GENETIC CONTROL OF THE DEVELOPMENT OF HAUSTORIA OF ERYSIPHE GRAMINIS F. SP. TRITICI ON WHEAT

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This is to certify that the

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GENETIC CONTROL OF THE DEVELOPMENT OF
HAUSTORIA OF ERYSIPHE GRAMINIS F. SP.
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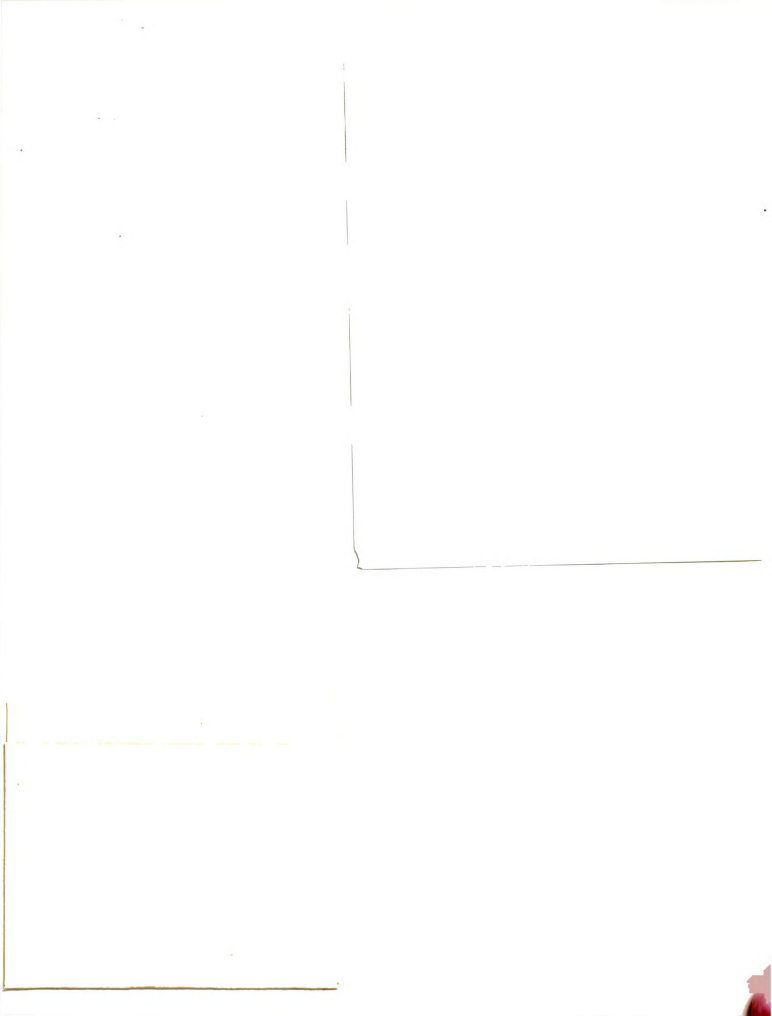

ABSTRACT

GENETIC CONTROL OF THE DEVELOPMENT OF HAUSTORIA OF ERYSIPHE GRAMINIS F. SP. TRITICI ON WHEAT

By

Mary Joy Haywood

Genes conferring resistance in wheat to Erysiphe graminis f. sp. tritici have been shown to affect the ontogeny of interactions between host and parasite. Direct microscopic observations made every 2 hr from 8 through 30 hr after inoculation, following fixation and staining of materials, with aniline blue, showed that, with the compatible Px/pmx genotype, 87% of the parasite units form primary haustoria by 30 hr after inoculation. With the incompatible genotypes, P1a/Pm1a, P2a/Pm2a, P3a/Pm3a, and P4a/Pm4a, the percentages of the parasite units that formed primary haustoria by 30 hr after inoculation were 15, 66, 18, and 3, respectively. The incompatible interaction P2a/Pm2a apparently does have an affect earlier than previously believed. The earlier work was based on the infection efficiency and development of elongating secondary hyphae (ESH). With the incompatible genotype, P3a/Pm3a, by 30 hr after inoculation, only 15% of the parasite units had haustoria greater than 35μ in length. A comparison of these results with the infection efficiency of 30% obtained by direct measurements of the elongating secondary hyphae, the percent of the

parasite units that produced ESH was smaller and had to include haustoria less than 35μ in length, thus, smaller haustoria are supporting the development of ESH in this genotype. The percent of the parasite units that produced ESH with the incompatible interactions, P1a/Pm1a and P4a/Pm4a are in close agreement with previous findings. The data also showed that haustorial development in the apparently incompatible interactions is not a clear-cut phenomenon. When some infected cells were penetrated, development of the parasite unit ceased rapidly. In other infected cells, the parasite unit appeared to develop normally for a few hours and development stopped, leaving a rudimentary haustorium. In these latter reactions, the host cells picked up the dye indicating mesophyll collapse.

