Disease Analysis through Genetics and Biotechnology

INTERDISCIPLINARY BRIDGES TO IMPROVED SORGHUM AND MILLET CROPS

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Iowa State University Press / Ames

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This book has been produced directly from camera-ready copy supplied by the editors.

♾ Printed on acid-free paper in the United States of America

First edition, 1995

Library of Congress Cataloging-in-Publication Data

Disease analysis through genetics and biotechnology: interdisciplinary bridges to improved sorghum and millet crops / edited by John F. Leslie and Richard A. Frederiksen.

p. cm.

Includes bibliographical references and index.

ISBN 0-8138-2125-8

1. Sorghum—Diseases and pests. 2. Millet—Diseases and pests. 3. Sorghum—Disease and pest resistance—Genetic aspects. 4. Millet—Disease and pest resistance—Genetic aspects. 5. Fungal diseases of plants. I. Leslie, John F. II. Frederiksen, Richard A.

SB608.S6D57 1995

633.1'7499—dc20 94-46977

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Fusarium Stalk Rot in Argentina

Laura M. Giorda, María José Martínez and Sofia Chulze

Abstract

Sorghum crop in Argentina is grown under dryland conditions in several distinct ecological regions. Water deficits may occur during the growing season in some of these regions. Lodging, root and stalk rot are usually associated with drought stress and photosynthetic stress induced by greenbug damage during grain filling. The fungal isolates most prevalent are species of *Fusarium* from the *Liseola* section with *Fusarium moniliforme* (= *Gibberella fujikuroi*) as the most serious causal agent of root and stalk rot and grain mold. Greenbug resistance is closely correlated with lodging resistance and is used as an indicator during selection. Research initiated on screening techniques for resistance on early growth stages of the sorghum plant is discussed. Areas of research needed in Argentina are listed.

Introduction

Forage and grain sorghum in Argentina are cultivated throughout the subtropical and temperate regions between latitudes 22-40° S and longitudes 56-66° W. Grain sorghum is grown primarily as a feed crop on approximately 800,000 hectares in dryland areas and is usually cultivated in a rotation system with either soybeans or peanuts. Sorghum production is concentrated in the states of Córdoba, Santa Fe, Entre Ríos, and La Pampa.

The major sorghum diseases in Argentina are grain mold, stalk

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and root rot (induced primarily by *Fusarium moniliforme* Sheldon), bacterial leaf stripe (*Pseudomonas andropogoni* Stapp), downy mildew (*Peronosclerospora sorghi* Weston & Uppal), and maize dwarf mosaic (MDMV). Research is being conducted at Instituto National de Tecnologia Agropecuraria (INTA) in coordination with National University of Córdoba on different aspects of these diseases.

Root and Stalk Rot Diseases in Sorghum

In Argentina, *Fusarium* root and stalk rot was identified from diseased sorghum plants in the states of Córdoba and La Pampa in 1963. Carrera [3] described the causal agent as *F. moniliforme*. Other species commonly associated with stalk rot in Argentina are *Macrophomina phaseolina* (Tassi) Goid (=*Sclerotium bataticola*), *Fusarium subglutinans* (Wollenweber and Reinking) Nelson, Toussoun & Marasas, *Fusarium graminearum* Schwabe (=*Gibberella zeae*), *Bipolaris sorokinia* (Sacc.) Shoemaker (=*Helminthosporium sativum* Pamm, King & Bakke), *Nigrospora sphaerica* (Sacc.) Mason, *Rhizoctonia solani*, *Pythium* spp., and *Tetraploa ellisii* Cooke [6]. However, the most prevalent and the most serious inducing agent of this disease is *F. moniliforme* [12]. Frezzi [6] determined that 85% of the fungal population from diseased plants with stalk rot symptoms collected from different fields in the states of Buenos Aires, Córdoba, Santa Fe, and Chaco was *F. moniliforme*.

Recently a survey was made of diseased plants from three distinct geographic/ecological areas of the Argentine sorghum region: north subtropical, central temperate, and south, where the climate is semiarid to arid with lower temperatures. These areas differ in soil characteristics, relative humidity and temperature. Diseased plants with root and stalk rot symptoms were collected at harvest time and *Fusarium* spp. identified and classified using the characters suggested by Nelson et al. [14]. These observations indicated that the *Fusarium* species associated with the disease were *F. moniliforme*, *F. proliferatum* (Matsushima) Nirenberg, *F. semitectum* Berk. and Rav., *F. scirpi* Lamb. and Fautr., and *F. nygamai* Burgess and Trimboli [2]. *F. proliferatum* predominated in diseased material collected in the southernmost region of the sorghum belt (Bordenave, Buenos Aires).

