MACROPHOMINA PHASEOLINA, CAUSAL ORGANISM OF CHARCOAL ROT OF SOYBEAN. I. LABORATORY TESTS FOR RESISTANCE. II. IMPLICATIONS OF CONIDIA IN EPIDEMIOLOGY. III. ANTIMICROBIAL ACTIVITY OF TOXIN(S).

by

CHARLES ALBON STANLEY PEARSON

B. S., Pennsylvania State University, 1980

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1982

Approved by:

Fred W. Schwenk
Major Professor

SPEC
COLL
LD
2668
.T4
1982
P42
c.2

ACKNOWLEDGMENTS

I wish to express my sincerest thanks to Fred Schwenk for his encouragement and many valuable suggestions during the course of my research. I also want to thank my committee members, Charles Kramer, William Schapaugh, and William Willis for their advice and review of this manuscript. Other professors which allowed me to use their equipment and/or offered suggestions include: Arun Chatterjee and Lowell Johnson - Department of Plant Pathology, Phillip Nordin - Department of Biochemistry, and Dallas Johnson and George Milliken - Department of Statistics; thank you. Special thanks go to Mary Roth and Sherry Wootke for their laboratory assistance, and to Dan Skinner for his help in "taming" the computer. Words can not adequately thank my wife, Diane, for encouragement and prayers over the last two years. I wish to thank our parents for their support and prayers. I gratefully thank God for hearing our prayers and for directing my path.

Trust in the Lord with all thine heart; and lean not unto thine own understanding. In all thy ways acknowledge him, and he shall direct thy path. Proverbs 3:5-6.

A11203 569923

CONTENTS

FIGURES AND TABLES

Macrophomina phaseolina, causal organism of charcoal rot of soybean. I. Laboratory tests for resistance. II. Implications of conidia in epidemiology. III. Antimicrobial activity of toxin(s).

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid. causes charcoal rot of over 500 plant species world wide (36). Under hot, dry environmental conditions many economically important crops suffer significant yield losses (13, 24, 28). In 1938 Vasudeva and Ashraf described charcoal rot of cotton as "the most destructive and limiting disease in Punjab", India (30). Livingston found charcoal stalk rot of corn and sorghum to cause "considerable" damage in Nebraska (23). He observed premature ripening and lodging in affected plants. Corn losses of greater than 50% were observed in some areas of Kansas during the favorable conditions in 1980; state losses were estimated at 5% (35).

Charcoal rot is extremely important to growers of dryland soybeans. Agarwal and Sarbhoy recorded soybean losses estimated at 77% in northern India (1). Meyer *et. al.* mention 50% losses in Yugoslavia (28). In Kansas charcoal rot is considered a major disease problem of soybean (35). In 1980, a very dry year, Sim found plants in nearly every field surveyed in the eastern one-third of Kansas in September and October that had this disease, with incidences of 50-100% common (35).

Charcoal rot of soybean may appear as a seedling blight or as a root and stem rot (9). Infected seedlings often develop reddish brown lesions that usually originate between the crown and the cotyledonary node, often just above soil level (9,36). Lesions turn brown to black and under hot, dry conditions seedlings may die. Under cool, moist conditions infected plants may survive, but disease symptoms can appear later as hot, dry conditions develop (9).

In older plants root and stem rot occurs after midseason with lower stems and tap roots often becoming light brown. Leaves turn yellow and wilt but remain attached. Plants prematurely ripen. Small black bodies (sclerotia) under the epidermis give tissues a greyish-black color (9). Symptom development and yield losses are most pronounced at 30-35 C as plants approach maturity under dry conditions (13, 24, 28).

Macrophomina phaseolina (Tassi) Goid. [= Rhizoctonia bataticola (Taub.) Butl.], the fungus which causes this disease, has two distinct structures: sclerotia, produced in root and stem tissues, enable the fungus to survive in crop debris for years (34, 41), and pycnidia which may be important in airborne spread (25). The sclerotia, black and smooth-to-irregularly shaped are composed of thick, darkened anastomosed mycelial cells. Size and shape of sclerotia are quite variable , not only among isolates but also within

isolates depending upon the substrate and environmental conditions (12). The pycnidial stage, considered uncommon on soybean (36), has been found on several hosts (36,43). The globose pycnidia initially develop immersed in host tissues but become erumpent at maturity. Pycnidiospores are hyaline, aseptate, rodshaped conidia (22) that tend to be extruded from pycnidia in a gelatinous matrix (12). Although Lutrell (25) suggested a possible role for conidia, critical epidemiological studies with conidia are lacking.

Control of charcoal rot with systemic fungicides in soybean has been studied by Kirkpatrick (19). His results as well as those of other investigators (5,16,37) indicate a general lack of control through seed treatment in the U.S. To date there are no chemicals commercially available for use on soybean against M. phaseolina.

Since this fungus has a large host range and sclerotia which may remain viable in the soil for many years, crop rotation or fallowing to starve inoculum does not appear feasible in control . Possible cultural control methods suggested are the addition of organic amendments to increase competitive microbial activity (11,15), reduced seeding rates to reduce moisture competition (36), and when possible, maintainence of high soil moisture (11).

Development of resistant cultivars, perhaps the most reasonable control method since irrigation is often impractial,

has been suggested by many investgators (1, 24, 30, 33, 43). In 1976 Agarwal and Sarbhoy (1), screened 1,569 germplasm lines/varieties under inoculated field conditions in India , and found 4 lines which were considered to show some resistance. They measured lesion length and deemed those lines/varieties resistant which had lesion lengths less than 10 cm. In 1980, Schapaugh and Schwenk (unpublished) screened 160 lines/varieties under natural field conditions in southeastern Kansas and found 11 with no obvious infection. None of the plants developed symptoms in a similar test in 1981, probably due to abundant and timely rainfall.

Field studies require large amounts of space, depend upon variable weather conditions and are restricted to one plant generation per year. For these reasons, laboratory techniques enabling year round testing under constant environmental conditions are desirable. This study describes attempts to develop such techniques, as well as some ideas on the epidemiology of conidia and possible roles of toxin(s).

I. Laboratory tests for resistance

Soybean germplasm has been evaluated under field conditions for resistance to Macrophomina phaseolina (1). Such tests are limited to one trial per year and depend upon variable environmental conditions, including presence of inoculum. Development of laboratory, growth chamber, and/or greenhouse evaluation procedures would enable breeders to screen plant materials year round under controlled environmental conditions, thus making comparisons among breeding materials less difficult. This paper describes several attempts at developing such tests.

Materials and Methods

Seed test. Four seeds of a line/variety were surface sterilized by stirring for 2 min in 0.8% sodium hypochlorite. Seeds were then placed in a circular pattern approximately 2 cm from the center of a 100x15 mm petri dish containing 25 ml Hoaglands medium (17) with 1.5% agar (HA). The center of each dish was inoculated with a 2 mm agar plug of mycelium taken from the advancing margin of a 2 day old culture of M. phaseolina grown on Difco lima bean agar (LBA) at standard growth conditions (SGC) of 30 C and 6460 lux continuous light. Control plates had surface sterilized seeds but no inoculum. Plates were incubated 4 days under SGC, and seeds and seedlings evaluated for discoloration and root growth.

Seedlings in agar. Two surface sterilized seeds of a variety were placed in a foil capped 1 l graduated cylinder with 300 ml HA. These seeds were maintained under SGC for 4 days and then a 2 mm agar plug with mycelium was placed on the HA between the two developing seedlings. Plants were evaluated one week later for symptom development.

Toothpick inoculation. Young (42) described a method for inoculating corn for ear and stalk rots using fungi grown on toothpicks. He listed several advantages for the method: a uniform amount of inoculum is introduced into the plants; different sections of the stalk can be inoculated with the same or different organism at one time; the point of inoculation is readily detected ; the spread of the pathogen from the peg is easily traced; and large populations can be rapidily inoculated.

Young's method has been used to inoculate older soybeans under field conditions (1, 39). We modified Young's procedure for laboratory use with soybean seedlings. Pieces approximately 1 cm long were cut from the ends of round toothpicks, placed in LBA, and autoclaved 15 min. These ends were removed from molten agar and placed on 10 ml solidified LBA in 100x15 mm petri dishes. For uniformity, toothpicks were arranged around the perimeter of the dishes with the pointed ends facing inwards. A 2 mm agar plug of mycelium was placed at the center of each dish, and the dishes

were wrapped with Parafilm (American Can Co.,Greenwich,CT) and incubated under SGC.

Soybean seeds were planted 1 cm deep in vermiculite in pots watered from the bottom with tap water containing 1 gm 20:20:20 commerical fertilizer/l, and maintained in a growth chamber at 28 C under 15000 lux continous light. After 4 days, emerged plants were inoculated by inserting an infested toothpick into each cotyledonary midvein. Noninfested toothpicks were used for controls. Plants incubated at 30 C and light at 6460 lux and 100% relative humidity were periodically rated for lesion development and seedling decline.

Mycelial and sclerotial inoculum. Soybean seed dextrose broth (SSDB), used as a growth medium for mycelial production, was prepared by boiling 10 gm soybean seed in 200 ml distilled water for 15 min. The liquid fraction was filtered through cheesecloth and adjusted to 200 ml. After adding 4 gm dextrose, the medium was autoclaved in a 500 ml erlenmeyer flask for 20 min. Media in cooled flasks was inoculated with a 2 mm plug of mycelium and incubated at room temperature on a rotary shaker at approx 75 RPM for 7 days. Difco potato dextrose broth (PDB) was used to obtain sclerotia under the same conditions.

Growth media with mycelium or sclerotia were blended in a Waring blendor for 20 sec at low speed and then centrifuged

at 2500 RPM for 2 min. The liquid fraction was decanted and the fungus collected. These inocula were used in several ways. Two ml mycelium or sclerotia,packed volume, were diluted in 23 ml distilled water. Seven-day-old plants grown under SGC were misted with these inocula, placed in plastic bags for 24 hr at room temperature, then removed from bags and returned to the growth chamber. Control plants were treated the same but were misted with water only. After 7 days plants were examined for lesion development.

Roots were inoculated with diluted inocula by applying 2 ml per 10 cm square pot containing eight 7-day-old seedlings in vermiculite. Seedlings were incubated under SGC for two weeks and then rated for root lesion development against noninoculated controls.

Undiluted mycelium or sclerotia were used to wound inoculate 4-7 day old seedlings. Inocula were injected into the cotyledon either along the midvein or several times along the hypocotyl using a 22 ga needle. Plants were incubated under SGC for 7 days and then examined for lesion development and seedling mortality.

Conidial inoculum. Several isolates of M. phaseolina that produce prolific numbers of pycnidia when grown under SGC were placed on LBA along side autoclaved soybean leaves. After 7 days leaves were peeled from the agar and placed in a Waring blendor with 100 ml distilled water and 1 ml 0.1%

Triton. Leaves were blended 30 sec at low speed and then filtered through a layer of Kimwipes (Kimberly-clark Corp., Neenah,WI) several times until the filtrate was relatively free of sclerotia and unbroken pycnidia. The filtrate was centrifuged at 2500 RPM for 5 min and the pellet collected. Spore concentration was determined using a Bright-Line hemacytometer (American Optical Co., Buffalo,NY), and $3x10^5$ spores/ml applied to leaves of 8-day-old Columbus and Crawford soybeans. After inoculation, plants were placed in plastic bags for 48 hr and then incubated 8 days at 30 C. Conidial suspensions of 10^4-10^5 spores/ml were used to inject plants in the same manner as with sclerotial and mycelial inocula.

