no abnormal clinical signs in sheep (Table 2). Ten doses of 5 g/kg of uninoculated, autoclaved corn meal caused no deleterious effects in the control sheep.

Moniliformin was the main mycotoxin detected in culture material of the three *Fusarium* species lethal to sheep (Table 2). Moniliformin levels in the highly toxic culture material of *F. acuminatum* MRC 3311 and *F. reticulatum* MRC 3636 were 4,200 and 4,800 mg/kg, respectively, and both of these isolates also produced small amounts of diacetoxyscirpenol (1.8 and 1.3 mg/kg, respectively). The toxic culture material of *F. avenaceum* MRC 3302 contained 520 mg/kg of moniliformin, but no diacetoxyscirpenol. Small amounts of zearealenone (2.5 and 2.8 mg/kg) were produced by *F. equiseti* and *F. graminearum* Group 1. Deoxynivalenol and T-2 toxin were not detected in any of the samples. *F. sambucinum* did not produce any of the mycotoxins analyzed.

On the basis of the moniliformin levels detected in the culture material of *F. acuminatum* MRC 3311, *F. avenaceum* MRC 3302, and *F. reticulatum* MRC 3636 (Table 2), sheep dosed with cultures of these three species received 21.0, 5.2, and 24.0 mg/kg, respectively, of moniliformin. Dosing of a sheep with a single dose

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**TABLE 1. Incidence of *Fusarium* spp. in seed of annual *Medicago* spp.**

<table>
<thead>
<tr>
<th><em>Fusarium</em> spp.</th>
<th>Incidence (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summer</td>
</tr>
<tr>
<td><em>F. acuminatum</em></td>
<td>22.3</td>
</tr>
<tr>
<td><em>F. avenaceum</em></td>
<td>3.7</td>
</tr>
<tr>
<td><em>F. equiseti</em></td>
<td>7.0</td>
</tr>
<tr>
<td><em>F. graminearum</em> Gr. 1</td>
<td>0.8</td>
</tr>
<tr>
<td><em>F. reticulatum</em></td>
<td>0.0</td>
</tr>
<tr>
<td><em>F. sambucinum</em></td>
<td>4.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Percentage of seeds from which each *Fusarium* sp. was isolated based on 242 seeds collected in summer and 400 in winter.

**TABLE 2. Toxicity of *Fusarium* spp. isolated from seed of annual *Medicago* spp.**

<table>
<thead>
<tr>
<th><em>Fusarium</em> spp.</th>
<th>Isolate no. (MRC)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Duckling toxicity (no. dead/no. tested)</th>
<th>Sheep toxicity&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Mycotoxins produced (mg/kg)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Times dosed (no.)</td>
<td>Total amount dosed (g)</td>
<td>Result</td>
</tr>
<tr>
<td><em>F. acuminatum</em></td>
<td>3311</td>
<td>4/4</td>
<td>135</td>
<td>145</td>
</tr>
<tr>
<td><em>F. avenaceum</em></td>
<td>3302</td>
<td>4/4</td>
<td>360</td>
<td>260</td>
</tr>
<tr>
<td><em>F. equiseti</em></td>
<td>3685</td>
<td>4/4</td>
<td>1,600</td>
<td>850</td>
</tr>
<tr>
<td><em>F. graminearum</em> Gr. 1</td>
<td>3655</td>
<td>4/4</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td><em>F. reticulatum</em></td>
<td>3636</td>
<td>4/4</td>
<td>1,500</td>
<td>1,500</td>
</tr>
<tr>
<td><em>F. sambucinum</em></td>
<td>3307</td>
<td>4/4</td>
<td>1,500</td>
<td>1,500</td>
</tr>
</tbody>
</table>

<sup>b</sup>South African Medical Research Council.

<sup>c</sup>Sheep were dosed by means of a stomach tube at a rate of 5 g of culture material per kilogram body weight per day.

<sup>d</sup>Deoxynivalenol and T-2 toxin were not detected in any of the samples.

<sup>e</sup>N.D. = Not detected.
of 10 mg/kg of crystalline moniliformin resulted in death within 18 hr. The main pathological change in this sheep, as well as the sheep dosed with moniliformin-containing culture material of F. acuminatum, F.avenaceum, and F. reticulatum was degeneration of the proximal tubules of the kidneys.

DISCUSSION

Four of the six Fusarium species (F. acuminatum, F.avenaceum, F. equisetii, and F. graminearum Gr. 1) isolated from medic seed previously have been reported to be associated with diseased root and crown tissue of annual Medicago species in South Africa (7). This is the first report of the occurrence of seedborne Fusarium species in medic in South Africa. The predominant species isolated from seed was F. acuminatum. This species previously has been isolated from medic seed in Australia (10,15). F. acuminatum is known to be commonly associated with diseased root and crown tissue of medic in South Africa, but is weakly pathogenic to M. truncatula with only some isolates causing preemergence damping-off (7). However, F. acuminatum is considered to be an important pathogen of medic in Victoria, Australia (2). F.avenaceum was isolated from medic seeds collected in summer and in winter and previously has been recorded from seed of M. truncatula (5,6,10) and other annual Medicago species (5,10,15) in Australia. F.avenaceum is an important and widespread pathogen of medic in South Africa (7) and Australia (2,10) and the role of seedborne inoculum in the pathogenesis of the root and crown rot caused by this organism requires further investigation. F. graminearum Gr. 1 is also pathogenic to M. truncatula ‘Jemalong’ in South Africa (7). This species was isolated from a low percentage of seeds collected in summer and has not previously been recorded from medic seed. Another Fusarium species isolated from medic seed for the first time was F. reticulatum, which was the predominant species associated with seed in winter. It is not known whether F. reticulatum is pathogenic to medic.

Cultures on corn of all the Fusarium isolates from medic seed, with the exception of some isolates of F. equisetii, were lethal to ducklings and three species (F. acuminatum, F.avenaceum, and F. reticulatum) to sheep. The main mycotoxin produced by these three species was moniliformin. Both F. acuminatum (12) and F.avenaceum (18,9,12) have been reported to produce moniliformin. This is the first report of moniliformin production and the toxicity of F. reticulatum to animals. The toxicological data obtained with F. reticulatum strain MRC 3636 and F. acuminatum strain MRC 3311 were, however, identical and the taxonomy of these two species (11) requires further investigation.

The finding that a single dose of 10 mg/kg of crystalline moniliformin caused rapid death and similar kidney lesions in a sheep confirms that moniliformin was responsible for the high degree of toxicity of F. acuminatum, F.avenaceum, and F. reticulatum to sheep. Although degeneration of the proximal tubules of the kidneys appears to be a constant pathological change in moniliformin intoxication of sheep, it is not necessarily a diagnostic lesion. Kidney lesions have not been reported in sheep grazing medic seedpods in nature.

These results indicate that toxigenic Fusarium species associated with medic seeds, and particularly high moniliformin-producing strains of F. acuminatum and F. reticulatum, present a potential danger to grazing sheep. Moniliformin has, however, not been reported to occur naturally in medic seeds and no field outbreaks of a mycotoxicosis in animals grazing medic seedpods have been reported.

LITERATURE CITED