Light is necessary at specific times during primary and secondary interactions for synchronous development of Erysiphe graminis f. sp. tritici on wheat. Synchronous development of E. graminis during secondary infection was increased when light periods (1.3 ergs cm^{-2} sec-1, incandescent and fluorescent) were altered between 26 and 58 hr after inoculation. With a light period from 20-36 hr and a dark period from 36-44 hr followed by another light period from 44-58 hr after inoculation, approximately 87% of the E. graminis conidia applied to wheat that produced primary haustoria also produced secondary haustoria.

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Establishing the optimal environmental conditions necessary for secondary haustorial development of Px/pmx provided a standard for determining the rate of development of the incompatible interactions from 38-58 hr after inoculation. By 58 hr after inoculation, 52% of the compatible Px/pmx interaction had formed secondary haustoria up to 35μ in length. The percent of secondary haustoria formed with the incompatible parasite/host genotypes, P1a/Pm1a, P2a/Pm2a, P3a/Pm3a, and P4a/Pm4a were 6, 28, 5, and 1, respectively. Greater than 75% of the host cells in each of the incompatible interactions had picked up the dye, indicating mesophyll collapse and necrogenic protoplasts. These results provide additional evidence that the different genotypes affect different stages in the ontogeny of the host/parasite interactions and that some of these interactions affect development earlier than previously reported.

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By

Mary Joy Haywood

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Dedicated

to

My Mother and Father

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INTRODUCTION

Plants that live in groups, or communities, compete with one another at all stages of development. As a result of this competition, certain plants, the more vigorous and better adapted ones, survive. Others, less vigorous, are eventually suppressed or eliminated. The host-parasite interactions of Erysiphe graminis f. sp. tritici on Triticum aestivum can be recognized as competition between two organisms. The obligately parasitic fungus, Erysiphe graminis f. sp. tritici em. Marchal, causes powdery mildew of cereals and is responsible for a significant reduction in grain yield in certain areas of the world (21, 50).

The most practical means of controlling this disease is the development and use of resistant cultivars. This has been accomplished by selection for mildew resistance, which has produced many host cultivars with varying degrees of resistance to powdery mildew. The inheritance of resistance to E. graminis has been described (2, 3), and near-isogenic wheat lines which differ by only single genes for resistance have been developed (4). Many isolates of the fungus that differ with respect to virulence are also obtainable, though highly isogenic strains are not yet available (4).

Primary infection of wheat by Erysiphe graminis f. sp. tritici has been divided into distinct morphological stages (26, 34, 36). Given the appropriate environmental conditions, the parasite on a susceptible host undergoes each stage of development in a highly synchronous manner, i.e., one-six hours, germination; six-12 hours, appresorial initials and mature appresoria; 12-20 hours, formation of haustorial bodies and secondary hyphal initials; 20-26 hours, formation of elongating secondary hyphae and development of haustorial appendages. Under the same environment, incompatible interactions indicate that the incompatibility of some interactions is determined as early as 20 hr after inoculation (47). The demonstration that incompatible genotypes affect primary infection stresses the importance of directing any physiological or biochemical studies toward the very earliest interactions between a host and its parasite.

The objectives of this research were to study some of the events in the transition from two independent organisms to two organisms that have or have not established compatible relationships during primary infection. This was done by: (1) determining the development of the primary haustorium as a function of time to determine the earliest time incompatibility is expressed; (2) establishing the environmental conditions necessary for synchronous development of secondary haustoria with compatible interactions, and (3) determining the formation of secondary haustoria as a function of time with the incompatible interactions.

MATERIALS AND METHODS

Inoculum Production

The culture, Michigan strain (MS-1) of Erysiphe graminis f. sp. tritici was used in all experiments. The MS-1 strain was collected in Michigan and maintained on Triticum aestivum L. 'Little Club' wheat in growth chambers. Little Club has no known genes for resistance to powdery mildew. The strain was checked periodically for purity by scoring infection types on a set of differential host lines (Table 1) (25, 46).

Wheat seedlings grown in the greenhouse for six days were dusted with conidia from plants inoculated seven days earlier. The stock culture was maintained under the following environmental conditions:

1. Light -- 700 to 800 ft-c (650 to 750 ft-c from white VHO fluorescent tubes and 50 ft-c from 25 watt incandescent bulbs), sixteen hour photoperiod/day.
2. Temperature -- 18 ± 1C during the light period and 17 ± 1C during the dark period.
3. Relative humidity -- $80 \pm 5\%$ during the light period and $95 \pm 5\%$ during the dark period.
4. Continuous air circulation.

Table 1. Near-isogenic lines with single Pm genes and infection types produced seven days after inoculation with MS-1

Near-isogenic line	Designation of Gene Involved	Former Gene Symbol	C. I. (1)	Infection Type (2)
Axminister x Cc^8 (3)	Pm1a (Axminister)	M1 $\frac{t}{}$	14114	0
Ulka x Cc^8	Pm2a (Ulka)	M1 $\frac{u}{}$	14118	2
Asosan x Cc^8	Pm3a	M1 $\frac{a}{}$	14120	3
Khapli x Cc^8	Pm4a (Khapli)	...	14123	0
Chancellor (Cc)	pm x	...	12333	4

(1) Cereal Investigation accession number.

