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BACTERIAL STRIPE AND BACTERIAL LEAF BLIGHT OF SORGHUM: EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON DISEASE DEVELOPMENT, TOLERANCE AMONG SORGHUM HYBRIDS, AND OVERWINTER SURVIVAL OF THE INCITANT BACTERIA

by

MUHAMMAD AFZAL AKHTAR

B. S. West Pakistan Agricultural University, Lyallpur, 1970
M. S. West Pakistan Agricultural University, Lyallpur, 1972

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Table of Contents

List of Tables

Introduction

Three major bacterial diseases occur on sorghum (Sorghum bicolor); bacterial stripe (6) (incited by Pseudomonas andropogonis (E. F. Smith) Stapp), bacterial streak (7) (incited by Xanthomonas campestris pv. holcicola (Elliott) Dye (28), and bacterial spot (11) (incited by Pseudomonas syringae pv. syringae (van Hall). Thirteen other bacterial diseases have been reported on sorghum although the authenticity of several reports are doubtful. Bacterial diseases have received limited attention and their etiology is poorly understood despite the fact they occur on sorghum throughout the world (22).

P. andropogonis is also a common pathogen on corn (Zea mays L.) (24,26,27) and numerous other plants (2,8,9,16). Bacterial stripe symptoms appear on sorghum as elongated purple to tan stripes that usually are delimited by leaf veins. The lesions may extend from the sheath tissue to the leaf apex in those plants infected early in the season and coupled with warm, moist climatic conditions. The lesion color is generally constant within the same host genotype as the pigment production is a host response to injury. A copious amount of exudate is usually produced on the lower side of the leaves. A stalk rot phase has been observed in several sorghum genotypes (Claflin-unpublished).

P. avenae Manns (Syn. P. alboprecipitans Rosen) (18) causes bacterial leaf blight of corn and oats. Sorghum was also shown to be a host under greenhouse conditions (8,9) but P. avenae has not been isolated from lesions of naturally infected plants.

Bacterial stripe and bacterial leaf blight severity are associated

with warm, moist conditions (6) although definitive environmental conditions were not researched. The incitant bacteria were assumed to overwinter in infested seed, leaves and stalks and the bacteria were thought to be seedborne (6).

P. andropogonis and P. stizolobii isolates are synonymous in biochemical, physiological, cultural and slab gel tests (8,26) although serological procedures have not been reported. P. avenae was shown to be a different species from P. andropogonis and P. stizolobii (8).

The use of the fluorescent antibody staining (FAS) technique was suggested in 1943 (17) as a potentially useful procedure for detection and identification of plant pathogenic bacteria. Morton (15) reported the superiority of the direct FAS over an agglutination test for identifying Xanthomonas vesicatoria from diseased tissue. An indirect test was used to identify Erwinia aroideae in host tissue and soil extracts (12). Other uses of an indirect or direct FAS include detecting P. phaseolicola in bean seeds (13,23), P. solanacearum in soil (10), E. atroseptica in infected stalk and tuber tissue (1), X. campestris in soil (19) and Corynebacterium sepedonicum in bacterial ring rot infected tubers (21). A comprehensive listing of advantages and disadvantages of FAS and other serological procedures for detecting plant pathogenic bacteria was recently published (20).

Several aspects concerning the etiology of bacterial stripe and bacterial leaf blight including the effects of temperature and relative humidity, germplasm evaluation, and the use of FAS to detect incitant bacteria in overwintered host debris and in seeds are reported herein.

