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Effect of nutrients and inoculum quantity on Pythium ultimum infection of cotton hypocotyls

Chin-chu Hsieh

To the Graduate Council:

I am submitting herewith a thesis written by Chin-chu Hsieh entitled "Effect of nutrients and inoculum quantity on Pythium ultimum infection of cotton hypocotyls." 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.

Leander F. Johnson, Major Professor

We have read this thesis and recommend its acceptance:

J.W. Hilty, H.E. Reed

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

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L Evans Reed

Vice Chancellor
Graduate Studies and Research

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EFFECT OF NUTRIENTS AND INOCULUM QUANTITY ON *PYTHIUM ULTIMUM* INFECTION OF COTTON HYPOCOTYLS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Chin-Chu Hsieh

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DEDICATION

This thesis is dedicated to my mother, Chin-Luan Wang Hsieh, whose encouragement and support is deeply appreciated.

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ABSTRACT

Seedling disease, caused by Pythium ultimum Trow., is one of the most destructive disease of cotton in Tennessee. The objective of this study was to evaluate the effect of the nutritional status of the pathogen on infection and disease development.

The nutritional status of P. ultimum mycelium used to inoculate cotton hypocotyls was varied by inoculating the pathogen on agar media containing various concentrations of nutrients. Sucrose, nitrate-nitrogen, ammonium-nitrogen and potassium in agar media were found to significantly affect vegetative growth and pathogenicity. Disease was more severe when inocula were grown on media with medium to high levels of these materials than when grown on media with low levels. Magnesium and phosphate at low or high levels in agar media did not affect pathogenicity. The pathogen grown on a nitrate-nitrogen medium produced significantly more severe disease than when grown on an ammonium-nitrogen medium.

Light transmission through blended agar cultures was used as a measure of inoculum quantity. There was a significant postive correlation among three factors: levels of sucrose, nitrogen, or potassium in the culture media, inoculum quantity, and disease severity.

In liquid media, P. ultimum became progressively more virulent as the concentration of nitrogen or sucrose was increased, but the weight of the hyphae increased sharply to a maxium at low to median levels of these nutrients. Nitrogen or carbohydrate deficient hyphae produced as much disease as did non-deficient hyphae, provided that the quantities of inocula were similar.

These results emphasize that sources of nutrients and the capacity of the pathogen to utilize these materials are important considerations in understanding disease development in nature.

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CHAPTER I

INTRODUCTION

Cotton seedling disease is a complex disease and can be caused by Pythium ultimum Trow., P. sylvaticum Campbell and Hendrix, P. irregulare Buisman, Rhizoctonia solani Kuhn., Thielaviopsis basicola (Berk. & Br.) Ferr., and Fusarium spp. (25, 26, 27). Of these, Pythium spp. and R. solani cause the most damage in Tennessee (2, 26). Species of Pythium were most frequently isolated from diseased plants at 13 C or lower; R. solani was most often isolated at 20 C or higher (26). Soil temperatures in Tennessee are usually lower than in more southern cotton-growing areas, therefore a high incidence of seedling disease caused by Pythium results in an annual major loss in yield of cotton in this state. In 1976, it caused a 20% reduction (12), 5% reduction in 1977 (13), and 15% reduction in 1978 (14). It was estimated that reduction in yield from seedling disease totaled 37,500 bales in 1978 in Tennessee.

The occurrence and severity of cotton seedling disease is dependent on the interaction of many factors in the environment, such as temperature, moisture, root exudation, soil solution, etc. Temperature and moisture relationships have been extensiverly studied (2, 3, 26, 42). Since control of this disease is not at present satisfactory, more basic studies are needed to learn about other factors that affect infection of seedlings. Several investigators have demonstrated the importance of pathogen nutrition on pathogenicity of fungi (6, 34, 40, 46, 50, 51, 54). However, very little research has been done to determine the influence of

nutrition of Pythium sp. on disease potential on cotton. The present study was made: (i) to determine the effect of nutrition on the pathogenicity of Pythium ultimum hyphae on cotton hypocotyls, and (ii) to determine the effect of inoculum quantity on virulence.

CHAPTER II

LITERATURE REVIEW

I. SYMPTOMS

Pythium ultimum Trow. is an important pathogen of cotton and causes pre-emergence and post-emergence seedling disease (2, 15, 16, 26). However, it also may stunt the young plants severely, and the length of time that symptoms persist is dependent on temperature and moisture after infection. The fruiting cycle of cotton may be delayed and yield reduced (43).

In addition to a root rot, the fungus may produce a post-emergent soft rot of the cortex of the hypocotyl below the surface of the soil. The affected area is almost colorless to light-brown until the decay of the tissues have become well advanced, at which time the necrotic area becomes darker. The lesions tend to involve the entire circumference of the hypocotyls. The fungal hyphae invades the vascular system causing wilting and death of seedlings (2).