(2) Infection type:

0-no observable mildew development
1-chlorotic flecking, no pustules
2-chlorosis, necrotic reaction
3-significant reduction in mildew development
4-abundant mildew development

(3) Cc^8 refers to the 8 backcrosses to the cultivar Chancellor.

Conidia produced on the sixth day after inoculation were used in all experiments.

All the wheat lines used in experiments were planted in three inch pots and maintained in the greenhouse for five days prior to inoculation.

Method of Inoculation

The 'rolling method' of inoculation (35) was used for all studies of morphological development of powdery mildew during primary and secondary infections. Healthy conidia, dusted onto clean slides, were transferred to the abaxial or adaxial side of leaves (26). This method provided a uniform distribution of single conidia, with approximately one hundred conidia per centimeter of length of leaf.

Environmental Conditions for Experiments

All experiments were carried out in Sherer-Gillett (Model CEL 512-37 and Model CEL 25-7) growth chambers. The following environmental conditions were used to obtain a high infection efficiency and synchronous growth of the parasite population during primary infection (0-30 hours) (12, 47):

1. Zero to one hour, inoculated plants were maintained in darkness at 18 $\pm$ 1 C and approximately 100% relative humidity (RH).

2. One to six hours after inoculation, plants were kept under 1.0×10^5 ergs $cm^{-2}sec^{-1}$ radiation (0.6×10^5 ergs $cm^{-2}sec^{-1}$ from white VHO fluorescent tubes and 0.4×10^5 ergs $cm^{-2}sec^{-1}$ from 25 watt incandescent bulbs) at 22 ± 1 C and $65 \pm 5\%$ RH.
3. Six to 20 hours after inoculation, plants were kept at the same temperature and RH as in the above, but with no light.
4. Twenty to 30 hours after inoculation, conditions were the same as in 2.

The above environmental conditions were used for all experiments unless otherwise stated.

Light intensity was measured with a YSI Kettering Model 65 Radiometer.

Temperature and relative humidity were monitored during experiments with wet and dry bulb thermometers and a recording hygrothermograph calibrated with a sling psychrometer.

The experimental environmental conditions established for synchronous development of secondary infection (38-58 hours after inoculation) are given in Chapter 2.

Gene Symbols and Designation of Parasite/Host Genotypes

Briggle's suggested terminology (2, 3) was used to designate the Pm genes used to designate genes conditioning reaction

to E. graminis f. sp. tritici. The symbols Pm1, Pm2, etc. have been used to designate genes at distinct loci. Genes considered to be either closely linked or allelic are followed by the letters a, b, c, ... Alternate alleles for each Pm gene will be referred to by their respective recessive designations, pm1, pm2, ... As the genotypes of the host lines are all homozygous for the designated gene, PmxPmx, or pmxpmx, they will be abbreviated and written as if haploid, Pmx, or pmx.

Parasite/host combinations are referred to by their respective genotypes which specify compatibility or incompatibility. Px/pmx is intended to imply compatibility specified by each gene pair and P1a/Pm1a is intended to imply that P1a/Pm1a specifies incompatibility but all other gene pairs in that host and parasite specify compatibility.

LITERATURE REVIEW

Approaches to research on the problem of obligate parasitism have been varied. These range from studies of gross chemical differences between host varieties resistant or susceptible to a given strain of pathogen to studies of the host-parasite interface by electron microscopy. Each approach has certain advantages, but none seem to be completely free from either conceptual or technical problems. It has become increasingly obvious in the review of research literature that there is no direct road to an understanding of infections caused by obligate parasites. Extensive studies of the disease have been aimed at understanding the genetics of disease development and elucidating its physiological and biochemical aspects. I will attempt to summarize some of the important developments that relate to the results reported in this study.

The powdery mildew fungus, Erysiphe graminis f. sp. tritici, is an obligate parasite. Erysiphe graminis is characterized morphologically by the ectoparasitic conidium, appresorium with an infection peg, and extensive mycelial growth on the surface of the host. In a susceptible host, the infection peg forms a haustorium inside the penetrated cell. The infection spreads via

formation of secondary hyphae from the original germ tube and formation of additional infection pegs and haustoria. Only the host epidermal tissue is infected with haustoria. In spite of this localization, the disease is conservatively estimated to produce a 1-2% reduction in yield per year (21, 22).

Powdery mildew disease development is reportedly affected by environmental conditions. Germination and subsequent development of conidia occur over a wide range of temperature and relative humidity. Several different optimal conditions for mildew development and the effects of various environmental factors on disease development have been reported (46) and need not be reiterated.

