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To the Graduate Council:

I am submitting herewith a thesis written by Paul J. Goodfellow entitled "Growth, reproduction, and toxin production of Phomopsis sojae Lehman in culture." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

James W. Hilty, Major Professor

We have read this thesis and recommend its acceptance:

Ernest Bernard, Leander Johnson

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Paul Joseph Goodfellow entitled "Growth, Reproduction, and Toxin Production of *Phomopsis Sojae* Lehman in Culture." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

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James W. Hilty, Major Professor

We have read this thesis and recommend its acceptance:

Ernest C Bernard

Leander F. Johnson

Accepted for the Council:

L Evans Roth

Vice Chancellor
Graduate Studies and Research

Ag-VetMed
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GROWTH, REPRODUCTION, AND TOXIN PRODUCTION OF *PHOMOPSIS SOJAE* LEHMAN IN CULTURE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Paul Joseph Goodfellow

June 1980

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ABSTRACT

The role of *Phomopsis sojae* in the soybean field and in seed decay has been well investigated. The production of toxins by this fungus, not previously reported, was examined in this study. Dilutions of *P. sojae* culture filtrates, derived from 14 - 29 day old cultures, significantly affected soybean seed germination; 1/10 and 1/100 dilutions inhibited soybean seedling root elongation. A dilutable phytotoxin was produced by *P. sojae* in culture.

The fungus grew well on six of seven media tested at temperatures ranging from 10 - 30°C. Pycnidial formation in culture occurred infrequently and depended on incubation periods of 35 days or longer.

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CHAPTER I

INTRODUCTION

Phomopsis sojae Lehman causes pod and stem blight of soybeans. The pathogen is most often associated with mature, senescent, and weak plants (6,50). It has been reported as the most important deteriorating agent of soybean seed (30,68). Infected seed have reduced viability and in some instances are unsuitable for sowing (24). Oil and flour derived from infected seed are of low quality and infected seed are undesirable for livestock feed (34,53).

Reduced germination of infected seed was observed in preliminary isolations of *P. sojae*. In tests in which five seeds were placed on an agar substrate, recovery of the fungus from a single seed was associated with death or reduced vigor of the others, indicating that the fungus produced a toxin which diffused through the agar to the other seeds. Although knowledge of toxins produced by a pathogen is a useful tool in studying host-parasite interactions (77), no investigations of toxin production by *P. sojae* have been conducted. The purpose of this study was to investigate production of *P. sojae* toxins active against soybeans. A study of fungal growth on artificial media was also conducted.

CHAPTER II

LITERATURE REVIEW

Pod and stem blight of soybean (*Glycine max* [L.] Merr.) was first reported in the United States in 1920 (103). The causal agent was erroneously described as *Phoma* sp. In 1923 Lehaman (57) redescribed the disease and named the pathogen *Diaporthe sojae* Lehman, the perfect state of *Phomopsis sojae* Lehman. Subsequently the disease was reported from many soybean producing states (4,9,27,39,52,56,62,66,69,70,81,88) and several foreign countries (7,37,63,75,78,104).

The taxonomy of the pod and stem blight fungus is an issue of considerable confusion. Until 1948 (94), stem canker of soybean was considered part of a pod and stem blight complex. In the early 1950's (1,13) stem canker was recognized as a distinct disease incited by *D. phaseolorum* (Cke. and Ell) Sacc. var. *caulivora* Athow and Caldwell. *Diaporthe sojae*, as described by Lehman, had previously been taken to the varietal status (93) and recognized as *D. phaseolorum* (Cke. and Ell.) var. *sojae* Wehm. The pathogens are closely related and many efforts to distinguish the two in culture and the diseases they cause have been made (1,35,36,51,75,82,83). Presently, *D. phaseolorum* var. *sojae* is accepted as the perfect state of *Phomopsis sojae* (24,65,75).

Sinclair and Shurtleff (75) described the pathogen and noted the infrequency of the perithecial state in nature. *P. sojae* has been noted very variable in culture (6,16,35,36,60,61,68,91).

Hosts of *P. sojae* other than soybean include lima bean (*Phaseolus limensis* Hacf.) (31), snapbean (*P. vulgaris* L) (59), cowpea (*Vigna sinensis* [Torner] Savi) (73), birdsfoot-trefoil (*Lotus corniculatus* L.) (97), peanut (*Arachis hypogaea* L.) (3), tomato (*Lycopersicon esculentum* Mill.) (8), pepper (*Capsicum frutescens* L.) (84), garlic (*Allium sativum* L.), onion (*A. cepa* L.), lespedeza (*Lespedeza* spp.) and lupine (*Lupinus* spp.) (75).

P. sojae is a weak pathogen of soybeans (2,18,36,59,60,94). Once believed to be of neglible economic importance (36,60), *P. sojae* is now an important consideration in soybean production (34).

Infection of the growing plant results in pod and stem blight symptoms (57,75), stem canker symptoms (55,75), lesions on cotyledons and emerging plants (68,89), and basal stem and root rot (28), but the latter have been observed only under experimental conditions (28). Infection may lead to premature death of plants, failure of young ovules to develop and seed decay (57). Yield losses due to pod and stem blight are in most instances minimal (10,23,34,39,43,48,54,88,98,99,100) and have been reported as zero (36). Others have reported severe losses (48,52,81).

Pod and stem blight is usually a disease of mature, senescent, and weak plants (6,15,36,44,49,50,62,92). Plants are predisposed to infection by mechanical injury (55,60,62,101), fungal diseases (12,55,59), virus infection (33), insect damage (14,44,46,60), and frost (44,64,80). An abundance of moisture at maturity and delayed harvest contribute to greater disease outbreak and seed infection (20,25,28,29,42,44,61,72,75,

98,102). Early maturing cultivars are more susceptible to infection than late cultivars (14,41,44,45,59,61,75).

P. sojae is the most frequently recovered (11,20,21,89,90,91) and most important fungus deteriorating soybean seed (17,30,68,75,79,89). *P. sojae* infected seed have reduced germination (20,23,24,25) and in some instances are unsuitable for sowing (24,87). The fungus colonizes the seed coat (7,22,38,71) and occasionally embryonic tissue (7,22,38). Production of a tissue-degrading substance as part of seed pathology has been suggested (71). Infected seed can be moldy and fissured (68, 71,75) or asymptomatic (34). Diseased seed are smaller and less dense than healthy seed, and oil and flour derived from infected seed are of low quality (34). Infected seed are undesirable as livestock feed (53) and seedpods are inedible (78).