In earlier literature on sorghum stalk rot in Argentina it is likely that *F. proliferatum* was not identified as a separate species and was included with *F. moniliforme*. There also have been changes in the type of crops grown and their rotation patterns since these earlier surveys were conducted. During the 1980s, soybeans replaced much of the maize and sorghum, and sorghum was relegated to the regions most limited in fertility and water availability. Monoculture of soybeans has been, at least in part, responsible for much of the recent soil deterioration in Argentina. Today, the cropping systems are changing again, usually to rotations between soybean and sorghum or maize. No-till and minimum tillage agronomic practices are also becoming widespread, especially in wheat and soybean cropping sequences. These changes in the crop production management system may be influencing the distribution and the composition of the pathogen population(s) and their incidence in plants with stalk rot disease symptoms. March et al. [11] noticed that the incidence of the disease was greatly reduced (71-88%) under minimum tillage when compared with conventional tillage practices. The minimum tillage cropping system decreases soil erosion and conserves soil moisture, both of which reduce stress to the crop. Other investigators also have indicated that certain cultural practices can play an important role in the control of Fusarium root and stalk rot [5].

Fusarium stalk rot reduces seed filling and also causes lodging, becoming a serious problem under some environmental conditions. Frezzi [6] and Maunder [12] reported yield losses of up to 80% in commercial sorghum fields in Argentina. Symptoms are not expressed during vegetative growth of the host, but are expressed under photosynthetic stress as the plants approach maturity. Trimboli and Burgess [18] showed, in greenhouse trials, that Fusarium stalk rot was associated with drought stress between flowering and mid-dough stages. In Argentina the two major factors that predispose sorghum plants to Fusarium stalk rot are water stress and photosynthetic stress caused by *Schizaphis graminum* Rond damage between flowering and mid-dough stages.

Fusarium infection may begin in the rootlets, under optimal soil moisture conditions, where it remains latent in symptomless plants until maturity. Once the sorghum plants reach maturity, necrosis of the roots takes place under environmental conditions favoring disease development, e.g. photosynthetic stress, and then extends centripetally inducing rotting and lodging. Frezzi [6] indicated that Fusarium stalk rot is a late season disease of mature plants and that the infection mechanism operates through the rootlets plugging the root system. Infection can also operate locally on the basal internodes. In such cases, the fungus is thought to enter the stalk at or near ground level, through wounds or natural cracks that extend into the stem.

Pande and Karunakar [15] reviewed the present status of knowledge and progress on different aspects of sorghum root and stalk rot diseases with emphasis on charcoal rot. Most of the literature focuses on the relationship between moisture stress during flowering or post-flowering stages and stalk rot, using screening and evaluation techniques based on