Plants also were inoculated by placing a droplet containing $1x10^5$ spores/ml in fully expanded flowers of 5 week old soybean plants grown under 13000 lux for 10 hr/day at 24 C. Plants were examined daily for signs and symptoms.

Stressed plant inoculation. Soybean seeds were germinated in vermiculite 4 days in a growth chamber with 14 hr of incandescent and fluorescent light at 13000 lux and 24 C day/20 C night temperatures. Seedlings were then transferred to 2-liter hydroponic tubs containing nutrient solution (17). Air was continuously bubbled through the solution; all solutions were changed weekly. Plants were water stressed after 7 days by the addition of polyethylene glycol 600 (PEG) (Sigma Chemical Co.,St.Louis,MO). Zero, -3, and

-6 bars tension were obtained by adding 0%, 2.5%, and 7.5% PEG, respectively, to the nutrient solution. Daylength was decreased to 12 hr to induce flowering. One week later, flowering plants were inoculated by injecting mycelium, grown on LBA, into incisions made with a scalpal at the base of the hypocotyl. The wounds were covered with petroleum jelly and the plants incubated for another week. Plants were then split longitudinally and internal lesion length measured.

Results

Seed test. A 0-5 scale was used to evaluate seeds and seedlings for discoloration and root development. Zero was used for seeds which did not germinate, while 5 was used for seeds that germinated and had root development equivalent to controls. In seed discoloration evaluation, 0 represented no discoloration and 5 complete discoloration. Table 1 lists the mean seed discoloration and root development of 4 seeds/line tested 2-4 times. Of the 212 lines/varieties tested, lines PI189926, PI404190, and PI398185 showed the least discoloration and best root development with respect to noninoculated controls.

Table 1 also lists the field results obtained from southeastern Kansas by Schapaugh and Schwenk (unpublished). Multiple correlations coefficients were used to determine if seed discoloration and/or root development could model field results. Root development had an $R^2=0.0006$.

TABLE 1. Response of soybean lines/varieties to Macrophomina phaseolina. Inoculum was placed on Hoagland's medium along with seeds and allowed to infect as seeds germinated across the agar; or inserted into cotyledons of 4-day-old seedlings after being cultured on toothpick tips impregnated with lima bean agar. Mature plant responses of some of these same lines/varieties to natural infection are also given for comparison.

		Germinating seeds on agar			Toothpick inoculation		
		Seed	Root development		Percent	Percent	Field
Brand	Line/variety	discoloration[1]	Rating[2]	Ratio[3]	lesioned[4]	killed[5]	trials[6]
	A-100	.	.	.	.	.	43.8
Acco	301	2.0	3.0	.	.	.	.
Acco	401	3.0	1.5	.	.	.	.
	Altona	2.4	2.2	.	100	45	.
	Amsoy 71	4.4	1.1	.	100	35	55.9
Agripro	AP350	2.0	1.5	.	100	65	.
Agripro	AP40	3.3	1.5	0.05	100	44	14.4
Asgrow	A3127	1.5	4.0	.	100	44	.
Asgrow	A3659	4.5	0.0	.	.	.	.
Asgrow	A3860	2.5	2.0	.	.	.	.
Asgrow	A4268	3.5	1.0	.	.	.	.
Asgrow	A5618	4.5	0.5	.	.	.	.
	Calland	3.5	2.0	.	100	50	.
	Clark 63	4.5	0.5	.	.	.	.
VR	Classic II	4.5	0.0	.	.	.	.
	Columbus	4.3	0.8	.	100	85	.
Coop	495	3.0	1.0	.	.	.	.
Coop	500	4.0	1.0	.	.	.	.
	Crawford	4.2	0.7	.	100	80	.
	Cumberland	.	.	.	85	15	.
	Cutler 71	3.8	1.2	.	100	56	.
Pfizer	CX272	4.0	1.0	.	.	.	.
Pfizer	CX290	3.0	1.0	.	.	.	.
Pfizer	CX350	2.5	3.0	.	100	40	.
Pfizer	CX355	2.5	2.0	.	.	.	.
Pfizer	CX380	3.0	1.0	.	100	42	.
VR	Delta	1.5	3.5	.	.	.	.
	Desoto	.	.	.	100	67	.
	Douglas	.	.	.	100	43	.

TABLE 1 (continued). Response of soybean lines/varieties to Macrophomina phaseolina. Inoculum was placed on Hoagland's medium along with seeds and allowed to infect as seeds germinated across the agar; or inserted into cotyledons of 4-day-old seedlings after being cultured on toothpick tips impregnated with lima bean agar. Mature plant responses of some of these same lines/varieties to natural infection are also given for comparison.

		Germinating seeds on agar			Toothpick inoculation		
		Seed	Root development		Percent	Percent	Field
Brand	Line/variety	discoloration[1]	Rating[2]	Ratio[3]	lesioned[4]	killed[5]	trials[6]
	Elf	4.1	1.1	.	100	20	.
	Essex	4.0	0.4	.	100	60	.
Pfizer	EC9821	2.0	2.0	.	.	.	.
	FC036541	1.5	4.0	.	.	.	.
	FC04002R	.	.	.	.	.	51.2
	FC04007B	1.0	4.5	0.92	100	20	73.8
	FC29333R	.	.	.	.	.	37.5
	FC31571	3.5	1.5	.	.	.	8.8
	FC31697	.	.	.	.	.	17.5
	FC31702	2.0	2.0	0.68	.	.	2.5
Fontanelle	5656	3.5	0.5	.	.	.	.
Fontanelle	5959	4.5	0.5	.	.	.	.
Fontanelle	6161	3.0	1.5	.	.	.	.
Fontanelle	6262	3.0	2.0	.	.	.	.
	Forrest	3.0	2.0	.	100	25	.
	Harosoy	.	.	.	100	67	.
	Harosoy 63	2.5	2.5	.	100	75	.
	Harrow	.	.	.	.	.	45.0
Migro	HP2020	2.0	3.0	.	.	.	.
Migro	HP3030	4.0	1.0	.	.	.	.
	Jogun	.	.	.	.	.	27.5
Jacques	J114	5.0	0.0	.	.	.	.
Jacques	J120	5.0	0.0	.	.	.	.
Jacques	J125	4.0	1.0	.	.	.	.
Kean	Topeka	4.0	1.0	.	.	.	.
Kean	K45	4.0	1.0	.	.	.	.
	Mack	5.0	0.5	.	100	70	.
	Mandarin	4.0	2.0	0.35	100	80	95.0
	Mandrell	.	.	.	.	.	45.0
	Marshall	5.0	0.5	.	.	.	.

TABLE 1 (continued). Response of soybean lines/varieties to Macrophomina phaseolina. Inoculum was placed on Hoagland's medium along with seeds and allowed to infect as seeds germinated across the agar; or inserted into cotyledons of 4-day-old seedlings after being cultured on toothpick tips impregnated with lima bean agar. Mature plant responses of some of these same lines/varieties to natural infection are also given for comparison.

		Germinating seeds on agar			Toothpick inoculation		
		Seed	Root development		Percent	Percent	Field
Brand	Line/variety	discoloration[1]	Rating[2]	Ratio[3]	lesioned[4]	killed[5]	trials[6]
McNair	500	3.0	2.0	.	.	.	.
MFA	Mosoy 380	4.5	0.0	.	.	.	.
MFA	Mosoy 382	4.0	1.0	.	.	.	.
MFA	Mosoy 480	3.0	1.0	.	.	.	.
Multivar	100	.	.	.	100	43	.
N.A.P.B.	1955	4.0	0.5	.	.	.	.
N.A.P.B.	5734	4.0	1.0	.	.	.	.
N.A.P.B.	8831	3.5	1.0	.	100	35	.
Northrup-King	Multivar 92	5.0	0.5	.	.	.	.
Northrup-King	Multivar 95 Exp	3.5	0.5	.	.	.	.
Northrup-King	Multivar 100	4.5	0.0	.	.	.	.
Northrup-King	S4055	3.0	2.0	.	.	.	.
Northrup-King	504677	3.0	2.0	.	94	6	.
	PI123577-2	3.0	1.0	.	.	.	.
	PI153309	2.0	2.0	0.26	100	5	45.0
	PI157437	3.0	2.0	.	.	.	3.2
	PI157457	4.0	1.5	.	.	.	3.8
	PI157460	1.5	2.5	.	.	.	.
	PI157477	3.0	3.0	0.52	100	20	55.0
	PI189926	0.5	5.0	.	.	.	7.5
	PI196155	.	.	.	.	.	10.0
	PL200593	2.0	1.0	0.23	84	5	60.0
	PI219782	4.0	2.0	.	.	.	.
	PI229738	.	.	.	.	.	23.8
	PI248402	.	.	.	.	.	6.2
	PI253661C	2.0	3.5	.	.	.	1.2
	PI253665A	3.0	2.5	.	100	100	0.0
	PI253665D-5	.	.	.	.	.	5.0
	PI264555	2.0	3.5	.	.	.	.
	PI274204	2.0	2.5	.	.	.	.

TABLE 1 (continued). Response of soybean lines/varieties to Macrophomina phaseolina. Inoculum was placed on Hoagland's medium along with seeds and allowed to infect as seeds germinated across the agar; or inserted into cotyledons of 4-day-old seedlings after being cultured on toothpick tips impregnated with lima bean agar. Mature plant responses of some of these same lines/varieties to natural infection are also given for comparison.

		Germinating seeds on agar			Toothpick inoculation		
		Seed	Root development		Percent	Percent	Field
Brand	Line/variety	discoloration[1]	Rating[2]	Ratio[3]	lesioned[4]	killed[5]	trials[6]
	PI274205	2.0	3.0	0.0	100	0	56.2
	PI283331	.	.	.	.	.	2.5
	PI283332	4.5	1.0	.	.	.	.
	PI283344	.	.	.	.	.	11.2
	PI339864A	4.0	1.0	.	.	.	3.8
	PI339868C	1.5	3.0	.	.	.	7.5
	PI339868D	1.0	4.0	.	.	.	.
	PI33995	1.0	4.0	.	.	.	.
	PI339981	2.0	2.5	.	.	.	12.5
	PI339983	2.5	2.5	.	.	.	.
	PI339990	4.5	1.0	.	.	.	.
	PI339995	.	.	.	.	.	13.8
	PI339997	4.0	1.5	.	.	.	2.5
	PI340002	3.5	1.5	.	.	.	15.0
	PI340012	2.0	2.0	0.43	80	10	5.0
	PI340015	4.0	2.0	.	.	.	.
	PI340027	3.5	2.0	.	.	.	5.0
	PI340037	1.5	3.5	.	.	.	.
	PI340041	3.5	1.5	.	.	.	.
	PI340048	3.0	3.0	.	.	.	.
	PI340053B	1.5	3.0	.	.	.	10.0
	PI360836	4.0	2.0	0.24	.	.	0.0
	PI360841	.	.	.	.	.	28.8
	PI360844	3.0	2.5	.	.	.	3.8
	PI360846	3.0	2.0	.	.	.	1.2
	PI361063	.	.	.	.	.	6.2
	PI372404C	.	.	.	.	.	55.0
	PI378670B	.	.	.	.	.	13.8
	PI385942	4.5	1.0	.	.	.	18.8
	PI391586	.	.	.	.	.	2.5

TABLE 1 (continued). Response of soybean lines/varieties to Macrophomina phaseolina. Inoculum was placed on Hoagland's medium along with seeds and allowed to infect as seeds germinated across the agar; or inserted into cotyledons of 4-day-old seedlings after being cultured on toothpick tips impregnated with lima bean agar. Mature plant responses of some of these same lines/varieties to natural infection are also given for comparison.

