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To the Graduate Council:

I am submitting herewith a thesis written by Philip Pryor Hunter entitled "Pathogenic and Cultural Variability in Helminthosporium maydis Nisikado and Miyake." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

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PATHOGENIC AND CULTURAL VARIABILITY IN
HELMINTHOSPORIUM MAYDIS
NISIKADO AND MIYAKE

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Philip Pryor Hunter
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ABSTRACT

Following the 1970 epiphytotic of southern corn leaf blight a new race of the causal pathogen, Helminthosporium maydis Race T, was designated. This new race is host specific for corn lines produced in the Texas (T) male-sterile cytoplasm. The studies presented in this paper were undertaken to assess the variability that exists in the H. maydis population. The objectives of the study were twofold. The first objective was to measure the cultural variability among several single spore isolates of H. maydis as to sporulation and linear growth using four media. The second objective as to estimate the variation in pathogenicity to corn lines with six male-sterile and normal cytoplasms among the same isolates, a) as to crude and concentrated pathotoxin production by seedling bioassay, and b) seedling inoculation of the lines in the greenhouse.

Significant variability in linear growth and sporulation existed for the H. maydis isolates tested. There was a significant, negative correlation between linear growth and sporulation. Lactose casein hydrolysate medium was further substantiated as a good medium for inducing sporulation in Helminthosporium maydis.

The variation among the isolates in optimum harvest date for pathotoxin production reiterates the need for this type of determination previous to any studies dealing with the pathotoxin produced by H. maydis. There were significant differences in cytoplasm reaction to both the crude and concentrated pathotoxin; however, the reactions were too variable for meaningful patterns. These results

support two conclusions. First, at the present time, seedling inoculation and field reaction is the best method for determining the susceptibility or resistance of male-sterile cytoplasms to H. maydis. Second, pathotoxin studies should be done with highly purified pathotoxin preparations and should be based on several replications using large quantities of seed. Also, the reaction of the cytoplasms to the pathotoxin should be studied using the same cytoplasms in several genotypes.

The isolates used in the seedling inoculation studies were all H. maydis Race T. Based on seedling reaction under the conditions in this study, male-sterile cytoplasms C and W were as susceptible as T cytoplasm to the isolates tested. Cytoplasms G, K, and M were as resistant as N cytoplasm to the same isolates. These results indicate that the H. maydis Race T population contains biotypes that are more virulent to particular male-sterile cytoplasms than to others.

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CHAPTER I

INTRODUCTION

The fungus Helminthosporium maydis Nisikado and Miyake (Cochliobolus heterostrophus Drechsler) causes the southern leaf blight disease of corn (SCLB). The original description of the fungus was made in 1925, by Dreschler (10). He described and named the perfect stage Ophiobolus heterostrophus. In 1934, Dreschler (11) set up a new genus, Cochliobolus, and placed the perfect stage of H. maydis in this genus, Cochliobolus heterostrophus. In 1926, the asexual stage was described by Nisikado and Miyake (54) and named Helminthosporium maydis.

In 1970, SCLB developed in epiphytotic proportions in the United States. Prior to 1970, the disease was one of minor importance. Occasional destructive outbreaks were localized on susceptible hybrids and inbreds (51). This situation was the result of the use of resistant corn lines in areas where the disease occurred and limiting its northward spread by cool weather (24).

The epiphytotic was the result of the simultaneous occurrence of three factors:

1. The weather conditions of high humidity and cool temperature favorable to the development of a new race of the causal pathogen.
2. The extensive monoculture of the host plant susceptible to the new race, corn lines produced in Texas male-sterile cytoplasm.

3. The presence and distribution of the new race of the fungus designated Race T (71, 26). This new race was host-specific for corn lines produced using Texas (T) cytoplasm for male-sterility. The old race has been designated as Race O.

Even with all its destructive consequences, the southern corn leaf blight epiphytotic of 1970 has served to focus attention on two important problems in plant breeding and plant pathology: genetic uniformity of crop species and pathogenic variability. The two problems go hand in hand. The production of large monocultures of genetically uniform crops places selection pressure on the pathogen in favor of more virulent types as in Flor's gene-for-gene concept (13). In addition, if virulent races already exist in the pathogen population, and favorable environmental conditions occur, then epiphytotics similar to that of SCLB can result. Not only must genetic diversity be maintained in crop species, but also the variability within the pathogen must be under constant scrutiny. To quote Dr. R. R. Nelson:

With the knowledge that isolates of H. maydis with Race T capacities have existed for many years, one cannot help but speculate as to the present existence of races capable of attacking other types of male-sterile cytoplasm. The possibility of this is sufficient justification to warrant intensive studies with isolates of the fungus that have been accumulated in recent years (51).

It was upon this concept that the present work was undertaken.

The objectives of this study were as follows:

1. To measure the cultural variability among several single spore isolates of Helminthosporium maydis as to sporulation and linear growth of mycelium using four culture media.
2. To estimate the variation in pathogenicity to corn lines

with six male-sterile and normal cytoplasms, among some of the same isolates, in two areas:

- a. Pathotoxin production as measured by seedling bioassay of crude and concentrated, purified cultural filtrates.
- b. Seedling inoculation of the lines in greenhouse studies.

Although the race characteristic of some of the single spore isolates used in this study was determined, the major emphasis was not to study the above objectives in H. maydis Race T. The approach used herein was to study the pathogenic variability of the isolates with the possibility of identifying new races of the pathogen.

CHAPTER II

LINEAR GROWTH AND SPORULATION ON FOUR MEDIA

Literature Review

Although a large amount of work has been done with cultural variability in the genus Helminthosporium, very little has been done with the species maydis. This is due primarily to the greater economic importance of several other diseases caused by Helminthosporium species, prior to 1970. Studies have dealt with nutritional requirements and how growth and sporulation are affected by nutrition. Malca and Ullstrup (37), working with H. carbonum and H. turcicum, found variation in sporulation greater than variation in growth in response to several carbon (C) and nitrogen (N) sources. Hedge, et al. (21) described differences among isolates of H. nodulosum as to their ability to utilize C and N from different sources. Maloul (38) determined that for H. carbonum and H. turcicum the C source in the medium affected which N source was used most effectively in spore production, and the amount and rate of growth determined to a large extent the factors which favored sporulation. Lactose was found to be the C source which stimulated sporulation greatest. The work of Kafi and Tarr (28), using five species of Helminthosporium, indicated that sporulation occurred over a lower range of C/N ratios than did vegetative growth. They also found variation in spore morphology with media and C source variation. Reddy (60) showed that lactose was poorly utilized by the same five

species, but the utilization was improved when half of the lactose was replaced by its hydrolytic components. Using 61 different C sources, Vidhyasekaran, et al. (79) found that maltose and D-xylose gave maximum sporulation. In a later work, Tarr and Kafi (74) used various inorganic and organic N sources. Nitrate, asparagine, glycine, and urea stimulated growth and sporulation, whereas NH_4NO_3 , $(\text{NH}_4)_2\text{SO}_4$, NaNO_2 , methionine, and lysine did not. They were working with the same five species used previously. Sreedharan and Menon (73) obtained similar results working with H. oryzae. Zaman and Ahmed (91) varied the sugar content of a basic medium and found that the mycelial growth of H. oryzae increased with an increase in sugar content up to 3%. Thind and Rawla (75) studied the need for trace elements using six Helminthosporium species. Iron, zinc, and manganese were essential for all six, copper was essential for four, and molybdenum and calcium were essential for two. Studying H. cynodontis, White and Johnson (87) found that linear growth was increased by trace amounts of zinc. Fukuki and Aragaki (15) studied the effect of temperature and light on culture color and sporulation of Race T and Race O of H. maydis. Under fluorescent illumination and 28°C , Race T cultures were white except for cinnamon rufous to russet centers and Race O cultures were dark olive gray. Race T isolates had optimum sporulation at 20°C under continuous lighting and 24°C under continuous darkness. Race O isolates had optimum sporulation at $20\text{--}28^\circ\text{C}$ in continuous lighting and 28°C in continuous darkness.

Materials and Methods

Cultural variability in linear growth and sporulation among

42 single spore isolates of H. maydis was studied using four culture media. Each isolate was transferred to two 90 mm Petri plates containing potato-dextrose agar and the cultures incubated for eight days at 24°C. These cultures served as the inoculum for the test. The four media used were: corn meal agar (CMA), lactose casein hydrolysate medium (LCHA), potato-dextrose agar (PDA), and V-8 juice agar (VJA). The formulation of each of these media is shown in Table 1. All media were autoclaved 20 minutes at 15 pounds pressure and each plate contained 20 ± 2 ml of each media. A seven millimeter cork borer was used to cut uniform discs from the margins of the inoculum cultures. These discs were transferred to the centers of five 90 mm test plates. These plates were numbered and randomized within a stack and each stack was randomized in an incubator. Percival E-54U environmental chambers served as incubators and were maintained at $26 \pm 2^\circ\text{C}$ and complete darkness throughout the test. After four days incubation, the largest colony diameter of three plates was determined. Each plate served as one replication. The plates and stacks were rerandomized. After 48 hours, two plates were chosen at random and spore counts made by flooding the plates with a 5% sucrose - 0.2% "Tween 20" solution and scraping the surface of the colony. Each plate served as one replication. The resulting spore suspension was poured through cheesecloth into a 100 ml beaker and the filtrate volume adjusted to 50 ml with suspending solution. The spore suspensions were agitated, and a medicine dropper sample transferred to a hemacytometer. Four 1 mm^3 grids were counted and averaged to give a spore concentration for each plate. These values were expressed in spores per milliliter. The

TABLE 1. Formulations of the four media used in sporulation and linear growth determinations of 42 single spore isolates of Helminthosporium maydis

Medium	Ingredients			
V-8 juice ¹	V-8 juice	100 ml		
	CaCO ₃	2 g		
	Agar	17 g		
	Deionized H ₂ O to make 1 liter			
	Place CaCO ₃ in V-8 juice and let stand for 30 minutes, then filter through three layers of double-fold cheesecloth			
Lactose casein hydrolysate ¹	Lactose	37.5 g	Fe(NO ₃)·9 H ₂ O	723.5 mg
	Casein		ZnSO ₄ ·7 H ₂ O	439.8 mg
	hydrolysate	3.0 g	MnSO ₄ ·4 H ₂ O	203.0 mg
	KH ₂ PO ₄	1.0 g	Dissolve one at a time in 1 liter of H ₂ O and add 1 N H ₂ SO ₄ to clear solution	
	MgSO ₄	0.5 g		
	Microelements	2.0 ml		
Corn meal ²	Agar	17 g		
	Corn meal	20 g		
	Deionized H ₂ O to make 1 liter			
	Cook corn meal at about 60°C for 1 hour in 500 ml H ₂ O and filter through cheesecloth; add filtrate to agar in 500 ml H ₂ O			
Potato-dextrose ¹	Fresh potatoes	200 g		
	Dextrose	15 g		
	Agar	15 g		
	Deionized H ₂ O to make 1 liter			

¹Tuite (76).

²Johnson and Curl (27).

values were adjusted for colony growth by dividing by the colony area to obtain a value in spores per millimeter squared.

Results and Discussion

The range and mean values for linear growth and sporulation are presented in Table 2. The means in Table 2 are overall means for all four media. Linear growth on LCHA, CMA, and VJA was not significantly different as determined by Duncan's multiple range test at the 5% level of probability. Linear growth on PDA was significantly less than on the other three media.

Generally, fungal isolates decrease in vigor and virulence the longer they are maintained culturally. In reexamining 116 isolates of H. maydis following the 1970 SCLB epiphytotic, Nelson (51) found decreases in pathogenicity when susceptible corn lines were inoculated. Although all of the isolates had previously been pathogenic to corn, only a few incited even necrotic leaf spots. Comstock and Scheffer (6) and Lim, et al. (32) have reported reductions in pathotoxin producing ability by repeated transfers of isolates over long periods of time. Wheeler, et al. (86), however, found no differences in pathotoxin production between isolates reisolated from host tissue and those same isolates maintained on agar for five months. The fact that PDA was the maintenance medium used with these isolates for 12 months prior to this experiment might explain the differences in growth on PDA. When sporulation is considered, however, this explanation does not apply. The sporulation of PDA was significantly greater than on the other three media. An alternative hypothesis exists to explain the high degree of sporulation on PDA. If the reproduction (asexual in this

TABLE 2. Range and mean values for linear growth and sporulation of 42 single spore isolates of *Helminthosporium maydis* on corn meal agar (CMA), lactose casein hydrolysate medium (LCHA), potato-dextrose agar (PDA), and V-8 juice agar (VJA)

Media	Linear Growth (mm) ¹		Media	Sporulation (Spores/mm ² X 1000) ²	
	Range	Mean		Range	Mean
LCHA	78.00-31.00	59.61a ³	PDA	9.180-0.000	2.893a
CMA	74.33-40.67	58.26a	LCHA	3.965-0.000	1.585b
VJA	80.67-36.00	58.20a	VJA	3.910-0.000	1.376b
PDA	71.67-20.00	52.69b	CMA	2.565-0.000	0.271c

¹Values are means for three replications of all 42 isolates.

²Values are means for two replications of all 42 isolates.

³Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

case) of an organism is stimulated by stress, then continued maintenance on the same medium could have been a form of stress; and the increased sporulation might have been a reaction to such stress. Visual observation during the 12 months that the isolates were maintained prior to this experiment does not support the latter hypothesis, however. For example, 14 of the isolates did not sporulate to any large degree during the entire period. A more plausible explanation for the data in Table 2 is that both growth and sporulation are variable characteristics of individual isolates. For this reason, meaningful generalizations about such characteristics within a large group of isolates are difficult. This is particularly true for this study since the media used were not highly defined.

The mean values for linear growth and sporulation on each media by sources are shown in Table 3. The term source refers to the original isolate from which the several single spore isolations were made. The original isolates were all from corn and from six geographical locations. The six sources came from the following locations in Tennessee: I - Johnson County, Z - Knox County, R - Henry County, C - Monroe County, G - Madison County, and X - Lawrence County. The single spore isolates from source I and Z had extremely low spore production while maintained on PDA. Tuite (76) lists lactose casein hydrolysate medium as a good medium for inducing sporulation in Helminthosporium spp. The isolates from sources I and Z had sporulation values on LCHA significantly greater than on the other three media. These results further substantiate LCHA as a good medium for inducing sporulation. Even though sporulation was increased in

TABLE 3. Linear growth and sporulation of 42 single spore isolates of *Helminthosporium maydis* on corn meal agar (CMA), lactose casein hydrolysate medium (LCHA), potato-dextrose agar (PDA), and V-8 juice agar (VJA) by sources

Source	Media	Linear Growth (mm) ²	Media	Sporulation (Spores/mm ² X 1000) ³
Source I	CMA	64.95a ⁴	LCHA	1.12a
	LCHA	65.38a	PDA	0.11b
	PDA	61.38a	VJA	0.06c
	VJA	63.04a	CMA	0.00d
Source Z	LCHA	56.29a	LCHA	2.41a
	PDA	55.48a	VJA	0.62b
	VJA	54.52ab	PDA	0.27b
	CMA	50.32b	CMA	0.03b
Source R	CMA	54.53a	PDA	5.08a
	LCHA	52.24a	LCHA	1.93b
	VJA	50.62a	VJA	1.65b
	PDA	33.38b	CMA	0.78b
Source C	PDA	64.11a	PDA	3.31a
	VJA	62.78a	VJA	2.05ab
	LCHA	62.67a	LCHA	1.39ab
	CMA	55.19b	CMA	0.31b
Source G	LCHA	52.43a	PDA	3.45a
	CMA	51.11a	VJA	1.98ab
	VJA	48.81ab	LCHA	0.90ab
	PDA	45.00b	CMA	0.29b
Source X	CMA	69.05a	PDA	4.90a
	VJA	68.63a	VJA	1.92ab
	LCHA	67.92a	LCHA	1.72ab
	PDA	57.75b	CMA	0.24b

¹A source refers to the geographic isolation from which the several single spore isolates were made.