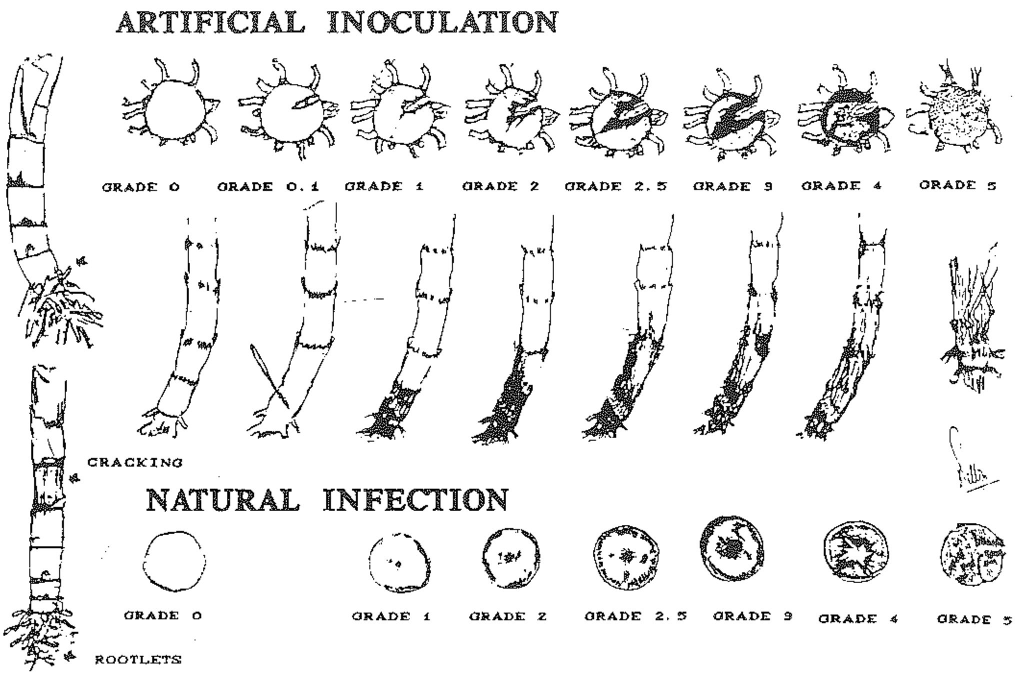

Figure 1. Rating scale for scoring Fusarium root and stalk rot.

induced water stress [1,8,16]. Little research, however, has been done on the predisposition of sorghum plants to stalk rot by greenbug stress [14]. Field observations from sorghum farms and commercial sorghum hybrid yield trials conducted at different experimental stations of INTA, suggested a close association between Fusarium stalk rot and greenbug incidence. To test this relationship an experiment was conducted at the Manfredi Experimental Station for two years with sorghum planted on two different dates (November and December) in each year [7, Giorda et al., unpublished). The incidence of *S. graminum* and Fusarium stalk rot was evaluated on 600 plants from 10 commercial grain sorghum cultivars with different levels of resistance to greenbug. This incidence was scored using a 0-5 scale. A rating of zero indicates no damage by aphids while a score of five denotes a susceptible cultivar with five or more dead leaves. *Fusarium* stalk rot severity was evaluated in longitudinal and transversal section according to a 0-5 scale, where a rating of zero means no symptoms and a rating of five corresponds to dead or lodged plants (Fig. 1). Half of the total plants (300 plants per cultivar) were sprayed with insecticide (Pirimicarb 50%, 75 g.a.i./ha) to control aphids. Within each treatment (sprayed

Table 1. Multiple correlation at two planting dates among yields, Fusarium stalk rot and *Shizaphis graminum*.

	November				December			
	Yi	Gr	Fm/l	Fm/t	Yi	Gr	Fm/l	Fm/t
Experiment 1								
Gr	-0.29[x]	1			-0.71*	1		
Fm/l	-0.26	0.48*	1		-0.69*	0.82*	1	
Fm/t	-0.28	0.44*	0.91*	1	-0.68*	0.80*	0.98*	1
Experiment 2								
Gr	-0.32	1			-0.53*	1		
Fm/l	-0.20	0.46*	1		-0.16	0.60*	1	
Fm/t	-0.49*	0.50*	0.99*	1	-0.12	0.53*	0.88*	1

Abbreviations: Yi = Yields; Gr = Greenbug damage; Fm/l = Damage by *F. moniliforme* longitudinal rating; Fm/t = Damage by *F. moniliforme* transversal rating.

[x]Pearson correlation coefficient (*) significant at $\alpha = 0.05/N = 600$.

and not sprayed with insecticide), 150 plants were inoculated with *F. moniliforme* using a toothpick technique. The other 150 plants were used as a control to check for natural *Fusarium* infection. Thus, a total of 300 plants were artificially inoculated. Yield per plant was obtained and correlated with the level of damage caused by greenbugs and *Fusarium* stalk rot (R'Pearson). Correlations between longitudinal and transversal Fusarium stalk rot scores and greenbug damage were always positive and significant, with higher correlations at the second planting date (Table 1). This high correlation indicates the importance of greenbugs in predisposing sorghum plants to *Fusarium* stalk rot.

Mughogho and Pande [13] working on charcoal rot indicated that any artificial inoculation technique is unsatisfactory because it does not closely simulate the natural infection process. Pande and Karunakar [15) also emphasize the uselessness of toothpick inoculations and similar methods because natural infection begins in the root and only later goes up to the stem. They state, "The level of disease development with toothpick inoculation is usually less than occurring naturally...".

Our results indicated, however, that the severity of stalk rot was greater following toothpick inoculation (Table 2). Since different infection mechanisms operate on the Fusarium root and stalk rot disease, the host

Table 2. Fusarium stalk rot of sorghum hybrids inoculated and naturally infected with *F. moniliforme* under two levels of greenbug infestation.