II. PLANT NUTITION AFFECTING DISEASE POTENTIAL

The effect of plant nutrtion on disease potential is very complex. Plant nutition status may be altered by a change of environment or by soil amendment (2, 4, 8, 9, 37). Hunter and Guinn (23) analyzed the concentrations of nutrients in cotton hypocotyls at different temperatures and found the concentrations of sugars and nitrogen decreased with age of the seedlings and increased with time at low temperatures.

In another study, the Cp/Rs ratio, the weight of total carbon in the whole plant (Cp) divided by the residual dry weight of the shoot (Rs), was associated with disease potential. High ratio were correlated with high disease potential (18). Maier (36) reported increased disease severity in nitrogen-starved pinto beans.

III. NUTRITION AFFECTING PATHOGENS

Three sources are available for providing required nutrients for soil pathogens: (i) endogenous nutrients in the pathogen, (ii) soil solution and organic materials in soil, and (iii) exudates from the host plant (45).

Hine (21) found that endogenous respiration of Pythium aphanidermatum was reduced by starvation. However, the utilization of stored materials in cells is a complicated process known to be influenced by a number of environmental and nutritional factors. Blakeman (7) stated that different isolates may be nutrient-independent or dependent with respect to exogenous nutrition requirement for conidial germination. Similar results were found by Clark and Lorbeer (10) with Bortrytis squamosa and B. cinerea. They believed this phenomenon was due to endogenous nutrition or endogenous inhibitors.

Endogenous nutrition does not always support germination (11). The energy for infection may depend more or less on the exogenous nutrition supply. Phillips (39) found that in high nutrient media the fungus, Fusarium roseum f. sp. cerealis, produced an apparent abundance of oil globules in the cytoplasm. In another study (24) endogenous nutrient

reserves were important in establishing a pathogenic relationship between a host and a soil-inhabiting pathogen.

Soil solution and organic materials in soil provide many nutrients for soil microorganisms. Several kinds of cations existing in the soil solution affect reproduction of P. ultimum. Yan and Mitchell (56) showed that Mg^{++} and K^{+} induced formation of sporangia only, but Ca^{++} induced formation of both sporangia and oogonia. Also, glucose, fructose, maltose and particularly sucrose induced germination of sporangia of P. ultimum in soil (1, 35). The fungus also utilized a wide range of amino acids and inorganic nitrogen sources (31, 32).

More severe cotton seedling disease caused by Rhizoctonia solani occurred in inoculated sterilized field soil than in sand (51). The author believed that the fungus needed the nutrients found in soil, but which were not present in sand.

Many investigators (20, 28, 29) have concluded that seed exudation is a primary factor in pre-emergence damping-off caused by Pythium spp. under environmental conditions favorable for pathogenesis.

Kraft and Erwin (33) found that when zoospores of Pythium aphanidermatum were supplemented with exudates, virulence of the fungus to mung bean seedlings was increased. Schroth, Hildebrand and Snyder (43, 44) demonstrated that seeds of plant varieties most susceptible to pre-emergence damping-off exuded greater quantities of amino acids and sugars during germination. Similar results obtained by Spence and Cooper (47) with Pythium spp., indicated that glutamic acid, fructose and glucose may be involved in the attraction of Pythium zoospores to cotton plants and in subsequent disease development.

IV. NUTRIENTS IN CULTURE MEDIA AFFECTING PATHOGENICITY

According to Garrett's definition of inoculum potential (17), the type and amount of nutrients available to the pathogen at the infection site could have a direct effect on establishment of infection. Recently studies have supported this. Weinhold *et al.* who did extensive research with *R. solani* Kuhn on cotton (51, 52, 53), found that when the pathogen was grown on high concentrations of aspargine, severe disease resulted. High concentration of glucose, however, interfered with the formation of infection cushions. They suggested that glucose interfered with pathogenesis through inhibition of pectinase enzyme production (52). Similarly, Toussoun *et al.* (48, 49) showed that *Fusarium solani* f. *phaseoli* favored saprophytic development on high concentrations of glucose. However , it was more parasitic when nitrogen was added.

Nitrogen is an important factor in pathogenicity. Kraft and Erwin (34) showed that nitrogen was necessary for infection of mung bean by *Pythium aphanidermatum* at low inoculum densities. Phillips (39) found that *Fusarium* sp. grown on media with high concentrations of nitrogen was more virulent. Maier (36) reported *F. solani* f. sp. *phaseoli* was able to use nitrate-nitrogen better for growth and conidial formation than ammonium-nitrogen, whereas ammonium-nitrogen was best utilized for pathogenesis. Therefore, it is difficult to correlate *in vitro* growth responses of many microorganisms with pathogenicity (55).

Addition of certain nutrients to inoculum may cause a change in fungal physiology and morphology. Banttari and Wilcoxson (5) showed with *Phoma herbarum* that nutrients stimulated the fungus to develop longer and

more vigorous germ tubes and a greater number of appressoria with penetration on a shorter time. However, in high aspargine agar media which was developed for maximum spore production by Helminthosporium maydis, there was decreased germination of spores, decreased formation of appressoria, and infectivity was lower (50).

