Fusarium Glume Spot of Wheat: A Newly Recorded Mite-Associated Disease in South Africa

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ABSTRACT

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Fusarium poae was frequently isolated from necrotic lesions on wheat glumes in South Africa. Glume infections were usually associated with the mycophageous mite *Siteroptes avenae*. Microscopic examination of *S. avenae* feeding on *F. poae* cultures revealed the presence of two elongated sporothecae containing microconidia of the fungus. Spray inoculation of adult wheat plants with a suspension of *F. poae* microconidia produced water-soaked lesions on leaves and black chaff-like symptoms and necrotic awns. When the suspension was injected through the glumes into florets, or when *F. poae*–fed mites were transferred to the glumes of uninfected plants, symptoms typical of those observed in the field were reproduced. A close association appears to exist between *S. avenae* and *F. poae* and evidence suggests that both the mite and fungus are responsible for causing Fusarium glume spot of wheat in South Africa.

Head blight or scab of wheat (*Triticum aestivum* L.) is one of the most destructive diseases in humid and semihumid areas (3, 7). *Gibberella zeae* (Schwein.) Petch (anamorph *Fusarium graminearum* Schwabe) has been reported as the most common cause of head blight (3). There are, however, reports of other species of *Fusarium* associated with head blight of wheat (3, 25,30,31). In South Africa, the occurrence of head blight was first reported in 1988 (24) but it was first noticed by the same authors on irrigated wheat in the Northwest province in 1980. Head blight has since spread to all wheat-growing areas in South Africa (19,24).

In recent years, wheat spikes displaying conspicuous necrotic glume spots (Fig. 1A) not typical of head blight were frequently observed in the winter wheat region of the Free State and Eastern Transvaal provinces, and on autumn-sown, irrigated spring wheat in Kwazulu-Natal, South Africa. Glumes of diseased plants had necrotic lesions surrounded by dark brown borders. These borders were often more pronounced toward the base of the glume. In general, only a few florets per spike were symptomatic. Awns of diseased spikes appeared healthy. The aim of this study was to identify the causal agent of wheat glume spot and its possible association with mites that were frequently observed on diseased spikes.

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MATERIALS AND METHODS

Isolation and identification of fungus and mite. Wheat spikes exhibiting necrotic spots were collected in northeastern Free State, Eastern Transvaal, and Kwazulu-Natal in South Africa. Glume segments from 102 spikes were incubated in moist chambers and fungi growing from these lesions were microscopically examined. No evidence of bacterial exudate or *Septoria* spp. was obtained in any of these isolations. A fungus consistently associated with the necrotic spots was provisionally identified as a *Fusarium* sp. Mycelium of these isolates was transferred to carnation leaf agar (CLA) (11,22). Conidia were transferred from the carnation leaf segments to McCartney bottles containing 5 ml of sterilized water. The bottles were shaken and 2% water agar (WA) plates flooded with this spore suspension. Flooded petri dishes were drained after 15 min and incubated for 24 h at 25°C in the dark. In total, 352 germinated conidia, representative of isolates obtained from all areas surveyed, were transferred to CLA and Difco potato dextrose agar (PDA) for identification.

Most of the *Fusarium* cultures originating from fungi that grew from lesions in moist chambers were infested with mites. Field observations also indicated that mites commonly occurred on wheat plants with glume spot symptoms. To establish a mite colony, gravid (fertilized) females were transferred to cultures of the *Fusarium* sp. most frequently isolated from lesions. The third mite generation in culture, including males that are required for identification (21), was used for determination of the species.

Inoculation. Inoculum of the *Fusarium* sp. derived from diseased glumes was prepared by culturing the fungus on PDA for 10 days at 25°C in the dark. Two mite-free agar cultures covered with mycelium were blended in 200 ml of sterile water for 10 s. The suspension containing microconidia and mycelial fragments was then filtered through sterile cheesecloth and either atomized onto Palmiet wheat plants, or 0.5 ml was injected into individual florets of 20 plants just before anthesis (growth stage 59) (29). Plants sprayed or injected with sterile water served as control treatments. Following an 18-h dew period, plants were transferred to a greenhouse where they were maintained at 20 to 25°C. Daylight was supplemented with photosynthetically active radiation from cool-white fluorescent tubes at 120 $\mu E\ s^{-1}\ m^{-2}$ each day.