	Longitudinal rating[x]			Transversal rating[x]		
	Untreated[y]	Treated	Mean	Untreated	Treated	Mean
November planting						
Artificial inoculation[z]	2.93	2.06	2.49A	3.50	2.60	3.05A
Natural infection	1.41	0.55	0.98B	1.48	0.54	1.06B
Mean	2.17A	1.31B		2.49A	1.57B	
December planting						
Artificial inoculation	3.54	2.59	3.06A	3.81	2.87	3.34A
Natural infection	2.92	1.22	2.07B	2.99	1.36	2.17B
Mean	3.23A	1.90B		3.40A	2.11B	

[x]Stalks were split longitudinally or transversally and rated on a 0-5 scale; 0 = no infection, and 5 = dead, shredded, and/or lodged (see Fig. 1). Rank transformation was performed on stalk rot scores for statistical analysis [4]. Data shown are ranks backtransformed to stalk rot scores. Means followed by a common letter are not significantly different ($P \leq 0.10$).

[y]Half of the plots were sprayed with Pirimicarb (50% 75 g a.i/ha) to control greenbugs.

[z]Of 600 plants, half were artificially inoculated with *F. moniliforme* and the rest were scored for natural infection.

response to natural or artificial inoculation may vary. Bramel-Cox et al. [1] indicated that inoculations seemed to be a better method for consistent selection of resistance to stalk rot based on lower coefficients of variation for disease scores (14-15%) compared to natural occurrence of the pathogens (C.V. over 60%).

Different screening techniques have been used to breed for stalk rot resistance [8,12,15,16]. Although the toothpick method was useful under our field and greenhouse conditions, we are now using a *Fusarium* disease nursery where materials are planted late in the season and subjected to greenbug infestation. Germplasm selected in this way is tested again under water stress conditions during anthesis. A new sorghum hybrid, Percheron INTA, highly resistant to lodging was released at the Bordenave Experiment Station in 1993. This high yielding cultivar has red grain and tan plant color and was selected for resistance to greenbugs under high aphid pressure.

Research in progress. At the present time, we are investigating the association of *Fusarium* root and stalk rot incited at early vegetative stages with field tolerance. The disease was induced by subjecting 4-leaf stage sorghum plants, previously infected with *F. moniliforme* to 7-9 days of complete darkness [Taleisnik and Giorda, personal communication]. Neither the inoculated nor the non-inoculated (control) plants grown under alternating 12 hr periods of light and dark exhibited any disease symptoms. Significant differences ($P \leq 0.05$) were observed in the percentage of lodging between inoculated and uninoculated plants subjected to darkness. The mean percentage of lodging within inoculated plants ranged from 25 to 85%, depending upon the cultivar, with genotypes B7904 and B35 having some of the lowest lodging percentages. None of the non-inoculated (control) plants lodged.

Discussion

Fusarium spp. are the most important fungi associated with root and stalk rot of sorghum in Argentina and most isolates belong to section Liseola [teleomorph: *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura]. Many of the strains in this section have the potential to produce significant levels of fumonisin B_1 [10]. Different approaches were taken to distinguish species within the section Liseola, most recently the ability to form the sexual stage when crossed with standard testers [9]. In *G. fujikuroi* six mating populations have been recognized with different abilities to produce fumonisin [10]. Following the taxonomic system proposed by Nelson et al. [14], members of both of the A and the F mating populations are *F. moniliforme*, members of the B and E populations are *F. subglutinans*, and members of the D population are *F. proliferatum*. Although these members of Liseola section were isolated from grain, basal nodes and roots of sorghum plants in Argentina, studies of populations from and mycotoxin production in sorghum are still lacking. Sydenham et al. [17] detected fumonisins in Argentinean corn field trials contaminated with *F. moniliforme*. This fungal pathogen of maize and sorghum also incites grain mold, head blight, and occasionally pokkah boeng and seedling blight in sorghum. At this time, these diseases including stalk rot are not the major constraints to sorghum cultivation, but rather the lower the value of the crop compared to other grains as maize, soybean and sunflower.

Future Research Needs

1. Investigate the composition of *Fusarium* populations under the crop

management regime(s) associated with grain mold and root and stalk rot diseases.
2. Identify strains to mating population and determine their potential to produce mycotoxins.
3. Conduct pathogenicity studies of representative isolates from different species of *Fusarium* found in the sorghum-growing areas.
4. Determine genetics of host resistance and susceptibility.
5. Study the mechanisms of resistance.
6. Develop effective screening techniques for identifying resistance in the host, including screening programs for early stages of plant growth.
7. Identify suitable markers to use when screening for resistance.

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