Several works have shown that previous culture of a pathogen might effect its enzyme systems. Culture filtrates of P. ultimum exhibited marked pectic enzyme activity and rapidly macerated susceptible snapdragon seedling root tissue. Presence of sitosterol in the culture medium of P. ultimum depressed pectic enzyme activity of the culture filtrates (38). Addition of sodium chloride in media stimulated the pectolytic activity of cultures of P. debaryanum Hess (19). Similarly, high concentration of glucose reduced Rhizoctonia cotton seedling disease through inhibition of pectinase enzyme production (52).

Nutritional control of pathogenicity was shown among biochemical mutants of Venturia inaequalis (CKE.) Wint. However, Kline et al. were unable to apply nutritional control in a wild type of the fungus (30). L-sorbose was found to inhibit Rhizoctonia solani in soil and protected cotton against seedling disease (22). Vitamin-amended media decreased virulence of Pellicularia filamentosa on cotton (46).

CHAPTER III

MATERIALS AND METHODS

I. SOURCES OF MATERIALS

Seeds of cotton, Gossypium hirsutum L., a mixture of 27 commercial cultivars, were obtained from P. E. Hoskinson, West Tennessee Experiment Station, Jackson, Tennessee. To minimize differences in seed quality, plants were grown to maturity, selfed and the resulting seed used in this study were acid delined, and those that floated in water were discarded. They were air-dried and stored at room temperature until used.

The fungus used for inocultion was Pythium ultimum Trow. which was isolated originally form diseased seedlings of cotton by L. F. Johnson.

II. THE EFFECT OF THE NUTRIENT STATUS OF THE FUNGUS ON VIRULENCE

Cotton was planted in 26 x 52 cm plastic trays containing autoclaved sand and incubated in temperature controlled plant growth chambers at 27 C for 8 days. In order to avoid hyphal spreading to neighboring plants, each plant was grown at least 3 cm from adjacent plants. It had been previously determined that seedlings become progressively more resistant to Pythium ultimum as the plants age (26,27). To reduce the error that could possibly occur due to the effect of the rate of emergence of seedlings on susceptibility, only seedlings emerging on the 4th, 5th and 6th day after planting were inoculated; seedling that emerged on the 7th and 8th day were removed and discarded.

The nutrient status of the inoculum was varied by culturing the fungus on Czapek's sucrose nitrate media and similar media containing various concentrations of nutrients. All media contained 15 g of agar and 15 mg $FeSO_4.7H_2O$ per liter. Concentration of nutrients per liter in each medium was as follows.

1. Control medium (Czapek's medium).

   $NaNO_3$ 2.0g

   K_2HPO_4 ..... 1.0g

   $MgSO_4.7H_2O$ 0.5g

   KCl 0.5g

   Sucrose 10.0g

2. Sucrose variable media

   $NaNO_3$ 2.0g

   K_2HPO_4 ..... 1.0g

   $MgSO_4.7H_2O$ 0.5g

   KCl 0.5g

   Sucrose ..... 0g, 1.0g, 10.0g, 20.0g and 30.0g

3. Nitrate variable media

   $NaNO_3$ ..... 0g, 0.5g, 1.0g, 2.0g and 4.0g

   K_2HPO_4 ..... 1.0g

   $MgSO_4.7H_2O$ 0.5g

   KCl 0.5g

   Sucrose 10.0g

4. Ammonium-N variable media

$NaNO_3$ ..... 2.0g, 0g, 0g, 0g, 0g, and 0g

NH_4Cl ..... 0g, 0g, 0.5g, 1.0g, 2.0g, and 4.0g

K_2HPO_4 ..... 1.0g

$MgSO_4.7H_2O$ 0.5g

KCl 0.5g

Sucrose 10.0g

Concentrations of $NaNO_3$ and NH_4Cl were paired in the order listed, e.g. concentration of $NaNO_3$ was 2.0 g/liter in the same medium which contained NH_4Cl at 0 g/liter.

5. Magnesium variable media

$NaNO_3$ 2.0g

K_2HPO_4 ..... 1.0g

$MgSO_4.7H_2O$ ..... 0g, 0.125g, 0.25g, 0.5g, and 1.0g

KCl 0.5g

Sucrose 10.0g

6. Potassium variable media

$NaNO_3$ 2.0g

K_2HPO_4 ..... 0g, 0.25g, 0.5g, 1.0g, and 2.0g

$Ca(H_2PO_4).H_2O$ ..... 0.78g, 0.585g, 0.39g, 0g, and 0g

$CaCl_2$ 0.3g

$MgSO_4.7H_2O$ 0.5g

Sucrose 10.0g

Concentration of K_2HPO_4 and $Ca(H_2PO_4).H_2O$ were paired in the order listed. To maintain the levels of chloride and phosphate in the control medium, $CaCl_2$ was substituted for KCl and $Ca(H_2PO_4)_2.H_2O$ was added.