²Values are means of all single spore isolates from the source replicated two times.

³Values are means of all single spore isolates from the source replicated three times.

⁴Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

isolates from sources I and Z on LCHA, the increase was not sufficient to give the quantities of spores needed for the spore suspensions used in the seedling inoculation experiment, and so these sources were omitted from this work.

The linear growth and sporulation of all 42 isolates are presented in Table 4. The values are means for all four media. The linear growth data are based on three replications and the sporulation data are based on two replications. The values for sporulation were calculated as spores per unit area (mm^2). Therefore, isolates producing large numbers of spores in a small area are represented by the larger values and vice-versa. R1, R5, R6, R7, G1, G5, C6, and X5 were the most prolific isolates, having sporulation values above the median (1.5355) for sporulation and below the median (55.61) for growth. As an example, the reader should compare isolate R7 with isolate X8. In proportion to X8, R7 should have produced $0.993 \text{ spores/mm}^2 \times 1000$,

$$\text{X8} - \frac{75.25\text{mm}}{44.25\text{mm}} = \frac{1.688 \text{ spores/mm}^2 \times 1000}{0.993 \text{ spores/mm}^2 \times 1000}$$

$$\text{R7} - 44.25\text{mm} = 0.993 \text{ spores/mm}^2 \times 1000$$

however, R7 produced $4.224 \text{ spores/mm}^2 \times 1000$, approximately four times more. For comparison the spores per milliliter and spores per millimeter squared mean values for each media are shown in Table 5.

Tables 6 and 7 present the data for linear growth and sporulation by sources. The values are means for all four media combined. There were significant differences among the single spore isolates within a source in two of the six sources for sporulation. There were significant differences among the single spore isolates within sources in all six of the sources as to linear growth. The data in Tables 5,

TABLE 4. Linear growth and sporulation of 42 single spore isolates of *Helminthosporium maydis* on corn meal agar (CMA), lactose casein hydrolysate medium (LCHA), potato-dextrose agar (PDA), and V-8 juice agar (VJA)

Isolate	Growth (mm) ^{1,3}	Isolate	Sporulation (Spores/mm ² X 1000) ^{2,3}
X8	75.25	R7	4.224
I3	74.17	R1	3.514
I4	72.50	R5	3.366
X4	69.92	X5	2.980
X3	69.00	X2	2.949
X1	68.17	G5	2.748
X7	66.33	C6	2.659
C1	65.67	X6	2.533
X2	65.17	X4	2.320
C2	65.08	G1	1.975
I2	65.00	X3	1.901
Z5	63.50	R2	1.829
C5	63.33	C1	1.818
C4	61.50	X8	1.688
I1	60.58	G2	1.674
C7	60.00	C5	1.671
X6	59.92	C7	1.660
I7	59.75	X7	1.649
I5	59.17	G4	1.595
Z6	57.58	X1	1.565
G2	56.08	R6	1.536
G1	55.75	C4	1.535
X5	54.92	G6	1.484
R6	53.75	R4	1.481
Z1	53.67	G3	1.415
C6	53.42	Z2	1.379
I8	53.25	C2	1.351
Z3	52.92	Z3	1.214
G3	52.25	Z1	1.179
Z7	51.42	I1	0.993
Z4	50.92	Z5	0.946
R4	49.92	I8	0.885
Z2	49.67	Z4	0.750
G6	49.58	G7	0.686
R5	49.42	R3	0.433
R2	49.17	I6	0.405
R3	48.83	Z6	0.345
G4	48.25	I2	0.000
R7	44.25	I3	0.000
G5	43.58	I5	0.000
G7	40.58	I7	0.000
R1	39.00	Z7	0.000

¹Values are means for three replications; all media combined.

²Values are means for two replications; all media combined.

³Means joined by the same line are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 5. Spores per milliliter and spores per millimeter squared values of the 42 single spore isolates of *Helminthosporium maydis* for corn meal agar (CMA), lactose casein hydrolysate medium (LCHA), potato-dextrose agar (PDA), and V-8 juice agar (VJA)

Isolate	CMA		LCHA		PDA		VJA		Overall	
	Spores/ml X 1000	Spores/mm ² X 1000	Spores/ml X 1000	Spores/mm ² X 1000	Spores/ml X 1000	Spores/mm ² X 1000	Spores/ml X 1000	Spores/mm ² X 1000	Spores/ml X 1000	Spores/mm ² X 1000
I1	0.250	0.105	13.750	3.865	0.000	0.000	0.000	0.000	3.438	0.993
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	0.000	0.000	6.625	1.620	0.000	0.000	0.000	0.000	1.656	0.405
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6	0.000	0.000	5.000	2.320	2.000	0.770	0.875	0.450	1.969	0.885
7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Z1	0.000	0.000	7.750	3.360	0.875	0.355	2.500	1.000	2.781	1.789
2	0.125	0.070	8.125	3.905	2.000	0.895	2.250	0.645	3.125	1.379
3	0.125	0.065	6.500	3.680	0.125	0.055	2.500	1.055	2.313	1.214
4	0.000	0.000	6.375	3.000	0.000	0.000	0.000	0.000	1.594	0.750
5	0.125	0.060	5.625	1.715	1.750	1.495	4.250	1.515	2.938	0.946
6	0.000	0.000	3.250	1.215	0.125	0.065	0.250	0.100	0.906	0.345
7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
R1	0.375	0.185	2.625	3.245	3.375	8.370	3.000	2.255	2.344	3.514
2	1.375	0.595	1.250	0.315	4.000	4.340	2.875	2.065	2.375	1.828
3	0.375	0.285	5.000	0.920	0.000	0.000	1.875	0.525	1.813	0.433
4	0.375	0.120	3.000	1.055	1.875	3.145	3.375	1.605	2.156	1.481
5	2.000	0.865	6.250	2.850	3.500	6.890	7.125	2.860	4.719	3.366
6	0.625	0.235	3.125	1.155	3.500	3.690	3.125	1.075	2.594	1.536
7	4.000	2.565	6.000	3.965	3.125	9.180	1.250	1.185	3.594	4.224
C1	1.375	0.715	6.000	1.540	4.250	2.580	9.000	2.435	5.156	1.818
2	1.375	0.500	4.500	1.190	7.750	1.950	7.000	1.765	5.156	1.351
4	0.375	0.125	2.875	0.960	11.500	3.100	5.250	1.955	5.000	1.535
5	0.500	0.210	6.875	2.195	11.500	2.905	4.375	1.375	5.813	1.671
6	1.125	0.730	4.125	1.305	11.750	6.205	8.000	2.395	6.250	2.659
7	0.000	0.000	2.875	1.135	10.000	3.145	6.000	2.360	4.719	1.660
G1	0.500	0.315	2.250	0.820	6.625	4.160	4.125	2.605	3.375	1.975
2	0.750	0.225	4.875	1.520	5.625	3.445	3.250	1.505	3.625	1.674
3	0.375	0.200	1.250	0.690	4.750	3.405	4.000	1.365	2.594	1.415
4	0.625	0.340	1.375	0.685	5.500	3.165	4.500	2.190	3.000	1.595
5	0.125	0.535	2.500	1.200	4.875	5.545	2.750	3.910	2.563	2.748
6	0.500	0.260	1.750	0.930	4.875	2.530	2.000	2.215	2.281	1.484
7	0.250	0.150	0.625	0.460	1.625	2.075	0.125	0.060	0.656	0.686
X1	1.375	0.335	2.250	0.665	12.875	3.545	6.625	1.715	5.781	1.565
2	0.250	0.060	8.375	1.925	11.000	7.665	7.625	2.145	6.813	2.949
3	1.125	0.340	8.125	1.845	11.875	3.945	6.250	1.475	6.844	1.901
4	1.250	0.280	6.875	1.525	18.250	5.010	9.125	2.465	8.875	2.320
5	0.375	0.100	9.625	2.900	13.750	7.085	3.750	1.835	6.875	2.980
6	0.375	0.195	3.875	1.280	9.875	6.795	7.000	1.860	5.275	2.533
7	1.125	0.390	4.750	1.840	10.125	2.745	5.375	1.620	5.344	1.649
8	0.875	0.245	7.125	1.790	6.125	2.485	11.750	2.230	6.469	1.688

TABLE 6. Linear growth of 42 single spore isolates of Helminthosporium maydis by sources

Source ¹	Isolate	Linear Growth (mm) ²
I	3	74.17a ³
	4	72.50a
	2	65.00b
	1	60.58c
	7	59.75c
	5	59.17c
	6	53.25d
Z	5	63.50a
	6	57.58b
	1	53.67bc
	3	52.92bc
	7	51.42c
	4	50.92c
	2	49.67c
R	6	53.75a
	4	49.92ab
	5	49.42ab
	2	49.17ab
	3	48.83ab
	7	44.25bc
	1	39.00c
C	1	66.67a
	2	65.08a
	4	63.33a
	5	61.50a
	7	60.00ab
	6	53.42b
G	2	56.08a
	1	55.75a
	3	52.25ab
	6	49.58ab
	4	48.25ab
	5	43.58ab
	7	40.58b

TABLE 6 (continued)

Source ¹	Isolate	Linear Growth (mm) ²
X	8	75.25a ³
	4	69.92ab
	3	69.00ab
	1	68.17b
	7	66.33bc
	2	65.17bc
	6	59.92cd
	5	54.92d

¹A source refers to the geographic isolation from which the several single spore isolations were made.

²Values are means of three replications; all media combined.

³Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 7. Sporulation of 42 single spore isolates of Helminthosporium maydis by sources

Source ¹	Isolate	Sporulation (Spores/mm ² X 1000) ²
I	1	0.993a ³
	6	0.885b
	4	0.405c
	2	0.000c
	3	0.000c
	5	0.000c
	7	0.000c
Z	2	1.379a
	3	1.214ab
	1	1.179abc
	5	0.946bc
	4	0.750c
	6	0.345d
	7	0.000e
R	7	4.224a
	1	3.514a
	5	3.366a
	2	1.829a
	6	1.536a
	4	1.481a
	3	0.433a
C	6	2.659a
	1	1.818a
	5	1.671a
	7	1.660a
	4	1.535a
	2	1.351a
G	5	2.748a
	1	1.975a
	2	1.674a
	4	1.595a
	6	1.484a
	3	1.415a
	7	0.686a

TABLE 7 (continued)

Source ¹	Isolate	Sporulation (Spores/mm ² X 1000) ²
X	5	2.980a
	2	2.949a
	6	2.533a
	4	2.320a
	3	1.901a
	8	1.688a
	7	1.649a
	1	1.565a

¹A source refers to the geographic isolation from which the several single spore isolates were made.

²Values are means of two replications; all media combined.

³Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

6, and 7 indicate that linear growth is a more variable character than sporulation. Since the single spore isolates were made using asexual spores, a high degree of genetic purity would be expected. Of particular interest is the high degree of difference among single spore isolates from the same source as to linear growth. These results indicate that variation exists even within isolates made from the same haploid source.

The data for linear growth and sporulation among the four media for each isolate are shown in Tables 8 and 9, respectively. Isolates Z4, R3, R4, R5, C4, G5, G7, X2, X4, and X6 had significantly different linear growth among the four media. Isolates I1, I6, Z2, R1, R4, C2, C5, G4, G7, X1, X3, X4, X5, and X6 had significantly different amounts of sporulation among the four media. Isolates R4, G7, and X6 had significant differences among the four media as to linear growth and sporulation.

Tables 10, 11, 12, and 13 show the linear growth and sporulation data for 42 single spore isolates on the individual media. The amount of variability on a given medium can be used as a measure of the extent to which that medium distinguishes differences among a group of isolates. The level of probability values on which the Duncan's multiple range tests were based are shown for each medium in Table 14. There were no differences among the four media in distinguishing variation in linear growth. The variation in sporulation among the isolates was best demonstrated by PDA, followed by LCHA. Corn meal agar did not distinguish any difference among the isolates as to sporulation. The 4.94% level of probability for VJA was slightly

TABLE 8. Linear growth of 42 single spore isolates of *Helminthosporium maydis* on four media - corn meal agar (CMA), lactose casein hydrolysate medium (LCHA), potato-dextrose agar (PDA), and V-8 juice agar (VJA)

Isolate	Growth (mm) ¹			
	CMA	LCHA	PDA	VJA
I1	56.00a ²	62.67a	61.33a	62.33a
2	62.33a	65.67a	66.67a	65.33a
3	76.00a	78.00a	69.33a	73.33a
4	71.67a	72.33a	71.67a	74.33a
5	66.67a	63.00a	38.67b	68.33a
6	50.67a	55.00a	57.67a	49.67a
7	65.67a	61.00a	64.33a	48.00a
Z1	52.67a	31.00a	38.33a	36.00a
2	45.00a	52.33a	52.33a	49.00a
3	47.67a	52.33a	57.67a	54.00a
4	46.67c	46.67c	52.33b	58.00a
5	58.67a	67.00a	66.00a	62.33a
6	56.67a	56.67a	59.00a	58.00a
7	47.33a	59.67a	44.33a	54.33a
R1	50.67a	31.00a	38.33a	36.00a
2	56.33a	49.33a	36.00a	55.00a
3	40.67c	59.67c	48.67b	46.33bc
4	63.67a	56.33ab	28.33d	51.33bc
5	58.33ab	56.00abc	23.67d	59.67a
6	58.33a	58.33a	33.67b	64.67a
7	55.67a	55.00a	25.00a	41.33a
C1	53.33a	69.00a	67.33a	73.00a
2	65.67a	67.67a	59.33a	67.67a
4	59.00bc	62.33ab	71.00a	53.67bc
5	62.33a	61.33a	67.33a	62.33a
6	44.33a	55.67a	53.67a	60.00a
7	54.00a	60.00a	66.00a	60.00a
G1	57.67a	59.33a	50.33a	55.67a
2	55.67a	62.33a	54.67a	51.67a
3	48.67a	53.33a	48.00a	59.00a
4	47.00a	53.00a	44.67a	48.33a
5	52.67a	51.00a	34.33b	36.33b
6	52.00a	50.67a	51.33a	44.33a
7	47.00a	37.33c	31.67c	46.33ab

TABLE 8 (continued)

Isolate	Growth (mm) ¹			
	CMA	LCHA	PDA	VJA
X1	69.33a ²	66.33a	68.00a	69.00a
2	75.00a	71.67a	41.33b	72.67a
3	66.67a	69.67a	64.67a	75.00a
4	76.33a	73.67a	65.67b	64.00b
5	62.33a	62.00a	47.33a	48.00a
6	70.00a	57.33b	41.33c	71.00a
7	61.33a	69.33a	66.00a	68.67a
8	79.33a	73.33a	67.67a	80.67a

¹Values are means of three replications.

²Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 9. Sporulation of 42 single spore isolates of *Helminthosporium maydis* on four media - corn meal agar (CMA), lactose casein hydrolysate medium (LCHA), potato-dextrose agar (PDA), and V-8 juice agar (VJA)

Isolate	Sporulation (Spores/mm ² x 1000) ¹			
	CMA	LCHA	PDA	VJA
I1	0.105b ²	3.865a	0.000b	0.000b
2	0.000a	0.000a	0.000a	0.000a
3	0.000a	0.000a	0.000a	0.000a
4	1.620a	0.000a	0.000a	0.000a
5	0.000a	0.000a	0.000a	0.000a
6	0.000b	2.320a	0.770b	0.450b
7	0.000a	0.000a	0.000a	0.000a
Z1	0.000a	3.360a	0.355a	1.000a
2	0.070b	3.905a	0.895b	0.645b
3	0.065a	3.680a	0.055a	1.055a
4	0.000a	3.680a	0.000a	0.000a
5	0.060b	1.715a	0.495b	1.515a
6	0.000a	1.215a	0.065a	0.100a
7	0.000a	0.000a	0.000a	0.000a
R1	0.185b	3.245b	8.370a	2.255b
2	0.595a	0.315a	4.340a	2.065a
3	0.285a	0.920a	0.000a	0.645a
4	0.120c	1.055bc	3.145a	1.605b
5	0.865a	2.850a	6.890a	2.860a
6	0.235a	1.155a	3.680a	1.075a
7	2.565a	3.965a	9.180a	1.185a
C1	0.715a	1.540a	2.580a	2.435a
2	0.500b	1.190ab	1.950a	1.765a
4	0.125a	0.960a	3.100a	1.955a
5	0.210c	2.195ab	2.905a	1.375bc
6	0.730a	1.305a	6.205a	2.395a
7	0.000b	1.135ab	3.145a	2.360ab
G1	0.315a	0.820a	4.160a	2.605a
2	0.225a	1.520a	3.445a	1.505a
3	0.200a	0.690a	3.405a	1.365a
4	0.340c	0.685c	3.165a	2.190b
5	0.535a	1.200a	5.345a	3.910a
6	0.260a	0.930a	2.530a	2.215a
7	0.150b	0.460b	2.075a	0.060b

TABLE 9 (continued)

Isolate	Sporulation (Spores/mm ² x 1000) ¹			
	CMA	LCHA	PDA	VJA
X1	0.335c ²	0.665bc	3.545a	1.715b
2	0.060a	1.925a	7.665a	2.145a
3	0.340c	1.845b	3.945a	1.475b
4	0.280c	1.525bc	5.010a	2.465b
5	0.100c	2.900b	7.085a	1.835bc
6	0.195b	1.280b	6.795a	1.860b
7	0.390a	1.840a	2.745a	1.620a
8	2.485a	2.230a	1.790a	0.245a

¹Values are means of two replications.

²Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 10. Linear growth and sporulation of 42 single spore isolates of Helminthosporium maydis on corn meal agar

Isolate	Mean Growth (mm) ^{1,3}	Isolate	Sporulation (Spores/mm ² X 1000) ^{2,3}
X8	79.33	R7	2.565
X4	76.33	R5	0.865
I3	76.00	C6	0.730
X2	75.00	C1	0.715
I4	71.67	R2	0.595
X7	70.00	G5	0.535
X1	69.33	C2	0.500
I5	66.67	X7	0.390
X3	66.67	G4	0.340
C2	65.67	X3	0.340
I7	65.67	X1	0.335
R4	63.67	G1	0.315
C5	62.33	R3	0.285
X5	62.33	X4	0.280
I2	62.33	G6	0.260
X7	61.33	X8	0.245
C4	59.00	R6	0.235
Z5	58.67	G2	0.225
R5	58.33	C5	0.210
R6	58.33	G3	0.200
G1	57.67	X6	0.195
Z6	56.67	R1	0.185
R2	56.33	G7	0.150
I1	56.00	C4	0.125
G2	55.67	R4	0.120
R7	55.67	I1	0.105
C7	54.00	X5	0.100
C1	53.33	Z2	0.070
G5	52.67	Z3	0.065
Z1	52.67	Z5	0.060
G6	52.00	X2	0.060
R1	50.67	I2	0.000
I6	50.67	I3	0.000
G3	48.67	I4	0.000
Z3	47.67	I5	0.000
Z7	47.33	I6	0.000
G7	47.00	I7	0.000
G4	47.00	Z1	0.000
Z4	46.67	Z4	0.000
Z2	45.00	Z6	0.000
C6	44.33	Z7	0.000
R3	40.67	C7	0.000

¹Values are means of three replications.

²Values are means of two replications.

³Means joined by the same line are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 11. Linear growth and sporulation of 42 single spore isolates of Helminthosporium maydis on lactose casein hydrolysate medium

Isolate	Growth (mm) ^{1,3}	Isolate	Sporulation (Spores/mm ² X 1000) ^{2,3}
I3	78.00	R7	3.965
X4	73.67	Z2	3.905
X8	73.33	I1	3.865
I4	72.33	Z3	3.680
X2	71.67	Z2	3.360
X3	69.67	R1	3.245
X7	69.33	Z4	3.000
C1	69.00	X5	2.900
C2	67.67	R6	2.850
Z5	67.00	I6	2.320
X1	66.33	C5	2.195
I2	65.67	X2	1.925
I5	63.00	X3	1.845
I1	62.67	X7	1.840
G2	62.33	X8	1.790
C4	62.33	Z5	1.715
X5	62.00	I4	1.620
C5	61.33	C1	1.540
I7	61.00	X4	1.525
C7	60.00	G2	1.520
Z7	59.67	C6	1.305
R3	59.67	X6	1.280
G1	59.33	Z6	1.215
R6	58.33	G5	1.200
X6	57.33	C2	1.190
Z6	56.67	R7	1.155
R4	56.33	C7	1.135
R5	56.00	R4	1.055
C6	55.67	C4	0.960
R7	55.00	G6	0.930
I6	55.00	R3	0.920
Z1	53.67	G1	0.820
G3	53.00	G3	0.690
G4	53.00	G4	0.685
Z3	52.33	X1	0.665
Z4	52.33	G7	0.460
Z2	52.33	R2	0.315
G5	51.00	I2	0.000
G6	50.67	I3	0.000
R2	49.33	I5	0.000
G7	37.33	I7	0.000
R1	31.00	Z7	0.000

¹Values are means of three replications.

²Values are means of two replications.

³Means followed by the same line are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 12. Linear growth and sporulation of 42 single spore isolates of Helminthosporium maydis on potato-dextrose agar

Isolate	Growth (mm) ^{1,3}	Isolate	Sporulation (Spores/mm ² X 1000) ^{2,3}
I4	71.67	R7	9.180
C4	71.00	R1	8.370
I3	69.33	X2	7.665
X1	68.00	X5	7.085
X8	67.67	R5	6.890
C5	67.33	X6	6.795
C1	67.33	C6	6.205
I2	66.67	G5	5.345
C7	66.00	X4	5.010
Z5	66.00	R2	4.340
X7	66.00	G1	4.160
X4	65.67	X3	3.945
X3	64.67	R6	3.680
I7	64.33	X1	3.545
I1	61.33	G2	3.445
C2	59.33	G3	3.405
Z6	59.00	G4	3.165
Z4	58.00	C7	3.145
I6	57.67	R4	3.145
Z3	57.67	C4	3.100
G2	54.67	C5	2.905
C6	53.67	X7	2.745
Z2	52.33	C1	2.580
G6	51.33	G6	2.530
Z1	51.00	X8	2.485
G1	50.33	G7	2.075
R3	48.67	C4	1.950
G3	48.00	Z2	0.895
X5	47.33	I6	0.770
G4	44.67	Z5	0.495
Z7	44.33	Z1	0.355
X6	41.33	Z6	0.065
X2	41.33	Z3	0.055
I5	38.67	R3	0.000
R1	38.33	I1	0.000
R2	36.00	I2	0.000
G5	34.33	I3	0.000
R6	33.67	I4	0.000
G7	31.67	I5	0.000
R4	28.33	I7	0.000
R7	25.00	Z4	0.000
R5	23.67	Z7	0.000

¹Values are means of three replications.

²Values are means of two replications.

³Means joined by the same line are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 13. Linear growth and sporulation of 42 single spore isolates of Helminthosporium maydis on V-8 juice agar

Isolate	Growth (mm) ^{1,3}	Isolate	Sporulation (Spores/mm ² X 1000) ^{2,3}
X8	80.67	G5	3.910
X3	75.00	R5	2.860
I4	74.33	G1	2.605
I3	73.33	X4	2.465
C1	73.00	C1	2.435
X2	72.67	C6	2.395
X6	71.00	C7	2.360
X1	69.00	R1	2.255
X7	68.67	X8	2.230
I5	68.33	G6	2.215
C2	67.67	G4	2.190
I2	65.33	X2	2.145
R6	64.67	R2	2.065
X4	64.00	C4	1.955
Z5	62.33	X6	1.860
I1	62.33	X5	1.835
C5	62.33	C2	1.765
C6	60.00	X1	1.715
C7	60.00	X7	1.620
R5	59.67	R4	1.605
G3	59.00	Z5	1.515
Z6	58.00	G2	1.505
Z1	57.33	X3	1.475
G1	55.67	C5	1.375
R2	55.00	G3	1.365
Z7	54.33	R7	1.185
Z3	54.00	R6	1.075
C4	53.67	Z3	1.055
G2	51.67	Z1	1.000
R4	51.33	Z2	0.645
I6	49.67	R3	0.525
Z2	49.00	I6	0.450
G4	48.33	Z6	0.100
I7	48.00	G7	0.060
X5	48.00	I1	0.000
Z4	46.67	I2	0.000
G7	46.33	I3	0.000
R3	46.33	I4	0.000
G6	44.33	I5	0.000
R7	41.33	I7	0.000
G5	36.33	Z4	0.000
R1	36.00	Z7	0.000

¹Values are means of three replications.

²Values are means of two replications.

³Means joined by the same line are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 14. Level of probability values for corn meal agar (CMA), lactose casein hydrolysate medium (LCHA), potato-dextrose agar (PDA), and V-8 juice agar (VJA) for linear growth and sporulation

Media	Sporulation	Linear Growth
CMA	5.29	0.01
LCHA	0.15	0.01
PDA	0.01	0.01
VJA	4.94	0.01

significant and only three groups of means were distinguished in Table 13.

The only work which can be used for comparison of the sporulation values reported in this study is that by Fukuki and Aragaki (15). Under the conditions used in the present study, these authors reported sporulation values of approximately 1.8 million spores per plate for both Race T and Race O. They did not report spores per milliliter or spores per millimeter squared values. Sixty millimeter Petri plates and V-8 juice medium were used in their study. The maximum value reported in this study on VJA is 11,750 spores/ml. Adjusted for the 50 ml quantity of the suspending solution, this value is 5.875×10^5 spores/plate. In view of the 90 mm plate size used in this study, these values are less than the maximum value reported by Fukuki and Aragaki.

The data for linear growth and sporulation on each medium by sources are shown in Tables 15, 16, 17, and 18. No significant differences among the single spore isolates of any of the sources as to sporulation occurred on corn meal agar. Three of the six sources - I, Z, and X - showed variation among the single spore isolates as to linear growth on corn meal agar. Lactose casein hydrolysate medium produced significant differences among the single spore isolates within sources in I, Z, G, and R for linear growth and I for sporulation. Sources I, G, and X had significant differences in linear growth on potato-dextrose agar. Sources I and Z had significant differences for sporulation on PDA. There were significant differences in linear growth in sources I, Z, R, G, and X on V-8 juice agar, but only source Z had significant differences in sporulation.

TABLE 15. Linear growth and sporulation of 42 single spore isolates of Helminthosporium maydis on corn meal agar by sources

Source and Isolate	Growth (mm) ¹	Source and Isolate	Sporulation (Spores/mm ² X 1000) ²
I3	76.00a ³	I1	0.105a ³
4	71.67ab	2	0.000a
5	66.67abc	3	0.000a
7	65.67abc	4	0.000a
2	62.33bc	5	0.000a
1	56.00cd	6	0.000a
6	50.67d	7	0.000a
Z5	58.67a	Z2	0.070a
6	56.67ab	3	0.065a
1	52.67b	5	0.060a
3	47.67c	1	0.000a
7	47.33c	4	0.000a
4	46.67c	6	0.000a
2	45.00c	7	0.000a
R4	63.67a	R7	2.565a
5	58.33a	5	0.865a
6	58.33a	2	0.595a
2	56.33a	3	0.285a
7	55.67a	6	0.235a
1	50.67a	1	0.185a
3	40.67a	4	0.120a
C2	65.67a	C6	0.730a
5	62.33a	1	0.715a
4	59.00a	2	0.500a
7	54.00a	5	0.210a
1	53.33a	4	0.125a
6	44.33a	7	0.000a
G1	57.67a	G5	0.535a
2	55.67a	4	0.340a
5	52.67a	1	0.315a
6	52.00a	6	0.260a
3	48.67a	2	0.225a
4	47.00a	3	0.200a
7	47.00a	7	0.150a

TABLE 15 (continued)

Source and Isolate	Growth (mm) ¹	Source and Isolate	Sporulation (Spores/mm ² X 1000) ²
X8	79.33a ³	X7	0.390a ³
4	76.33ab	3	0.340a
2	75.00ab	1	0.335a
6	70.00abc	4	0.280a
1	69.33abc	8	0.245a
3	66.67bc	6	0.195a
5	62.33c	5	0.100a
7	61.33c	2	0.060a

¹Values are means of three replications.

²Values are means of two replications.

³Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 16. Linear growth and sporulation of 42 single spore isolates of *Helminthosporium maydis* on lactose casein hydrolysate medium by sources

Source and Isolate	Growth (mm) ¹	Source and Isolate	Sporulation (Spores/mm ² X 1000) ²
I3	78.00a ³	I1	3.865a ³
4	72.33ab	6	2.320ab
2	65.67bc	4	1.620bc
5	63.00bc	2	0.000c
1	62.67bc	5	0.000c
7	61.00bc	3	0.000c
6	55.00c	7	0.000c
Z5	67.00a	Z2	3.905a
7	59.67ab	3	3.680a
6	56.67b	1	3.360a
1	53.67b	4	3.000a
2	52.33b	5	1.715a
3	52.33b	6	1.215a
4	52.33b	7	0.000a
R3	59.67a	R7	3.967a
6	58.33a	1	3.245a
4	56.33ab	5	2.850a
5	56.00ab	6	1.155a
7	55.00ab	4	1.055a
2	49.33b	3	0.920a
1	31.00c	2	0.315a
C1	69.00a	C5	2.195a
2	67.67a	1	1.540a
4	62.33a	6	1.305a
5	61.33a	2	1.190a
7	60.00a	7	1.135a
6	55.67a	4	0.960a
G2	62.33a	G2	1.520a
1	59.33ab	5	1.200a
3	53.33b	6	0.930a
4	53.00b	3	0.690a
5	51.00b	4	0.685a
6	50.67b	1	0.820a
7	37.33c	7	0.460a

TABLE 16 (continued)

Source and Isolate	Growth (mm) ¹	Source and Isolate	Sporulation (Spores/mm ² X 1000) ²
X4	73.67a ³	X5	2.900a ³
8	73.33a	2	1.925a
2	71.67a	3	1.845a
3	69.67a	7	1.840a
7	69.33a	8	1.790a
1	63.33a	4	1.525a
5	62.00a	6	1.280a
6	57.33a	1	0.665a

¹Values are means of three replications.

²Values are means of two replications.

³Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 17. Linear growth and sporulation of 42 single spore isolates of *Helminthosporium maydis* on potato-dextrose agar by sources

Source and Isolate	Growth (mm) ¹	Source and Isolate	Sporulation (Spores/mm ² X 1000) ²
I4	71.67a ³	I6	0.770a ³
3	69.33a	2	0.000b
2	66.67ab	3	0.000b
7	64.33ab	4	0.000b
1	61.33ab	5	0.000b
6	57.67b	1	0.000b
5	38.67c	7	0.000b
Z5	66.00a	Z2	0.895a
6	59.00a	5	0.495ab
4	58.00a	1	0.355bc
3	57.67a	6	0.065bc
2	52.33a	3	0.055bc
1	51.00a	4	0.000c
7	44.33a	7	0.000c
R3	48.67a	R7	9.180a
1	38.33a	1	8.370a
2	36.00a	5	6.890a
6	33.67a	2	4.340a
4	28.33a	6	3.680a
5	23.67a	4	3.145a
7	25.00a	3	0.000a
C4	71.00a	C6	6.205a
1	67.33a	7	3.145a
5	67.33a	4	3.100a
7	66.00a	5	2.905a
2	59.33a	1	2.580a
6	53.67a	2	1.950a
G2	54.67a	G5	5.345a
6	51.33a	1	4.160a
1	50.33a	2	3.445a
3	48.33a	3	3.405a
4	44.67ab	4	3.165a
5	34.33bc	6	2.530a
7	31.67c	7	2.075a

TABLE 17 (continued)

Source and Isolate	Growth (mm) ¹	Source and Isolate	Sporulation (Spores/mm ² X 1000) ²
X1	68.00a ³	X2	7.665a ³
8	67.67a	5	7.085a
7	66.00ab	6	6.795a
4	65.67ab	4	5.010a
3	64.67ab	3	3.945a
5	47.33bc	1	3.545a
2	41.33c	7	2.745a
6	41.33c	8	2.485a

¹Values are means of three replications.

²Values are means of two replications.

³Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

TABLE 18. Linear growth and sporulation of 42 single spore isolates of Helminthosporium maydis on V-8 juice agar by sources

Source and Isolate	Growth (mm) ¹	Source and Isolate	Sporulation (Spores/mm ² X 1000) ²
I4	74.33a ³	I6	0.450a ³
3	73.33a	1	0.000a
5	68.33ab	2	0.000a
2	65.33ab	3	0.000a
1	62.33b	4	0.000a
6	49.67c	5	0.000a
7	48.00c	7	0.000a
Z5	62.33a	Z5	1.515a
6	58.00ab	3	1.055ab
1	57.33ab	1	1.000ab
7	54.33b	2	0.645bc
3	54.00bc	6	0.100cd
2	49.00cd	4	0.000d
4	46.67d	7	0.000d
R6	64.67a	R5	2.860a
5	59.67ab	1	2.255a
2	55.00abc	2	2.065a
4	51.33abc	4	1.605a
3	46.33bcd	7	1.185a
7	41.33cd	6	1.075a
1	36.00d	3	0.525a
C1	73.00a	C1	2.435a
2	67.67a	6	2.395a
5	62.33a	7	2.360a
6	60.00a	4	1.955a
7	60.00a	2	1.765a
4	53.67a	5	1.375a
G3	59.00a	G5	3.910a
1	55.67ab	1	2.605a
2	51.67ab	6	2.215a
4	48.33ab	4	2.190a
7	46.33bc	2	1.505a
6	44.33bc	3	1.365a
5	36.33c	7	0.060a

TABLE 18 (continued)

Source and Isolate	Growth (mm) ¹	Source and Isolate	Sporulation (Spores/mm ² X 1000) ²
X8	80.67a ³	X4	2.465a ³
3	75.00ab	8	2.230a
2	72.67ab	2	2.145a
6	71.00ab	6	1.860a
1	69.00ab	5	1.835a
7	68.67ab	1	1.715a
4	64.00b	7	1.620a
5	48.00c	3	1.475a

¹Values are means of three replications.

²Values are means of two replications.

³Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test.

Pearson product moment correlation coefficients between linear growth and sporulation, using all media and isolates combined, were negative and significant at the 1% level of probability. As linear growth increased sporulation decreased. The correlation between linear growth and sporulation on CMA and VJA was negative but not significant. On LCHA the correlation was negative and significant at the 5% level of probability. The PDA correlation was negative and significant at the 1% level of probability. The correlation between linear growth and sporulation was negative and significant at the 5% level for the following isolates: R2, R5, R6, G7, X2, X5, and X6. The correlation was negative and significant at the 1% level for isolates R4, G1, and G6. Positive correlation at the 5% level was found for isolate C7. Isolate I4 had a positive correlation at the 1% level. There were no significant correlations between linear growth and sporulation for the remaining isolates.

CHAPTER III

PATHOTOXIN AND SEEDLING PATHOGENICITY

Literature Review

The term pathogenicity has been defined in recent literature by several authors. Roberts and Boothroyd (62) give the following definition, "The ability of the pathogen to cause disease." Day (8) regards pathogenicity as a general attribute of a species. Watson (83) interprets pathogenicity as a general term defined by such characteristics of the pathogen-host relationship as lesion size and in vivo spore production and growth rate. The term virulence is used in conjunction with pathogenicity. Virulence is the measure of relative pathogenicity (62) or the pathogenicity of a particular pathogen strain on a particular host genotype (8). Watson (83) states that virulence implies "that either the host has no genes for resistance...or the gene or genes for resistance in the host are matched in the fungus by corresponding genes for virulence." Upon these two terms, Watson impinges a third term, aggressiveness. An aggressive strain is one which has certain characters, e.g., survival ability, that make it more fit to compete. These characters may or may not be involved in pathogenicity. The genes that control aggressiveness are not the same as those that control virulence, but genes for aggressiveness and pathogenicity may be the same.

A few studies of pathogenic variability have been made using H. victoriae, H. sativum, and H. carbonum (12, 43, 48, 49, 50, 88).

Two pathogenic races of H. carbonum are recognized (78). Prior to the 1970 epiphytotic, Yu (90) and Orillo (56) reported on the pathogenicity of H. maydis.

In 1969 Nelson (52) demonstrated that 13 different genes contribute to the control of pathogenicity of H. maydis to nine gramineous species. Studying the segregation of the ascospore progeny of a cross of isolates of Race T and Race O, Lim and Hooker (34) reported the race of the H. maydis isolates to be monogenic in inheritance and virulence to be polygenic in inheritance.

Throughout this chapter repeated reference will be made to cytoplasmic male-sterility. Sterility in plants is the result of the production of non-functional gametes. This production is the result of either gene action (nuclear) or cytoplasmic factors or both that cause the abortion or modification of any of several flower parts, e.g., pollen. When pollen is rendered non-functional by cytoplasmic factors, the sterility is referred to as cytoplasmic male-sterility (cms). Certain male-sterile cytoplasms have the ability to be "restored" to male fertility when pollinated by a male parent carrying "restorer" genes (2). The first report of cytoplasmic male-sterility in corn was by Rhoades (61). The Texas source of male-sterility (cms-T) was isolated and identified by Rogers and Edwardson (63).

Scheifele (67) has made an extensive study of the susceptibility of corn to southern leaf blight as it is related to cytoplasmic male-sterility. Discussing cytoplasmic male-sterility related disease susceptibility to H. maydis Race T, Scheifele has hypothesized that the genetic systems of the organelles in the cytoplasm completely

override the nuclear genetic system, modified to some extent by weak modifier genes in the nucleus. Scheifele postulates the following mode of action for his hypothesis.

The organelles, presumably mitochondria, in the normal cytoplasm contain genes which control the synthesis of certain proteins making up the mitochondrial ribosomes; the normal proteins evidently insensitive to the pathotoxin of H. maydis Race T...whereas the ribosomal proteins of mitochondria from T...male-sterile cytoplasms being inactivated, resulting in a destruction of the organelle's protein synthesizing apparatus essential to the integrity of certain structures, conceivably the mitochondria themselves.

Further credence is given to this postulation by two other factors. First, the conclusion of Arntzen, et al. (3) that organelle membranes (e.g., mitochondria) may be the primary site of action for H. maydis Race T pathotoxin. Second, the mitochondrial membranes are the location of the ribosomes associated with the mitochondria.

Scheifele suggests that the disease reaction conditioning genetic system in the cytoplasm is separate from that controlling male-sterility. Supporting this idea is the occurrence of both male-sterility and H. maydis Race T resistance in the majority of the other male-sterile cytoplasms. Although the conditioning of disease reaction to H. maydis Race T by fertility restoring genes has been reported (see below), nuclear genes conditioning H. maydis Race T reaction have not been observed to modify pollen abortion or fertility restoration.

In 1970 Hooker, et al. (26) first reported observing increased susceptibility of T cytoplasm corn to SCLB in central Illinois in 1969. This observation was also made in 1969 by Scheifele, et al. (69). Several reports of a similar situation were reported as early as 1961 in the Phillippines (1, 39, 80, 81). The same inbreds used in the

Phillippine studies were tested in the U. S. in 1963 and no differences were found (71); however, the workers later determined their inoculum to be Race 0.

Following the 1970 epiphytotic, Nelson, et al. (51) studied the past occurrence of Race T of H. maydis using several isolates the senior author had maintained for 4-13 years. None of the 116 isolates that had been maintained culturally showed any of the characteristics of Race T. Twenty-seven isolates were reisolated from the original material. Of these, 24 were classified as Race T. Ten of these isolates were originally collected on corn and 17 on other hosts. In a later work, Nelson (53) re-cultured 165 isolates from original diseased material. These isolates, which dated from 1958 to 1967, were of world-wide distribution and were from several host species. Of these 165 isolates, 150 were designated Race T and 15 Race 0 on the basis of seedling reaction. The results of these two studies led Nelson to conclude that Race T had predominated the H. maydis population for many years. In 1957 Nelson (41) reported H. maydis to be heterothallic and identified two mating types, A and a. Since that time, he has done extensive work with the genetics of H. maydis (42, 44, 45, 46, 47). In the study using 165 re-cultured isolates of H. maydis, Nelson found the majority of the Race T isolates to be of one mating type. The Race 0 isolates were similar in mating type to the minority mating type of the Race T isolates. Nelson suggested that this indicates that Race 0 isolates may be defunct Race T isolates. These findings also suggest that the origin of H. maydis Race T was diverse in time and location.

Hooker (24) believes that these past isolates did not have all the characteristics of Race T nor was the Race T of the 1970 epiphytotic a single biotype. The epiphytotic of 1970 was probably caused by a mixture of biotypes having similar pathotoxin and pathogenic characteristics. Both of these authors, Nelson (51) and Hooker (24), emphasize the importance of Race T development in supporting the conclusion that new races of plant pathogens may develop independently of the selection pressure of existing susceptible or resistant host populations.

Leonard has also studied the origin of H. maydis Race T (29, 31). From the data in several papers (14, 25, 39, 68, 69, 71, 81, 82) reporting on the past occurrence of Race T, Leonard suggests that Race T originated in the Midwest, perhaps in Iowa. Race T was probably introduced in the Midwest on infected seed, because mutation from Race 0 or from a weak Race T to a virulent Race T would have been more likely in the South where H. maydis had long been present. The H. maydis population in the Midwest was predominately mating type A. Leonard proposes that Race T in the South arose by crosses between Race T in the Midwest, mating type A, and Race 0 in the South, mating type a. The A mating type carried linked genes for Race T virulence. The a mating type carried linked genes for adaptation to the southern environment, since Race 0 was well adapted in the South. The resulting a mating type progeny had linked genes for Race T virulence and Race 0 adaptation. Leonard (31) has reported a change in the mating type composition of the H. maydis population in the South from 80% A mating type and 20% a mating type in 1970 to 47% A and 53% a in 1971. This shift is due to the linked genes for adaptation of the a mating type progeny of the cross outlined previously.

In contrast to Nelson and Hooker, Leonard maintains that Race T is of recent origin in the U. S. He presents three lines of evidence to support this belief. First, the association of H. maydis and T male-sterile cytoplasm was not observed in the U. S. until 1969. Second, the predominance of mating type A in the Race T population in the Midwest indicates an imbalance characteristic of young populations. Third, the rapid increase of mating type a in the South supports the origin of Race T there through crosses with Midwestern types. To explain the conflict between his theory of recent origin and Nelson's re-culturing of Race T isolates from early collections, Leonard gives two possible explanations. First, the Race T isolates of Nelson could have arisen as contaminants. Leonard (30) has shown that such contamination by airborne Helminthosporium spp. can easily occur in the time required for stored isolates to resume growth and sporulation. Second, there may be two types of Race T. One type is an older, geographically widespread type which lacked the ability to infect cms-T corn. The second type is one which has arisen only recently in the U. S. in the manner described above for the Midwest and South.

The two races of H. maydis, Race T and Race 0, have been characterized by Hooker (24). Symptomologically, Race 0 is limited to the leaf, where it produces small parallel-sided lesions. Race T attacks not only the leaf area, but also the leaf sheath, husk, and ear. Lesions of Race T are larger than Race 0, spindle-shaped and tan in color with chlorotic borders. Race T is host-specific for corn lines with the Texas (T) cytoplasmic male-sterility factor. Male-sterile cytoplasm Q, P, and HA have also been reported as susceptible. Race 0 has no cytoplasmic specificity. Race T has a higher optimum temperature

for growth and a higher reproductive rate than Race 0. Race T produces a host-specific pathotoxin in large amounts in vitro and in vivo. Race 0 produces low amounts of a pathotoxin that is not host-specific.

Resistance to H. maydis Race T or Race 0 is expressed in several ways: lesion number, size, and sporulation; Race T pathotoxin resistance; time of ear death; and time of plant death (24). Resistant lines have small lesion number, size, and few spores are produced in these lesions. The elongation of primary roots of germinating seedlings of resistant lines are not inhibited by the pathotoxin of Race T. Earlier premature death of ears and plants occurs in Race T susceptible lines.