The primary infection process of E. graminis on the plant surface is conveniently divided into distinct morphological stages: 1) germination, 2) production of 'club-shaped' appresorial initials, 3) formation of mature appresoria, 4) penetration of the cuticle and epidermal cells, 5) formation of haustoria, 6) development of elongating secondary hyphae (ESH), 7) initiation of secondary infections, and 8) sporulation. Each stage of the infection process differs in its requirements for temperature, relative humidity, and light (24, 27, 28, 36, 43). Under optimal conditions, over 80% of the parasite population move through the stages of primary infection with a high degree of synchrony (27, 28, 34, 36). The production of elongating secondary hyphae

(ESH) has been used as the criterion for the establishment of a functional relationship in primary infection between host and parasite (27, 28). For each ESH that formed on the host surface, a haustorium was produced in the epidermal cell (27, 28). The production of ESH by the parasite indicates that a functional relationship had been formed and that transfer of nutrients and other essential materials from host to parasite occurs. The percentage of conidia applied that produced ESH is defined as infection efficiency (10, 11, 12).

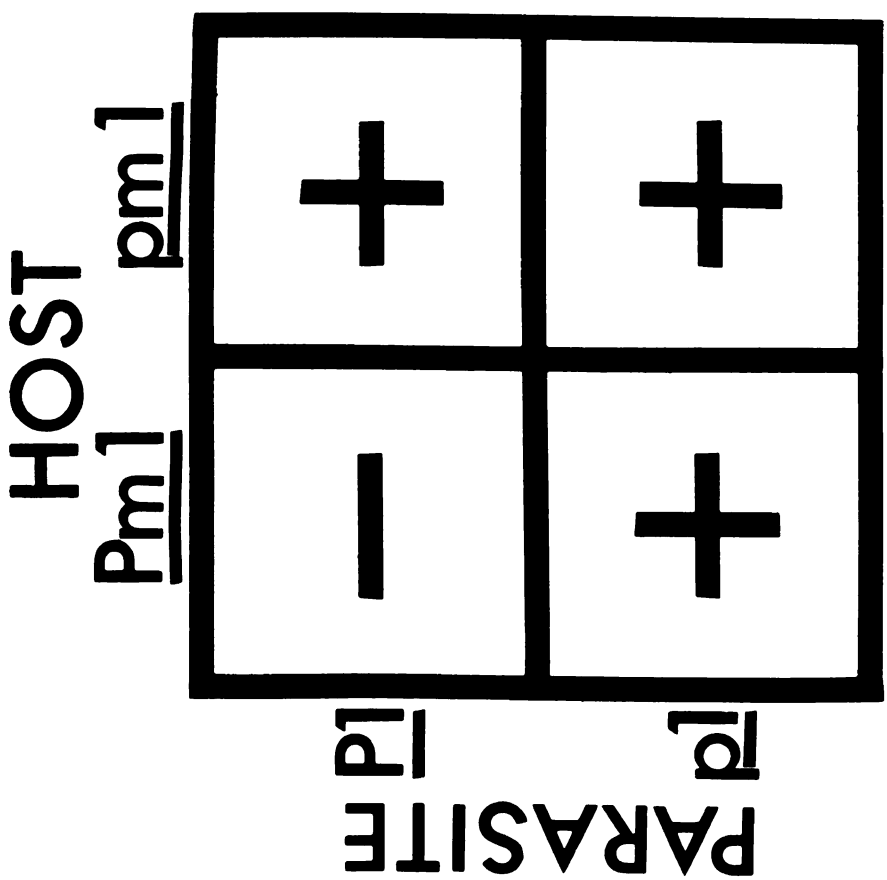
The formation of a functional haustorium is the crucial step in the development of a compatible host-parasite relationship, but it is very important to know the earliest stages of interaction between host and parasite and to determine if there is a sequence of events which is critical to the establishment of a compatible or incompatible relationship.

A number of physiological changes are known to occur following inoculation of wheat with E. graminis. Respiration (1), photosynthesis (44), and translocation patterns of organic molecules (9, 40, 53) have been reported to change two to 10 days after inoculation. Previous studies (47) have shown that the establishment of compatibility or incompatibility often occurs before 24 hr after inoculation. Conidia placed on non-host plants germinated, formed appresoria, and attempted to penetrate epidermal cells, but they did not form either haustoria or ESH

(26, 51). Corner (8) observed that the development of powdery mildew is arrested before ESH or haustoria are formed in the resistant hosts. These interactions between the incompatible parasite and host may lead to the death of the infected cell and adjacent cells and act as a barrier to additional infections by the parasite units (6, 8).

It is clear from many investigations (15, 16, 33, 38) that the interactions between host and parasite are controlled by complementary genes possessed by both host and parasite. Flor (13, 14) found that the ability of Melamspora lini to grow and produce disease symptoms on genetically different flax lines was determined by specific corresponding genes in the pathogen. The finding of one gene in the pathogen for each gene in the host led to the development of the gene-for-gene hypothesis which states that for each R gene in the host that conditions resistance there is a corresponding P gene in the parasite that conditions avirulence. The P gene interacts with the R gene to determine incompatibility, i.e., low infection type. Incompatibility results only when a P gene in the parasite interacts with a specific R gene in the host, e.g., P1/R1. Compatibility is specified with the other possible parasite/host genotypes, P1/r1, p1/R1, and p1/r1. With two alleles at one locus in a parasite, P or p and two alleles at one locus in the host, R or r, there are four possible interactions (Figure 1).