Seed become infected by colonization of the maturing pod (5,14,42,46, 47). Systemic infection has been suggested (44,49), but is a matter of uncertainty (50). The major sources of inoculum are crop debris (28,36, 50,75,86), weed hosts (17), volunteer soybean plants (26), and infected seed (74,75).

CHAPTER III

MATERIALS AND METHODS

A. BIOASSAY FOR PHYTOTOXIN PRODUCTION

Phomopsis sojae was maintained on potato-dextrose sugar (PDA). For phytotoxin production, the fungus was cultured at room temperature for 14, 21, 23, and 29 days in stationary culture in 400 ml R_x bottles containing 200 ml liquid Fries' medium. The medium was supplemented with 0.1% yeast extract, prepared as described by Tuite (85). Cultures were established with 7 mm discs cut with a cork borer from 10-12 day old mycelium growing on PDA.

The bioassay for phytotoxin activity was a modification of that used by Luke and Wheeler (58). After the appropriate incubation period, a cell-free culture filtrate was obtained by passing the culture medium under vacuum through a 0.45μm Metricel® membrane filter in a Millipore® filter apparatus. The culture fluid was diluted 1/10, 1/100, 1/1000 and 1/10,000 with White's nutrient solution (96). Forrest cultivar soybean seeds were sterilized with propylene oxide for 24 hours at the rate of 0.5 ml per liter volume, followed by a six minute soak in 0.525% NaOCl and two sterile distilled water-rinses. Seeds were placed individually in 60 × 15 mm plastic Petri dishes. Approximately 10 of the culture fluid dilution was poured into Petri dishes and seeds were allowed to incubate one week at 25°C ± 2°C. Thirty seeds per concentration of cell-free filtrate and a control of White's solution constituted

a replicate. The bioassay was replicated three times for each culture age.

After one week in solutions the seeds were rated for germination using 0 for seeds that failed to germinate, and 1 for germinating seeds. Root length in cm was also measured. Seeds failing to germinate were assigned a root length of 0.00 cm. Contaminated seeds were disregarded.

Germination data were analyzed with a SAS FUNCAT procedure (32) to produce minimum chi-square estimates for a factorial randomized complete block design. Data were tested for significance of effects of dilution of cell-free filtrate, effects of culture age from which filtrates were derived, and replicate effects. Effects of the source culture age-dilution treatment interactions were also tested. All tests were conducted at the 5 percent level of significance.

Data on root lengths were analyzed with a SAS GLM procedure (32) for general linear models. An unbalanced analysis of variance for a factorial randomized complete block design was conducted (32). Data were tested for significance of dilution of cell-free filtrate effects, effects of source culture age, replicate effects, and the effects of source culture age-dilution treatment interactions at the 5 percent level. SAS LSMEANS statement and STDERR option (34) were used to generate least squares means and standard errors for root lengths for each cell-free filtrate dilution, culture age, and dilution-culture age interaction.

B. GROWTH CHARACTERIZATION AND SPORULATION STUDIES

Growth and sporulation of *Phomopsis sojae* on seven agar media at five temperatures were compared. Agar media tested were corn meal (CMA), lima bean (LBA), 2% water agar (WA), potato-dextrose agar (PDA), Czapek's agar (CA), BBL ™potato-dextrose agar (PDAb) and a soybean seed decoction agar (SSA). Incubation temperatures were 30, 25, 18, 15, and 10°C, all ±2°C. CMA was prepared as described by Johnson and Curl (40). LBA, PDA, and CA were prepared as described in Tuite's manual (85), PDAb and WA as directed on labels. SSA was prepared by modifying Edgerton's bean pod agar with 30 g soybean seeds instead of dried bean pods (19,85). A cork borer was used to cut uniform discs from margins of 12 day old PDA cultures of *P. sojae*. The discs were transferred to the centers of 90 mm Petri dishes containing approximately 20 ml of the media. Radii of cultures in three replicates of each medium and temperature were recorded daily for one week. Cultures were examined periodically for fruiting structures and discarded after 20 days.

Data on radii and colony area ($\pi radius^2$) after 48-hour incubation were analyzed with an analysis of variance for a factorial randomized complete block design with the SAS ANOVA procedure (32). Data were tested for significance of temperature, medium, and replicate effects, as well as the effects of medium-temperature interaction. Mean radii and area for each temperature-medium combination were separated with the SAS DUNCAN procedure (32). All analyses were performed at the 5 percent level of significance.

Further attempts to induce pcynidial formation included a modification of Papavizas' technique (67) in which the fungus was first cultured on a rich growth medium and transferred to a sporulation medium low in nutrients. The fungus was cultured on sporulation media with and without exposure to light. *P. sojae* was cultured 8 days on Czapek-Dox agar (CDA) and potato-dextrose agar with 1 g of yeast extract per liter (PDA-YE). Seven-mm discs from each were transferred to 8 plates of each of the following:

1. Flentje soil extract agar (SEA);
2. Soil extract with 0.1g yeast extract per liter (SEA-YE);
3. 2% water agar (WA).

Petri dishes were sealed with cellophane tape to retain moisture. All media were prepared as described by Tuite (85).

Four plates of each sporulation medium were arranged on a counter top and illuminated continuously with a small desk lamp for 35 days. Another 4 plates per treatment were incubated in the dark at 25°C ± 2°C during the same period. Cultures on growth media were also retained 35 days. All cultures were examined for development of fruiting structures.

Methods of Sloan, Routien, and Miller (76) were followed in a further attempt to induce sporulation through use of coconut milk. Twenty 90mm Petri dishes each of potato-glucose agar and alphacel medium, prepared as described by Sloan et al. (74), were initiated with 7mm discs taken from 22 day-old cultures of *P. sojae* on PDA. Cultures were examined for development of fruiting structures after 25 days

incubation at room temperature. From preliminary investigations it appeared that a long period of maturation of cultures was necessary for pycnidial formation. Fifteen 90mm Petri dishes containing approximately 20 ml of PDA were initiated as previously described, sealed with Parafilm® and placed in a single layer in plastic bags. The cultures received intermittent periods of illumination for 40 days, after which they were examined for pycnidial development.