Resistance to Race 0 has been present in several inbreds and hybrids for many years (24). Resistance to Race T is present in lines with normal (N) cytoplasm and in the following male-sterile cytoplasm: B, C, CA, D, EK, F, G, H, I, IA, J, K, L, M, ME, MZ, PS, R, RB, S, SD, TA, TC, VG, El Salvador, and CIMMYT (72, 51). Resistance to Race T within corn lines with T male-sterile cytoplasm has been investigated (24). Inbreds with the greatest resistance to Race 0 also had the greatest resistance to Race T when produced on cms-T. This resistance was not equal to that of normal (N) cytoplasm lines. Similar results were obtained with the same inbreds in crosses to Race 0 susceptible cms-T testers. Some resistance has been found in crosses of the same inbreds with a mixture of broadly based synthetics. Some resistance has been obtained through induced mutation of T male-sterile cytoplasm lines (24). Hilty and Josephson (23) observed the seedling reaction of cms-J and cms-T with the restorer factor (Rf) in several inbred lines. Male-sterile cytoplasm J was as resistant as normal cytoplasm.

Inbred line Ky21-TRf was rated as resistant to H. maydis Race T. This result was later found to be in error (L. M. Josephson, unpublished). Similar findings were reported by Gracen and Grogan (19) in field tests using the pathotoxin of H. maydis Race T. The inbred line AyESY 103-3-TRf was resistant to Race T; and inbred lines Ay 172-25, Pa 83, Au X65T, A 97, Ay X138, and SD 105 in TRf were partially resistant. The last four lines were partially resistant in the cms-T condition.

Prior to any discussion of a toxin produced by a plant pathogen there must be a definition of the concept of a "toxin."

Wheeler and Luke (84) have outlined four criteria for a microbial metabolite to be a toxin:

- a. The toxin when applied in concentrations expected in the diseased plant, produces all the symptoms characteristic of the disease in a susceptible host.
- b. The pathogen and toxin have similar suscept specificity.
- c. The ability of the pathogen to produce the toxin is directly proportional to its ability to produce the disease.
- d. A single toxin is involved.

These authors have defined three groups of toxins - phytotoxin, vivotoxin, and pathotoxin. Phytotoxin is a general term applied to all products of living organisms toxic to plants. Vivotoxin was first defined by Diamond and Waggoner (9) as "a substance produced in the infected host by the pathogen and/or its host, which functions in the production of disease, but is not itself the disease-producing entity." They listed three requirements for a vivotoxin:

- a. Reproducible separation from the diseased plant.
- b. Purification or chemical characterization.
- c. Reproduction of at least a portion of the disease syndrome by placing the toxin in a healthy plant (64).

Wheeler and Luke retained this term as a second group for phytotoxic compounds isolated from diseased plants and chemically characterized. The third group defined by Wheeler and Luke was pathotoxins. Pathotoxins are phytotoxins which have been shown "to play an important causal role in disease."

Goodman, et al. (16) classified the toxic metabolites produced by pathogenic fungi into four groups. (1) Vivotoxins produce part of the disease syndrome and must be isolated from diseased tissue - *Fusarium* wilt toxin, lycopersamin (*Fusarium oxysporum* f. *lycopersici*). (2) Phytotoxins which induce few or none of the symptoms caused by the living pathogen are not host specific, and there is no relationship between toxin production and the pathogenicity of the causal organism - alternaric acid (*Alternaria solani*). (3) Several facultative parasites secrete "toxic" substances which are either enzymes or growth regulators - *Verticillium albo-atrum*. (4) Pathotoxins are metabolites which are produced in culture and are able to produce all the characteristic disease symptoms in susceptible plants. Pathogen and pathotoxin are similar in host range and virulence is directly proportional to toxin production - Victorin (*H. victoriae*).

Scheffer and Pringle (64) have separated plant pathogen produced toxic substances which are involved in disease into two classes, primary and secondary determinants of disease. Primary determinants

may either be host-specific or non-host-specific, but they are essential to the pathogenicity of the organism. Secondary determinants are substances that contribute to virulence but not pathogenicity.

The most recent classification of toxic substances in plant disease was by Graniti (20). The system offered by Graniti is presented in Table 19 and is the system that will be used herein. The term pathotoxin will be used in this paper for the metabolite produced by H. maydis for two reasons. First, although there is some controversy between Hooker and Pringle and Scheffer over the usage of the term pathotoxin, the classification system of Graniti allows the terms primary determinant of disease (Pringle and Scheffer) and pathotoxin (as defined by Wheeler and used by Hooker) to be used interchangeably. Second, pathotoxin is the term that has been used most widely in the literature dealing with H. maydis and it will be retained for consistency. A pathotoxin is defined as a toxic metabolite produced in culture and in the plant having the same host-specificity as the pathogen, producing the same symptoms on susceptible hosts as the pathogen and is a primary determinant of pathogenicity. Additional information concerning toxins may be found in the following reviews: Brian (5), Wood (89), Braun (4), Cruickshank (7), Scheffer and Samaddar (66), Wheeler and Hanchey (85), Pringle (58), and Scheffer and Yoder (65).

Several papers have been published dealing with the production of the crude cultural filtrates and their bioassay for H. maydis pathotoxin. The term crude filtrate as used in this study means the liquid expressed from H. maydis cultures, either liquid or solid media,

TABLE 19. Substances involved in plant disease

Pathogen-Derived Substances	
Phytotoxins (and Vivotoxins)	Phytoaggressins (and Vivoaggressins)
Chemical nature - low molecular and high molecular weight compounds; inorganic compounds; organic compounds: aliphatic, aromatic, alicyclic, heterocyclic	Toxicity and chemical nature - compounds toxic in excessive concentrations (alcohols, organic acids); non-toxic compounds (pigments, polysaccharides, polypeptides)
Suscept specificity - host-specific; non-host-specific; taxaspecific	Mode of action - enzymes (cutinases, macerating enzymes, phenoloxidases, lignin-degrading enzymes); growth regulators (growth promoters; growth inhibitors); metabolic determinants (nucleic acids; tumor inducing principle
Substrate specificity - active on meristems, parenchyma, leaves, stems, roots, flowers	
Virulence of the pathogen - primary determinants (pathotoxins); secondary determinants	
Mode of action - antimetabolites; enzyme inhibitors; chelating agents; pseudoplasmodysing agents	
Host reaction - chlorotic toxins; necrotic toxins (necrotoxins); wilting toxins (marasmins)	

Revised from: Graniti, A. 1972. The evolution of the toxin concept in plant pathology, p. 10. In R. K. S. Wood, A. Ballio, and A. Graniti [ed.]. Phytotoxins in plant disease. Academic Press, New York.

and used as they are for bioassay. There were early reports of the production by H. maydis of one or more toxins which were not host-specific (55, 57) and the production of several secondary metabolites (22). Immediately previous to the 1970 SCLB epiphytotic, Smedegard-Petersen and Nelson (70) produced crude filtrates using Fries' modified liquid medium plus 2.0% yeast extract. These filtrates were bioassayed by floating leaf sections on various dilutions of the filtrates in Petri plates and determining the amount of watersoaking, chlorosis, and necrosis present after 48 hours. They found the hybrid Pa290 susceptible and inbred Tr resistant to the pathotoxin in the filtrates. Since this work was prior to 1970 no information was given as to the race of the two isolates used in the test. Based on their studies with lines similar to those of Smedegard-Petersen and Nelson, Comstock and Scheffer (6) have concluded that the isolates which Smedegard-Petersen and Nelson used were Race 0.

Following the 1970 SCLB epiphytotic, Hooker, et al. (25) reported the production of a pathotoxin by H. maydis Race T in culture. Wheeler, et al. (86) used Fries' modified medium plus 0.1% yeast extract to produce the pathotoxin. Their seedling bioassay of the crude filtrates confirmed the differential susceptibility of corn lines with normal (N) and Texas (T) male-sterile cytoplasm. The seedling bioassay used was based on the inhibition of the elongation of the primary roots of germinating corn seedlings, with the results being expressed as the percent inhibition of treated seedlings versus control seedlings. This type of seedling bioassay has subsequently been used by other authors and was used in the present study. Subsequent

reference to a seedling bioassay in this paper will refer to the method just described.

The remainder of the work with the pathotoxin produced in culture has been done using "concentrated" pathotoxin preparations. The term concentrated describes pathotoxin preparations obtained by applying one of a number of extraction procedures to the crude cultural filtrates. Hooker, et al. (25) in their original pathotoxin work, produced the crude filtrates on a synthetic medium and concentrated these by an extraction procedure. Seedling bioassay of the resulting concentrates also revealed host-specificity to N and cms-T corn lines. In a later study Lim, Hooker, and Smith (32) cultured H. maydis on a potato-sucrose medium and on PDA. Both the crude and concentrated filtrates were assayed. The order of the degree of host-specificity was concentrated filtrates (potato-sucrose and PDA) greater than PDA crude filtrates which were greater than potato-sucrose crude filtrates. Comstock and Scheffer (6) used a more detailed extraction procedure. The resulting concentrated pathotoxin solution, used in a seedling bioassay, showed host-specificity in 23 of 36 Race T isolates. The Race 0 isolate used showed no host specificity. Arntzen, et al. (3), by a series of microfilters, concentrated crude filtrates from cultures grown on Fries' modified medium plus 0.1% yeast extract. The concentrated toxin gave a very rapid and distinct host-specific reaction in bioassay.

Several papers have dealt with characteristics of H. maydis isolates in liquid culture during pathotoxin production. Under similar conditions, different workers have reported on the optimum date at

which to harvest H. maydis cultures for maximum toxin production. Smedegard-Petersen and Nelson (70) list 13-14 days; Wheeler, et al. (86), 13-18 days; and Comstock and Scheffer (6), 12-15 days. The works by Smedegard-Petersen and Nelson and Wheeler, et al. found the pH of the culture medium to drop to a low of about pH 3.0 (10 days) and rise to 7.5-8.0 as the mycelium began to lyse. Maximum pathotoxin production was correlated with pH 6.5 by Wheeler at approximately 15 days. Smedegard-Petersen and Nelson found maximum pathotoxin production 4-6 days following minimum pH. The measurement of the changes in mycelial dry weight of H. maydis cultures has been made by Smedegard-Petersen and Nelson (70). The mycelium growth followed the changes in pH, being maximum at the minimum pH and decreasing as the mycelium lysed and the pH increased. Comstock and Scheffer (6) studied pathotoxin production on five defined media: Czapek's solution, Richard's solution, Hooker's shake culture medium, Fries' solution plus 0.1% yeast extract, and Fries' solution plus 2% yeast extract. The greatest pathotoxin production was on Fries' solution plus 0.1% yeast extract.

Some of the chemical characteristics of the pathotoxin of H. maydis have been determined. The pathotoxin has been described as follows: thermostable and stable in acid and slightly alkaline solutions (6, 8, 33, 70); dialyzable (6, 70); negative in charge (70); paper chromatographic R_f 0.9 (6, 33); thin layer chromatographic R_f 0.95 (33); ninhydrin positive (70, 33); absorption maximum 270 nm (33); fluoreses light yellow under 366 nm ultraviolet irradiation (33); H_2O , methanol, and butanol soluble (33); and carbon tetrachloride and carbon disulfide insoluble (33). These characteristics indicate that the toxin is a low molecular weight polypeptide (6).

Smedegard-Petersen and Nelson (70) and Lim and Hooker (34), using ascospore progeny of compatible H. maydis crosses, found pathotoxin production to be monogenic in inheritance, with the quantity of toxin produced being polygenically inherited.

The host-specificity of the pathotoxin produced by Race T isolates of H. maydis has been well established both in culture and in the field (6, 17, 19, 33, 70, 86). Corn lines with cms-T are highly sensitive to the pathotoxin, whereas lines with normal cytoplasm are not. Using the seedling bioassay, Hooker, et al. (25) described cms-S and cms-C as resistant and cms-P as susceptible to the pathotoxin of H. maydis Race T. Gracen, et al. (17, 18) tested several male-sterility sources in four inbred lines by injecting seedlings in the field with the pathotoxin. They found the male-sterile cytoplasm 474, HA, P, and RS susceptible and cytoplasm B, C, CA, CH, D, EP, F, G, H, I, IA, J, K, L, LF, M, ML, MY, NT, OY, R, RB, SG, TA, TC, W, and 181 resistant.

Two studies have dealt with the effect of H. maydis Race T pathotoxin on cellular organelles and subcellular processes. Miller and Koepe (40) reported that the pathotoxin of H. maydis Race T caused irreversible swelling of mitochondria from susceptible seedlings. The addition of pathotoxin in the reaction medium reduced the rates of O₂ uptake, malate-pyruvate oxidation, and increased the rate of oxidation of extramitochondrial NADPH and succinate. Following the addition of the pathotoxin, additions of ADP did not stimulate respiration. These effects were not observed with mitochondria from H. maydis resistant seedlings. The authors concluded that the mitochondria might be the primary site of pathotoxin action and that the observed effects were

concerned with mitochondrial membrane changes. Arntzen, et al. (3), in a more recent paper, confirmed the mitochondrial swelling and respiratory changes of the earlier work. However, no inhibition of cellular ^{32}P and/or O_2 uptake by the pathotoxin was found even though root inhibition occurred. Uptake of ^{32}P and O_2 is a measurement of respiratory processes at the cellular level. From this it was concluded that the mitochondria are not the primary site of action of the toxin. The reductions of cellular ATP were deemed secondary effects because they occurred only at high pathotoxin concentrations. Although the pathotoxin inhibition of root growth was rapid, there was no rapid or high leakage of PO_4 or ions from pathotoxin treated cells. This indicated no cell membrane disruption, which was confirmed by EM examination. The authors concluded that the pathotoxin causes no general membrane disruption, but that organelle membranes may be disrupted.

In the early work by Smedegard-Petersen and Nelson (70) and in recent work by Lim and Hooker (35), the workers were able to isolate toxic materials from H. maydis diseased tissue. Lim and Hooker found that the toxic fraction from diseased leaves of susceptible plants was active and host-specific in bioassay. The leaf extracts were similar in chemical characteristics to the pathotoxin produced in culture. Only small amounts of the pathotoxin were extracted from resistant plants and this was not host-specific.

Lindberg (36) studied pathotoxin production by diseased isolates of H. maydis Race T and found that pathotoxin production was not affected by a transmissible disease. Pathotoxin production was induced in one isolate by infecting it with the disease.

Materials and Methods

Forty-two single spore isolates from the six sources were used in the cultural variability work. For practical reasons fewer isolates could be used in the pathotoxin and seedling variability work. These isolates were chosen at random from each source. Isolates I5, Z7, C6, X2, G1, and R4 were used in the optimum pathotoxin harvest date and crude pathotoxin filtrate work. Isolates I5, X2, G1, and R4 were used in the concentrated pathotoxin experiments. Isolates X2, G1, R4, and C6 were used in the seedling pathogenicity tests.