Materials and Methods

A. Hybrid evaluation:

Twenty-six grain sorghum hybrids were evaluated for tolerance to Pseudomonas andropogonis and P. avenae during the 1982 and 1983 growing seasons at the Rocky Ford Experimental Farm near Manhattan, KS. The seeds were planted during the first week of June in 7.6 m rows spaced 76.2 cm apart and seeds spaced 11.0 cm apart. The experimental design was a randomized complete block with four replications for each entry and treatment. Carbofuran 15 G (FMC Corporation, Philadelphia, PA) was applied (1.12 kg (a.i.)/ha) in a 17.8 cm band at planting for insect control. The plots were furrow-irrigated and cultivated as needed. Three plants from each row of each entry were inoculated with P. andropogonis (ATCC #23061, American Type Culture Collection, Rockville, MD) and P. avenae (PA 137, International Collection of Phytopathogenic Bacteria, Davis, CA) at 30, 40, and 50 days after planting in 1982 and 30 and 40 days in 1983. This approximated, respectively, Stage 3 (growing point differentiation; 7-10 leaves), Stage 4 (flag leaf visible in whorl)and stage 5 (boot stage) (25).

Bacterial stripe and bacterial leaf blight symptoms were evaluated on a 0-5 rating scale where: 0 = no visible symptoms, 1 = 1-10, 2 = 11-25, 3 = 26-50, 4 = 51-75, and 5 = 76-100% of the leaf area exhibiting symptoms. Notes were taken 10 days after each inoculation and again at physiological maturity. Duncan's multiple range test was used for statistical analysis.

B. Determination of temperature and relative humidity:

Six grain sorghum hybrids from Cargill Seeds, Minneapolis, MN; Pioneer Seeds, Johnston, IA; Funk Seeds, Bloomington, IL, and Taylor-Evans Seed Co., Tulia, TX were used to ascertain the temperature and relative humidity effects on disease development and to determine the virulence of various isolates of Pseudomonas andropogonis, P. avenae and P. stizolobii. Four seeds of each entry were planted in 7.6 cm plastic pots containing vermiculite. Ten day-old plants were inoculated by scratching the leaf surface with a 20-gauge needle and then depositing a drop of the bacterial inoculum on the injured tissue. Inoculated plants were covered (48 hours) with polyethylene bags and placed in a growth chamber (Model E15, Controlled Environments Ltd., Winnipeg, Canada). The chamber was maintained at various maximum-minimum temperatures and the desired relative humidity (RH) levels with a day length of 14 hours. Plants were evaluated 7 days after inoculation with a 0-5 scale as described previously. A randomized complete block design with four replications was used and the data analyzed with Duncan's multiple range test.

C. Inoculation procedure:

Stock suspensions of P. andropogonis and P. avenae were streaked on yeast extract-dextrose-calcium carbonate-agar (YDC) medium (5) and incubated for 96 hours at 28 C. The cells were removed with a sterile rubber spatula, suspended in 0.01 M phosphate buffer (pH 7.2) and adjusted to an optical density of 0.7 and 0.8 (OD = Klett-Summersons reading X 0.002) at 600 nm with a Klett-Summersons (Model 800-3) colorimeter. This approximated 1.9×10^8 colony forming units (CFU)/ml and 2.2×10^8 CFU/ ml for P. andropogonis and P. avenae, respectively.

Fresh inocula were prepared for each inoculation and the bacterial suspensions were maintained under refrigeration (4 C) until utilized in the field.

D. Preparation of antisera:

Stock cultures of Pseudomonas andropogonis (ATCC 23061) and P. avenae (ICPB PA 137) were streaked on (YDC) medium and incubated for 96 hours at 28 C. The cells were removed from the medium with a spatula and suspended in sterile 0.01 M phosphate buffered saline, pH 7.2 (PBS). Bacterial cells were washed (3X) by centrifuging at 12,000 rpm (SS-34 rotor) for 20 min, the supernatant was discarded and the pellet resuspended in sterile PBS. Bacteria were killed and proteins fixed by dialyzing (Spectrapor, Spectrum Medical Industries, Los Angeles, CA) in a 2% glutaraldehyde (Sigma Chem. Corp.,St. Louis, MO) solution for 3 hours at room temperature (1). Glutaraldehyde was removed by dialyzing the suspension in PBS at 4 C for 24 hours with 5-6 changes of buffer. One ml of the bacterial suspension (3.0×10^9 CFU/ml) was mixed with an equal volume of Freund's incomplete adjuvant (Difco, Detroit, MI) and emulsified with a Spex mixer-mill (No. 5100, Spex Industries, Metuchen, NJ) for 2 min.