		Germinating seeds on agar			Toothpick inoculation		
		Seed	Root development		Percent	Percent	Field
Brand	Line/variety	discoloration[1]	Rating[2]	Ratio[3]	lesioned[4]	killed[5]	trials[6]
	PI393538	3.0	2.0	.	.	.	.
	PI398185	0.5	4.0	.	.	.	.
	PI398191	2.5	2.0	.	.	.	3.8
	PI398251	3.0	2.5	.	.	.	.
	PI398297	1.5	3.5	0.16	100	5	10.0
	PI398312	2.5	2.0	.	.	.	12.5
	PI404161	1.5	2.5	.	.	.	.
	PI404173A	.	.	.	.	.	0.0
	PI404178	3.0	2.0	.	.	.	0.0
	PI404190	0.5	4.5	.	.	.	5.0
	PI404196B	.	.	.	.	.	2.5
	PI404729	3.5	2.0	.	.	.	.
	PI406707	4.0	1.0	.	.	.	17.5
	PI406709	3.0	2.0	0.17	100	20	16.2
	PI407727	1.5	3.5	.	.	.	1.2
	PI407731	4.0	1.5	.	100	100	0.0
	PI407733	2.5	2.5	.	.	.	0.0
	PI407740	2.0	2.0	0.20	100	50	15.0
	PI424217A	4.0	1.0	.	.	.	.
	PI424217B	1.5	3.0	.	.	.	.
	PI424219A	3.0	2.0	.	.	.	.
	PI424219B	3.0	2.5	.	.	.	.
	PI424220A	4.0	2.0	.	.	.	.
	PI424220B	4.0	1.5	.	.	.	.
	PI424221A	3.0	3.0	.	.	.	.
	PI54608-2	3.0	2.5	.	.	.	15.0
	PI54609	1.0	2.0	0.27	100	64	8.8
	PI54610-4	3.5	2.0	.	.	.	.
	PI54615-2	2.0	3.5	.	.	.	.
	PI60296-2	4.0	2.0	.	.	.	6.2

TABLE 1 (continued). Response of soybean lines/varieties to Macrophomina phaseolina. Inoculum was placed on Hoagland's medium along with seeds and allowed to infect as seeds germinated across the agar; or inserted into cotyledons of 4-day-old seedlings after being cultured on toothpick tips impregnated with lima bean agar. Mature plant responses of some of these same lines/varieties to natural infection are also given for comparison.

		Germinating seeds on agar			Toothpick inoculation		
		Seed	Root development		Percent	Percent	Field
Brand	Line/variety	discoloration[1]	Rating[2]	Ratio[3]	lesioned[4]	killed[5]	trials[6]
	PI68449	2.0	2.0	0.42	100	65	.
	PI68499	.	.	.	.	.	65.0
	PI68600	.	.	.	.	.	41.2
	PI68621	3.0	2.0	0.72	100	100	11.2
	PI68692	2.0	2.0	0.32	100	100	78.8
	PI68761-3	.	.	.	.	.	17.5
	PI69995	2.0	3.0	0.51	100	20	53.8
	PI70076	1.0	3.0	0.56	100	63	42.5
	PI73541	.	.	.	.	.	7.5
	PI79587	2.0	2.0	0.70	.	.	0.0
	PI79627	.	.	.	.	.	37.5
	PI79692	2.0	1.0	0.73	100	63	15.0
	PI79693	1.0	3.0	0.26	100	90	65.0
	PI79696	2.0	2.0	.	.	.	.
	PI79712	.	.	.	.	.	8.8
	PI79726	.	.	.	.	.	46.2
	PI79743	2.0	2.0	.	.	.	18.8
	PI79760	3.0	2.0	0.22	100	71	55.0
	PI79870-4	3.0	2.5	.	.	.	2.5
	PI79872	1.5	2.5	0.53	100	75	25.0
	PI79874-1	.	.	.	.	.	18.8
	PI80461	3.0	2.0	.	.	.	22.5
	PI80471-1	.	.	.	.	.	43.8
	PI80479	1.0	3.5	.	.	.	30.0
	PI80834-1	2.0	3.0	0.24	100	50	76.2
	PI80847-2	3.0	2.0	.	.	.	.
	PI81029-1	3.0	3.0	.	.	.	.
	PI81041	2.0	2.0	.	.	.	20.0
	PI81042-1	.	.	.	.	.	30.0
	PI83893	.	.	.	.	.	1.2

TABLE 1 (continued). Response of soybean lines/varieties to Macrophomina phaseolina. Inoculum was placed on Hoagland's medium along with seeds and allowed to infect as seeds germinated across the agar; or inserted into cotyledons of 4-day-old seedlings after being cultured on toothpick tips impregnated with lima bean agar. Mature plant responses of some of these same lines/varieties to natural infection are also given for comparison.

		Germinating seeds on agar			Toothpick inoculation		
		Seed	Root development		Percent	Percent	Field
Brand	Line/variety	discoloration[1]	Rating[2]	Ratio[3]	lesioned[4]	killed[5]	trials[6]
	PI84656	.	.	.	.	.	25.0
	PI84666	.	.	.	.	.	7.5
	PI85340	3.0	2.0	0.23	95	32	57.5
	PI85559	2.0	3.0	.	.	.	7.5
	PI85630	2.0	1.0	0.17	50	0	7.5
	PI86004	.	.	.	.	.	18.8
	PI86026	3.0	1.0	.	.	.	.
	PI86073	.	.	.	.	.	27.5
	PI86114R	.	.	.	.	.	38.8
	PI86136	.	.	.	100	0	17.5
	PI86144	.	.	.	40	0	3.8
	PI86445	.	.	.	.	.	12.5
	PI86449	4.0	1.5	.	.	.	23.8
	PI86456	2.5	3.0	.	.	.	6.2
	PI86469	1.5	2.0	.	100	50	47.5
	PI86502	1.0	3.0	.	.	.	6.2
	PI86740	2.5	2.0	0.38	50	0	17.5
	PI86972-2	1.5	3.0	.	.	.	.
	PI87026	2.0	3.0	0.87	100	80	1.2
	PI87457	.	.	.	.	.	3.8
	PI87465-2	.	.	.	.	.	1.2
	PI87588	.	.	.	.	.	11.2
	PI87631-3	5.0	0.5	.	.	.	.
	PI87634	4.5	0.0	0.19	63	0	5.0
	PI88302-1	3.0	2.0	.	86	86	1.3
	PI88305	.	.	.	.	.	7.5
	PI88306	.	.	.	.	.	0.0
	PI88310	.	.	.	.	.	2.5

TABLE 1 (continued). Response of soybean lines/varieties to Macrophomina phaseolina. Inoculum was placed on Hoagland's medium along with seeds and allowed to infect as seeds germinated across the agar; or inserted into cotyledons of 4-day-old seedlings after being cultured on toothpick tips impregnated with lima bean agar. Mature plant responses of some of these same lines/varieties to natural infection are also given for comparison.

		Germinating seeds on agar			Toothpick inoculation		
		Seed	Root development		Percent	Percent	Field
Brand	Line/variety	discoloration[1]	Rating[2]	Ratio[3]	lesioned[4]	killed[5]	trials[6]
	PI88349	.	.	.	.	.	11.2
	PI88350	.	.	.	.	.	8.8
	PI88359	2.5	2.8	0.19	100	66	35.0
	PI88445	2.0	3.0	0.62	100	5	10.0
	PI88447	3.5	2.0	0.21	50	5	20.0
	PI88447-3	.	.	.	.	.	13.8
	PI88499	2.0	2.0	.	.	.	6.2
	PI88806	3.0	5.0	0.67	78	11	40.0
	PI88809	3.5	4.0	0.17	35	5	32.5
	PI88988-2	.	.	.	.	.	36.2
	PI89002	.	.	.	.	.	26.2
	PI89009	.	.	.	.	.	37.5
	PI89010	4.0	1.5	0.21	100	5	25.0
	PI89128	.	.	.	.	.	17.5
	PI89154-2	2.0	2.0	.	.	.	.
	PI89769	1.5	3.0	.	.	.	0.0
	PI90258	5.0	2.0	0.14	100	25	2.5
	PI90566-1	.	.	.	.	.	5.0
	PI90579	3.0	5.0	0.41	95	16	67.5
	PI90723	3.0	5.0	.	70	0	1.2
	PI91113	3.0	3.0	0.22	100	47	2.5
	PI91142	.	.	.	.	.	22.5
	PI91152	.	.	.	.	.	13.8
	PI91153-4	3.5	2.5	.	.	.	.
	PI91159-4	3.0	1.0	0.19	100	55	66.2
	PI91161	1.0	4.0	0.53	78	28	32.5
	PI91166	.	.	.	.	.	22.5
	PI91169	.	.	.	.	.	27.5
	PI91174	2.0	3.0	0.80	100	83	75.0
	PI91641	2.0	1.5	.	.	.	21.2

TABLE 1 (continued). Response of soybean lines/varieties to Macrophomina phaseolina. Inoculum was placed on Hoagland's medium along with seeds and allowed to infect as seeds germinated across the agar; or inserted into cotyledons of 4-day-old seedlings after being cultured on toothpick tips impregnated with lima bean agar. Mature plant responses of some of these same lines/varieties to natural infection are also given for comparison.

		Germinating seeds on agar			Toothpick inoculation		
		Seed	Root development		Percent	Percent	Field
Brand	Line/variety	discoloration[1]	Rating[2]	Ratio[3]	lesioned[4]	killed[5]	trials[6]
	PI91730-1	.	.	.	.	.	26.2
	PI91731-1	2.5	3.0	.	.	.	.
	PI91750	2.0	2.0	.	.	.	7.5
	PI92560	.	.	.	.	.	18.8
	PI92593	.	.	.	.	.	28.8
	PI92601-2	.	.	.	.	.	40.0
	PI92640	.	.	.	.	.	28.8
	PI92642	3.5	1.0	0.35	53	0	48.8
	PI92645	.	.	.	.	.	28.8
	PI92654	2.0	4.0	0.36	75	0	38.8
	PI92707-5	.	.	.	.	.	33.8
	PI92718-2	.	.	.	.	.	5.0
	PI92728	3.0	3.0	0.32	100	56	70.0
	PI96194P	.	.	.	.	.	17.5
	PI97797	.	.	.	.	.	21.2
	Pomona	4.0	1.2	.	100	65	.
Ring Around	Mitchell 450	3.5	2.0	.	.	.	.
Ring Around	RA-402	3.5	1.0	.	.	.	.
Ring Around	RA-480	3.5	0.5	.	.	.	.
	Sanga	2.5	3.0	.	.	.	.
SRF	250	3.0	2.0	.	100	60	.
SRF	707743	3.0	3.5	.	.	.	.
SRF	745897	2.0	4.0	.	95	5	.
S Brand	S 48	3.0	2.0	.	.	.	.
S Brand	S 49	3.5	2.0	.	.	.	.
S Brand	S 50A	3.0	2.5	.	.	.	.
S Brand	S 51	2.0	2.5	.	.	.	.
S Brand	S 55	2.0	1.0	.	100	47	.
S Brand	S 57	3.0	0.5	.	91	19	.
S Brand	S 58	3.0	1.5	.	.	.	.