7. Phosphate variable media

$NaNO_3$ 2.0g

K_2HPO_4 ..... 0g, 0.25g. 0.5g, 1.0g, and 2.0g

KCl ..... 1.5g, 1.25g, 1.0g, 0.5g, and 0g

$MgSO_4.7H_2O$ 0.5g

Sucrose 10.0g

Concentration of K_2HPO_4 and KCl were paired in the order listed. To maintain the levels of potassium in the control medium, concentrations of KCl were increased.

Agar disks, 5 mm in diameter, cut with a cork borer from 5-day-old petri dish cultures, were used as inocula. A small hole adjacent to each seedling hypocotyl to be inoculated was made with a jet of sterile water from a plastic wash bottle. An agar disk of the fungus was placed against the hypocotyl in the hole and the sand was pressed gently against the disk to hold it in place (27).

Inoculated plants were incubated for 7 days at 18 C and then disease severity ratings were made as follows: 1-no symptoms; 2- a few pin-point dark spots, or a faint diffuse discolored area on the hypocotyl; 3- a necrotic area less than 1 cm; 4- a necrotic area 1 cm or more in length; 5- plant wilted with cotyledons dropping; and 6- plant dead. Thirty plants were inoculated individually with the fungus at each nutrient treatment level. All tests were repeated. The data were subjected to an analysis of variance and 5% levels of significance were determined with Duncan's Multiple Range Test.

III. EFFECT OF NUTRIENTS ON GROWTH OF P. ULTIMUM

Growth of P. ultimum on agar media containing different concentration of nutrients was determined indirectly by measuring light transmission through blended cultures.

The fungus was grown on agar media containing various levels of nutrients in petri dishes. The media and hyphae of eight 5-day-old cultures of each nutrient-treatment were combined with 100 ml of distilled water and blended in a Waring Blendor for 1-2 minutes. Blended cultures were placed in cuvets and light transmission was determined at 620 nm with a Bausch and Lomb Spectronic 20 spectrophotometer. The control medium was Czapek's sucrose nitrate agar without the fungus. The quantity of light transmitted was related to growth.

IV. INOCULUM QUANTITY AND DISEASE SEVERITY

These experiments were designed to determine if the quantity of mycelium in the inoculum plug affected disease severity. P. ultimum was grown on sucrose variable and on nitrate variable liquid media in round, glass, screw-capped bottles. The inside diameter of each bottle was approximately 50 mm, and 100 ml of medium were added to each bottle. After 5 days of incubation at room temperature the mycelial mats were removed from the bottles and spread out on filter paper. To vary the amount of inoculum, 1 to 4 mycelial mats were placed in layers on the filter paper; all of the mats on a particular paper were from media of a similar nutrient level. Filter papers with mats were cut into 1-cm-square segments; each segment was inoculum for a single hypocotyl. Inoculation was effected as previously described. In addition, wet weight of single layers of mats collected in small beakers were determined.

CHAPTER IV

RESULTS

I. SUCROSE

The virulence, light transmission and inoculum weight of P. ultimum in sucrose variable media are presented in Table 1. When grown on Czapek's agar medium containing 0-1 g sucrose/liter, the fungus was weakly virulent. When grown on media containing 10-30 g sucrose/liter, virulence was markedly increased. Light transmission through blended cultures was determined and used as a measure of inoculum quantity. There was a significant negative correlation ($r = -0.965$) between disease severity and light transmission. Wet weight of mycelium varied indirectly with light transmission as concentrations of sucrose were increased, except that at 1 g/liter of sucrose, weight of mycelium was higher than would be expected when inoculum quantity was measured by light transmission. Mycelial weights in 1-30 g/liter of sucrose did not differ significantly.

The effect on disease severity of increasing amounts of inoculum from sucrose variable media is illustrated in Figure 1. The amount of mycelium was so small in the medium not supplemented with sucrose that distinct mycelial mat layers were not obtained, and therefore not tested. When the quantity of inoculum was doubled, tripled, or quadrupled by adding together layers of mycelial mats from liquid cultures , disease severity increased within each level of sucrose. With a single inoculum mat, disease severity increased generally as the concentration of sucrose was increased.

TABLE 1. PATHOGENICITY AND QUANTITY OF PYTHIUM ULTIMUM ON SUCROSE VARIABLE MEDIA

CONCENTRATION OF SUCROSE (g/l)	DISEASE INDEX[a]	INOCULUM QUANTITY	
		LIGHT TRANSMISSION[b] (%)	WET WEIGHT[c] (g/FLASK)
0	1.35 ab	36.5	0.104
1	1.62 b	21.0	0.469
10	4.02 c	4.0	0.460
20	4.49 c	1.0	0.444
30	4.57 c	0.8	0.449
CONTROL[d]	1.00 a	40.0	-

[a]Disease index is the mean of 60 plants tested in 2 repeated experiments. Disease index is a measure of disease severity based on hypocotyl symptoms, where 1 = no symptoms and 6 = dead plant. Disease index values followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($p = 0.05$).