In view of the heterothallic nature of *P. sojae* (75) a number of strains of the fungus were isolated from soybean seeds obtained from the Plant Sciences Farm, The University of Tennessee, Knoxville. They were mated with the laboratory strain in an attempt to induce perithecia of *Diaporthe phaseolorum* var. *sojae*. Pods of Pickett 71, Centennial, Bedford, Forrest, McNair 500 and Essex cultivars with pod and stem blight symptoms were hand shelled. Fifty seeds from each were sterilized 3 minutes in 0.525% NaOCl solution followed by 2 rinses in sterile distilled water. Seeds were placed 5 per PDA plate and after 5 days *P. sojae* isolates were identified on the basis of gross culture morphology, coded as to cultivar of origin and transferred to PDA. When cultures were 7 to 14 days old a 7mm disc from each was placed on the periphery of a 90mm Petri dish containing approximately 20ml of PDA. A similar disc of the laboratory strain of *P. sojae* was placed of the opposite periphery. All dishes were sealed with Parafilm®. After 29 days incubation at room temperature, cultures were observed for perithecial development.

CHAPTER IV

RESULTS AND DISCUSSION

A. BIOASSAY FOR PHYTOTOXIN PRODUCTION

Chi-squares from analysis of germination data are given in Table 1.

Table 1. Chi-squares for Germination of Soybean Seeds Treated With Culture Filtrates of *Phomopsis sojae*.

Sources	df	Chi-square	Probability
Intercept	1	390.86	0.0001
Culture Age	3	3.55	0.3144
Dilution of Culture Filtrate	4	2.77	0.5963
Replication	2	0.94	0.6245
Dilution-Culture Age Interactions	12	21.95	0.0381
Residual	37	24.20	0.9480

There were no significant differences in germination between seeds treated with different dilutions of culture filtrate or filtrates from cultures of different ages. Significant differences in germination existed among interactions of culture filtrate dilutions and culture ages. The significant differences may have been due to lower germination demonstrated in the 14 day – 1/10 dilution treatment combination (Table 2). Results given in Table 2 indicate that if a toxin is produced by *P. sojae*, it inhibits germination when at high concentration and when the fungus

Table 2. Mean Percent Germination of Soybean Seeds Treated With Culture Filtrates of *Phomopsis sojae*.

Age of Source Culture (Days)	Dilution of Culture Filtrate	% Germination
14	1/10	77[1]
14	1/100	96
14	1/1,000	93
14	1/10,000	97
14	Control	93
21	1/10	98
21	1/100	91
21	1/1,000	97
21	1/10,000	92
21	Control	96
23	1/10	94
23	1/100	96
23	1/1,000	89
23	1/10,000	90
23	Control	93
29	1/10	97
29	1/100	99
29	1/1,000	93
29	1/10,000	88
29	Control	85

[1]Averages for 3 replicates.

cultures are 14 days old. Further studies on the effects of 1/10 dilutions of *P. sojae* culture filtrates from 5-14 days may be warranted.

There were significant differences in root lengths between different culture filtrate dilutions and between interactions of dilution — source-culture age. However, there were no differences in root length between treatments with filtrates from cultures of different ages. Filtrate dilutions of 1/10 and 1/100 reduced root length, but greater dilutions did not (Figure 1). Results were similar from 14, 21, 23, and 29 day old cultures (Figure 2), but in general, older cultures had a greater inhibitory effect on root length. Filtrates from the 14 and 21 day cultures were effective only at the 1/10 dilution. Filtrate dilutions of 1/1,000 and 1/10,000 from cultures 21,23, and 29 days old stimulated root elongation (Figure 2). The general trend was one of reduction of root length at 1/10 and 1/100 dilutions, and stimulation at 1/1000 and 1/10,000 dilutions (Figure 3).

These experiments indicate that *P. sojae* produces in culture, a toxin which inhibits soybean seedling root elongation. Filtrates of *P. sojae* cultures diluted 1/10 and 1/100 have an apparent inhibitory effect on root elongation. Culture filtrates diluted 1/1000 and 1/10,000 apparently stimulate root elongation.

The capacity of very dilute culture filtrate solution to stimulate root elongation is probably the result of several interacting factors. Dilution of the proposed phytotoxin beyond a critical level of biological activity would explain loss of inhibitory effects. Nutrients present in the medium that supported *P. sojae* and byproducts of the fungus' metabolism may account in part for apparent stimulation of root elongation.