Two ml of the emulsified antigens were injected subcutaneously into New Zealand white rabbits on a weekly basis. Normal serum was obtained from each animal prior to the inoculation sequence. After the fourth injection, blood was taken by bleeding the marginal ear vein. The titers were determined in agglutination tests by reacting successive two-fold antisera (AS) dilutions with the antigen (AG). The titer was the highest dilution at which discernable agglutination

occurred. Injections were continued on a weekly schedule until the titer was equal to or greater than 1:2560.

E. Conjugation procedure:

The conjugation procedure was similar to that reported by others (1,14). Antisera were diluted to 1% protein (10 mg/ml) by adjusting the absorbance with 0.1 M carbonate buffer (pH 9.5) in a Coleman 124 Spectrophotometer (Hitachi-Perkin, Japan) at 280 um. The equation, absorbance X dilution factor/1.3, was used to determine the quantity of protein. To facilitate conjugation, the AS pH was increased to 9.5 with 1 N NaOH. Five mg of fluorescein isothiocyanate (FITC) (Calbiochem, LaJolla, CA 92037) was dissolved in 8 ml of 0.1 M carbonate buffer (pH 9.5) and then added dropwise to the AS while stirring. The FITC-AS reaction continued for 6 hours with slow stirring in the dark at room temperature. Unconjugated FITC was removed by centrifuging at 15,000 rpm for 20 min. The supernatant was then passed through Sephadex G-25 (Pharmacia, Piscataway, NJ) gel filtration columns to remove smaller particles of unconjugated FITC. The pH of conjugated AS was lowered to pH 7.2 by dialyzing against 100-fold volumes of PBS in the cold for 72 hours. Conjugated AS were filter-sterilized (0.2 um pore size, Gelman, Ann Arbor, MI 48106) and stored at 4 C in serum bottles without preservatives.

F. Staining procedures:

Antisera were tested by smearing microscope slides with a suspension of the homologous antigens obtained from 48-hour-old colonies on YDC media. The slides were air-dried and a drop of conjugated sera was placed on the smear. The slides were incubated in a moist chamber

for 30 minutes in the dark at room temperature (1,3) and then rinsed in 3 changes of PBS (10 minutes/rinse). PBS was removed by immersing the slides (ca. 30 sec) in sterile double distilled water. A drop of PBS-buffered glycerol (0.02 M, pH 7.6, 1:9 v/v) was used for the mounting medium.

The prepared slides were examined with an Olympus microscope equipped with a 100 W mercury power light source. A FITC 8.7 excitation filter, 0530 barrier filter and a B 200 354 dichroic mirror were found to provide the maximum fluorescence with minimal background problems.

G. Overwintering Studies:

Sorghum hybrids were inoculated with isolates of P. avenae (ICPB PA 137) and P. andropogonis (ATCC 23061) during the 1982 and 1983 growing seasons to evaluate germplasm for sources of tolerance. The plants were left standing in the field without tillage to avoid disturbing the plant materials. Leaves, stalks and seeds of inoculated plants were collected every two weeks from February until mid-May. The specimens were washed with running tap water for 30 min and then briefly rinsed in distilled water. The leaves and stalks were cut into 0.2 g samples and 30 seeds comprised one sample and were ground (individually) in a mortar containing 3 ml of sterile distilled water. The homogenates were filtered through 2 layers of cheesecloth, placed in a beaker and incubated at 28 C for 24 hours. Several drops of the suspension were placed on a slide, air-dried, and stained as previously described. Pathogenicity of the overwintered bacterial cells were determined by culturing the organisms on YDC media. Colonies similar to those produced by P. avenae and P. andropogonis were harvested and suspended in PBS. The suspension was inoculated into sorghum plants (Redlan, IS

413) under greenhouse conditions and evaluated after 10 days. Controls consisted of selecting noninoculated plants in the field.