Optimum pathotoxin harvest date. Previous to the concentration and bioassay of the pathotoxin, a study was made to determine the optimum date at which to harvest the cultures for maximum pathotoxin production. Twenty 250 ml Erlenmeyer flasks containing 50 ml of Fries' modified medium plus 0.1% yeast extract (32, 59, 86) were "seeded" with 7 mm cork borer discs of mycelium only of the six single spore isolates of H. maydis. The pH of the medium was adjusted to 6.0. The flasks were placed on laboratory benches. Two flasks per isolate were harvested at three day intervals from day three to day 30. Each flask served as one replication. At each harvest date the cultures were expressed through double fold cheesecloth. The mycelium was dried at 75°C for 24 hours and weighed. Ten milliliters of the crude filtrate was used for a pH measurement and the remainder was frozen for later use in a bioassay. The crude pathotoxin was bioassayed by floating three leaf sections on 20 ml of the filtrates in Petri plates on laboratory benches. The filtrates were diluted 1:100, 1:500, and 1:1000 with sterile deionized H₂O. After 48 hours the tissue in each

dilution was rated as resistant (+++) or susceptible (---) or any combination of + or -. The rating was based on the amount of water-soaking present on the leaf section.

Crude pathotoxin pathogenicity. Three day old cultures of six single spore isolates of H. maydis grown on potato-dextrose agar (PDA) were used as inoculum in the test. Seven millimeter cork borer discs of mycelium only were used to "seed" five 250 ml Erlenmeyer flasks per isolate, each flask containing 115 ml $\pm$ 2% of Fries' modified liquid medium plus 0.1% yeast extract, pH 6.0. The flasks were placed on laboratory benches at 24-26°C and left undisturbed for 20 days. At this time the flasks were harvested by expressing the cultures through double fold cheesecloth. The resulting crude filtrates were bioassayed for pathotoxin by measuring their ability to inhibit the elongation of primary roots of corn seedlings. Inbred Ky 21 having six male-sterile cytoplasms - T, C, G, M, W, and K - and normal (N) cytoplasm was used. The seeds were washed for one hour in running tap water, surface disinfected with 1:10 Chlorox-H₂O, and allowed to soak 24 hours in sterile deionized H₂O. Following the imbibition of H₂O the seeds were disinfected with 0.1% HgCl₂ solution and rinsed three times with sterile deionized H₂O (39). The seeds were then placed embryo down on paper towels in 8 X 15 inch baking pans. The towels were moistened with a 1×10^{-4} M CaCl₂ solution. The pans were covered with Saran Wrap and placed in an incubator at 29°C under continuous darkness. After 48 hours those seeds with a primary root length of 4-6 mm were selected for use in the bioassay. Two 90 mm Petri plates with five seeds per plate served as two replications for each isolate-cytoplasm-

dilution combination. The crude filtrates of each isolate were diluted 1:100, 1:500, and 1:1000 with sterile deionized H₂O. Twenty milliliters of each dilution were added to each plate and the plates placed in an incubator in a random pattern at 29°C and continuous darkness. Ten plates with five seeds of each cytoplasm per plate in 20 ml of sterile Fries' medium served as controls. At the end of 48 hours, the length of the primary root of each seedling was measured. A mean length for the five seeds in each replication was determined. A mean for the ten control plates was also determined. The percent inhibition was calculated as follows:

Percent Inhibition =

$$\frac{\text{Control Mean Length (mm)} - \text{Treatment Mean Length (mm)}}{\text{Control Mean Length (mm)}} \times 100$$

Concentrated pathotoxin pathogenicity. Similar work to that using the crude pathotoxin was done using four single spore isolates of H. maydis in which the concentrated pathotoxin was used in the seedling bioassay. This work varied from the above in that 20, 250 ml Erlenmeyer flasks each containing 100 ml of Fries' medium were used for each isolate. The flasks were harvested at various dates depending on the optimum harvest date determined in the liquid culture characteristics work given previously. The crude filtrates were expressed through double fold cheesecloth. These crude filtrates were concentrated by methods similar to those of Lim and Hooker (33). Approximately two liters of the crude material were concentrated to 100 ml (one-twentieth the original volume) in a flash evaporator at 45°C under vacuum. This concentrate was mixed with an equal volume of methanol and placed at

4°C for 24 hours. The solution was filtered to remove the precipitate and washed with 75% methanol. The filtrates were bulked and evaporated to dryness in a flash evaporator. The dried material was resuspended in 100 ml (one-twentieth the original volume) of deionized H₂O. This solution was dialysed against 3000 ml of deionized H₂O for 72 hours at 10°C. The dialysate was concentrated to 50 ml (one-fortieth the original volume) under vacuum in a flash evaporator and extracted four times with equal volumes of butanol. The butanol phases were bulked, concentrated to dryness by flash evaporation, and dissolved in 500 ml deionized H₂O. This solution was used in a seedling bioassay as outlined above with the exception that the seedlings were inbred K55 having the same six male-sterile cytoplasms and normal cytoplasm.

Seedling pathogenicity. Seedlings of the corn inbred Ky 21 produced in normal (N) and six male-sterile cytoplasms - T, C, G, M, and K - and four single spore isolates of H. maydis were used to study pathogenic variability in a greenhouse test. The four isolates were cultured on PDA for the length of time for optimum sporulation by each isolate. The plates were then flooded with a 5% sucrose, 0.2% "Tween 20" solution and the spores suspended by scraping the surface of the plate. The suspension was placed in a 100 ml beaker and the volume adjusted to 50 ml with the suspending medium. A spore concentration was determined by taking two samples with a medicine dropper and transferring them to a hemacytometer. Four 1 mm³ grids were counted for each sample and these averaged to give a concentration for the solution. The spore suspensions for each isolate were adjusted to 20,000 spores/ml. Two 4-inch pots for each cytoplasm-isolate combination were

planted with three seeds per pot. Each pot served as one replication. The plants were inoculated at the 3-4 leaf stage by spraying them with the spore suspensions using an atomizer. The pots were then placed in a darkened mist chamber in a random pattern for 24 hours, then removed from the mist. After 72 hours the second and third leaves of each plant were removed by clipping with a razor blade at the sheath. The leaves were placed in a plant press and dried for two weeks. At this time the leaves were photographed, and the photographs enlarged to 5 X 7 inches. A total area for each leaf was determined using an area meter. The diseased area was determined by planimetry of the photographs. All values were adjusted to a constant size and a percent diseased area computed for each leaf.

Results and Discussion

The results of Duncan's multiple range analysis among the cytoplasms for the seedling inoculation work are presented in Table 20. In this analysis and all subsequent analyses of percentage data, the percentages were transformed to arcsin values and the analyses performed on the transformed data. The maximum percent diseased area occurred on corn with T cytoplasm for all the isolates. The minimum diseased area occurred in N cytoplasm with isolates R4, X2, and C6. The minimum diseased area did not occur in N cytoplasm with isolate G1; however, it was not significantly different from K, the lowest value. These results indicate that the H. maydis isolates used in this test were Race T. Cytoplasms C and W were not significantly different from T cytoplasm in percent diseased area for isolates X2 and R4. Although cytoplasms C and W were significantly different from each other and

TABLE 20. Differences in percent diseased area among the normal and six male-sterile cytoplasm of corn seedlings infected with four single spore isolates of Helminthosporium maydis

Isolate	Cytoplasm ¹						
R4	T 49.75a ²	C 46.50a	K 22.25ab	W 19.75ab	G 6.00bc	M 1.00bc	N 0.75c
X2	T 49.75a	C 48.25a	W 28.25a	G 2.75b	M 2.25b	K 1.75b	N 1.25b
G1	T 48.00a	W 47.50a	C 6.75b	G 5.25b	N 3.50b	M 2.50b	K 1.50b
C6	T 39.25a	W 22.25b	C 15.00c	G 7.75d	K 5.00de	M 4.75de	N 2.50e

¹Values are means of two replications.

²Means followed by same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test. The analysis of variance and Duncan's were performed on arcsin transformations of the percent data.



from T cytoplasm for isolate C6, these three cytoplasms had percent diseased area values significantly higher than the other cytoplasms tested. For isolate G1 cytoplasms T and W were not significantly different from each other, but were significantly greater than the other cytoplasms, including C. These results indicate that cytoplasms C and W were as susceptible as T cytoplasm to H. maydis isolates used in this test. Table 21 presents the same data as Table 20 except that Duncan's analysis was performed among the four isolates on each cytoplasm. There were no significant differences among the isolates for C, W, and T cytoplasm. There were significant differences among the isolates with all the other cytoplasms, including N cytoplasm. However, these differences were among very low percent diseased area values.

Based on the results of the optimum harvest date study, the change in mycelial dry weight and medium pH with time are presented in Table 22. The changes for each isolate are plotted against time in Figures 1, 2, 3, 4, 5, and 6. The optimum harvest date for each isolate was as follows: Z7 - day 17, C6 - day 21, I5 - day 23, R4 - day 23, G1 - day 24, and X2 - day 26. The optimum harvest date for each isolate occurred approximately five days after maximum mycelial dry weight production occurred and minimum medium pH was reached. Only isolate Z7 had an optimum harvest date which occurred within the 13-18 day range reported in previous papers. The optimum harvest dates of the five other isolates were from three to eight days later than the day 18 maximum previously reported. These differences emphasize further the need for preliminary work to determine the optimum harvest date before any studies dealing with the pathotoxin are undertaken.

TABLE 21. Differences in percent diseased area among the four single spore isolates of Helminthosporium maydis infecting corn seedlings with normal and six male-sterile cytoplasms

Cytoplasm	Isolate ¹			
	R4	X2	G1	C6
T	49.75a ²	49.75a	48.00a	39.25a
N	G1 3.50a	C6 2.50ab	X2 1.25bc	R4 0.75c
C	X2 48.25a	R4 46.50a	C6 15.00a	G1 6.75a
G	C6 7.75a	R4 6.00a	G1 5.25a	X2 2.75b
M	C6 4.75a	G1 2.50ab	X2 2.25ab	R4 1.00b
W	G1 47.50a	X2 28.25a	C6 22.25a	R4 19.75a
K	R4 22.25a	C6 5.00ab	X2 1.75b	G1 1.50b

¹Values are means of two replications.

²Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test. The analysis of variance and Duncan's were performed on arcsin transformations of the percent data.

TABLE 22. Mycelial dry weight and medium pH changes with time of six single spore isolates of Helminthosporium maydis on Fries' modified liquid medium

Isolate																	
R4			G1			Z7			X2			I5			C6		
Day	Dry Weight (g)	pH	Dry Weight (g)	pH	Dry Weight (g)	Dry Weight (g)	pH	Dry Weight (g)	Dry Weight (g)	pH	Dry Weight (g)	Dry Weight (g)	pH	Dry Weight (g)	Dry Weight (g)	pH	
0	0.00	6.0	0.00	6.0	0.00	0.00	6.0	0.00	0.00	6.0	0.00	0.00	6.0	0.00	0.00	6.0	
3	0.10	5.5	0.07	5.5	0.08	0.09	5.3	0.09	0.09	5.4	0.09	0.13	5.4	0.13	0.13	5.2	
6	0.21	4.9	0.31	5.0	0.19	0.28	4.5	0.28	0.28	4.7	0.20	0.29	4.7	0.29	0.29	4.7	
9	0.23	4.9	0.28	4.7	0.31	0.40	4.0	0.40	0.40	4.4	0.28	0.48	4.2	0.48	0.48	4.1	
12	0.56	3.8	0.47	3.9	0.33	0.56	4.0	0.56	0.56	3.9	0.36	0.47	4.0	0.47	0.47	4.2	
15	0.41	4.2	0.62	3.7	0.22	0.60	5.9	0.60	0.60	3.7	0.36	0.76	3.9	0.76	0.76	3.4	
18	0.50	3.9	0.62	3.6	0.22	0.60	5.8	0.60	0.60	3.6	0.37	0.72	3.9	0.72	0.72	3.4	
21	0.46	3.8	0.61	3.5	0.22	0.63	5.6	0.63	0.63	3.5	0.24	0.59	6.6	0.59	0.59	3.5	
24	0.52	3.8	0.59	3.8	0.21	0.57	7.0	0.57	0.57	3.5	0.24	0.58	7.3	0.58	0.58	4.0	
27	0.39	4.0	0.52	4.0	0.16	0.48	7.0	0.48	0.48	4.1	0.17	0.51	7.7	0.51	0.51	4.0	
30	0.36	4.1	0.57	4.0	0.15	0.39	7.1	0.39	0.39	4.5	0.18	0.49	7.9	0.49	0.49	3.9	

1 Values are means of two replications.

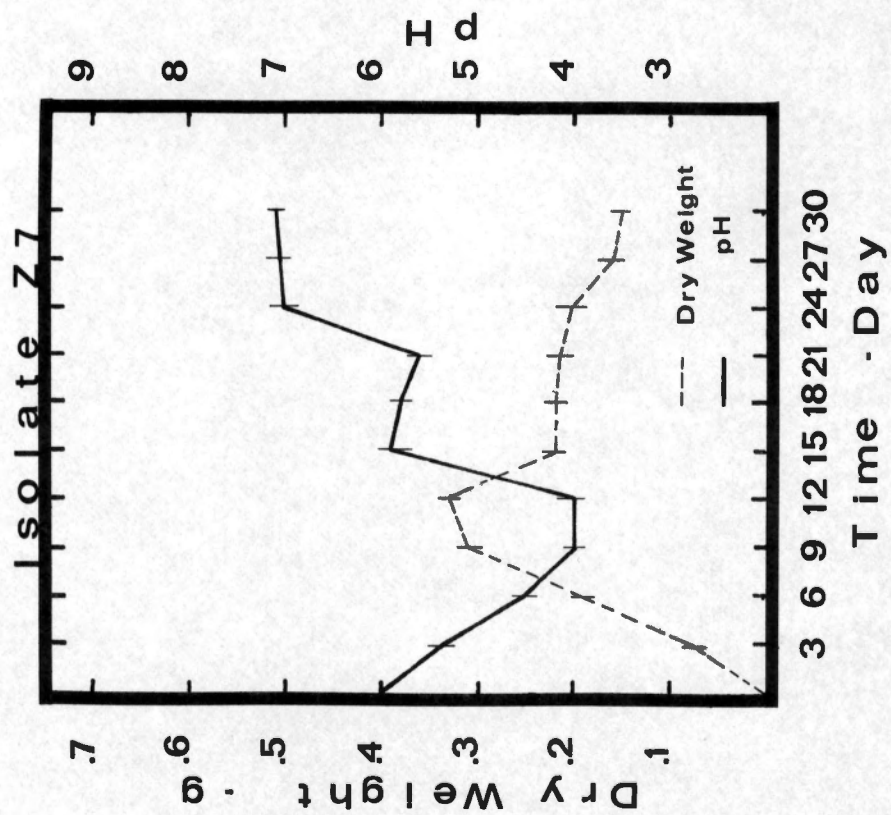


FIGURE 1. The change in mycelial dry weight and medium pH with time for isolate Z7.