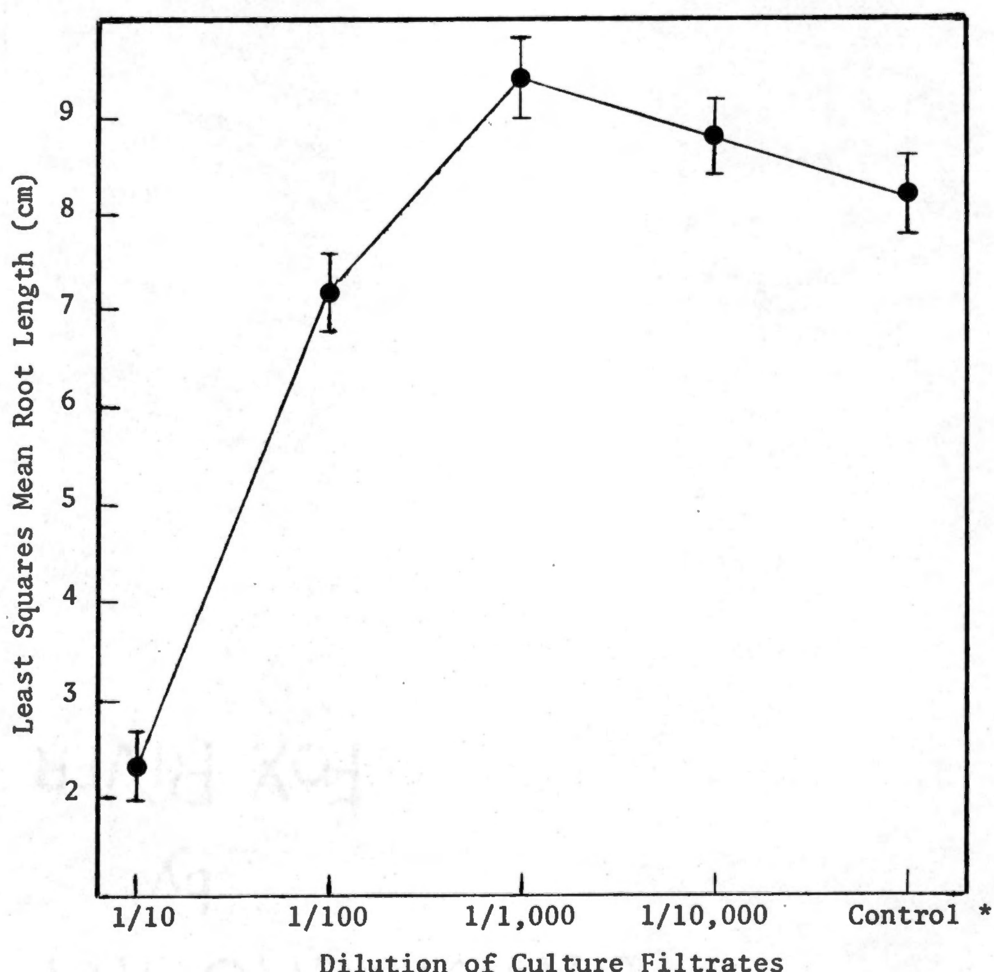

Figure 1. Effect of *Phomopsis sojae* Culture Filtrates on Root Length of Soybean Seedlings. White's Nutrient Solution as Control; Vertical Bars Equal Standard Errors of Means.

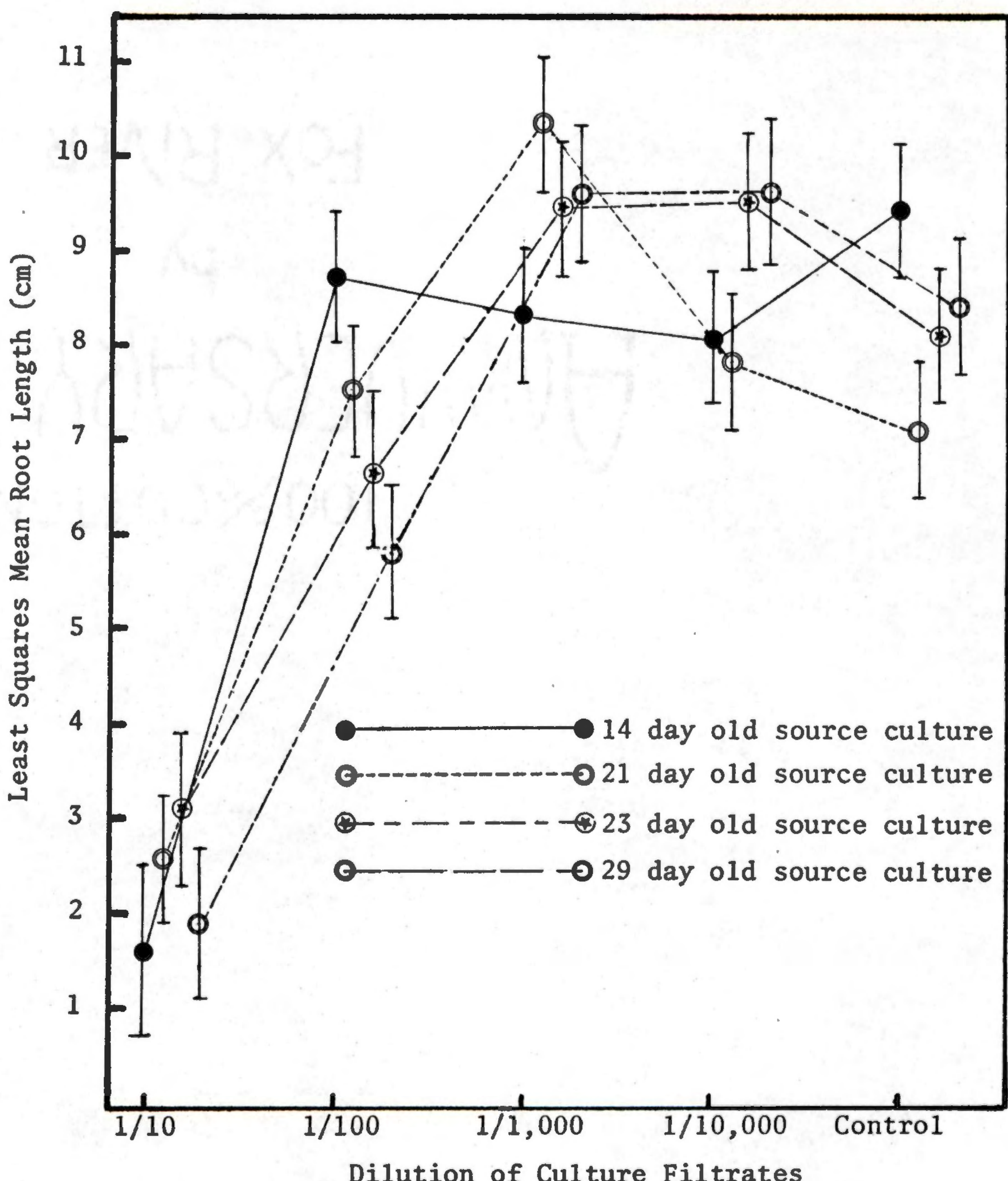

Figure 2. Effects of Culture Age on Root Lengths of Soybean Seedlings. White's Nutrient Solution as Control; Vertical Bars Equal Standard Errors of Means.