H. Ouchterlony Double Diffusion Test:

Ouchterlony plates were prepared with 1.0% Bacto-agar (Difco Laboratories, Detroit, MI) in 0.01 M PBS (pH 7.2) and 0.02% sodium azide (3). A template was used to cut seven wells of equal diameter (5 mm) with a distance of six mm between wells. The antigen was prepared by harvesting 96-hr old bacterial cells from culture plates and then grinding 2 ml of the bacterial suspension (10^8-10^9 CFU/ml) in a test tube containing 1 cc of glass beads (0.17-0.18 mm diameter) at top speed of a Vortex-Genie mixer (Scientific Industries, Inc., Bohemia, NY) for 2 minutes. The AS was diluted 1:8 and placed in the center wells. Disrupted cells of the homologous antigen (10 µl) were placed in the remaining three alternate outer wells. Plates were incubated in a moist chamber at 26 C for 48 hours.

Results

Higher disease readings for P. andropogonis were observed in the moderate temperature regime (26-20 C) with high (90%) relative humidity although not significantly different with most strains at 30-24 C (Table 1). Limited disease development occurred at 36-24 C and almost no disease symptoms were observed at 22-16 C. With one exception (26-20 C; 90% RH); the corn strain (ATCC 23062) of P. andropogonis yielded readings similar to the sorghum strain (ATCC 23061). Although the clover strain (ICPB Pa 132) was negative in Ouchterlony double diffusion tests (Table 4) with antisera prepared to isolates of P. andropogonis and P. stizolobii, pathogenicity of this strain on sorghum was comparable to the corn and sorghum isolates in most respects. P. stizolobii strains were similar to those of P. andropogonis in pathogenicity tests.

Disease severity was enhanced when the P. avenae-inoculated plants were maintained under hot moist conditions (Table 1). Differences between strains of P. andropogonis and P. stizolobii and those of P. avenae were especially pronounced at 36-24 C temperatures and 90% RH. Reasons for the inconsistency in results from those plants inoculated with strain ATCC 19860 are unknown as ICPB Pa 117 was deposited in the collection and designated as ATCC 19860.

Inoculating sorghum plants in an early growth stage (7-10 leaves) resulted in greater disease severity (Table 2). Readings were less for those plants inoculated with P. avenae and overall ratings were less in 1983 and is attributable to the prolonged periods of hot and dry climatic conditions.

No grain sorghum hybrids were immune to P. andropogonis, however, most possessed tolerance levels that would likely be acceptable in commercial grain sorghum production (Table 3). Lesser ratings were observed for those hybrids inoculated with P. avenae. In addition, ratings were noticeable less in 1983 and was due to climatic conditions experienced during the growing season.

P. andropogonis and P. avenae cells were detected by FAS in grain sorghum leaf, stalk and seed samples. The samples were taken every two weeks from mid-February until May and a total of 70 samples for each P. andropogonis and P. avenae were evaluated and all were positive. Non-inoculated plants served as controls and were negative. As expected, more of the incitant bacteria were observed in leaf tissue than in stalk or seed samples. Even though a positive fluorescence was observed with the seed samples, the fluorescence was relatively weak (+1) in comparison to those observed in leaf tissue (+3) at dilutions of 1:8 and 1:16. Neither incitant bacteria were recovered from seed samples when attempts were made to culture on YDC medium whereas the incitants were easily recovered from stalk and leaf tissue. Seeds were collected from plants inoculated with P. andropogonis and planted in the greenhouse. Nearly 11% (48 out of 420 seeds) of the plants exhibited bacterial stripe symptoms 3 weeks after planting. To test pathogenicity, the isolates of P. andropogonis and P. avenae were inoculated on leaves of sorghum plants in the greenhouse. Positive symptoms were observable within 10 days.