[b]Transmission of light (620 nm) through 8 blended agar cultures in 100 ml water measured with a Bausch & Lomb Spectronic 20 spectrophotometer.

[c]Weight of mycelium and spores from a 250 ml flask containing 100 ml media.

[d]Sterile Czapek's agar medium.

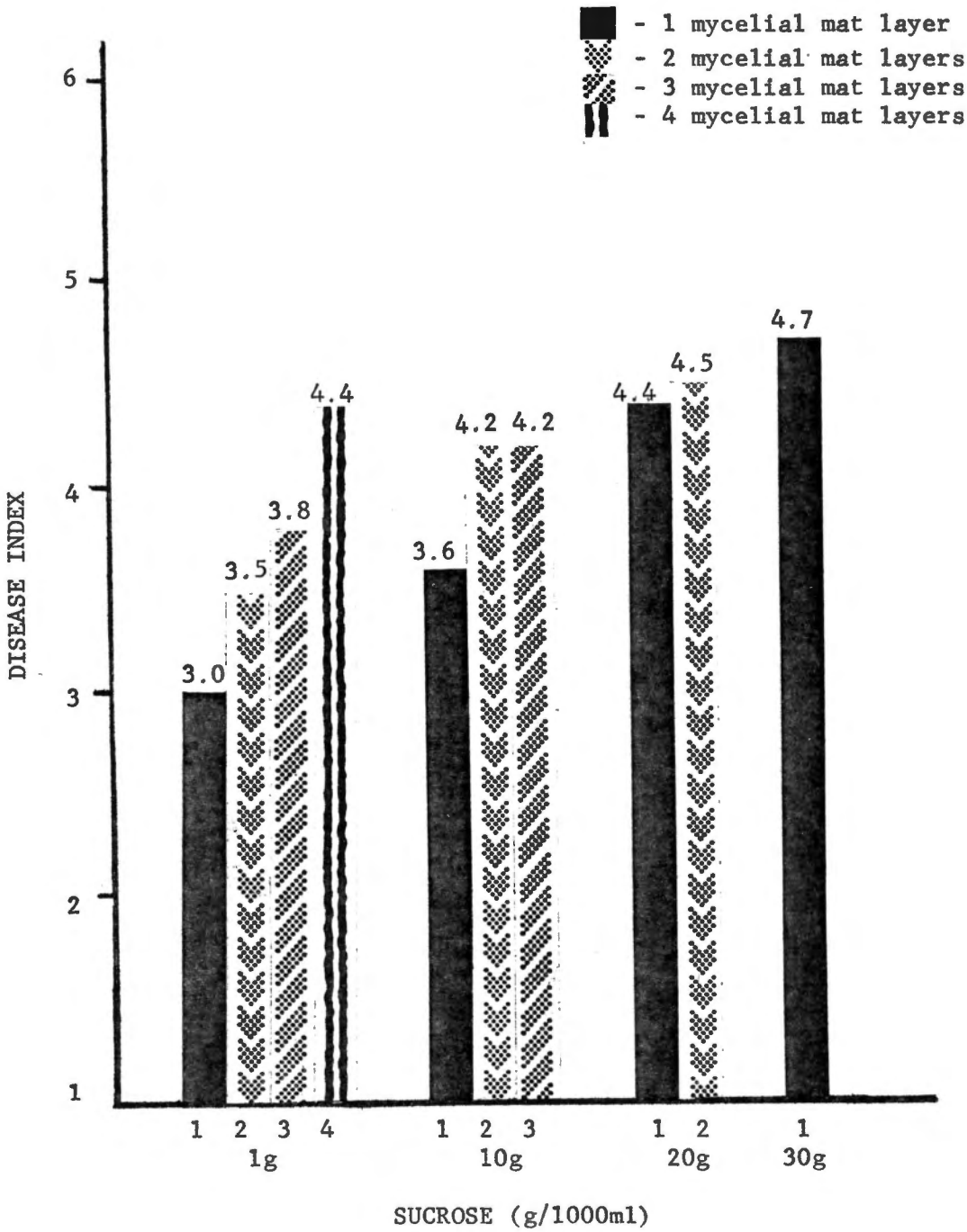

FIGURE 1. The effect of P. ultimum quantity and sucrose concentration in the culture medium on disease severity of cotton seedlings. Each mat layer was obtained from a flask containing 100 ml culture media. Each disease index value is the mean of 20 plants in 2 repeated experiments. L.S.D. (0.05) = 0.69.

Two or three layers of mycelium grown on media containing 1 g sucrose/liter caused disease symptoms similar to that caused by 1 layer from 10 g sucrose/liter.

II. NITRATE-NITROGEN

P. ultimum was less virulent when grown on media containing low concentrations (0-0.5 g/liter) of sodium nitrate (Table 2) than when grown on media containing high concentrations (1.0-4.0 g/liter). Correlation between light transmission and disease severity was significantly negative ($r = -0.976$). The differences of mycelial wet weight in 0.1 g to 4.0 g $NaNO_3$/liter Czapek's liquid media was small, but the amount of mycelium on the medium without nitrate added was considerably smaller. Wet weight of mycelium generally correlated with disease severity.