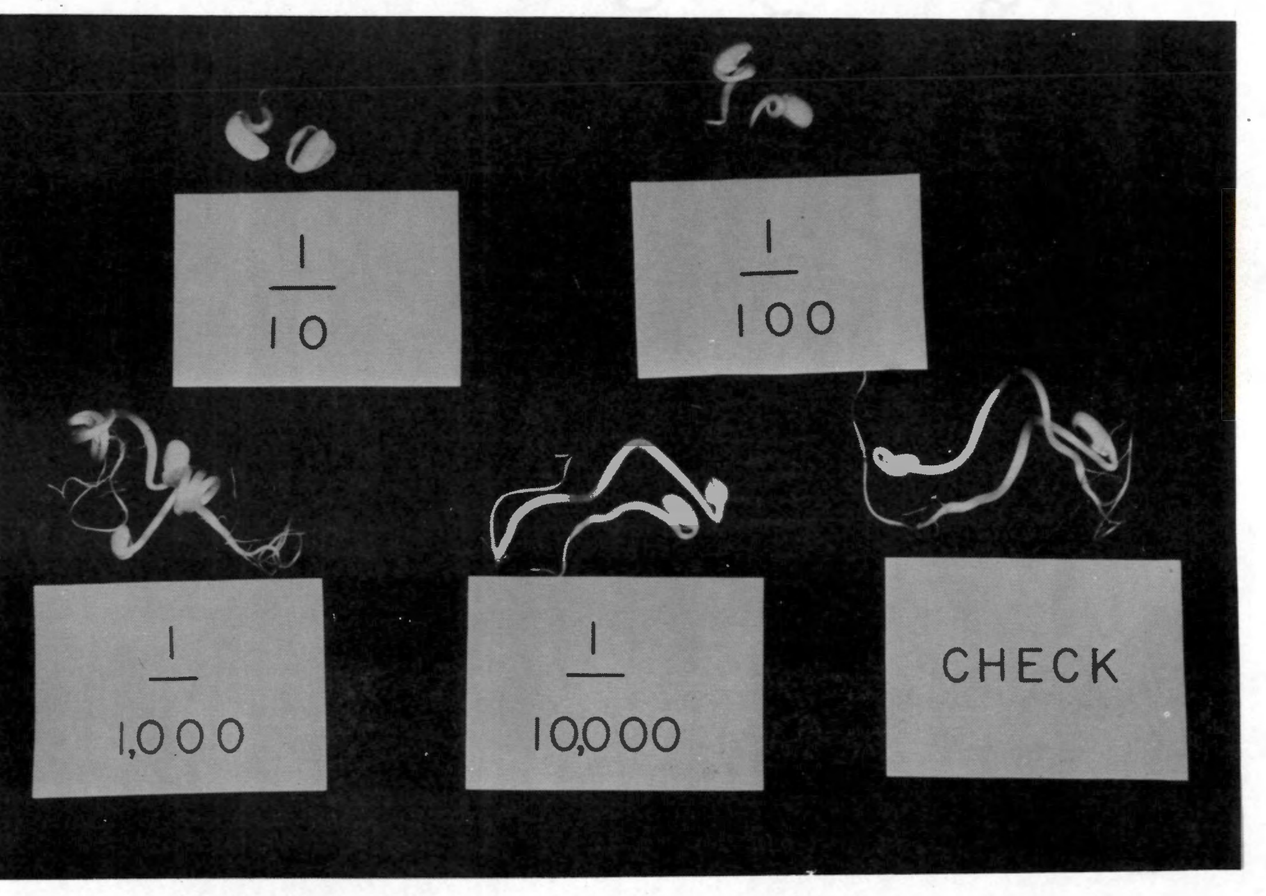

Figure 3. Germinating Soybean Seeds Treated with 29-Day-Old Culture Filtrate Dilutions of *Phomopsis sojae*.

Further investigation of the apparent *P. sojae* phytotoxin is warranted. The nature and role of the biologically active compound or compounds in seed pathology is of both economic and academic interest. Phytotoxins do not necessarily play any role in relation to disease (95). Production of tissue degrading substances by *P. sojae* has, however, been suggested (71) and production of mycotoxins by this fungus has also been reported (53). Further studies might include similar bioassays using a number of recently isolated cultures of the fungus, investigations of the biological activity of culture filtrates on various developmental stages of the plant, and chemical characterization of the biologically active compound or compounds.

B. GROWTH CHARACTERIZATION AND SPORULATION STUDIES

Sporulation of *P. sojae* in culture was infrequent and occurred only after long periods of incubation. Culture morphology varied with medium and temperature (Figures 4 and 5). *P. sojae* failed to produce pycnidia on potato-dextrose, cornmeal, lima bean, bottled potato-dextrose, Czapek's, soybean seed decoction or water agar after 25 days growth at temperatures tested. Some degree of stromatization was observed in all cultures.

Significant differences in growth as measured by both radius and culture area were found among agar media, temperatures, and their interactions (Table 3, 4). The optimum temperature for growth on all media was 25°C. Significant differences in growth existed between commercially prepared and fresh potato-dextrose agar. Fresh