The corn and sorghum isolates of P. andropogonis were positive against antisera prepared to P. andropogonis and P. stizolobii (Table 4). The clover isolate, ICPB pa 132 was negative to both P.

andropogonis and P. stizolobii antisera. These results are similar to those reported by others (8,9). Homologous antisera was not prepared to strain ICPB Pa 132. Potential serovars are conceivable when isolates are collected from widely diverse host plants. On the other hand, ICPB Pa 132 reacted in pathogenicity tests similar to the corn and sorghum isolates (Table 1).

Discussion

Bacterial stripe, bacterial streak and bacterial spot diseases of sorghum are common in Kansas although they do not appear to cause any substantial losses in yields. Attempts were made to measure yields in those hybrids inoculated with P. andropogonis and P. avenae but were inconsistent due to extensive bird damage in the plots. As outlined by Elliott and Smith (6), environmental conditions consisting of warm and moist conditions are necessary for disease severity. This confirms our observations in the hybrid evaluation plot as limited disease development occurred in 1983 when prolonged periods of hot and dry conditions persisted. In 1982, conditions were favorable for disease development and extensive secondary infections were noted on those leaves above the ones that were inoculated. Sorghum genotypes produce various reactions to injuries and the predominant lesion colors are purple and tan. In ascertaining readings, purple lesions are more distinct and easier to evaluate than tan lesions and may account for some hybrids having a higher reading than others.

Although our data showed that growth stage 3 was most vulnerable to P. andropogonis and P. avenae, it is probably irrelevant in screening procedures whether the plants are inoculated at this growth stage. Climatic conditions consisting of timely rainfall, mild temperatures overcast skies, and frequent dews after inoculation are vital for extensive disease development to occur.

Most of the hybrids possessed acceptable levels of tolerance to P. andropogonis and all hybrids would most likely be tolerant to P. avenae (Table 3). Unfortunately, obtaining yield data to determine the effect

of bacterial incitants on potential yield losses was impossible. High disease development based on foliar readings may not always correlate to yield losses (4). P. avenae is a less aggressive pathogen than P. andropogonis in our field evaluations and does not appear to pose a threat. P. avenae would most likely be present in sorghum when that crop is rotated with corn as the number of overwintering incitants would be enhanced and corn appears to be the preferred host.

Both P. andropogonis and P. avenae were recovered from stalk tissue of overwintered plants. The leaf sheath surrounding the stalk provides an ideal environment for the bacteria and it appears that the bacteria enter the stalk either directly or as a result of wounding at or near the node. P. andropogonis has been recovered from stalk tissue of severely infected and/or lodged plants in the field (L. E. Claflin - unpublished). Significant losses are most likely a result of stalk, peduncle or inflorescence infections and not from foliar infections.

Under normal cultural practices, the sorghum stalks are grazed after harvest, particularly in those areas vulnerable to soil erosion; stalks may remain undisturbed until tillage commences in the spring, or may be disced, moldboard plowed, or chisel plowed shortly after harvest to take advantage of the freezing-thawing action. The bacteria are less likely to survive in those situations where the stalks, leaves and seeds are incorporated into the soil after harvest or in those fields where animals were allowed to graze. Conversely, more cells would be expected where the debris is left undisturbed. In a continuous sorghum monoculture, the role of volunteer seed in infection sites within a field is a distinct possibility as nearly 11% of the seed from infected plants resulted in infections. The role of seed in disseminating the

disease may not be an important factor. Those seeds planted in the greenhouse were not treated with seed protectants and were stored only several months before planting. Long term storage and seed treatments may cause significant decline in numbers. Although the seed was washed 30 min prior to FAS staining, bacteria outside of the seed coat may adhere to the testa or in the hilum which may account for the low numbers with the remainder washed off. No efforts were made to determine if the bacteria were within the seed.