Figure 1. The four possible parasite/host genotypes involving a single gene pair governing compatibility of host and parasite. Pm1 and pm1 are alternate alleles in the host. P1 and p1 are alternate alleles in the pathogen. P1/Pm1 specifies incompatibility (-) while P1/pm1, p1/Pm1, and p1/pm1 specify compatibility (+).



A

Figure 1

The basic scheme (41) was proposed to be used as a biological test to study physiological and biochemical effects of disease development. This test is useful in studying disease development, especially with powdery mildew which follows the gene-for-gene relationship (32, 37, 39).

CHAPTER I

GENETIC CONTROL OF PRIMARY HAUSTORIAL DEVELOPMENT OF ERYSIPHE GRAMINIS ON WHEAT

Introduction

A high percentage of spores of Erysiphe graminis (DC.) Merat f. sp. tritici em. Marchal placed on a leaf of wheat will germinate, produce appresoria, haustoria, and elongating secondary hyphae (ESH) if given the appropriate environmental conditions, and if the parasite/host genotype specifies compatibility (27, 28, 47).

Genes conferring resistance in wheat to E. graminis are expressed at different stages in the ontogeny of interactions between host and parasite. The effects of these genes have been determined by measuring the production of ESH during primary infection.

The production of ESH greater than 10μ has been used as the criterion of development of a functional relationship between wheat and E. graminis. It has been shown that for each ESH greater than 10μ, a normal haustorium was always present (48).

However, no direct studies had been done on the development of haustoria during primary infection in either the compatible or incompatible interactions.

The objectives of this research were: 1) to determine the rate of development of primary haustoria of E. graminis on 5 near-isogenic lines of wheat containing Pm genes, and 2) to correlate these results with the development of elongating secondary hyphae.

Materials and Methods

The development of haustorial bodies was measured by direct microscopic observations. Measurements were made every two hours from eight through 30 hr after inoculation. Environmental conditions were used which permitted synchronous development of the ectoparasitic portion of the parasite (27, 28, 29). Conidia were applied via 'rolling technique' (35). Every two hours after inoculation, beginning at hour 8, strips of the abaxial epidermis were taken and fixed in Carnoy's solution for five minutes. Extractable chlorophyll was removed by two or three rinses of 100% methyl alcohol. The epidermal strips were placed in 0.06% aniline blue, specific for fungal material and protoplasts (7), and incubated at 35 C for one hour to intensify the dye in the fungal tissue. The tissue was transferred to 85% lactic acid for an additional hour, then allowed to destain in

fresh 85% lactic acid at room temperature for one or two days. The dark blue-stained haustoria were easily observable with bright field microscopy (see Figure 2 and interpretive drawing, Figure 3).

The microscopic determinations and observations were done with Bausch & Lomb and Zeiss phase contrast microscopes. The photomicrographs of the complete parasite unit was taken with a Zeiss Photomicroscope II.

Spatial relationships, higher resolution, and a greater depth of focus, were accomplished by use of the scanning electron microscope (SEM), Model AMR 900 (Figure 4).

Since wheat leaves possess a cuticle layer which is almost impermeable to most liquids, the following procedure gave good results for SEM: 1 cm length inoculated leaf sections were fixed in 3:3 Glutaraldehyde (25%)/Acrylic Aldehyde (100%) solution for 1 hr under vacuum, followed by a phosphate buffer rinse. The tissue was then fixed in 2% OsO_4 for 1 hr and dehydrated via the iso-amyl acetate technique (18, 30, 31). A Model Bomar SPC-900/Ex critical point drying instrument was then used to remove any excess water from the tissue. The tissue was then mounted, coated and examined with the SEM.

The data are presented as percent of total number of conidia applied to the leaf that have produced haustoria of various lengths at different times during primary infection. The

Figure 2. Photomicrograph of a primary haustorium, with a compatible genotype, Px/pmx, in wheat epidermal cell at 26 hr after inoculation.

(A) Conidium
(B) Appresorium
(C) Point of penetration
(D) Haustorial body with appendages

Figure 3. Drawing of a primary haustorium with a compatible genotype, Px/pmx, at 26 hr after inoculation depicting infection structures and extensive hyphal growth. The haustorial body has the finger-like projections.