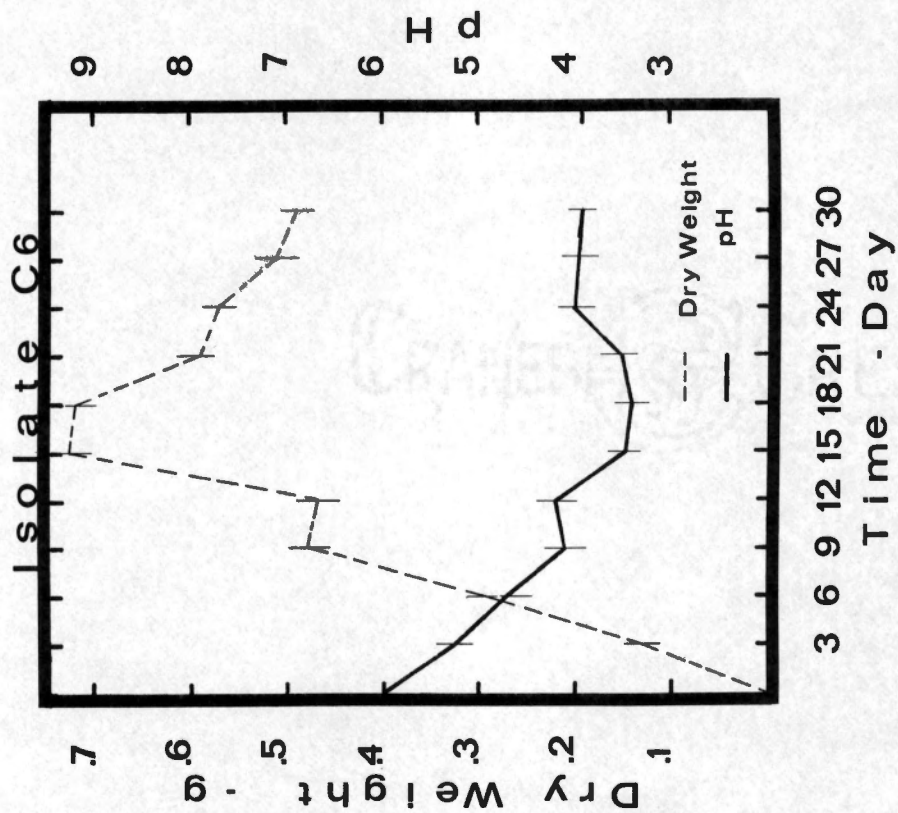


FIGURE 2. The change in mycelial dry weight and medium pH with time for isolate C6.

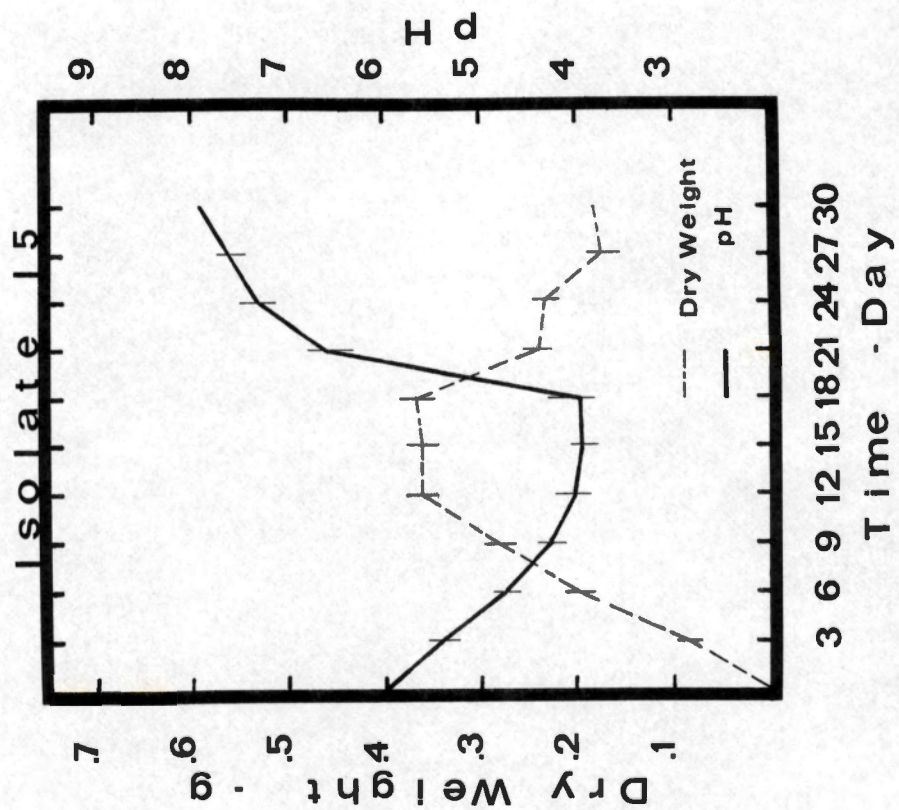


FIGURE 3. The change in mycelial dry weight and medium pH with time for isolate I5.

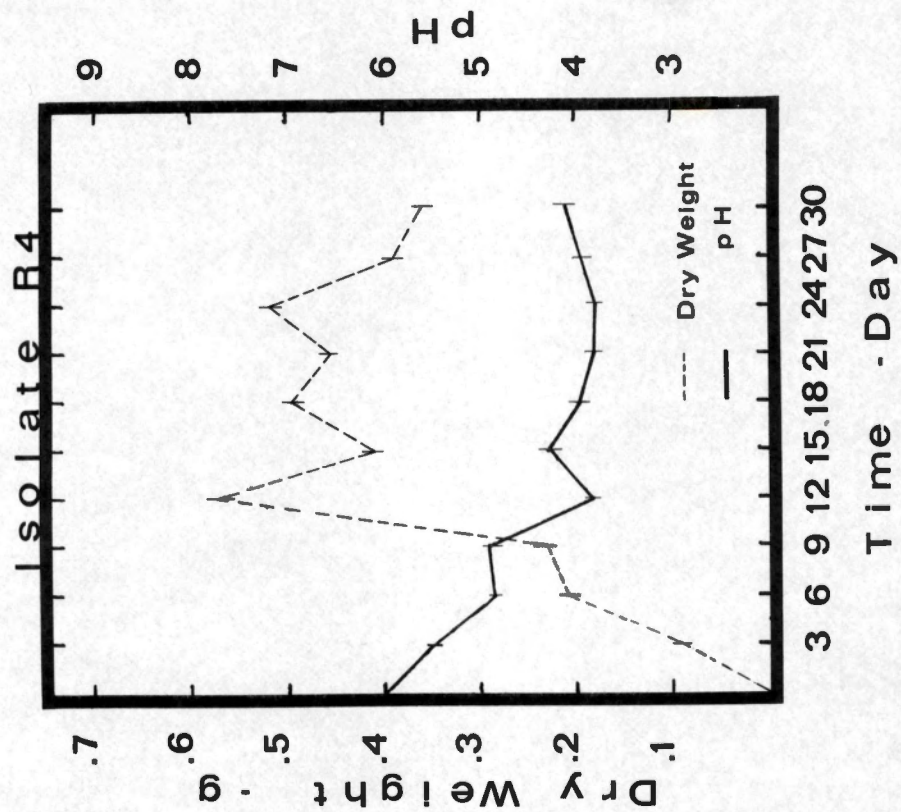


FIGURE 4. The change in mycelial dry weight and medium pH with time for isolate R4.

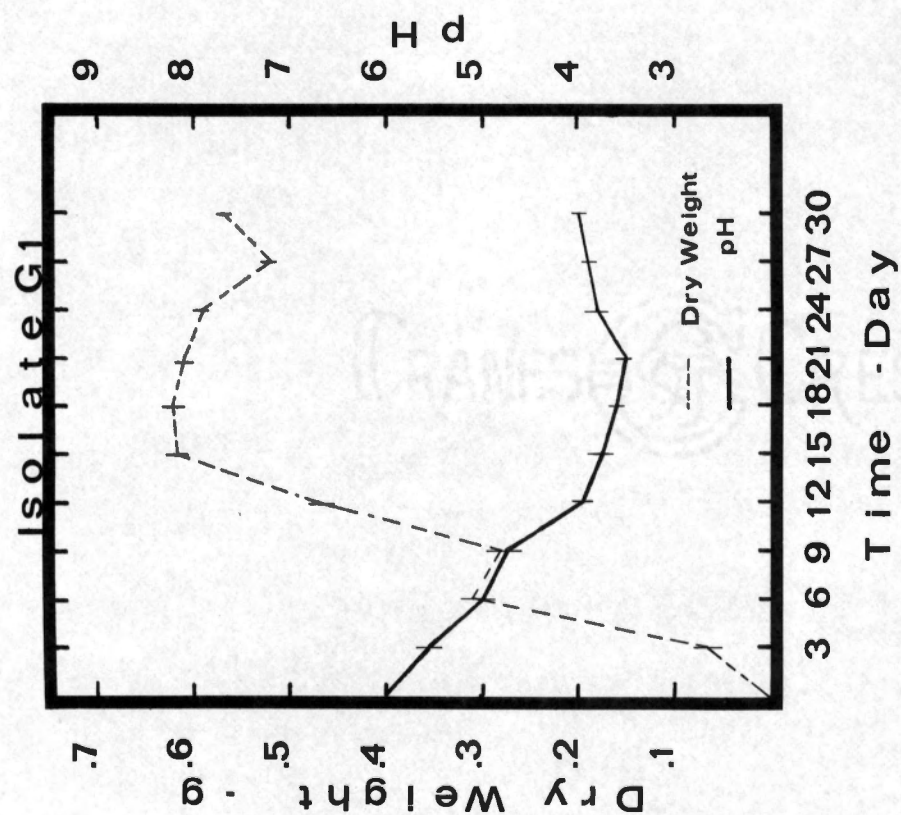


FIGURE 5. The change in mycelial dry weight and medium pH with time for isolate G1.

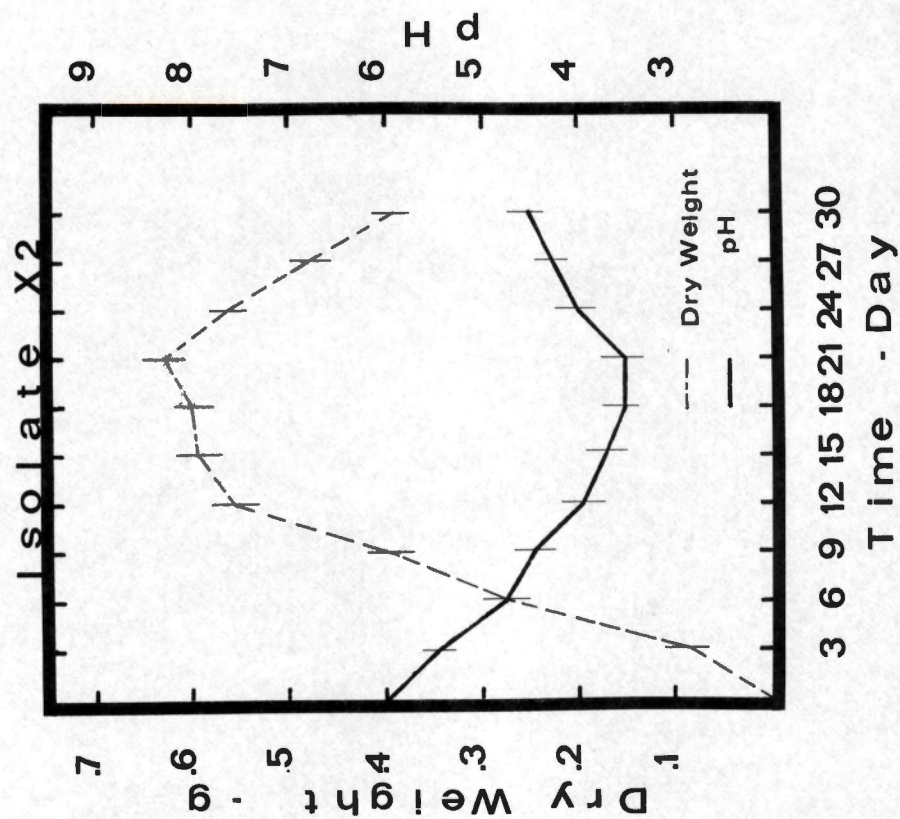


FIGURE 6. The change in mycelial dry weight and medium pH with time for isolate X2.

Since the pathotoxin tests using the crude filtrates were conducted prior to the determination of the optimum harvest date, all the flasks were harvested after 20 days. The percent inhibition of primary roots of corn seedlings with normal and six male-sterile cytoplasms by the pathotoxin of the six isolates is presented in Table 23. The pathotoxin of each isolate was diluted 1:100, 1:500, or 1:1000. The dilution end point (DEP) is that dilution that causes 50% or greater inhibition. The DEP values for each isolate with all cytoplasms are shown in Table 24. Isolates X2, R4, C6, and G1 were used because they were also used in the seedling pathogenicity work. Isolates I5 and Z7 were included because they were non-sporulating isolates during the cultural characteristics studies. Cytoplasm W had dilution end points for all isolates comparable to cytoplasm T, with the exception of isolate G1. Isolates I5 and Z7 inhibited cytoplasms W and T, respectively, at the 1:1000 dilution. Isolate C6 inhibited normal cytoplasm at a dilution of 1:100.

Table 25 presents Duncan's multiple range analysis among the cytoplasms for each isolate using the percent inhibition values obtained in the seedling bioassay. The percent inhibition of cytoplasm T was highest with isolates Z7, I5, and G1. The percent inhibition of cytoplasm T was not significantly different from the highest cytoplasm with isolates X2 and C6. Cytoplasm G had the highest percent inhibition by isolate R4, and cytoplasm T was significantly less than cytoplasm G. With all isolates except X2, I5, and C6, cytoplasm N was inhibited significantly less than the highest cytoplasm. For isolate X2 cytoplasm N was not significantly different from the cytoplasm with

TABLE 23. Percent inhibition of roots of corn seedlings with normal and six male-sterile cytoplasms by the pathotoxin of six single spore isolates of Helminthosporium maydis at three dilutions

Isolate	Dilution ²	Cytoplasm ¹						
		T	N	C	G	M	W	K
X2	1	64.6	10.0	46.2	5.6	57.9	72.8	21.5
	2	24.8	0.0	0.0	3.2	45.7	25.2	2.5
	3	8.4	0.0	0.0	0.0	16.4	0.0	0.0
R4	1	53.6	20.0	59.2	97.2	57.9	53.3	36.8
	2	45.2	5.2	0.0	18.8	45.7	29.8	23.2
	3	13.2	0.0	0.0	0.0	16.4	21.9	6.1
Z7	1	79.4	24.4	76.9	36.4	43.6	78.0	54.3
	2	74.4	6.0	22.3	23.2	17.1	55.6	47.2
	3	58.8	0.0	19.3	0.0	0.0	26.3	7.9
I5	1	86.8	44.4	43.9	16.8	0.0	80.7	11.1
	2	80.8	13.0	16.2	0.0	0.0	65.2	0.0
	3	49.6	6.4	15.4	0.0	0.0	56.3	0.0
C6	1	84.0	50.0	41.6	40.0	42.1	86.3	45.0
	2	50.4	32.4	0.0	12.8	0.0	61.9	15.0
	3	0.8	0.0	0.0	9.2	0.0	45.6	0.0
G1	1	94.4	28.8	57.0	32.0	40.0	46.0	51.0
	2	70.8	12.0	44.6	17.6	7.9	36.0	11.4
	3	16.8	4.0	44.6	0.0	0.0	28.2	0.0

¹Values are means of two replications.