The direct FAS test resulted in a rapid and reliable technique for identifying the causal agents in disease diagnosis and to prove the overwintering survival of the pathogens. Bacterial diseases in sorghum may be difficult to diagnose as they may closely mimic other sorghum diseases, abiotic problems including scorch, and insect injury. In addition, it is often impossible to distinguish these bacterial diseases from each other as the symptoms may be expressed differently on different genotypes. It is imperative that the causal agent be identified instead of relying on symptom diagnoses in seed certification and breeding programs.

Ouchterlony double diffusion tests showed that P. andropogonis (except ICPB Pa 132) and P. stizolobii were serologically identical. Our results confirm cultural, physiological, biochemical, and slab gel tests conducted by others (8,9,26). P. andropogonis is the appropriate nomenspecies as it has priority over P. stizolobii (8).

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Table 1. Influence of temperature and relative humidity on bacterial diseases of sorghum incited by various species and strains of Pseudomonas andropogonis, P. avenae and P. stizolobii.

Bacterial species and strain no.	Host	Donor-Location	Disease ratings					
			36-24[x] 90% RH	30-24 90% RH	30-24 75% RH	30-24 50% RH	26-20 90% RH	22-16 50% RH
P. andropogonis:								
Pa 116	Sorghum bicolor	Ulstrup-Indiana	0.0[y]a[z]	1.6 c	1.0 b	1.3 b	1.4 b	0.0 a
Pa 132	Trifolium repens	Goto-Japan	0.0 a	1.1 b	2.4 cd	1.7 c	2.1 c	0.0 a
Pa 133	Sorghum bicolor	Goto-Japan	1.1 b	1.6 c	1.7 c	0.3 a	2.0 c	0.3 a
Pa 140	Sorghum bicolor	Goto-Japan	0.9 b	0.7 ab	2.0 c	2.0 c	3.0 d	0.0 a
ATCC 23061	Sorghum bicolor	Goto-Japan	1.3 b	1.6 bc	0.6 ab	0.7 ab	2.9 d	0.1 a
ATCC 23062	Zea mays	Ulstrup-Indiana	1.3 b	1.3 b	0.6 ab	1.1 b	1.9 c	0.1 a
P. avenae								
Pa 117	Zea mays	Ulstrup-Florida	1.7 c	2.6 d	1.3 b	1.7 c	2.3 cd	0.0 a
Pa 137	Zea mays ssp. mexicana	Goto-Japan	2.6 d	3.1 d	2.1 c	2.0 c	3.0 d	0.3 a
ATCC 19860	Zea mays	Ulstrup-Florida	2.3 cd	0.3 a	1.7 c	0.0 a	1.1 b	0.1 a
P. stizolobii								
P.s. 187	Bouganvillea spp.	Hayward-Hawaii	1.4 b	0.3 a	0.9 b	1.4 b	1.9 c	0.0 a
P.s. 280	Trifolium repens	Hayward-Hawaii	0.0 a	1.3 b	0.4 a	1.4 b	2.0 c	0.0 a

[x] Maximum temp corresponds to day cycle (14 hours).

[y] Rating scale of 0-5 where 0 = no symptoms; 1 = 1-10; 2 = 11-25; 3 = 26-50; 4 = 51-75; 5 = 76-100% of the leaf area affected. Each number represents an average of 12 plants from each of 6 hybrids.

[z] Numbers with the same letter are not significantly different at the 0.05 level as determined by Duncan's multiple range test.

Table 2. Effect of inoculating grain sorghum plants at various growth stages with Pseudomonas andropogonis and P. avenae

Growth Stage[X]	P. andropogonis		P. avenae	
	1982	1983	1982	1983
3	2.45[Y]b[Z]	1.76 a	1.39 a	1.09 a
4	1.97 c	1.11 b	1.01 b	0.20 b
5	1.51 d		1.04 b	
9	2.84 a		1.25 a	

[X]Growth stages approximated where 3 = growing point differentiation (7-10 leaves); 4 = flag leaf visible; 5 = boot stage; 9 = physiological maturity.