TABLE 1 (continued). Response of soybean lines/varieties to Macrophomina phaseolina. Inoculum was placed on Hoagland's medium along with seeds and allowed to infect as seeds germinated across the agar; or inserted into cotyledons of 4-day-old seedlings after being cultured on toothpick tips impregnated with lima bean agar. Mature plant responses of some of these same lines/varieties to natural infection are also given for comparison.

		Germinating seeds on agar			Toothpick inoculation		
		Seed	Root development		Percent	Percent	Field
Brand	Line/variety	discoloration[1]	Rating[2]	Ratio[3]	lesioned[4]	killed[5]	trials[6]
S Brand	S 606	3.5	1.5	.	.	.	.
S Brand	S 62A	3.0	1.0	.	.	.	.
S Brand	S 68	3.5	1.0	.	.	.	.
	Tracy	4.7	0.5	.	100	5	.
	Vinton	.	.	.	100	5	.
VR	Delta	3.5	0.5	.	.	.	.
VR	Eagle	4.5	0.5	.	.	.	.
VR	8027	3.0	1.0	.	.	.	.
	Wells	5.0	0.0	.	.	.	.
	Williams	3.6	0.9	.	100	90	.
Wilson	3130	3.0	2.5	.	100	90	.
Wilson	3340	4.0	1.0	.	82	47	.
Wilson	3550	4.0	0.5	.	94	6	.
Asgrow	XP5474 Exp	3.5	1.0	.	.	.	.
		s=0.96	s=0.88	s=0.36	s=1.33	s=1.42	s-value unpublished[6]

[1]Based on a 0-5 scale with 0= no discoloration and 5= complete discoloration.

[2]Based on a 0-5 scale with 0= no growth and 5= growth as control.

[3]Means of 4 observations in each of two replications; Ratio= $\frac{\text{Mean root length inoculated}}{\text{Mean root length control}}$.

[4]Number of plants lesioned when inoculated in the cotyledons with infested toothpicks divided by the total number of plants inoculated.

[5]Number of plants killed when inoculated in the cotyledons with infested toothpicks divided by the total number of plants inoculated.

[6]Percent infection. Data from Schapaugh and Schwenk (unpublished). Field plots were in Columbus, KS (1980).

A model using discoloration had an R^2=0.0349. The two-variable model had an R^2=0.0660. Root length also was measured and a root development ratio calculated from the mean root length of inoculated and control seedlings (Table 1). The correlation coefficient using the root development ratio was R^2=0.0070.

Seedlings in agar. Altona, Amsoy 71, and Columbus soybeans were tested using this method. Plants grew well in the medium but symptoms did not develop on inoculated plants. The method was somewhat cumbersome and would not allow large numbers of plants to be tested at one time. For these reasons experimentation with this procedure was discontinued.

Toothpick inoculation. Table 1 lists the percent lesion development and seedling mortality of lines/varieties 9 days after inoculation. Results given are means of two trials per line/variety. These results were compared to those from the 1980 field trials. Multiple correlation coefficents were calculated; percent lesioned had an R^2=0.1565 and percent killed an R^2=0.1822. The two variable model had an R^2=0.2770. A five variable model including all variables listed in table 1 had an R^2=0.1150.

Three soybean cultivars were inoculated, using infested toothpicks, with five isolates of M. phaseolina (Table 2). Isolates CR77-6 and CR80-12 were from soybean, CR80-SB was

TABLE 2. Response of three soybean varieties inoculated in the cotyledons with infested toothpick tips containing one of five isolates of *Macrophomina phaseolina*.

Isolate	Altona		Amsoy 71		Columbus	
	% Lesioned	% Killed	% Lesioned	% Killed	% Lesioned	% Killed
CR77-6	100.0	28.6	75.0	12.5	100.0	100.0
CR80-SB	100.0	92.9	83.3	50.0	100.0	100.0
CR80-SW	84.6	69.2	80.0	20.0	100.0	100.0
CR80-PC	72.7	00.0	10.0	00.0	66.7	16.7
CR80-12	28.6	00.0	00.0	00.0	100.0	00.0

TABLE 3. Response of four soybean varieties to several isolates of Macrophomina phaseolina when mycelium was applied by misting plants.

	Experiment 1 (% Killed)				Experiment 2 (% Killed)			
Isolate	Amsoy 71	Columbus	Essex	Mandarin	Amsoy 71	Columbus	Essex	Mandarin
CR77-6	21	71	92	39	54	88	38	75
CR80-5	21	0	16	87	85	64	53	19
CR80-7	8	0	0	25	17	18	0	13
CR80-13	0	25	62	13	60	27	43	31
CR80-14	17	50	67	19	21	50	13	88
CR80-15	10	44	31	31	42	63	44	25
CR81-10	0	67	7	22	27	7	14	19
Control	0	0	0	0	0	0	0	0

from strawberry, CR80-SW from sweetcorn, and CR80-PC from popcorn. The results given are means of two replicates each having 10-16 inoculated plants /treatment.

Mycelial and sclerotial inoculum. Plants misted with inocula showed some lesion development after 7 days, but no plants were killed. The experiment was then repeated and the relative humidity maintained at 100% for the entire incubation period. Table 3 lists the results from inoculating 4 soybean cultivars with 7 isolates of M. phaseolina using mycelial inoculum. Sclerotial inoculum yielded similar results.

When Agriopro 40, Altona, Columbus, Cutler 71, and Tracy soybean seedlings were inoculated by adding mycelium or sclerotia to vermiculite, no symptoms developed. If inocula were added to pots at the time of planting, damping off was common.

Injection of inocula into cotyledons resulted in lesion development similar to that observed when using the toothpick method. During inoculation it was common for the needle to become clogged with plant material and/or sclerotia. Cotyledons often cracked or were broken from trying to apply inocula. Injection into the hypocotyl resulted in necrosis at the site of entry but the fungus in general did not produce lesions or kill plants.

Conidial inoculum. About 6% of both Columbus and Crawford soybeans developed lesions when conidia were injected into cotyledons, but no plants were killed. Plants misted with conidia yielded similar results.

Many flowers of Agripro 40, Amsoy 71, Columbus, Crawford, and Cutler 71 inoculated with conidia aborted within 3 days. Some flowers inoculated with CR77-6 continued to develop. Necrotic streaks originating from the point of attachment were observed on developing pods two weeks from inoculation. One week later, pods were harvested and split longitudinally. Pod sections and seeds, surface sterilized in 0.8% sodium hypochlorite for 1 min, were placed on LBA. M. phaseolina was recovered from all pod sections and 3 of 8 seeds.

Stressed plant inoculation. Three cultivars, Altona, Amsoy 71, and Columbus, were tested using this procedure (Figure 1). Four plants of each variety subjected to each level of stress were inoculated with CR77-6, split longitudinally and lesion length measured in millimeters. Figure 1 suggests that as water stress is increased, lesion length is also increased. Statistically differences among cultivars were significant ($P<0.01$) and there was no interaction ($P=0.24$). When a one-way treatment structure was used to analyze lesion length in cultivars at each level of stress,

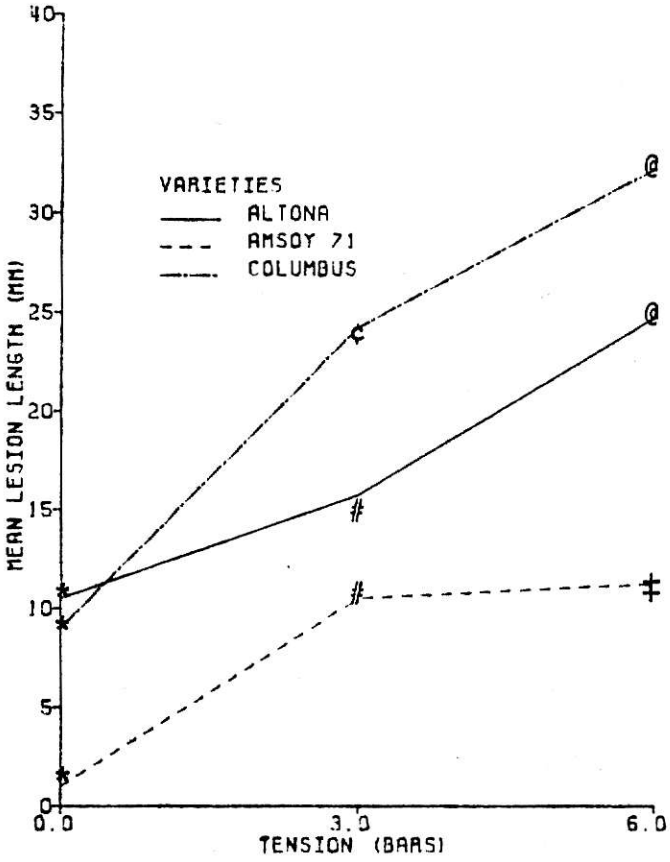

Figure 1. Mean lesion length (mm) of three soybean varieties inoculated with Macrophomina phaseolina CR77-6 at three levels of drought stress. Means with the same symbols are not significantly different (P=0.05). Symbols designate differences within each level of stress.

significant differences among cultivars were not observed at 0 bars ($P>0.07$), but were observed at -3 bars and -6 bars ($P=0.05$).

Discussion

Charccal rot of soybean can becone very serious under hot, dry conditions (35). Control through resistance has been considered a reasonable approach to this problem (1,24,30,33,43), but no such varieties are yet identified (36). Identification of resistant genotypes has been difficult (1) because stress is very important in disease development (12). In this study we tried to develop a screening technique with seedlings that could predict late season field responses. Our results indicate that simple inoculation procedures, such as the seed test or toothpick procedure, do not predict field response. Physiologic differences between young, vegetatively-growing plants and older, reproducing plants which may undergo such stress as those associated with flowering, may explain our results. M. phaseolina also can produce enzymes and toxins (10) which may affect seedlings more quickly and differently than older plants.

Using PEG to stress plants may lead to the development of a good screening program. It is interesting to note that the three varieties tested varied in their response to water

stress. Field grown plants no doubt undergo stress at least at some time during the growing seasion. Our other screening procedures did not include overt stress as a factor. Differences in infection seen in field trials may have been confounded by differences in stress. More work in this area is needed. The PEG procedure requires large amounts of space and nutrient solution. Since lesion length is not always easily measured, other response variables that could aid in screening should be investigated. A better understanding of the relationship between drought tolerance and disease susceptibility is needed.

M. phaseolina has been shown to vary greatly in cultural characteristics among isolates (12). Inoculations of three varieties with five isolates of M. phaseolina indicate that isolates may vary in their ability to lesion and kill plants. CR80-PC and CR80-12 tended to be less agressive than the other isolates tested. Such differences may be related to differences in enzyme and/or toxin production by isolates. Percent plants killed by an isolate did not correspond to percent plants lesioned. Such differences might be reduced if plants were water stressed during inoculation and incubation with infested toothpicks. Aerial application of mycelium yielded highly variable results; the effect of stress on plants thus inoculated also needs to be examined.