When the quantity of inoculum was increased by adding layers of mycelia from liquid cultures, disease became more severe at each level (Figure 2). Three layers of mycelia from media containing 0.1 g/liter of $NaNO_3$ caused as much disease as did one layer from media with 4.0 g/liter.

III. AMMONIUM-NITROGEN

Mycelium grown on media with no nitrogen added caused significantly less disease than did mycelium grown on media containing ammonium nitrogen (Table 3). P. ultimum grown on media to high concentrations (0.5-2.0 g/liter) of NH_4Cl produced severe disease symptom. P. ultimum grown on media containing 2.0 g/liter of $NaNO_3$ produced significantly

TABLE 2. PATHOGENICITY AND QUANTITY OF PYTHIUM ULTIMUM ON NITRATE-NITROGEN VARIABLE MEDIA

CONCENTRATION OF $NaNO_3$ (g/l)	DISEASE INDEX[a]	INOCULUM QUANTITY	
		LIGHT TRANSMISSION[b] (%)	WET WEIGHT[c] (g/FLASK)
0	1.90 b	29.5	0.182
0.1	-	-	0.377
0.5	4.74 c	1.8	0.389
1.0	5.15 d	2.2	-
2.0	5.23 de	2.2	0.418
4.0	5.60 e	2.2	0.354
CONTROL[d]	1.00 a	40.0	-

[a]Disease index is the mean of 60 plants tested in 2 repeated experiments. Disease index is a measure of disease severity based on hypocotyl symptoms, where 1 = no symptoms and 6 = dead plant. Disease index values followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($p = 0.05$).

[b]Transmission of light (620 nm) through 8 blended agar cultures in 100 ml water measured with a Bausch & Lomb Spectronic 20 spectrophotometer.

[c]Weight of mycelium and spores from a 250 ml flask containing 100 ml media.

[d]Sterile Czapek's agar medium.