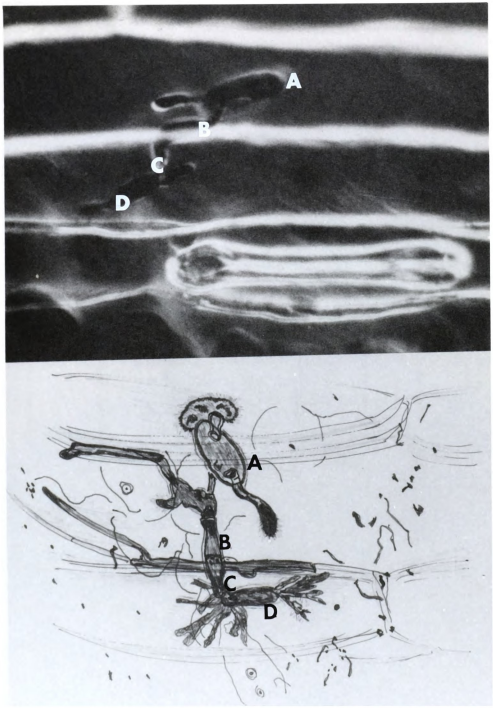


Figure 2 and Figure 3

Figure 4. The ectoparasitic structures of Erysiphe graminis on wheat observed with a Scanning Electron Microscope.

(A) Conidium

(B) Appresorium with characteristic 'beak-like' appearance

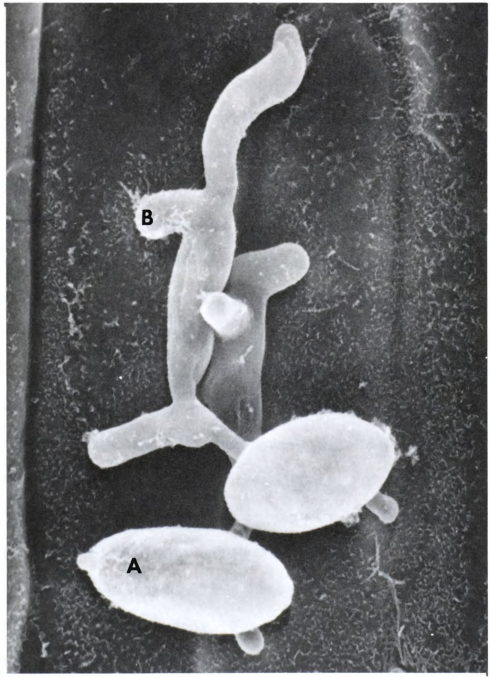


Figure 4

experiments were repeated on four different days. When near-isogenic lines were compared, all lines were tested on the same day.

Results

It is imperative that a common base be established to allow for meaningful interpretation of results in relation to the work done by previous experimenters (27, 28, 34, 42). Optimum environmental conditions for germination, formation of appresorial initials, formation of mature appresoria, and production of secondary hyphae were used (25). Figure 5 shows that the development of the parasite population was in close agreement with previous findings (25).

The rate of haustorial development was determined for five different parasite/host genotypes with standard environmental conditions (Table 2). At eight hours (Figure 6-A) after inoculation with the compatible genotype, Px/pmx, (Chancellor), all of the parasite units applied had attempted penetration and/or formed haustoria 5μ or less in length. By 16 hr, 21% had formed haustoria 5μ in length, 56% had haustoria 5-15μ long, and 25% had haustoria 15-25μ long. Formation of appendages on the haustorial bodies was evident at 18 hr after inoculation in all the compatible and incompatible interactions. By 30 hr after inoculation with the compatible genotype, the percentage of parasite units

Figure 5. Development of Erysiphe graminis f. sp. tritici during primary infection of wheat leaves

(A) Germination
(B) Formation of appresorial initials
(C) Formation of mature appresoria
(D) Formation of secondary hyphal initials
(E) Formation of elongating secondary hyphae (ESH)> 10μ in length

(----------) Martin (25)

(_____) results obtained in this study

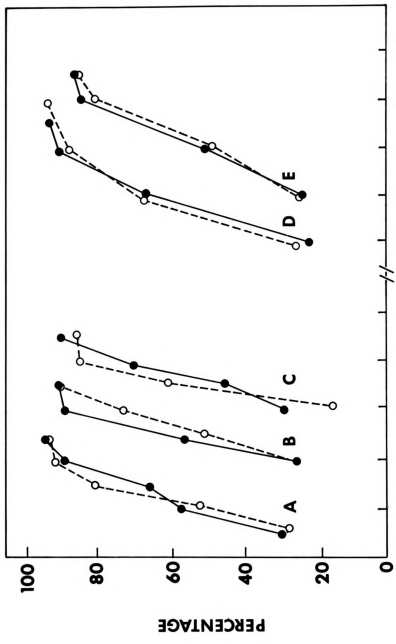

PERCENTAGE
100
80
60
40
20
0
0
2
4
6
8
10
20
22
24
26
28
TIME AFTER INOCULATION (hr)
A
B
C
D
E

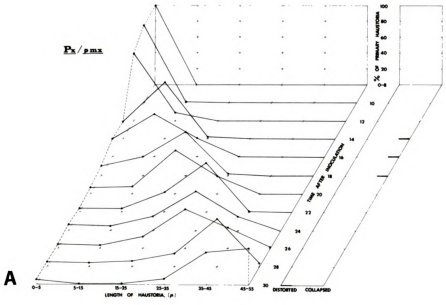
Figure 5

Table 2. Effect of genotype of host and pathogen on development of haustoria through primary infection