Flowers inoculated with conidia aborted or produced seed containing the fungus. It would be interesting to determine if conidia can infect floral parts of soybean under field conditions.

II. Implications of conidia in epidemiology

Macrophomina phaseolina (Tassi) Goid., the causal organism of charcoal rot, can produce sclerotia and pycnidia. The sclerotial form, commonly seen on diseased plant tissues, serves in the long-term survival of the fungus (33, 40) and therefore has been of interest to many investigators (11, 12, 31, 33, 34, 39). The pycnidial form, although sporadically observed on several hosts (8, 9, 25, 43), plays a role in the disease cycle that is not well understood. Since the fungus does not commonly sporulate on ordinary media, techniques have been developed for pycnidial induction. Knox-Davies (20, 21) was able to induce pycnidial formation by growing the fungus on autoclaved peanut meal in 2% water agar under longwave ultraviolet light. Sporulation was also obtained when isolates were grown on filter paper impregnated with an ether extract of peanut meal. Thus it was suggested that most isolates of M. phaseolina require an ether-soluble precursor of a photoactivated sporulation intermediary and that variation in the ability of isolates to sporulate reflects the variation in their capacity to synthesize the precursor.

Chidambaram and Mathur (8), obtained pycnidia by growing isolates on 1.5% water agar with autoclaved leaf pieces under alternating 12 hr cycles of light and darkness. They

found leaves of barley, oats, and wheat to be better substrates than pearl millet or sorghum for pycnidial production. Sporulation was best under near ultraviolet light.

Watanabe (38) tested pycnidial formation in 50 isolates of M. phaseolina obtained from kidney bean seeds or soil in Japan. Pycnidia were induced by growing the fungus on water agar with dried kidney bean hypocotyl segments which were sterilized in propylene oxide. In this study, Watanabe's three "best" isolates formed pycnidia 56% of the times they were tested. Fifty-eight percent of the isolates studied formed pycnidia or pycnidial initials. Pycnidial production was found to vary with light and temperature.

In the present study, 29 Kansas isolates of M. phaseolina were evaluated for their ablity to sporulate; conidial survival and germination also were determined under a variety of environment conditions.

Materials and Methods

Pycnidial production. Twenty-nine Kansas isolates, obtained from soybean in 1977, 1980, and 1981 were screened for pycnidial production. Balsam wood splints were cut to approx 5 x 80 mm and autoclaved in Difco lima bean agar (LBA). While agar was still molten, two splints were placed 5 mm apart on 10 ml solidified LBA in 15 x 100 mm petri dishes. The plates were inoculated at center with a 2

mm plug of mycelium from the advancing margin of a 2 day old culture of the fungus grown on LBA. The plates were wrapped with Parafilm (American Can Co.,Greenwich,CT) and then incubated at 30 C with 12 hr light at 6460 lux. After 7 days, the numbers of pycnidia per field were counted in 5 randomly selected fields per splint at 20x magnification.

To determine pycnidial stability, two field isolates were subcultured via single spores and eight of these single-spore (SS-1) isolates plus the two field isolate were tested for pycnidial production. Two of these single-spored isolates from each field isolate were again subcultured via single spores and each of these isolates (SS-2) were tested twice for pycnidial production.

Environmental effects. Experiments were initiated in November to determine if conidia could survive Kansas winters. LBA-splint plates were inoculated with 2 mm plugs of mycelium plus sclerotia. Plates were wrapped with two layers of Parafilm and one layer of masking tape and immediately placed at approx 10 m, 2 m, and 0 m above ground level. Plates were recovered in March and conidia tested for viability by incubating them in water for 2-4 hr.

In another experiment, LBA-splints with mature 7 day old pycnidia were placed in nylon bags and placed in the field 2 m above , or approx 5 cm below ground level in November. Splint samples were recovered in November, December, January, and March. Conidial viability was determined by plac-

ing splints in water for several hrs and then looking for germinated spores that had been released from the pycnidia.

To futher determine the effects of temperature, conidia of two isolates were isolated from leaf tissue as previously described (part I) and placed in 0.5% Agarose VII (Sigma Chemical Co.,St. Louis, MO) with 1% sucrose. Plates containing conidia were wrapped in foil and then incubated in a growth chamber at 4, 10, 15, 17, 20, 25, 30, 35, 40, or 45 C for 8-12 hrs. Percent germination was then determined.

Results

Pycnidial production. Poor sporulation in several isolates caused the pycnidial production distribution to be skewed. Nonparametric analysis (22) was done by ranking the data and then analyzing variances by rank; the 29 Kansas isolates are listed by rank in Table 1. Those isolates which did not produce pycnidia are included in the table but were not used when calculating Duncan's multiple range test .

Pycnidial production was found to be stable in CR77-6 and CR81-6 after each isolate was single spored eight times through two generations.

Environmental effects. LBA-splint plates recovered in March contained numerous pycnidia with viable conidia. These conidia, when grown on LBA, produced sclerotia

TABLE 1. Pycnidial production of Macrophomina phaseolina grown on lima bean agar at 30 C and 6460 lux.

Isolate	Mean Number of pycnidia[1]	Mean rank[2]
CR77-6	129.90	213.43 a[3]
CR80-1	112.10	201.70 ab
CR81-3	48.80	183.85 bc
CR80-8	22.20	170.05 cd
CR81-10	19.10	155.00 de
CR81-9	17.00	134.15 ef
CR81-4	10.60	132.75 ef
CR81-7	8.80	124.35 fg
CR80-13	9.10	123.70 fg
CR80-4	7.60	106.00 gh
CR80-3	5.10	95.45 hi
CR81-5	4.40	89.55 hi
CR81-8	6.90	87.45 hi
CR81-6	3.70	85.40 hi
CR81-2	2.90	77.60 i
CR81-11	3.60	70.35 i
CR80-6	1.00	42.70 j
CR80-10	0.50	34.95 j
CR80-7	0.40	34.20 j
CR81-13	0.40	34.10 j
CR80-11	0.30	32.90 j
CR80-2	0.00	*
CR80-9	0.00	*
CR80-12	0.00	*
CR80-14	0.00	*
CR80-15	0.00	*
CR80-16	0.00	*
CR81-1	0.00	*
CR81-12	0.00	*

[1]Means were calculated from four wooden splints each having five observations.

[2]Ranks and mean ranks were calulated using SAS (Statistical Analysis System, SAS Institute Inc.,Box 8000, Cary, NC 27511).

[3]Duncan's multiple range test on rank, P=0.05;data was also significant at P=0.01. Isolates with "*" were not included in the Duncan's test.

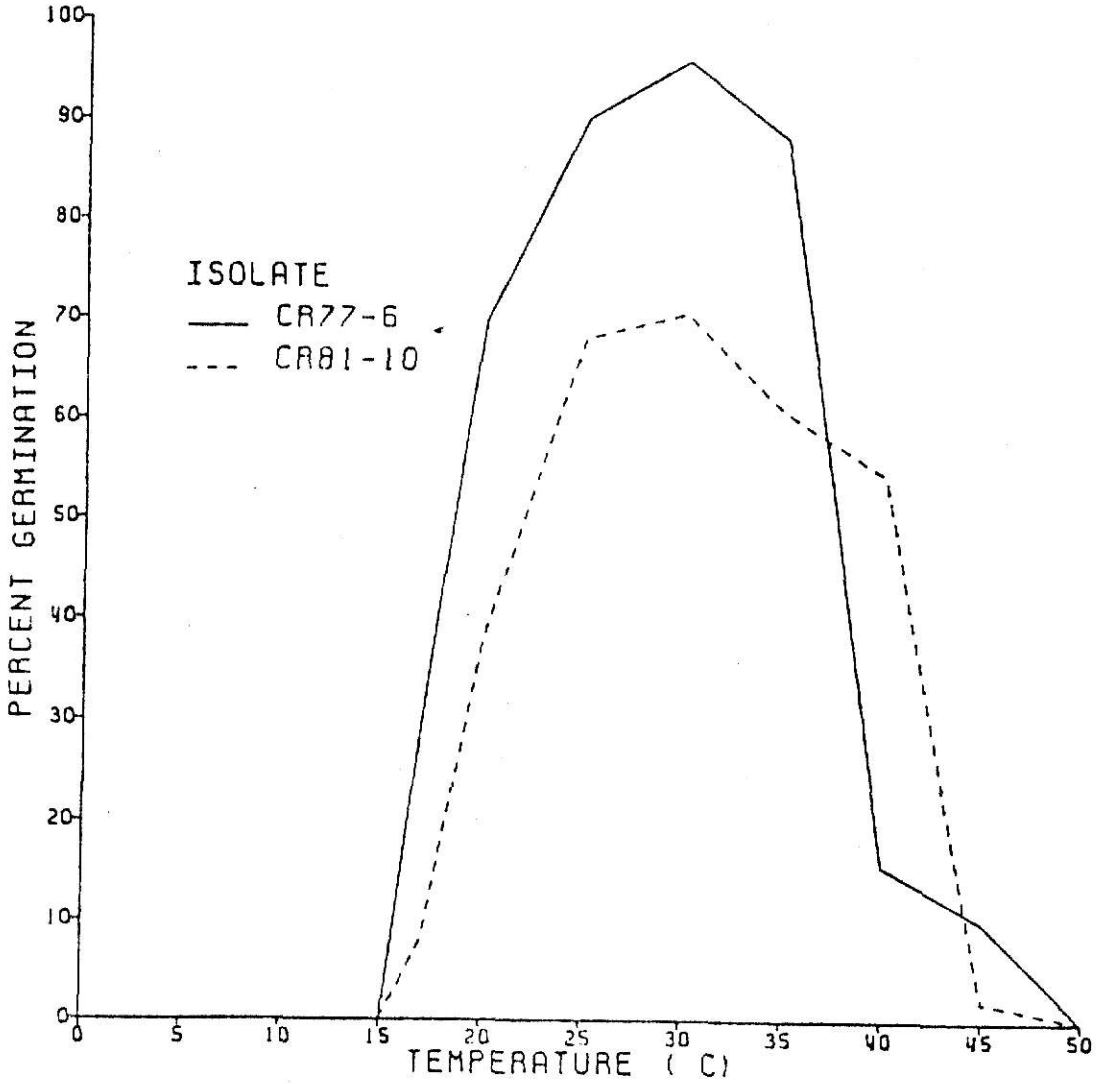

Figure 1. Response of Macrophomina phaseolina conidia to temperature. Isolated conidia were placed in 0.5% agarose VII with 1.0% sucrose, wrapped in foil, and incubated at various temperatures for 8-12 hr. Spores were considered germinated when the germ tube length was greater than conidium width. Percent germination was calculated from two replications at each temperature, each with sample size of 100.