[Y]Rating scale of 0-5 where 0 = no symptoms; 1 = 1-10; 2 = 11-25; 3 = 26-50; 4 = 51-75; 5 = 76-100% of leaf area affected. Each number represents an average of 3 plants of 26 hybrids inoculated at each stage of growth.

[Z]Numbers within a column not followed by a common letter are significantly different at the 0.05 level as determined by Duncan's multiple range test.

Table 3. Response of grain sorghum hybrids to Pseudomonas andropogonis and P. avenae

Entry	P. andropogonis		P. avenae	
	1982	1983	1982	1983
Pioneer X6390	2.68[x]a[y]	1.88 a	1.18 bcd	0.88 ab
Pioneer 878	2.62 ab	1.75 ab	1.56 ab	0.88 ab
Pioneer 8442	2.56 abc	1.63 abc	1.18 bcd	0.50 b
Pioneer X3082	2.50 abcd	1.38 bcde	1.31 bcd	0.75 ab
Cargill Exp. 2	2.43 abcde	1.63 abc	1.37 abcd	0.75 ab
Taylor-Evans Y45	2.43 abcde	1.50 abcd	1.81 a	1.00 a
Pioneer 894	2.43 abcde	1.50 abcd	1.43 abc	0.50 b
Pioneer 8272	2.37 abcdef	1.13 de	1.12 bcd	0.50 b
Cargill Exp. 3	2.31 abcdef	1.63 abc	1.43 abc	0.88 ab
Taylor-Evans Dinero-R	2.31 abcdef	1.63 abc	0.93 cd	0.75 ab
Pioneer 988	2.31 abcdef	1.38 bcde	1.06 cd	0.50 b
Pioneer X5139	2.31 abcdef	1.63 abc	1.00 cd	0.50 b
Cargill Exp. 1	2.25 abcdefg	1.25 cde	1.25 bcd	0.50 b
Cargill Exp. 5	2.25 abcdefg	1.25 cde	1.31 bcd	0.50 b
Funk G499	2.25 abcdefg	1.38 bcde	1.12 bcd	0.75 ab
Funk G550	2.25 abcdefg	1.63 abc	1.25 bcd	0.63 ab
Taylor-Evans Exp. 8163	2.18 abcdefg	1.63 abc	1.06 cd	1.00 a
Funk G611	2.18 abcdefg	1.00 e	1.00 cd	0.63 ab
Funk G522A	2.12 bcdefg	1.25 cde	1.00 cd	0.63 ab
Funk G522DR	2.06 cdefg	1.50 abcd	1.18 bcd	0.75 ab
Taylor-Evans 66R	2.00 defg	1.50 abcd	1.18 bcd	0.63 ab
Taylor-Evans 66B	1.93 efg	1.50 abcd	1.12 bcd	0.50 b
Taylor-Evans Y-101-R	1.83 efg	1.63 abc	1.00 cd	0.50 b
Pioneer 8479	1.93 efg	1.13 de	1.06 cd	0.50 b
Cargill Exp. 4	1.75 gh	1.25 cde	1.00 cd	0.50 b
Funk G623	1.75 gh	1.63 abc	0.93 cd	0.63 ab

[x] Rating scale of 0-5 where 0 = no symptoms; 1 = 1-10; 2 = 11-25; 3 = 26-50; 4 = 51-75; 5 = 76-100% of the leaf area affected. Each number represents an average of 3 plants inoculated at 3 different stages of growth.

[y] Means within a column followed by the same letter are not significantly different at the 0.05% level according to Duncan's Multiple Range Test.