Genotypes (parasite/host)	Hours after inoculation	% of parasite units with haustoria of a given length (μ) 0-5	5-15	15-25	25-35	35-45	45-55	Distorted	Collapsed	Total
Px/pmx										
	8	100								304
	10	99	1							267
	12	90	10							388
	14	22	73	3					2	256
	16	21	56	25					2	221
	18	13	25	57	4				1	228
	20	8	9	57	27					239
	22	4	5	27	63	2				232
	24	7	5	19	50	20				235
	26	5	3	14	50	26	2			189
	28	5	4	8	18	59	6			185
	30		1	1	8	43	44			209
P1a/Pm1a										
	8	100								299
	10	100								298
	12	91	8						2	314
	14	69	21	2				5	3	221
	16	38	25	33				2	2	231
	18	50	8	29	4			6	3	219
	20	29	7	28	24			7	5	249
	22	25	10	24	11	2		24	5	236
	24	46	11	14	8	4		19	2	250
	26	34	3	6	11	4		42		252
	28	41	4	6	12	8	1	33		217
	30	33	3	8	19	8	7	26		265
P2a/Pm2a										
	8	100								322
	10	98							1	313
	12	92	8						1	411
	14	53	35	7				1	4	230
	16	35	18	47					2	260
	18	18	9	63	8			2	1	234
	20	17	6	34	44					222
	22	23	4	16	51	5				241
	24	16	4	14	32	31		2	2	239
	26	24	2	3	41	33	2			239
	28	21	2	5	9	42	18	2	1	221
	30	19		3	7	35	31	4		198
P3a/Pm3a										
	8	100								277
	10	98	1							277
	12	91	9							317
	14	23	69	4				3	2	223
	16	47	23	30						261
	18	26	15	50	7				1	216
	20	36	5	34	22				3	221
	22	27	4	30	22			13	4	284
	24	23	1	12	37	9		16	1	268
	26	23	3	10	19	6		32		244
	28	34	2	4	15	8	2	34	2	200
	30	35	2	6	12	11	7	29		251
P4a/Pm4a										
	8	100								346
	10	98	2							224
	12	90	10							372
	14	52	38	8	1				2	233
	16	58	20	21					2	205
	18	51	7	30	8			4	2	271
	20	70	4	9	5			6	5	230
	22	44	6	20	10			11	8	291
	24	46	2	11	15	4		14	9	295
	26	64	3	4	3	1		16	8	231
	28	49	2	4	3	3	1	38		198
	30	60	2	4	3	2	1	18	10	199

Figure 6. Effect of five different parasite/host genotypes on the development of primary haustoria of *Erysiphe graminis* f. sp. *tritici* on wheat

(A) Genotype Px/pmx
(B) Genotype P1a/Pm1a
(C) Genotype P2a/Pm2a
(D) Genotype P3a/Pm3a
(E) Genotype P4a/Pm4a


A
Px / pmx
% OF TERMINAL HAUSTORIA
0-5
10
12
14
16
18
20
22
24
26
28
30
HOST CELL INFECTION
0-3
5-15
15-25
25-35
35-45
45-55
LENGTH OF HAUSTORIA, µ
DISTORTED
COLLAPSED

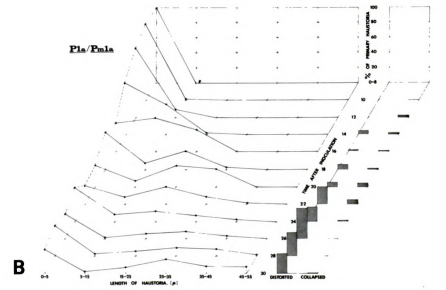

B
Pla/Pmla
% OF TERMINAL HAUSTORIA
0-5
10
12
14
16
18
20
22
24
26
28
30
HOST CELL INFECTION
0-5
5-15
15-25
25-35
35-45
45-55
LENGTH OF HAUSTORIA, µ
DISTORTED
COLLAPSED

Figure 6

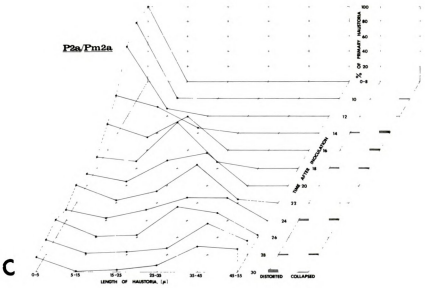


Figure 6 (cont'd.)