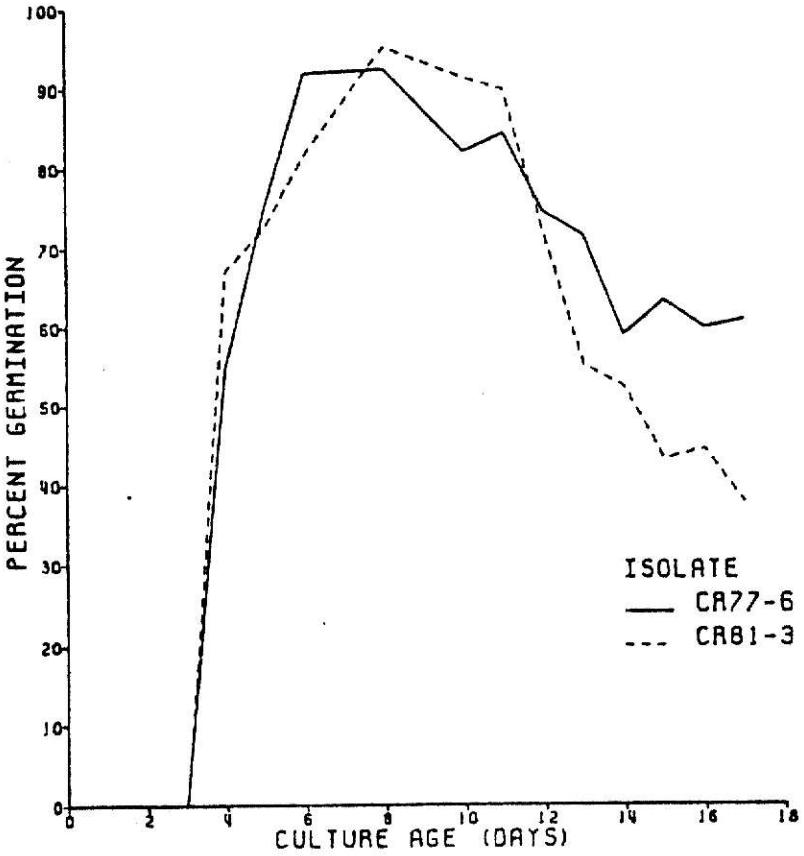

Figure 2. The effect of age on the germination of Macrophomina phaseolina conidia. Isolated conidia of various ages were placed in 0.5% agarose VII with 1.0% sucrose and incubated at 30 C for 8-12 hr. Percent germination was calculated from two replications for each treatment, each with sample size of 100.

characteristic of M. phaseolina. LBA-splints retrieved from nylon bags throughout the winter had viable conidia. Although not quantified , bags removed from soil appeared to contain more viable conidia then did bags in ambient air.

Conidia from isolates CR77-6 and CR81-10 were incubated under various temperature regimes to determine minimum, optimum, and maximum temperatures required for germination (Figure 1). Conidia of neither isolate would germinate at temperatures below 16 C. However, these same conidia would germinate if they were later exposed to temperatures between 17 and 40 C. The optimum temperature for germination was 30-35 C, whereas 45-50 C was lethal.

Figure 2 shows the effects of culture age on conidial germination of CR77-6 and CR81-3. Spore germination was optimum in 6-8 day old cultures. Conidial germination in 17-day-old cultures of CR77-6 and CR81-3 was 61% and 38%, respectively. After 30 and 40 days conidia still germinated, although percent germination was not determined.

Discussion

The role of conidia of M. phaseolina in disease spread and development is not well understood. Several workers have induced pycnidial production and have indicated the importance of light and temperature in sporulation (8, 20,

21, 38). Under standardized conditions of 30 C and 6460 lux of light, 21 of 29 Kansas isolates sporulated in culture. The number of pycnidia produced differed significantly among isolates. CR77-6 and CR81-6 were stable in pycnidial production. CR81-10, CR80-7, CR81-13, and CR80-11 were not tested for stablity but did not sporulate well and appeared unstable under the given conditions. Eight isolates did not sporulate at all. Although it is possible that under a different set of growing conditions these might produce pycnidia, we have chosen to classify them as nonsporulators, i.e., Rhizoctonia bataticola (12).

The ability of conidia to overwinter is suggested by the ability of spores to survive freezing in the field and in the laboratory. It is possible that sclerotia serve in overwintering with pycnidia production starting in the early spring. But since pycnidia are not commonly found in the field more studies are needed in this area.

Optimum temperatures for spore germination correspond to those conducive for disease development (12, 36). This indicates that conidia are suited to infect plants even under temperatures commonly encountered in Kansas during late spring and summer. Reports of finding pycnidia in the field have been scattered. Our attempts to locate pycnidia have been fruitless. Sporulation of this organism does not occur under dry conditions commonly associated with the

disease. It is possible that sporulation , and infection with conidia , only occur under moist or very humid conditions such as those found in Kansas during the early spring.

Conidium viability was optimum in 6-8 day old cultures. Such information is useful if spores are to be used for inoculum when screening plants for resistance. Although conidia are hyaline and appear sensitive to desiccation and sunlight, they are protected in a gelatinous matrix. This matrix may attract insects which could carry spores long distances. The association of insects with the charcoal rot disease of sunflower has recently been studied (40). Others have suggested insects as dispersal agents (25). Possible associations of insect vectors also needs more study.

III. Antimicrobial activity of toxin(s)

Macrophomina phaseolina (Tassi) Goid. causes charcoal rot in over 500 plant species (36). In 1968 Mathur (26) exposed cut sunflower seedlings to culture filtrates of two isolates of this fungus. He found that, unless autoclaved, filtrates from both isolates could macerate tissue. Autoclaved and unautoclaved culture filtrate also produced symptoms on cuttings similar to those observed when plants were inoculated with the fungus, i.e., necrosis, wilting , and yellowing. From this investigation, Mathur concluded that toxin(s) and pectolytic enzymes played a role in the pathogenesis of M. phaseolina (= Sclerotium batiticola).

In 1969 Chan and Sackston (6) determined that the toxin could be translocated in the plant. Attempts also were made to characterize the toxin; it was soluble in methanol, relatively insoluble in other organic solvents, and resistant to autoclaving. This information led to the conclusion that the toxin was not a protein or enzyme but perhaps was a polar compound.

In 1973 Chan and Sackston (7) tested 21 plant species in 13 families against toxin from four isolates of S. batiticola. The toxins were not host specific but several important hosts, i.e., cotton, sorghum, and soybean, did not develop symptoms.

In 1974 Dhingra and Sinclair (10) isolated and partially purified toxin from culture filtrates and soybean seedlings inoculated with M. phaseolina. Toxins from both sources were soluble in water and aqueous pyridine, insoluble in organic solvents, autoclavable, and chromatographically similar with absorption maxima at 255 nm.

This paper reports isolation and antimicrobial activity of toxin produced by M. phaseolina. The response of 14 soybean lines/varieties to the toxin is also reported.

Materials and Methods

M. phaseolina was grown at 30 C in soybean seed extract broth (SSEB) with 0.5% carboxymethylcellulose and 0.5% pectin (Sigma Chemicial Co, St.Louis,MO) as described by Dhingra and Sinclair (10). After 15 days, culture filtrate was passed through an Amicon stirred cell ultrafiltration system with a PM10 membrane (Amicon Corp.,Lexington,MA). Fractions were then tested for phytotoxicity by placing 10 day old Columbus soybean seedlings, grown in vermiculite and excised at ground level while under water, into each test solution . After 24 hr plants were rated for desiccation.

The purification procedure outlined by Dhingra and Sinclair (10) was modified. One liter of culture filtrate was rotary-evaporated at 60 C to approx 60 ml. This material

was then dialized against 4 l of double-distilled water (DDW) for 72 hr at 4 C with constant stirring. The water was changed at 24 hr intervals. The dialysate was collected, reduced to 70 ml, and the pH adjusted to 2.0 with 6N HCl. After centrifugation at 11,000 g for 20 min, the supernatant was mixed with 300 ml reagent grade isopropanol, stirred for 30 min, and the precipitate collected on Whatman No.42 filter paper. The precipitate was dissolved in 20 ml DDW and the extraction repeated twice. The solution was then extacted with 250 ml reagent grade methanol and stirred 30 min. The suspension was centrifuged at 5,000 g and the pellet collected. The pellet was resuspended in DDW, dried in a rotary evaporator at 50 C, and resuspended in 4 ml DDW.

This solution was washed through a 2.2 x 9.0 cm Dowex-50 H^+ cclumn with 75 ml DDW, dried at 50 C in a rotary evaporator, resuspended in DDW and then reduced to 4 ml. This material was passed through a 2.2 x 35 cm Sephadex G-50 column, which had been calibrated with $8x10^3$, $5x10^5$, and $2x10^6$ molecular weight Dextrans, at a flow rate of 15 ml/hr. Sixty-five 2-ml fractions were collected and assayed against 10 day old Columbus soybean seedlings. Active fractions were pooled, dried at 50 C, and resuspended in 4 ml DDW. Anthrone, ninhydrin, and Lowery tests were done for carbohydrate and protein detection. Absorption peaks also were determined.

Toxin taken through the Dowex-50 column, but not the Sephadex G-50 column, was tested for activity against bacteria. One-day-old cultures of bacteria were transferred to 10 ml Luria broth (LuB) (29) and incubated at 28 C in a shaking water bath until log phase. One ml bacteria and 2 ml LuB with 0.6% agar at 40 C were mixed. One ml of this mixture was then pipetted on to LuB containing 1.5% agar and incubated 4 hr. Five two-fold dilutions of toxin were made and tested against 14 bacteria obtained from the bacteriology laboratory, Department of Plant Pathology, Kansas State University. Two plates of each bacteria were spotted with 10 ul of each dilution of toxin, plus water and a HCl-NaOH solution at pH 3.4. Plates were incubated 12-14 hr and then rated for zones of inhibition.

Pseudomonas phaseolicola PDDCC3612 and Staphylococcus aureus were used to determine toxin action on actively growing cells. These organisms were grown in Klett flasks with 5 ml LuB at 28 C in a shaking water bath. Klett readings were taken hourly with the toxin being added after 2 hr incubation to give concentrations of approx 10, 5, 2.5, 1, and 0,6 mg/ml. Controls included flasks with no toxin as well as flasks containing a buffer solution equlivant to the pH of the toxin.

To determine if toxin could inhibit fungal spore germination, conidia of several fungi were placed into wells of a Boerner slide and 0.1 ml of 5 mg/ml toxin added. Water and

buffer were used for controls. Slides were incubated at room temperature in a moist chamber for 12 hr and then examined for germinating spores.

Results

Culture filtrate retained above the Amicon PM10 membrane caused leaf curling in Columbus soybean seedlings within 2 hr after exposure. Noninoculated media became viscous when treated in the same manner probably due to concentration of carboxymethylcellulose and pectin. After 24 hr both the putative toxin and control caused complete desiccation in soybean cuttings.

Culture filtrate dialyzed, extracted, and separated on a Dowex 50 column was inhibitory to Gram + and Gram - bacteria as well as to several fungi (Table 1). Viscous controls did not affect these organisms. To determine the minimum inhibitory concentration (MIC) of this material and pH effects on bacterial growth, broth cultures were used and bacterial growth monitored with a Klett meter (Figure 1). P. phaseolicola had a MIC of 10 mg/ml while S. aureus had a MIC of 5 mg/ml. After 8-9 hr these organisms , removed from their respective MIC's by centrifugation, were resupended in LuB. Klett readings remained unchanged 12 hr later, indicating the bactericidial nature of the toxin(s). Since a buffer at pH 3.4 did not inhibit P. phaseolicola (Figure 1A), pH does not appear to be the inhibitory quality of the toxin(s).

Using bacterial inhibition for a bioassay, five isolates of M. phaseolina were tested and found to produce toxin. The five isolates listed in decreasing order of toxin production, determined by weight of material extracted using the above procedure, are CR77-6, CR81-13, CR81-8, CR80-6, and CR80-17. All isolates produced material which inhibited Serratia marsecens and S. aureus.