Mite/glume spot relationship. To establish the role of mites in the symptomology of glume spot, mite-infested *Fusarium* cultures were grown on PDA. A mite population was obtained by transferring gravid females from wheat spikes with glume spot symptoms to *Fusarium* cultures. Petri dishes with third generation colonies of *Fusarium*-fed mites and lids ajar were placed within the canopy of healthy Palmiet plants on a bench in a greenhouse.

Additional colonies of *Fusarium*-fed mites were killed in an ethyl acetate vapor and microscopically examined for the presence of fungi. For light microscopy, mites were mounted in Hoyer's solution (13) and examined using interference and bright-field illumination technology. For scanning electron microscopy, specimens were fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (pH = 7.0) and post-fixed in 1% osmium tetroxide in the same buffer. Mites were dehydrated in an ethanol series, critical point dried, and coated with gold/palladium using a Biorad sputter coater. Specimens were examined with an ISI 100A scanning electron microscope.

Seedborne association of *Fusarium* sp. Twenty-nine seed samples of the commercial wheat cultivars Flamink and Molopo (one sample each), Molen (three samples), Betta (four samples), SST102

(five samples), Karee (six samples), and SST124 (nine samples) were collected in eastern Free State. Two hundred kernels per sample were randomly selected. Of these, 100 kernels were surface sterilized for 1 min in a mixture of 1% sodium hypochlorite and 16.5% sodium chloride. Ten kernels were placed per petri dish containing WA. The plates were incubated for 5 days at 25°C in the dark. Single conidial colonies from all infected kernels were transferred to PDA and CLA. These cultures were examined to differentiate between the *Fusarium* sp. consistently associated with glume spot and other fungi.