Table 4. Ouchterlony double diffusion reactions of antisera prepared to Pseudomonas andropogonis, P. avenae, and P. stizolobii and various strains of these organisms

Bacterial species and strain number[x]	Host	Donor-location	Antisera				
			P. andropogonis ATCC 23061	P. andropogonis ATCC 23062	P. andropogonis Pa 133	P. avenae (ICPB-Pa 137)	P. stizolobii (ICPB Ps 186)
P. andropogonis:							
ICPB Pa 116	sorghum bicolor	Ullstrup-Indiana	0[y]	--	0	0	0
ICPB Pa 120	Zea mays	Wehlburg-Florida	--	--	0	0	--
ICPB Pa 132	Trifolium repens	Goto-Japan	--	--	--	--	--
ICPB Pa 133	Sorghum bicolor	Goto-Japan	++	++	++	--	++
ICPB Pa 140	Sorghum bicolor	Goto-Japan	++	++	0	--	++
ATCC 23061	Sorghum bicolor	Goto-Japan	++	++	++	--	++
ATCC 23062	Zea mays	Ullstrup-Indiana	+	+	+	--	+
P. avenae:							
ICPB Pa 134	Zea mays	Goto-Japan	--	--	--	++	--
ICPB Pa 137	Zea mays ssp. mexicana	Goto-Japan	--	0	--	++	--
P. stizolobii:							
ICPB Ps 186	Stizolobium spp.	Lelliott-Rhodesia	++	--	++	--	++
ICPB Ps 187	Bouganvillea spp.	Hayward-Hawaii	--	0	0	0	--
ICPB Ps 280	Trifolium repens	Hayward-Hawaii	--	0	0	0	--

[x] ICPB = International Collection of Phytopathogenic Bacteria, Davis, CA 95615; ATCC = American Type Culture Collection, Rockville, MD.

[y] ++ = reaction of identity, + = faint reaction, - = no reaction, 0 = not tested.

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BACTERIAL STRIPE AND BACTERIAL LEAF BLIGHT OF SORGHUM: EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON DISEASE DEVELOPMENT, TOLERANCE AMONG SORGHUM HYBRIDS, AND OVERWINTER SURVIVAL OF THE INCITANT BACTERIA

by

MUHAMMAD AFZAL AKHTAR

B. S. West Pakistan Agricultural University, Lyallpur, 1970
M. S. West Pakistan Agricultural University, Lyallpur, 1972

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Department of Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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Abstract

Bacterial stripe and bacterial leaf blight of sorghum (Sorghum bicolor) is incited by Pseudomonas andropogonis (Syn. P. stizolobii) and P. avenae (Syn. P. alboprecipitans), respectively. Symptoms on sorghum appear as elongated purple to tan stripes on leaves and leaf sheaths and, may result in stalk decay.

Bacterial stripe was favored by moderate temperatures (26-20 C) with high relative humidity (90%). High (36-24 C) and low (22-16 C) temperatures limited disease development of bacterial stripe. Corn strains of P. andropogonis resulted in disease readings similar to the sorghum and clover strains. In contrast to bacterial stripe, bacterial leaf blight was favored by hot (36-24 C) moist (90% RH) conditions.

Disease severity was increased when sorghum plants were inoculated at an early (7-10 leaves) growth stage. Twenty-six grain sorghum hybrids were screened for tolerance to P. andropogonis and P. avenae. No hybrids were immune to P. andropogonis although most would be acceptable in grain sorghum production. All hybrids possessed more tolerance to P. avenae than P. andropogonis.

Overwintered P. andropogonis and P. avenae cells were detectable by fluorescent antibody stain in leaf, stalk and seed samples. Nearly 11% of the seeds obtained from plants inoculated with P. andropogonis exhibited bacterial stripe symptoms 3 weeks after planting.

Most of the corn and sorghum strains of P. andropogonis were positive against antisera prepared to P. andropogonis and P. stizolobii in Ouchterlony, double diffusion tests. Those P. andropogonis negative isolates included one strain from the following; corn (ICPB Pa 120), sorghum (ICPB Pa 116), clover (ICPB Pa 132), and the negative P.

stizolobii isolates were from clover (ICPB Ps 280) and *Bouganvillea* (ICPB Ps 187).