Fourteen soybean lines/varieties were subjected to the partially purified CR77-6 toxin used in MIC determinations at 5 mg/ml. Seedling cuttings were rated for desiccation after 15 hr in toxin. All lines/varieties showed some degree of desiccation when rated against plants in water controls. Amsoy 71, Columbus, PI79693, and PI404173A completely desiccated while Essex, Mandarin, and PI360836 showed only slight cupping of leaves; responses of Williams, PI79587, PI80834, PI407733, PI88302, PI31702, and PI91174 were intermediate.

Toxin passed through a Sephadex G-50 column was eluted at the same volume as Blue Dextran (Figure 2). Anthrone and Lowery tests were positive while the ninhydrin test was negative. Absorption maximum was 210 nm. High performance liquid chromatography (HPLC) of trifluroacetic acid hydrolized toxin (2) yielded nine sugars including 2-deoxyribose, rhanose, manose, fructose, fucose, galactose, xylose, glucose, and two unidentified compounds. Protein

TABLE 1. Effect of toxin produced by *Macrophomina phaseolina* CR77-6 on microorganisms.

Organisms	Test solutions		
	Buffer[a]	Water	Toxin
Corynebacterium insidiosum	-[b]	-	+
Enterobacter aerogens	-	-	+
Erwinia amylovora 178	-	-	+
Erwinia amylovora 225	-	-	+
Erwinia amylovora v. *alfalfae* 216 Lg	-	-	+
Erwinia cararovora v. *atroseptia*	-	-	+
Ercherichia coli C600	-	-	+
Ercherichia coli K12	-	-	+
Pseudomonas phaseolicola PDDCC3612	-	-	+
Pseudomonas solanacearum	-	-	+
Pseudomonas syringae 955	-	-	+
Salmonella typhimrium LT2	-	-	+
Serratia marcesens	-	-	+
Staphylococcus aureua	-	-	+
Drechslera tritici-repentis	-	-	+
Macrophomina phaseolina CR77-6	-	-	-
Mucor spp.	-	-	+
Phomopsis sojae	-	-	+
Phytophthora megasperma f.sp. *glycinea*	-	-	+
Tricoderma viride	-	-	+

[a]Buffer was HCl-NaOH mixed to give pH 3.4 which is the same as that of the toxin.

[b]-= no inhibition and += inhibition.

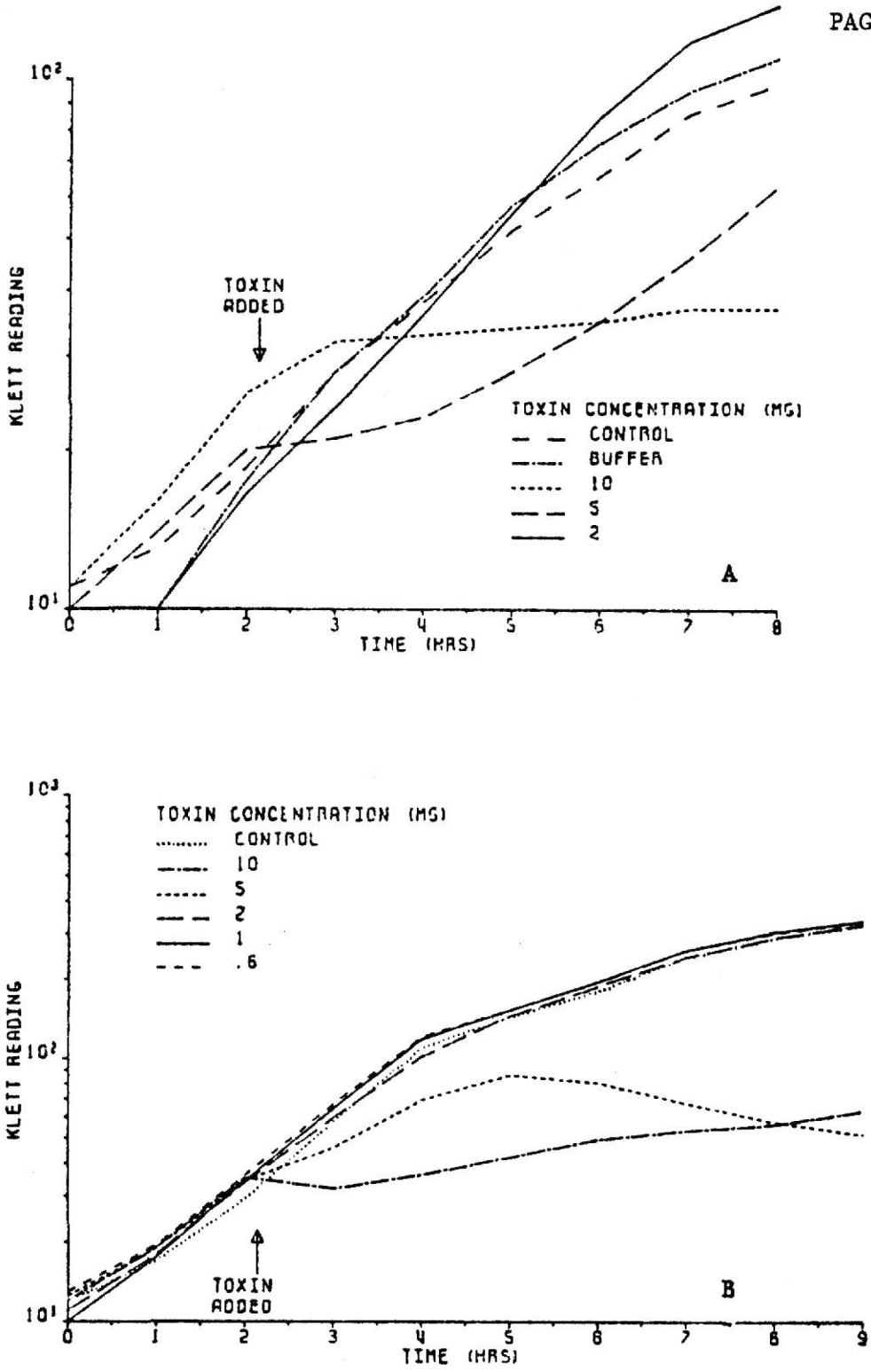

Figure 1. Antibacterial activity of Macrophomina phaseolina CR77-6 toxin. A, Pseudomonas phaseolicola grown in Luria broth and exposed to toxin after 2 hr active growth. B, Staphylococcus aureus grown in Luria broth and exposed to toxin after 2 hr active growth. Both bacteria were incubated at 28 C in a shaking water bath. Controls contained no toxin and the buffer was a HCl-NaOH mix at pH 3.4; the same pH as the toxin.

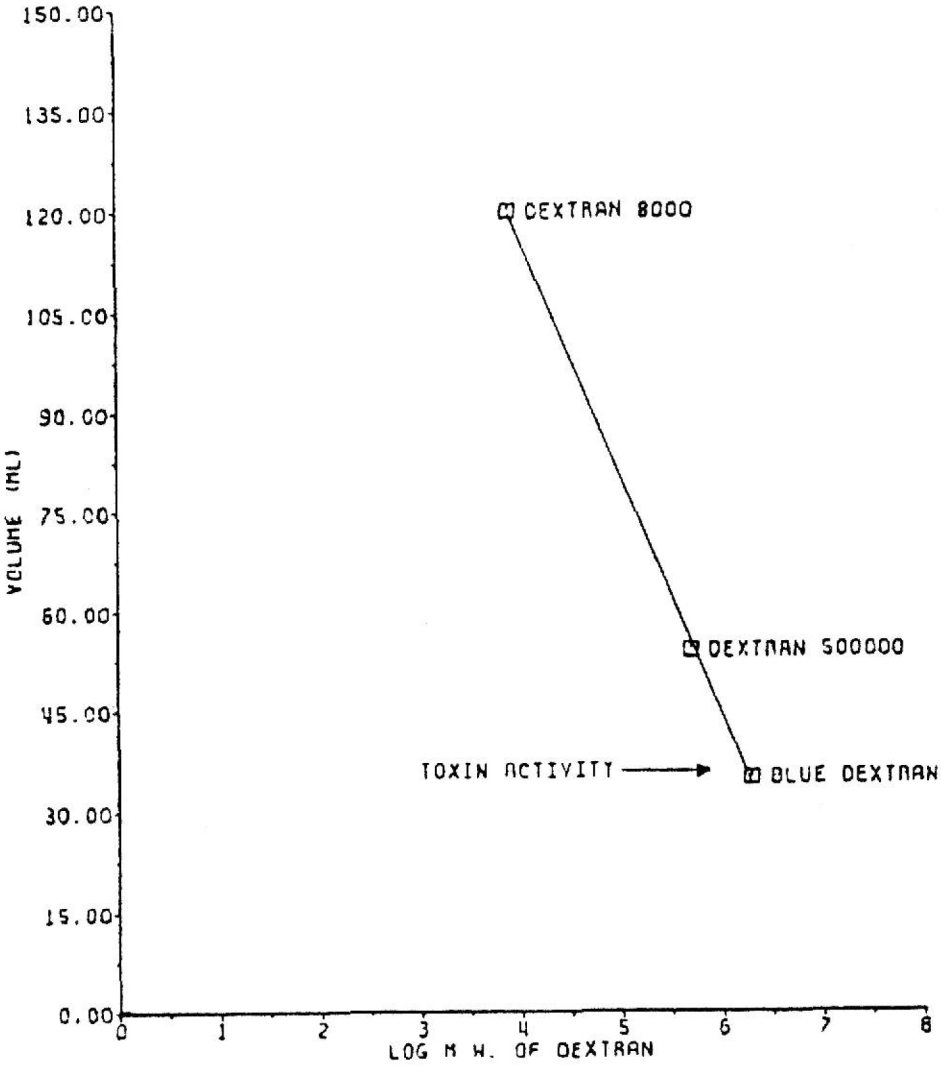

Figure 2. Molecular weight determination of Macrophomina phaseolina CR77-6 toxin. Dextrans were used to calibrate a Sephadex G-50 column (2.2 x 35 cm) at a flow rate of 15 ml/hr. The molecular weight of the toxin was similar to that of Blue Dextran (2×10^6).

determinations were not done but a positive Lowery test indicated proteinaceous material present in the toxin(s).

Discussion

Phytotoxins produced by M. phaseolina have been demonstrated to play a role in the pathogenesis of charcoal rot (5, 26). These toxins have been shown to be nonspecific, affecting many plant species (6). Our results indicate that toxins commonly produced by this fungus inhibit bacteria and some fungi. Such activity could theoretically aid in the maintainance of a food base by decreasing competition through antibiosis.

Minimum inhibitory concentrations of 5-10 mg/ml seem very high. When testing for bacterial activity, CR77-6 toxin had not yet been passed through the Sephadex G-50 column. These MIC's were probably inflated by the presence of other materials with the toxin. In later experiments a 10^3 (approx 200 ug) dilution of purified toxin inhibited S. marcesens. Such a concentration is probably closer to that found in vivo.

Absorption maximum at 210 nm, a positive anthrone test, and HPLC show that carbohydrate is associated with the toxin. Protein may also be part of the toxin since the Lowery test was positive (the ninhydrin test , a much less sensitive test for protein, was negative).