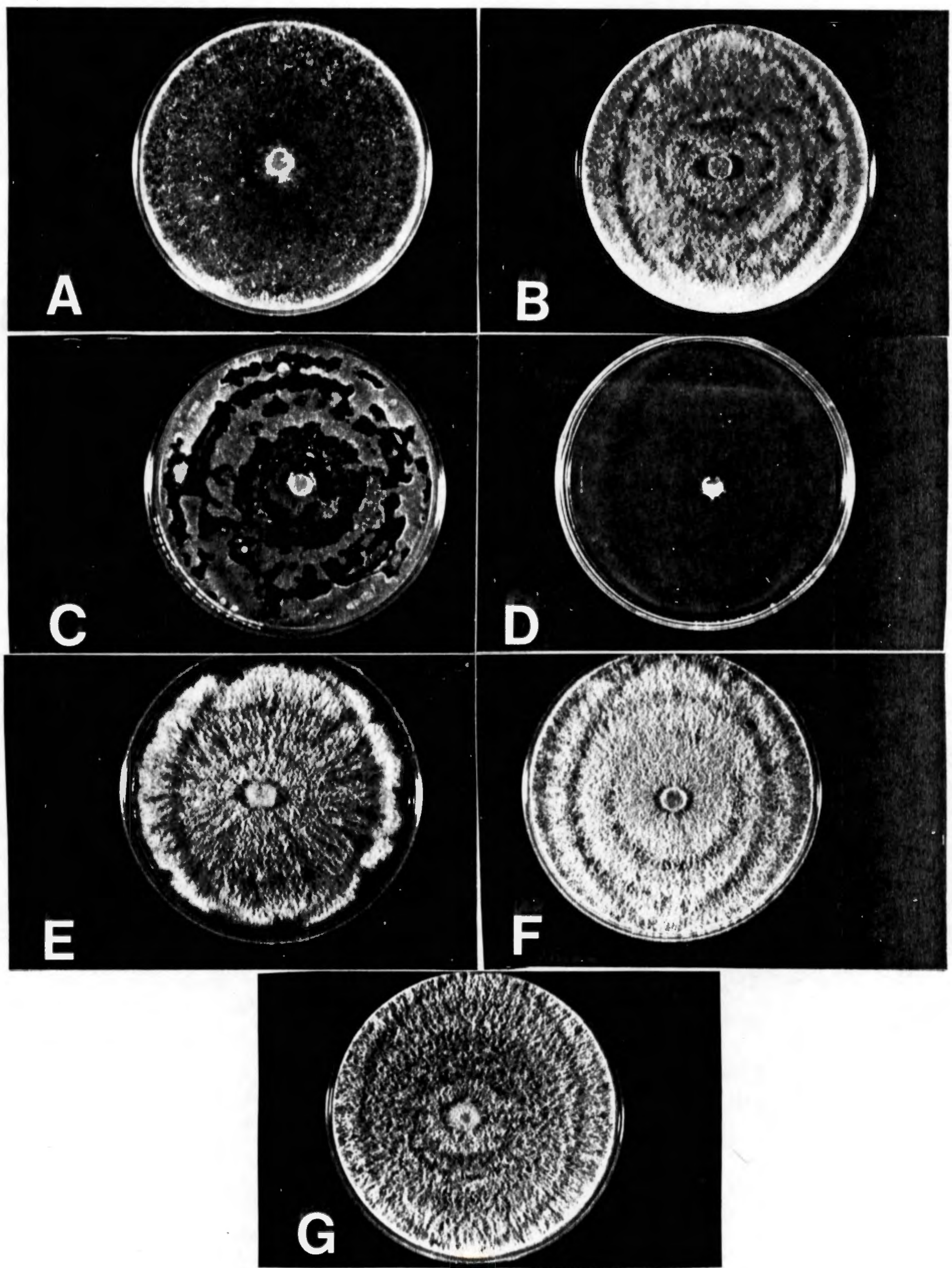

Figure 4. Ten Day Old Cultures of *Phomopsis sojae* on Seven Media at 25°C. (A) C, (B) SSA, (C) CMA, (D) WA, (E) PDA, (F) PDAb, (G) LBA.

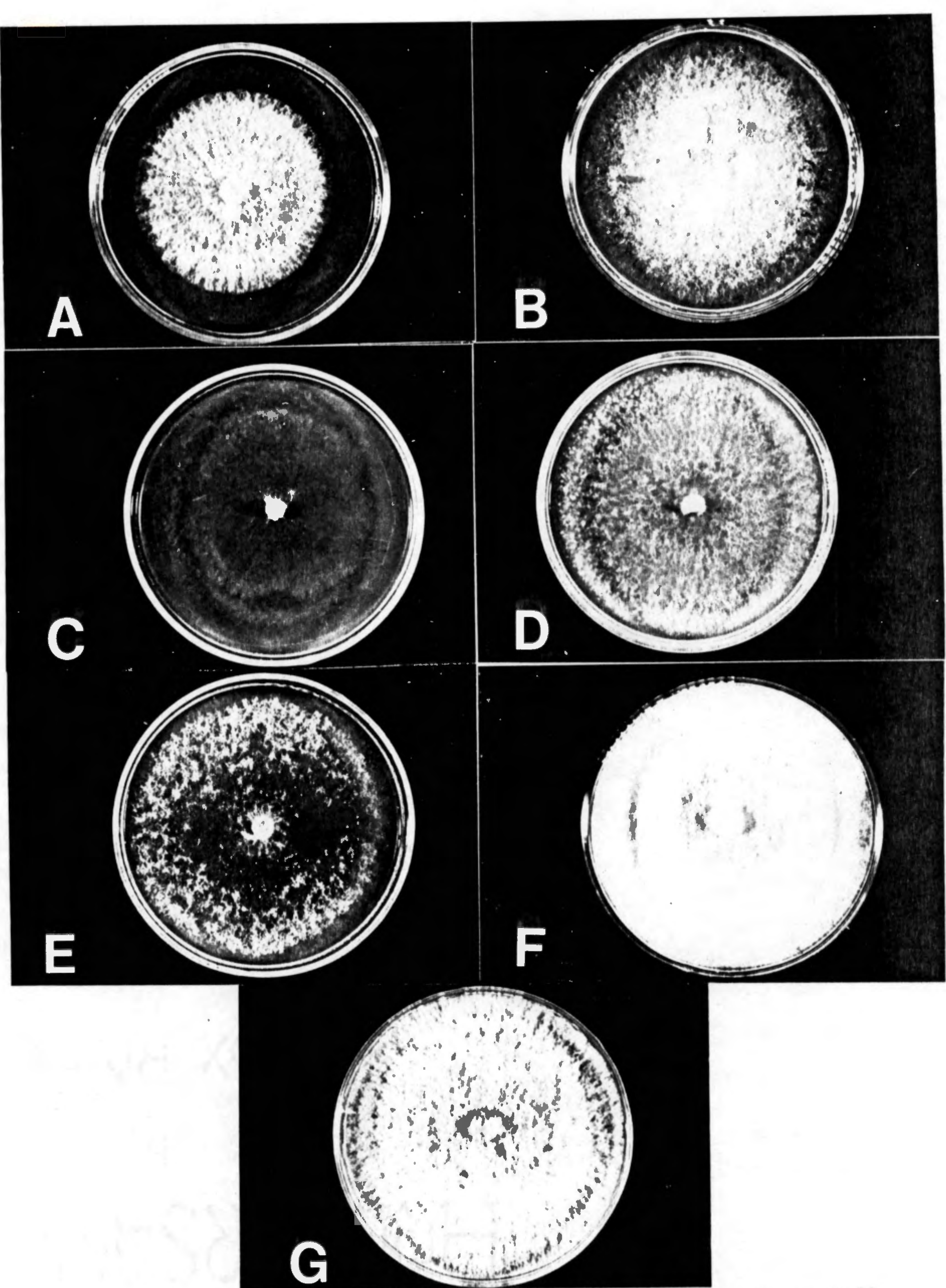

Figure 5. Ten Day Old Cultures of *Phomopsis sojae* on Seven Media at 10°C. (A) C, (B) SSA, (C) CMA, (D) WA, (E) PDA, (F) PDAb, (G) LBA.