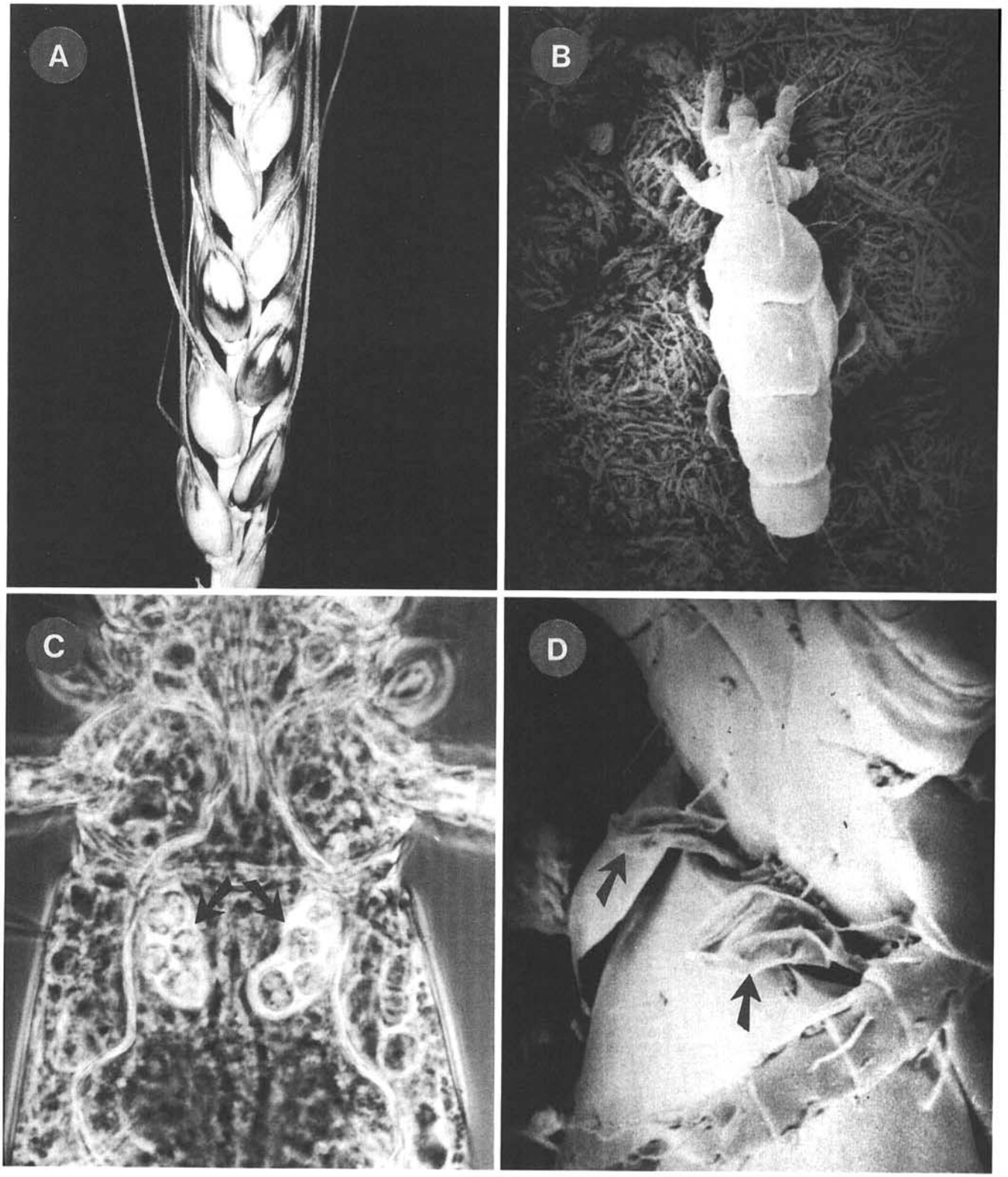

Fig. 1. Wheat spike displaying typical glume spot symptoms observed under field conditions **(A)**; scanning electron micrograph of the dorsum of the mite *Siteroptes avenae* consistently associated with Fusarium glume spot of wheat **(B)**; two kidney-shaped, sac-like structures identifiable as sporothecae (arrows) containing microconidia of *Fusarium poae* **(C)**; ventral view of sporothecae (arrows) protruding behind the podosomal plate **(D)**.

RESULTS

Isolation and identification. The *Fusarium* sp. associated with glume spot of wheat was identified as *Fusarium poae* (Peck) Wollenweb. and deposited (PREM 49277; PPRI 3414) in the National Collection of Fungi, Pretoria, S.A. The identification of *F. poae* was based on the abundant production of ampulliform (napiform) to globose microconidia on doliform monophialides (4,22) and the characteristic fruity aroma of the cultures (22). The color of the cultures from below varied from white-yellow to salmon (4,22). The mite (Fig. 1B) was identified as *Siteroptes* (= *Pediculopsis*) *avenae* (Muller), a species belonging to the *S. cerealium* complex (28).

Inoculation. Upon removal of the spray-inoculated plants from the dew chamber, leaves exhibited extensive water-soaked lesions. Leaves and awns became necrotic within 6 days after inoculation. This resulted in premature defoliation of plants. Furthermore, glumes displayed symptoms similar to basal glume rot or black chaff caused by bacterial pathogens of wheat (30). Typical glume spots, almost resembling the description of basal glume rot given by Zillinsky (32), developed only on those florets inoculated with a syringe. Lesions were confined to the inoculated glume and lemma and did not spread to glumes of adjacent florets. *Fusarium poae* was re-isolated from all tissue displaying symptoms. Other than the inoculation marks on florets injected with sterile water, no visible symptoms developed on any of the control treatments.

Mite/glume spot relationship. Symptoms typical of those observed in the field (Fig. 1A) were reproduced 3 weeks after petri dishes with fungus-fed mites had been placed among healthy plants. Up to six spikelets per ear became diseased. *Fusarium poae* was isolated from all tissues showing symptoms.

Using light microscopy, two kidney-shaped, sac-like structures identifiable as sporothecae (Fig. 1C) containing microconidia of *F. poae* were clearly visible in female mites. Most mites examined with the scanning electron microscope had their sporothecae retracted. In a few cases sporothecae could be observed protruding behind the podosomal plate (Fig. 1D). Conidia had apparently been released from these inverted sporothecae.