²Dilution 1 = 1:100, 2 = 1:500, 3 = 1:1000.

TABLE 24. The dilution end points (DEP)¹ of the crude filtrates of six single spore isolates of Helminthosporium maydis determined in seedling bioassay of corn seedlings with normal and six male-sterile cytoplasms

Cytoplasm	Isolate					
	X2	R4	Z7	I5	C6	G1
T	1:100	1:100	1:500	1:500	1:500	1:500
N	N ²	N	N	N	1:100	N
C	N	1:100	1:100	N	N	N
G	N	1:100	N	N	N	N
M	1:100	1:100	N	N	N	N
W	1:100	1:100	1:500	1:1000	1:500	N
R	N	N	1:100	N	N	1:100

¹DEP refers to the dilution which had greater than 50% inhibition of growth of the primary root.

²N = less than 50% inhibition.

TABLE 25. Differences among the cytoplasms in percent inhibition of the primary roots of corn seedlings with normal and six male-sterile cytoplasms by the crude pathotoxin of six single spore isolates of Helminthosporium maydis at 1:100 dilution

Isolate	Cytoplasms ¹						
X2	W 72.3a ²	T 64.4a	M 57.9a	C 46.2a	K 26.5a	N 10.0b	G 5.6b
R4	G 97.2a	C 59.3ab	T 53.6b	W 53.3b	M 50.0b	K 37.8bc	N 20.0c
Z7	T 79.2a	C 76.9a	W 73.0a	K 54.3ab	M 43.6b	G 36.4b	N 24.4b
I5	T 86.8a	K 80.7ab	N 44.4bc	C 43.9cd	M 16.8cde	G 11.1de	W 0.0e
C6	W 86.3a	T 84.0a	N 50.0ab	K 45.0ab	M 42.2b	C 41.6b	G 40.0b
G1	T 94.4a	K 50.7b	W 46.0b	C 44.6b	M 40.0b	G 32.0b	N 28.8b

¹Values are means of two replications.

²Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test. The analysis of variance and Duncan's were performed on arcsin transformations of the percent data.

the lowest percent inhibition. The percent inhibition of cytoplasm N by isolate I5 was significantly different from the lowest cytoplasm. For isolate C6, the percent inhibition value for cytoplasm N was not significantly different from cytoplasm T (maximum value) or from cytoplasm G (minimum value). Unlike the seedling inoculation results, there was no definite pattern of cytoplasm reaction to the crude pathotoxin. Cytoplasms T, C, W, and K had significantly greater percent inhibition values for isolates X2 and Z7. These same cytoplasms had the highest values for isolate G1, although these differences were not significant. The reactions of the cytoplasms to the crude pathotoxin of isolates R4, I5, and C6 were variable. Duncan's analysis among the isolates on each cytoplasm is presented in Table 26. There were no significant differences among the isolates on cytoplasms T and C. There were significant differences among the isolates on the other cytoplasms.

The results of Duncan's multiple range analysis among the cytoplasms for each isolate used in the concentrated pathotoxin experiment are presented in Table 27. The data are extremely variable. The pattern of cytoplasm reaction was similar to the seedling inoculation and crude filtrate results in isolate G1. The cytoplasm reaction for the other three isolates ranged from no significant differences for isolate I6 to significantly less percent inhibition of cytoplasm T with isolate X2. Duncan's analysis among the isolates for each cytoplasm is presented in Table 28. There were no significant differences among the isolates for cytoplasms M, W, and K. The inhibition by the isolates was similar for cytoplasms T and C and N and G.

TABLE 26. Differences among the isolates in percent inhibition of primary roots of corn seedlings with normal and six-male sterile cytoplasms by the crude pathotoxin of six single spore isolates of Helminthosporium maydis at 1:100 dilution

Cytoplasm	Isolate ¹					
T	G1 94.4a ²	I5 86.8a	C6 84.0a	Z7 79.2a	X2 64.4a	R4 53.6a
N	C6 50.0a	I5 44.4a	G1 28.8a	Z7 24.4a	R4 20.0a	X2 10.0b
C	Z7 76.9a	R4 59.3a	X2 46.2a	G1 44.6a	I5 43.9a	C6 41.6a
G	R4 97.2a	C6 40.0b	Z7 36.4b	G1 32.0b	I5 11.1c	X2 5.6c
M	X2 57.9a	R4 50.0a	Z7 43.6a	C6 42.2a	G1 40.0a	I5 16.8b
W	C6 83.1a	Z7 73.0a	X2 72.3a	R4 53.3b	G1 46.0b	I5 0.0c
K	I5 80.7a	Z7 54.3a	G1 50.7a	C6 45.0b	R4 36.8b	X2 26.5b

¹Values are means of three replications.

²Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test. The analysis of variance and Duncan's were performed on arcsin transformations of the percent data.

TABLE 27. Differences among the cytoplasms in percent inhibition of the primary roots of corn seedlings with normal and six male-sterile cytoplasms by the concentrated pathotoxin of four single spore isolates of Helminthosporium maydis at 1:100 dilution

Isolate	Cytoplasm ¹						
X2	G 87.5a ²	W 49.0a	M 46.5a	N 45.5a	K 44.0a	C 36.0a	T 0.0b
G1	T 99.5a	K 47.5b	C 46.5b	W 25.5bc	G 18.5bc	N 15.0bc	M 0.0c
I5	T 100.0a	C 92.0a	N 61.5a	M 61.0a	K 60.0a	W 47.5a	G 45.5a
R4	T 92.0a	N 84.0ab	G 56.0ab	M 48.0b	K 47.0b	C 0.0c	W 0.0c

¹Values are means of two replications.

²Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test. The analysis of variance and Duncan's were performed on arcsin transformations of the percent data.

TABLE 28. Differences among the isolates in percent inhibition of the primary roots of corn seedlings with normal and six male-sterile cytoplasms by the concentrated pathotoxin of four single spore isolates of Helminthosporium maydis at 1:100 dilution

Cytoplasm	Isolate ¹			
	I5	G1	R4	X2
T	100.0a ²	99.5ab	92.0b	0.0c
N	R4 84.0a	I5 61.5a	X2 45.5a	G1 15.0b
C	I5 92.0a	G1 46.5b	X2 36.0b	R4 0.0c
G	X2 87.5a	R4 56.0a	I5 45.5a	G1 18.5b
M	I5 61.0a	R4 48.0a	X2 46.5a	G1 0.0a
W	X2 49.0a	I5 47.5a	G1 25.5a	R4 0.0a
K	I5 60.0a	G1 47.5a	R4 47.0a	X2 44.0a

¹Values are means of two replications.

²Means followed by the same letter are not significantly different at the 5% level of probability as determined by Duncan's multiple range test. The analysis of variance and Duncan's were performed on arcsin transformations of the percent data.

Comparisons of the crude and concentrated pathotoxin preparations are presented in Table 29. Data for the four isolates which were common to both experiments are presented for the normal and six male-sterile cytoplasms. Fifteen of the isolate-cytoplasm combinations had greater percent inhibition values with the concentrated toxin. This relationship would be expected. The remaining 13 combinations had greater percent inhibition with the crude pathotoxin. Comstock and Scheffer (6) have reported the accumulation of "staling products" by fungi grown in culture. Examples of staling products are various salts and organic acids. In cultural filtrates these materials can accumulate to toxic levels. Staling products may have been responsible for the percent inhibition values being greater with the crude filtrates than with the concentrated pathotoxin. The use of crude filtrates allows a more rapid bioassay and the use of a greater number of isolates due to the complicated procedures involved in concentrating the pathotoxin. Based on the above comparisons of crude and concentrated preparations bioassays using crude filtrates should be based on large numbers of seed and several replications to insure that the results will realistically represent the reaction of the host genotype or cytoplasm being studied.

Tables 30, 31, 32, and 33 present the relative pathogenicity of isolates X2, G1, R4, and I5, respectively, as determined by crude and concentrated pathotoxin and seedling inoculation. No values for seedling inoculation are presented for isolate I5 because it was one of the non-sporulating isolates. Isolates X2 and R4 were inconsistent in cytoplasm reaction among the three methods of measurement. The

TABLE 29. Percent inhibition of the primary roots of corn seedlings with normal and six male-sterile cytoplasms by the crude and concentrated pathotoxin of four single spore isolates of Helminthosporium maydis

Cytoplasm	Isolate ¹							
	X2		G1		I5		R4	
	Crude	Concentrated	Crude	Concentrated	Crude	Concentrated	Crude	Concentrated
T	64.4	0.0	94.4	99.5	86.8	100.0	53.6	92.0
N	10.0	45.5	28.8	15.0	44.4	61.5	20.0	84.0
C	46.2	36.0	44.6	46.5	43.9	92.0	59.3	0.0
G	5.6	87.5	32.0	18.5	11.1	45.5	0.2	56.0
M	57.9	46.5	40.0	0.0	16.8	61.0	50.0	48.0
W	72.3	49.0	46.0	25.5	0.0	47.5	53.3	0.0
K	26.5	44.0	50.7	47.5	80.7	60.0	37.8	47.0

¹Values are means of two replications.

TABLE 30. Relative pathogenicity of isolate X2 of *Helminthosporium maydis* to corn seedlings with normal and six male-sterile cytoplasms as determined by seedling bioassay of crude and concentrated pathotoxin and seedling inoculation

Pathotoxin (% Inhibition) ¹					Percent Diseased Area ¹
Cytoplasm	Crude	Cytoplasm	Concentrated	Cytoplasm	
W	72.3	G	87.5	T	49.75
T	64.4	W	49.0	C	48.25
M	57.9	M	46.5	W	28.25
C	46.2	N	45.5	G	2.75
K	26.5	K	44.0	M	2.25
N	10.0	C	36.0	K	1.75
G	5.6	T	0.0	N	1.25

¹Values are means of two replications.

TABLE 31. Relative pathogenicity of isolate G1 of *Helminthosporium maydis* to corn seedlings with normal and six male-sterile cytoplasms as determined by seedling bioassay of crude and concentrated pathotoxin and seedling inoculation

Pathotoxin (% Inhibition) ¹					Percent Diseased Area ¹
Cytoplasm	Crude	Cytoplasm	Concentrated	Cytoplasm	
T	94.4	T	99.5	T	48.0
K	50.7	K	47.5	W	47.5
W	46.0	C	46.5	C	6.75
C	44.6	W	25.5	G	5.25
M	40.0	G	18.5	N	3.50
G	32.0	N	15.0	M	2.50
N	28.8	M	0.0	K	1.50

¹Values are means of two replications.

TABLE 32. Relative pathogenicity of isolate R4 of *Helminthosporium maydis* to corn seedlings with normal and six male-sterile cytoplasms as determined by seedling bioassay of crude and concentrated pathotoxin and seedling inoculation

Pathotoxin (% Inhibition) ¹				
Cytoplasm	Crude	Cytoplasm	Concentrated	Percent Diseased Area ¹
G	97.2	T	92.0	49.75
C	59.3	N	84.0	46.50
T	53.6	G	56.0	22.25
W	53.3	M	48.0	19.75
M	50.0	K	47.0	6.00
K	37.8	C	0.0	1.00
N	20.0	W	0.0	0.75

¹Values are means of two replications.

TABLE 33. Relative pathogenicity of isolate I5 of Helminthosporium maydis to corn seedlings with normal and six male-sterile cytoplasms as determined by seedling bioassay of crude and concentrated pathotoxin

Pathotoxin (% Inhibition) ¹			
Cytoplasm	Crude	Cytoplasm	Concentrated
T	86.8	T	100.0
K	80.7	C	92.0
N	44.4	N	61.5
C	43.9	M	61.0
M	16.8	K	60.0
G	11.1	W	47.5
W	0.0	G	45.5

¹Values are means of two replications.

relative pathogenicity is generally the same for all three means of determination for isolate G1.

The lack of consistent relative pathogenicity patterns between the pathotoxin bioassay and seedling inoculation casts doubt on the reliability of pathotoxin seedling bioassays as a means of determining pathogenicity. Throughout the literature dealing with the pathotoxin of H. maydis Race T, various authors have cited the host-specificity of the pathotoxin and the pathogen as being the same. However, none of these studies quantified the seedling reactions in order that correlation coefficients for pathotoxin and seedling reaction could be determined. All previous studies have been based on resistance or susceptibility of the genotype. In this study Pearson product moment correlations were determined for the variables: crude and concentrated pathotoxin and crude and concentrated pathotoxin and seedling reaction. All correlation coefficients were positive but not significant.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Significant variability in linear growth and sporulation existed for the H. maydis isolates tested. There was a significant, negative correlation between linear growth and sporulation. Lactose casein hydrolysate medium was further substantiated as a good medium for inducing sporulation in Helminthosporium maydis.

The variation among the isolates in optimum harvest date for pathotoxin production reiterates the need for this type of determination previous to any studies dealing with the pathotoxin produced by H. maydis. The isolates used in the seedling inoculation studies were all H. maydis Race T. Based on seedling reaction under the conditions used in this study, male-sterile cytoplasms C and W were as susceptible as T cytoplasm to the isolates tested. Cytoplasms G, K, and M were as resistant as N cytoplasm to the same isolates. These results indicate that the H. maydis Race T population contains biotypes that are more virulent to particular male-sterile cytoplasms than to others. These differences may have been caused by genotype differences due to the lack of complete homozygosity in the inbred used. To confirm the reaction of cytoplasms C and W studies need to be made in which other inbreds and hybrids with these cytoplasms are subjected to these same isolates under both greenhouse and field conditions.

There were significant differences in cytoplasm reaction to both the crude and concentrated pathotoxin; however, the reactions were too

variable for meaningful patterns. These results support two conclusions. First, at the present time, seedling inoculation and field reaction is the best method for determining the susceptibility or resistance of male-sterile cytoplasms to H. maydis. Second, pathotoxin studies should be done with highly purified pathotoxin preparations and should be based on several replications using large quantities of seed. Also, the reaction of the cytoplasms to the pathotoxin should be studied using the same cytoplasms in several genotypes. Further studies need to be done in developing more accurate and efficient methods for purifying the pathotoxin. Once these have been developed, studies need to be made in which the highly purified pathotoxin is bioassayed and the results correlated with quantified seedling reaction of the same lines used in the bioassay. The results of such a study would give evidence as to the reliability of the pathotoxin as an indicator of pathogenicity.

The variability in cultural characteristics and pathogenicity observed in this study reiterate the need for cytoplasmic diversity in the nation's corn crop and continued observance of the variability in the H. maydis population.

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