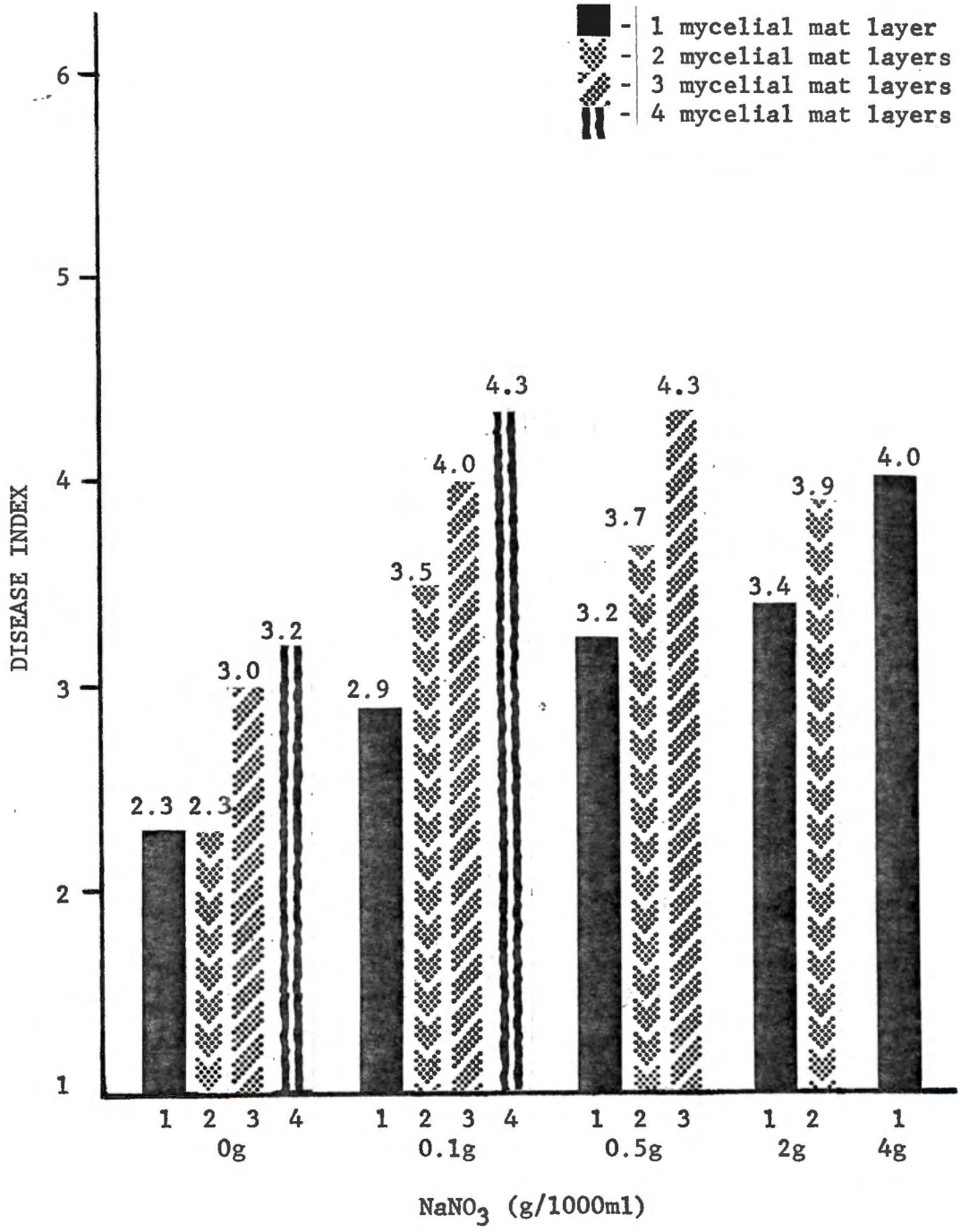

FIGURE 2. The effect of P. ultimum quantity and sodium nitrate concentration in the culture medium on disease severity of cotton seedlings. Each mat layer was obtained from a flask containing 100 ml culture media. Each disease index value is the mean of 20 plants in 2 repeated experiments, L.S.D.(0.05) = 0.68.

TABLE 3. PATHOGENICITY AND LIGHT TRANSMISSION OF PYTHIUM ULTIMUM IN AMMONIUM-NITROGEN VARIABLE MEDIA

CONCENTRATION OF NITROGEN		DISEASE INDEX[a]	LIGHT TRANSMISSION[b]
$NaNO_3$ (g/l)	NH_4Cl (g/l)		(%)
2.0	0	5.22 d	2.0
0	0	1.98 b	18.0
0	0.5	4.03 c	2.8
0	1.0	3.88 c	3.0
0	1.5	4.02 c	3.0
0	2.0	4.05 c	3.5

[a]Disease index is the mean of 60 plants tested in 2 repeated experiments. Disease index is a measure of disease severity based on hypocotyl symptoms, where 1 = no symptoms and 6 = dead plant. Disease index values followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($p = 0.05$).

[b]Transmission of light (620 nm) through 8 blended agar cultures in 100 ml water measured with a Bausch & Lomb Spectronic 20 spectrophotometer.

more severe disease on cotton seedlings than when grown on media containing 2.0 g/liter of NH_4Cl. Light transmission was slightly lower through P. ultimum on the nitrate medium than on the ammonium medium, but this difference did not appear to be significant.

IV. MAGNESIUM

Deletions or additions of $MgSO_4.7H_2O$ to Czapek's agar did not cause significant differences in virulence of P. ultimum (Table 4).

TABLE 4. PATHOGENICITY AND LIGHT TRANSMISSION OF PYTHIUM ULTIMUM IN MAGNESIUM VARIABLE MEDIA

CONCENTRATION OF MAGNESIUM $MgSO_4.7H_2O$ (g/l)	DISEASE INDEX[a]	LIGHT TRANSMISSION (%)[b]
0	4.30 a	6.0
0.125	4.27 a	5.5
0.250	4.23 a	5.0
0.500	4.30 a	5.0
1.000	4.20 a	5.0

[a]Disease index is the mean of 60 plants tested in 2 repeated experiments. Disease index is a measure of disease severity based on hypocotyl symptoms, where 1 = no symptoms and 6 = dead plant. Disease index values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (p = 0.05).

[b]Transmission of light (620 nm) through 8 blended agar cultures in 100 ml water measured with a Bausch & Lomb Spectronic 20 spectrophotometer.

Similarly, there was no apparent significant differences in the quantity of light transmitted through mycelia on media with no magnesium added and different concentrations of magnesium in the media.

V. POTASSIUM

The influence of potassium on pathogenicity and pathogen growth are presented in Table 5. P. ultimum on media without a potassium nutrient added, caused significantly less disease, and its light transmission was 3 to 5 times higher than on medium to high concentrations of potassium. There was no significant difference in disease severity caused by inocula

TABLE 5. PATHOGENICITY AND LIGHT TRANSMISSION OF PYTHIUM ULTIMUM IN POTASSIUM VARIABLE MEDIA

CONCENTRATION OF POTASSIUM		DISEASE INDEX[a]	LIGHT TRANSMISSION (%)[b]
$Ca(H_2PO_4)_2.H_2O$ (g/l)	K_2HPO_4 (g/l)		
0.780	0	3.22 a	10.0
0.585	0.25	4.77 b	2.0
0.390	0.50	4.85 b	2.0
0	1.00	4.78 b	3.0
0	2.00	5.05 b	3.0

[a]Disease index is the mean of 60 plants tested in 2 repeated experiments. Disease index is a measure of disease severity based on hypocotyl symptoms, where 1 = no symptoms and 6 = dead plant. Disease index values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (p = 0.05).