Table 1. Percentage seed of different wheat cultivars infected with *Fusarium poae*

Cultivar	No. of samples	Percentage of infected seed[a]	
		Non-sterilized	Sterilized
SST124	9	2.44	2.33
Karee	6	2.67	1.17
SST102	5	3.20	1.00
Betta	4	0.25	0
Molen	3	1.70	0.67
Molopo	1	0	0
Flamink	1	4.00	0
Mean		2.21	1.21

[a] Percentage was calculated from 100 seeds per sample.

Seedborne association of *F. poae*. The isolation frequencies of *F. poae* from field grown seed samples are presented in Table 1. A mean of 2.21% of all the seed examined before surface sterilization, and 1.21% after sterilization, was colonized by *F. poae*.

DISCUSSION

Fusarium poae occurs widely in soils in temperate regions and has been characterized as a saprophyte or weak parasite of grasses and herbaceous plants (9,18). *Fusarium poae* was not isolated in a comprehensive survey of *Fusarium* spp. associated with plant debris in local soils (17), but has been isolated from sugarcane (5) and corn (20) in South Africa. The occurrence of *F. poae* on commercial wheat seed in the present study is in accordance with other reports of the recovery of the fungus from cereal grains (9,10,12,14,16,18) or blighted wheat spikes (1,25,31). Wiese (30) described *F. poae* as a weak parasite of the roots and crowns of wheat. *Fusarium poae* has also occurred in smutted barley spikes (6) and Sturz and Johnston (26) demonstrated that the fungus can be isolated from symptomless wheat and barley spikes.

The mite *Siteroptes cerealium* (Kirchner) (= *Siteroptes graminum* [Reuter]) in association with *F. poae* has been reported to cause silver-top of grasses (23,28) and bud rot of carnations (8). Silver-top disease is ubiquitous on wild and cultivated grasses in Europe, Canada, and the U.S. More than 30 species of grasses, including wheat, barley, and rye, serve as hosts. Economically, it has been considered an important disease and seed production losses as high as 100% have been reported (23, 28). Although three species of *Siteroptes* have been associated with the *S. cerealium* complex (28), no reference to the occurrence of these species in South Africa could be found. Therefore, this is the first report of the occurrence of *S. avenae* and its relationship with *F. poae* causing glume spot of wheat in South Africa.

Some controversy regarding the feeding habits of *Siteroptes* spp. exists. Krantz and Lindquist (15) stated that *Siteroptes* spp. feeding on cultivated grains, grasses, and other herbaceous plants have been reported to cause malformed, stunted, or silvered leaves of infested plants. Other studies showing that *Siteroptes* spp. can be sustained on *F. poae* cultures for several generations provided evidence that these mites are in fact fungivorous (15,28). Agrios (2) stated that the mites feed on decomposed host tissue as well as the fungus. Krantz and Lindquist (15) concluded that *Siteroptes* spp. feed primarily on fungi and that phytophagy is probably only incidental. They did, however, recognize the significance of phytophagy in mites that colonize aerial substrates of higher plants compared with those inhabiting soil litter.

Laboratory studies have confirmed the specificity and mutualistic association between *S. avenae* and *F. poae* (27). In preliminary studies of the preference of *S. avenae* for different *Fusarium* spp., the presence of gravid females was conspicuous in *F. poae* cultures. The occasional mite, but no gravid females, was observed in cultures of *F. graminearum*, *F. crookwellense* L. W. Burgess, P. E. Nelson & T. A. Toussoun, *F. culmorum* (Wm. G. Smith) Sacc., and *F. moniliforme* J. Sheld. (G. H. J. Kemp, *unpublished*).

Instances of sporothecae aiding fungus dispersal were previously recorded for mites of the *S. cerealium* complex, which includes *S. cerealium*, *S. avenae,* and *S. graminisugus* (Hardy) (28). *Siteroptes avenae* and *S. graminisugus*, in contrast to *S. cerealium*, are known to possess sporothecae behind the propodosomal plate (Fig. 1D). They include two elongate, sleeve-like sacs closed at one end. Usually the sporothecae are withdrawn inside the body. The microconidia of *F. poae* are probably gathered into the sporothecae when the mites crawl over mycelium, and then are discharged by eversion of the sporothecae due to changes in internal body pressure (28). Observations made in the present study are consistent with previous reports (15,28) that concluded that these mites are essentially fungivorous and that nourishment obtained from green plants is not necessary for their survival.

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