Table 3. Effects of Agar Media and Incubation Temperature on Radial Growth on *Phomopsis sojae*.

Medium	Incubation Temperature (°C))	Mean Colony Radius (mm^2)[1]
PDA	25	45.00 a
LBA	25	41.17 b
CMA	25	38.83 b
PDAb	25	34.67 c
SSA	25	29.83 d
PDA	30	29.83 d
CA	25	28.50 de
PDA	18	27.50 def
LBA	30	27.50 def
LBA	18	25.67 ef
WA	25	25.00 fg
PDAb	18	23.17 g
CMA	18	22.83 g
CMA	30	22.50 gh
CA	18	19.50 hi
PDA	15	19.00 i
CA	30	19.00 ij
PDAb	30	18.50 ijk
LBA	15	18.33 ijk
LBA	10	18.33 ijk
SSA	30	18.17 ijk
SSA	18	17.83 ijk
WA	18	17.83 ijk
PDAb	15	16.83 ijkl
CMA	15	16.67 ijkl
PDA	10	15.50 jklm
WA	30	15.33 jklm
CMA	10	15.17 klm
CA	15	14.17 lmn
PDAb	10	14.00 lmn
SSA	15	13.83 lmn
SSA	10	12.17 mno
CA	10	10.83 no
WA	15	10.33 o
WA	10	9.17 o

[1]Values are means for three replicates.

[2]CMA: Corn Meal Agar; LBA: Lima Bean Agar; WA: 2% Water Agar; PDA: Potato-dextrose Agar; CA: Czapek's Agar; PDAb: BBL Potato-dextrose Agar; SSA: Soybean Seed Decoction Agar.

Table 4. Effects of Agar Media and Incubation Temperature on Growth of *Phomopsis sojae*.

Medium	Incubation Temperature (°C)	Mean Colony Area (mm^2)[1]
PDA	25	6358.5 a
LBA	25	5323.6 b
CMA	25	4735.4 c
PDAb	25	3920.8 d
SSA	25	2794.9 e
PDA	30	2794.9 e
CA	25	2552.6 ef
PDA	18	2385.6 ef
LBA	30	2375.1 ef
LBA	18	2074.0 fg
WA	25	1064.6 fg
PDAb	18	1699.0 gh
CMA	18	1637.8 ghi
CMA	30	1590.1 ghij
CA	18	1194.5 hijk
PDA	15	1135.1 hijk
CA	30	1135.1 hijk
PDAb	30	1076.2 ijkl
LBA	10	1056.6 ijkl
LBA	15	1055.6 ijkl
SSA	30	1052.1 ijkl
SSA	18	1000.9 jklm
WA	18	999.3 jklm
PDAb	15	891.0 klmn
CMA	15	877.1 klmn
PDA	10	761.2 klmn
WA	30	738.4 klmn
CMA	10	722.5 klmn
PDAb	10	640.6 klmn
CA	15	636.6 klmn
SSA	15	606.3 klmn
SSA	10	465.5 lmn
CA	10	369.7 mn
WA	15	335.5 mn
WA	10	265.1 n

[1]Values are means for three replicates.

[2]CMA: Corn Meal Agar; LBA: Lima Bean Agar; WA: 2% Water Agar; PDA: Potato-dextrose Agar; CA: Czapek's Agar; PDAb: BBL Potato-dextrose Agar; SSA: Soybean Seed Decoction Agar.

potato-dextrose agar and lima bean agar media were both suitable for growth of *P. sojae* at the temperatures tested.

Growth of *P. sojae* on Czapek-Dox agar and potato-dextrose agar (PDA) supplemented with yeast extract was rapid and luxurious. Considerable stromatization occurred on both media. Pycnidial development was observed on PDA supplemented with yeast extract after a 35-day incubation. Growth and stromatization of cultures with and without exposure to light were limited on water agar, soil extract agar (SEA) and SEA supplemented with yeast extract. Water agar and soil extract agars were not suitable for development of pycnidia.

Alphacel medium and potato-glucose agar (PGA) containing coconut milk supported growth of *P. sojae*. The fungus grew more luxuriously on PGA than on alphacel medium. Sporulation was not observed on either medium after 25 days of growth. No conclusions can be made as to whether the coconut milk-supplemented media are suitable for *P. sojae* reproduction. Failure to develop fruiting structures may have been due to the short period over which the experiment was conducted.

Pycnidia were observed in 40 day old potato-dextrose agar (PDA) cultures (Figure 6). PDA has been reported as suitable for reproduction (68), but only 2 of 15 cultures developed fruiting bodies. Infrequent sporulation and sterility of *P. sojae* in culture has been reported (28).

P. sojae was isolated from seeds of 5 of 6 cultures tested (Table 5).

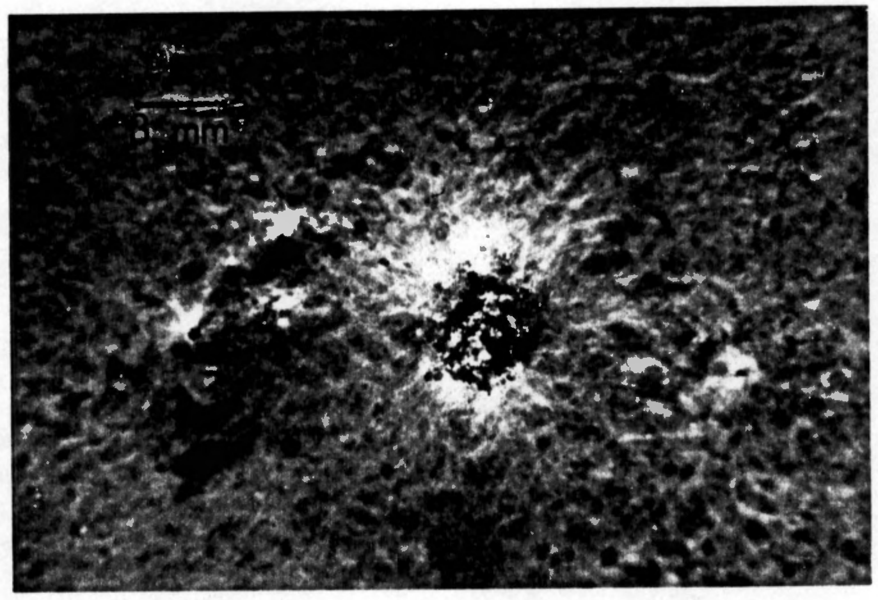

Figure 6. *Phomopsis sojae* Pycnidia in 45 Day Old Culture on PDA — Yeast Extract Supplemented Medium.