[b]Transmission of light (620 nm) through 8 blended agar cultures in 100 ml water measured with a Bausch & Lomb Spectronic 20 spectrophotometer.

grown on medium to high concentrations of potassium.

VI. PHOSPHATE

The addition or deletion of phosphate had no significant effect on disease severity or on light transmission (Table 6).

TABLE 6. PATHOGENICITY AND LIGHT TRANSMISSION OF PYTHIUM ULTIMUM IN PHOSPHATE VARIABLE MEDIA

CONCENTRATION OF PHOSPHATE		DISEASE INDEX[a]	LIGHT TRANSMISSION (%)[b]
KCl (g/l)	K_2HPO_4 (g/l)		
1.50	0	4.60 a	6.0
1.25	0.25	4.65 a	7.0
1.00	0.50	4.74 a	5.5
0.50	1.00	4.84 a	9.0
0	2.00	4.84 a	5.0

[a]Disease index is the mean of 60 plants tested in 2 repeated experiments. Disease index is a measure of disease severity based on hypocotyl symptoms, where 1 = no symptoms and 6 = dead plant. Disease index values followed by the same letter are not significantly different according to Duncan's Multiple Test ($p = 0.05$).

[b]Transmission of light (620 nm) through 8 blended agar cultures in 100 ml water measured with a Bausch & Lomb Spectronic 20 spectrophotometer.

CHAPTER V

DISCUSSION

It is customary to think such physical factors as temperature and humidity as the most important features of the environment that affect plant disease development. Nutrients, however, are also a part of this environment, and may be fully as significant. An understanding of the relationships between nutrient factors and disease potential is necessary for developing more effective control measures for Pythium seedling disease of cotton.

Several kinds of nutrients affect the growth of P. ultimum (1, 29, 33, 52), however, there have been no studies of the effect of nutrients on P. ultimum infection of cotton. The present study has shown that concentrations of sucrose, nitrate-nitrogen, ammonium-nitrogen, and potassium in culture media significantly affect virulence. It was found that medium to high concentrations of nutrients generally encourage more abundant mycelial growth and resulted in severe disease. This was similar to evidence obtained by other investigators with various plant pathogens. Berger and Hanson (6) showed that V-8 juice media influenced the amount of spore production of Cercospora and increased disease incidence. Similar results were obtained by Banttari and Wilcoxson (5) with Phoma herbarum var. medicaginis, and Maier (36) with Fusarium solani f. sp. phaseoli. The increased quantity of inoculum with doubled, tripled and quadrupled mycelial mats caused more severe disease. It appears that more inoculum was necessary for causing severe cotton seedling disease by P. ultimum at low nutrient concentrations. All these results

strongly support Garrett's definition of inoculum potential (14); the type and amount of nutrients available to the pathogen at the infection site could have a direct effect on establishment of infection.

Mellano et al. (38) found that culture filtrates of P. ultimum exhibited marked pectic enzyme activity. Gupta (19) also reported that addition of sodium chloride in media stimulated the pectolytic activity of P. debaryanum Hess. Did the high concentrations of sucrose (20-30 g/liter) or sodium nitrate (4 g/liter) merely enhance growth of hyphae or also induce physiological changes such as pectic enzyme activity? When hypocotyls were inoculated with single mycelial mats from liquid cultures, disease severity increased progressively as nitrogen or sucrose levels were increased. Mycelial weight, however, increased sharply at low levels of these nutrients and was not directly correlated to disease severity. Nutrient levels, therefore, affected disease severity independently of inoculum quantity.

In a low concentration of sucrose (1 g/liter), the fungus grew better in liquid media than on agar media. Therefore, the type of medium must be considered in evaluting the influence of pathogen nutrition on disease severity.

Nutrients in soil including the soil solution, organic materials in soil and, root exudates provide many nutrients for soil microorganisms. If the virulence of P. ultimum can be changed by addition or deletion of certain nutrients in culture, it is reasonable to assume that a similar effect occurs in soil. Delaying fertilization of cotton field for 3-4 weeks after planting could result in low levels of nitrogen and potassium during the critical period when seedlings are most susceptible. It is suggested that such a procedure could reduce cotton seedling disease caused by P. ultimum.

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VITA

Chin-Chu Hsieh was born in Tainan, Taiwan, Republic of China, on December 25, 1953. She attended elementary school in Tainan, and was graduated from Tainan First Girl High School in July 1972. The following September she passed the Entrance Examination of University in Taiwan, and entered Chung-Hsing University. She received a Bachelor of Science degree in Plant Pathology in June 1976. In the summer of 1976, she became a laboratory assistant in Ta-Gan-Sun Food Company, and after one year became a research assistant at the Taiwan Agriculture Institute. In the fall of 1978, she began study towards a Master's degree in the Department of Entomology and Plant Pathology at the University of Tennessee, Knoxville.

The author was elected a member of Gamma Sigma Delta, the Honor Societh of Agriculture in May, 1980.