All soybean lines/varieties exposed to toxin 15 hr showed some degree of desiccation. Although differences in sensitivity to toxin may exist, they are not easily differentiated. Since the toxin is nonspecific (7) the results were not unexpected.

Futher studies to determine toxin concentration *in vivo* would be useful. In our study SSEB was used to culture the fungus; this undefined medium could confound HPLC results. Characterization to determine specific components of the toxin needs futher exploration and defined media could aid in this task.

Literature cited

1. Agarwal,D.K.,and Sarbhoy,A.K. 1976. Evaluation of soybean germplasm for resistance against Macrophomina phaseolina. Indian Phytopath.29:190-191.

2. Barr,J.,and Nordin,P. 1980. Microdetermination of neutral and aminosugars found in glycoproteins. Anal. Biochem. 108:313-319.

3. Bega,R.V., and Smith,R.S. 1961. Time-temperature relationships in thermal inactivation of sclerotia of Macrophomina phaseoli. Phytopathology 52:632-635.

4. Bouslama,M. 1982. Stress tolerance in soybeans (Glycine max (L) Merr.). Ph.D. thesis, Kansas State Univ. 75 pp.

5 Borum,D.E., and Sinclair,J.B. 1968. Evidence for systemic protection against Rhizoctonia solani with Vitavax in cotton seedlings. Phytopathology 58:976-980.

6 Chan,Y.H., and Sackston,W.E. 1969. Mechanisms of pathogenesis in Sclerotium bataticola on sunflower. 1. Production and translocation of a necrosis-inducing toxin. Can. J. Bot. 51:1147-1151.

7. Chan,Y.H., and Sackston,W.E. 1973. Nonspecificity of the necrosis-inducing toxins of Sclerotium bataticola. Can. J. Bot. 51:690-692.

8. Chadambaram,P., and Mathur,S.B. 1975. Production of pycnidia by Macrophomina phaseolina. Trans. Br. Mycol. Soc. 64 (1):165-168.

9. Crall,J. 1948. Charcoal rot of soybean caused by Macrophomina phaseolina Ashby. Ph.D. thesis, Univ. MI, 148 pp.

10 Dhingra,O.D., and Sinclair,J.B. 1974. Isolation and partial purification of a phytotoxin produced by Macrophomina phaseolina. Phytopath. Z. 80:35-40.

11. Dhingra,O.D., and Sinclair,J.B. 1975. Survival of Macrophomina phaseolina sclerotia in soil effects of soil moisture, carbon:nitrogen ratios, carbon sources, and nitrogen concentrations. Phytopathology 65:236-240.

12. Dhingra,O.D., and Sinclair,J.B. 1978. Biology and pathology of Macrophomina phaseolina. Imprensa Universitaria, Universidade Federal De Vicosa, Brazil, 160 pp.

13. Edmunds,L.K. 1964. Combined relation of plant maturity, temperature, and soil moisture to charcoal rot development in grain sorghum. Phytopathology 54:514-517.

14. Gangopadhyay,S., Wyllie,T.D., and Ludders,V.D. 1970. Charcoal rot disease of soybean transmitted by seeds. Plant Dis. Reptr. 54:1088-1091.

15. Ghaffar,A., Zentmeyr,G.A., and Erwin,D.C. 1969. Effect of organic amendments on severity of Macrophomina root rot of cotton. Phytopathology 59:1267-1269.

16. Gray,L.E., and Sinclair,J.B. 1970. Uptake and translocation of systemic fungicides by soybean seedlings. Phytopathology 60:1486-1488.

17. Hoagland,D.R., and Arnon,D.I. 1950. The water culture method for growing plants without soil. Calfornia Agr. Exp. Sta. Cir. 347.

18. Kendricks,J.B. 1933. Seedling stem blight of field beans caused by Rhizoctonia bataticola at high temperature. Phytopathology 23:949-963.

19. Kirkpatrick,B. 1970. Studies on Macrophomina phaseoli and control by systemic fungicides in soybean. S. Thesis. Univ. Illinois, Urbana. 43 pp.

20. Knox-Davies,P.S. 1965. Pycnidium production by Macrophomina phaseoli. S. Afr. J. Agric. Sci. 8:205-218.

21. Knox-Davies,P.S. 1966. Futher studies on pycnidium production by Macrophomina phaseoli. S. Afr. J. Agric. Sci. 9-595-600.

22. Kulkarni,N.B., Patil,B.C., and Ahmed,L. 1965. Studies on the pycnidial formation by Macrophomina phaseoli (Maubl.) Ashby. Development of pycnidia and pycnidiospores. Mycopath. Myco. Appl. 28:337-341.

23. Lehman,E.L. 1975. Nonparametrics: statistical methods based on ranks. Holden-Day, San Francisco, pp. 1747-1758.

24. Livingston,J.E. 1945. Charcoal root of corn and sorghum. Nebraska Agr. Exp. Sta. Research Bull 136.

25. Lutrell,J.B. and Garren,K.H. 1952. Blights of snapbean in Georgia. Phytopathology 42:607-613.

26 Mathur,S.B. 1967. Pycnidia formation in Sclerotium bataticola Taub. on sunflower. Phytopath. Z. 58:137-140.

27. Mathur,S.B. 1968. Production of toxins and pectolytic enzymes by two isolates of Sclerotium bataticola Taub. and their role in pathogenesis. Phytopath. Z. 62:327-333.

28. Meyer,W.A.,Sinclair,J.B., and Khare,M.N. 1974. Factors affecting charcoal root of soybean seedlings. Phytopahtclogy 64:845-849.

29. Miller,J.H. 1972. Experiments in molecular genetics. Cold Springs Harbor, New York. 466 pp.

30 Orellana,R.G. 1970. The response of sunflower genotypes to natural infection by Macrophomina phaseoli. Plant Dis. Reptr. 54:891-893.

31. Reichert,I., and Hellinger,E. 1947. On the occurrence, morphology, and parasitism of Sclerotium bataticola. Palestine J. Botany VI:107-147.

32. Sahai Vasudeva,R., and Ashaf, M. 1938. Studies on the root-rot disease of cotton in Punjab. VII. Further investigation of factors influencing incidence of disease. Indian J. Agri. Sci. IX;595-608.

33. Short,G.E., Wyllie,T.D., and Ammon,V.D. 1978. Quantitative enumeration of Macrophomina phaseolina in soybean tissues. Phytopathology 68:736-741.

34. Short,G.E., Wyllie,T.D., and Bristow,P.R. 1980. Survival of Macrophomina phaseolina in soil and in residue of soybean. Phytopathology 70:13-17.

35. Sim,T.,IV. 1976,1977,1978,1980,1981. Summar(ies) of plant disease conditions in Kansas. Ks. State Bd. Agric.,Div. Entom.

36. Sinclair,J.B. 1982. Compendium of soybean diseases, second edition. Am. Phytopathol. Society. P. 30.

37 Tachibana,H. 1969. Evaluation of chemical seed treatment for soybeans. Phytopathology 59:1052 (Abstr.).

38. Wantanabe,T. 1072. Pycnidium formation by fifty different isolates of Macrophomina phaseoli originated from soil and kidney bean seed. Ann. Phytopath. Soc. Japan 382:108-110.

39. Wyllie,T.D., and Calvert,O.H. 1969. Effect of flower removal and pod set on formation of sclerotia and infection of Glycine max by Macrophomina phaseoli. Phytopathology 59:1243-1245.

40. Yang,S.M., and Owen,D.F. 1982. Symptomology and detection of Macrophomina phaseolina in sunflower plants parasitized by Cylindrocopturus adspersus larvae. Phytopathology 72:819-821.

41. Young,D.J., Gilbertson,R.L., and Alcorn,S.M. 1982. A new record for longevity of Macrophomina phaseolina sclerotia. Mycologia 74:504-505.

42. Young,H.C, 1943. The toothpick method of inoculating corn for ear and stalk rot. Phytopathology 33:16.

43. Young,P.A. 1949. Charcoal rot of plants in east Texas. Texas Agr. Exp. Sta. Bull. 712.

Appendix 1. Kansas isolates of *Macrophomina phaseolina* used in these studies.

Isolate	Date obtained	Host	County
CR77-6	9/77	Soybean (cv. Liberity)	Franklin
CR80-PC	8/80	Popcorn	Jefferson
CR80-SB	8/80	Strawberry	?
CR80-SW	8/80	Sweetcorn	Riley
CR80-1	7/80	Soybean (cv. Dare)	Cherokee
CR80-2	7/80	Soybean	Riley
CR80-3	7/80	Soybean (cv.Agripro 40)	Barton
CR80-4	9/80	Soybean	Riley
CR80-6	9/80	Soybean	Riley
CR80-7	9/80	Soybean (cv. Cumberland)	Brown
CR80-8	9/80	Soybean	Morris
CR80-9	9/80	Soybean	Bourbon
CR80-10	9/80	Soybean	Miami
CR80-11	9/80	Soybean	Osage
CR80-12	9/80	Soybean	Cherokee
CR80-13	9/80	Soybean	Cherokee
CR80-14	9/80	Soybean	Allen
CR80-15	9/80	Soybean	Linn
CR80-16	9/80	Soybean	Anderson
CR80-17	9/80	Soybean	Pottawatomie
CR80-18	9/80	Soybean	Brown
CR81-1	3/81	Soil	Cherokee
CR81-2	3/81	Alfalfa	Riley
CR81-3	4/81	Soybean	Riley
CR81-4	7/81	Soybean	Cherokee
CR81-5	7/81	Soybean	Barton
CR81-6	7/81	Soybean	McPherson
CR81-7	7/81	Soil	Cherokee
CR81-8	7/81	Soil	Cherokee
CR81-9	7/81	Soybean	Cherokee
CR81-10	8/81	Soybean	Cherokee
CR81-11	8/81	Soybean (cv. Cutler 71)	Barton
CR81-12	9/81	Soybean (cv. Essex)	Cherokee
CR81-13	9/81	Soybean (cv. Cumberland)	Marshall
CR81-14	9/81	Soybean	Butler

MACROPHOMINA PHASEOLINA, CAUSAL ORGANISM OF CHARCOAL ROT OF SOYBEAN. I. LABORATORY TESTS FOR RESISTANCE. II. IMPLICATIONS OF CONIDIA IN EPIDEMIOLOGY. III. ANTIMICROBIAL ACTIVITY OF TOXIN(S).

by

CHARLES ALBON STANLEY PEARSON

B. S., Pennsylvania State University, 1980

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1982

Several laboratory inoculation techniques were used with seeds or seedlings to try to predict field response of soybean to Macrophomina phaseolina. None did, either alone or in combination. Induced drought stress increased symptom severity of growth chamber grown plants, and may be useful in determining levels of resistance to the fungus.

Kansas isolates of M. phaseolina were tested for pycnidial production. The number of pycnidia produced differed signficantly among isolates, but was stable within an isolate and single-spored sub-isolates.

Optium temperatures for spore germination corresponded to those conducive for disease development. Conidia were viable throughout the winter of 1980 suggesting that spores could be important as primary inoculum.

Partially purified toxin(s) produced by CR77-6 inhibited bacteria and fungi, and caused soybean seedlings to wilt. This material contained both carbohydrate and protein, and had a molcular weight of 2×10^6. Such toxin(s) could aid M. phaseolina in maintainance of a food base by decreasing competition throught antibosis.