TABLE 5. Isolation of *Phomopsis sojae* from Surface-Sterilized Seeds of Selected Soybean Cultivars.

Cultivar	Number of seeds harboring *P. sojae* (out of 50/cultivar)
Bedford	5
York	4
Forrest	6
Essex	34
Pickett 71	0
McNair 500	8

Perithecia developed in 8 of 57 matings (Figure 7). Fruiting bodies developed along the line of interaction between the two fungal cultures. Successful matings involved 5 isolates from Essex cultivar and one isolate each from Forrest, McNair 500, and Bedford cultivars.

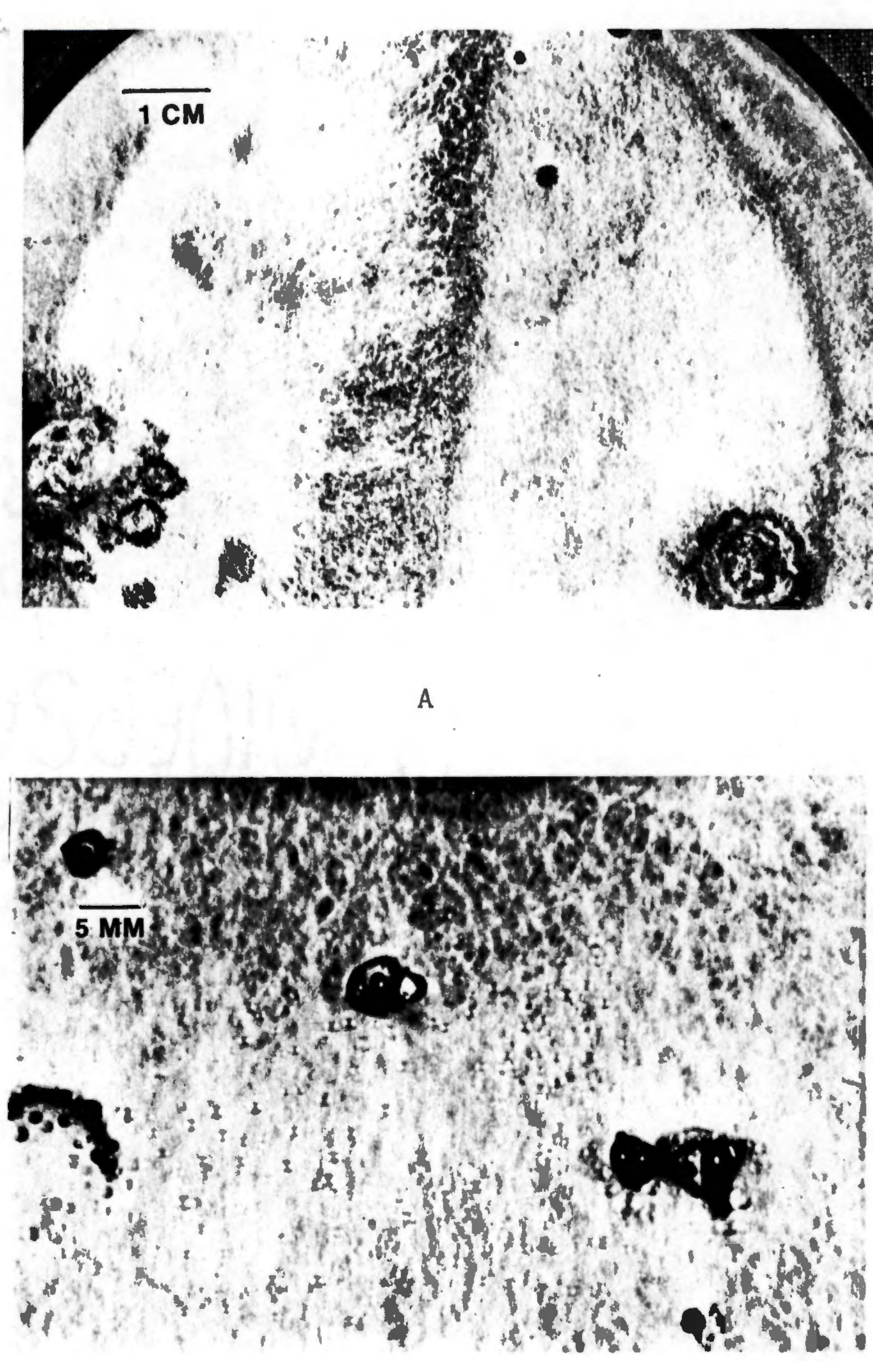

Figure 7. *Phomopsis sojae* Perithecia Produced on PDA. (A) Line of Interaction of Fungal Strains, (B) Close Up of Perithecia Arising in Stroma.

CHAPTER V

SUMMARY AND CONCLUSIONS

There were significant differences in germination of soybean seeds treated with dilutions of *Phomopsis sojae* culture filtrates from cultures 14, 21, 23, and 29 days old. Culture filtrates of 1/10 and 1/100 dilutions inhibited root elongation of treated seeds. Inhibition was attributed to phytotoxin production by *P. sojae*. The relationship of phytotoxins to plant disease is often a matter of uncertainty (95). Investigation into the production and role of the *P. sojae* phytotoxin in disease was suggested. The author surmises that toxin production is in fact a part of phytopathogenesis in *P. sojae* infection of soybean.

P. sojae grew well on six of seven media tested. Potato-dextrose agar was the best medium at temperatures ranging from 10-30°C. Growth on water agar was limited. The fungus grew at all five temperatures, but grew best at 25°C and poorly at 10°C.

Sporulation in culture was infrequent. Long periods of growth appeared to be necessary for development of fruiting bodies. Potato-dextrose agar supplemented with yeast extract was found to be suitable for pycnidial development. The perfect stage of the pathogen was also observed in culture.

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