

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

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ABSTRACT

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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. 1 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 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 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 medic seeds 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.

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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% novostreptomycin, 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% novostreptomycin in divided petri dishes. Cultures were incubated at 20 C under a light bank with one 40W cool-white fluorescent and one 40W black light (Philips TL 40W/80 RS F40BLB) 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. subglutinans* (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 used 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). Zearalenone was extracted and the extracts purified by the standard procedure of the Association of Official Analytical Chemists (4). Quantification of zearalenone was done by HPLC using a fluorescence detector and confirmation by thin-layer chromatography. The trichothecenes 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 medic (*M. littoralis* and *M. truncatula*) seed (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.

Toxicogenicity. 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* Gr. 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 zearalenone (2.5 and 2.8 mg/kg) were produced by *F. equiseti* and *F. graminearum* Gr. 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

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

<i>Fusarium</i> spp.	Incidence (%) ^a	
	Summer	Winter
<i>F. acuminatum</i>	22.3	4.5
<i>F. avenaceum</i>	3.7	2.3
<i>F. equiseti</i>	7.0	1.3
<i>F. graminearum</i> Gr. 1	0.8	0.0
<i>F. reticulatum</i>	0.0	6.5
<i>F. sambucinum</i>	4.1	1.0

^aPercentage of seeds from which each *Fusarium* sp. was isolated based on 242 seeds collected in summer and 400 in winter.

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

<i>Fusarium</i> spp.	Isolate no. (MRC) ^a	Duckling toxicity (no. dead/no. tested)	Sheep toxicity ^b		Mycotoxins produced (mg/kg) ^c			
			Times dosed (no.)	Total amount dosed (g)	Result	Moniliformin	Diacetoxyscirpenol	Zearalenone
<i>F. acuminatum</i>	3311	4/4	1	145	Death in 2 hr	4,200	1.8	N.D. ^d
<i>F. avenaceum</i>	3302	4/4	2	360	Death in 48 hr	520	N.D.	N.D.
<i>F. equiseti</i>	3659	4/4	10	1,600	No effect	N.D.	N.D.	2.5
<i>F. graminearum</i> Gr. 1	3655	4/4	10	850	No effect	20	N.D.	2.8
<i>F. reticulatum</i>	3636	4/4	1	160	Death in 18 hr	4,800	1.3	N.D.
<i>F. sambucinum</i>	3307	4/4	10	1,500	No effect	N.D.	N.D.	N.D.

^aSouth African Medical Research Council.

^bSheep were dosed by means of a stomach tube at a rate of 5 g of culture material per kilogram body weight per day.

^cDeoxynivalenol and T-2 toxin were not detected in any of the samples.

^dN.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. equiseti*, 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 medics 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 medics 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 medics 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 medics 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 medics.

Cultures on corn of all the *Fusarium* isolates from medic seed, with the exception of some isolates of *F. equiseti*, 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* (1,8,9,12) 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.

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