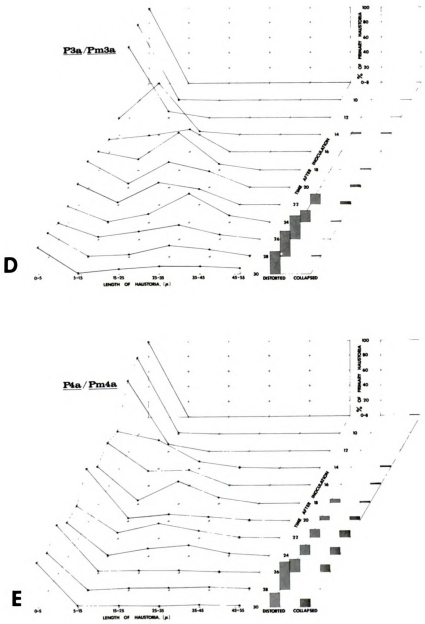


Figure 6 (cont'd.)

with haustoria of a given length was: 5% 0-5μ, 2% 5-25μ, 8% 25-35μ, 43% 35-45μ, and 44% 45-55μ.

There was not a significant number of collapsed ectoparasitic structures by 30 hr after inoculation and no evidence of distorted haustoria within the host cells. The mesophyll cells showed no sign of necrosis and none of the host cells had picked up the dye. Progressive development of the parasite units is obvious by the extension of the peaks of increased haustorial development up to 30 hr after inoculation.

With the incompatible genotype, P1a/Pm1a, (Figure 6-B), the percentage of parasite units with haustoria of a given length was: 33% 0-5μ, 3% 5-15μ, 8% 15-25μ, 19% 25-35μ, 8% 35-45μ, and 7% 45-55μ, by 30 hr after inoculation. An additional 26% of the parasite units were distorted (Figure 7). Distortion was evident in the observation that haustoria tended to "ball up" (Figure 8). The highest proportion of distorted haustoria was at 26 hr after inoculation. Cells with distorted haustoria picked up the dye more quickly than the other cells. By 30 hr after inoculation, approximately 95% of the infected cells were readily infiltrated with the dye. Collapse of the ectoparasitic portion of the parasite was greatest at approximately 20-22 hr after inoculation (Figure 6-B).

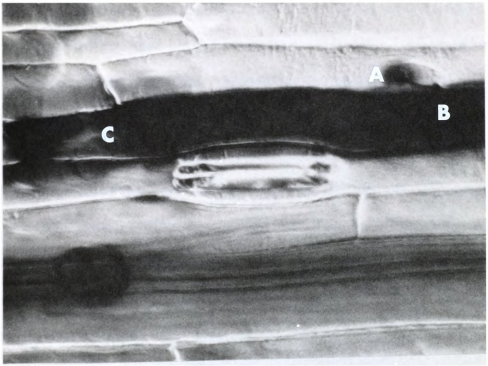
With the incompatible genotype, P2a/Pm2a, (Figure 6-C), 19% of the parasite units had formed haustoria 5μ or less in

Figure 7. Photomicrograph of the penetration process of the incompatible interaction, P1a/Pm1a, on wheat at 26 hr after inoculation. The incompatible interactions, P2a/Pm2a, P3a/Pm3a, and P4a/Pm4a, show similar results.

(A) Conidium
(B) Point of penetration of the host by the parasite unit
(C) Uptake of the aniline blue by infected host cell depicting mesophyll collapse

Figure 8. Drawing of the penetration process of the incompatible interaction P1a/Pm1a, with similar results for P2a/Pm2a, P3a/Pm3a, and P4a/Pm4a

(A) Penetration and failure of the parasite unit to develop haustoria
(B) Mesophyll collapse and uptake of aniline blue



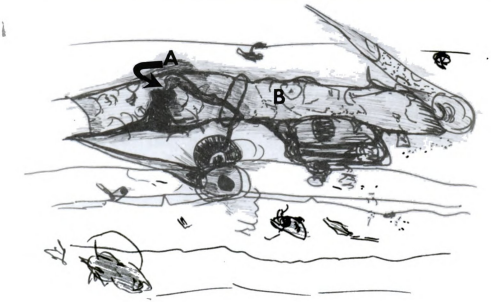


Figure 7 and Figure 8

length and 66% had haustorial bodies 35-55μ in length by 30 hr after inoculation: only 4% of haustoria were distorted and relatively few of the host cells had picked up the dye. Another interesting point was observed. With the incompatible P2a/Pm2a genotype, during appresorial development, the length of the appresorium is much longer when compared with the appresorial development of the other compatible and incompatible genotypes.

The development of haustoria with the P3a/Pm3a (Figure 6-D), genotype showed that at 30 hr after inoculation, 35% 0-5μ, 2% 5-15μ, 6% 15-25μ, 12% 25-35μ, 18% 35-55μ in length, and 2% were distorted. The highest percentage of haustoria was reached 28 hr after inoculation and almost all infected host cells had picked up the aniline blue (see Figure 7 and interpretive drawing in Figure 8).

The P4a/Pm4a (Figure 6-E), showed that very few of the parasite units had haustoria longer than 5μ in length by 30 hr after inoculation. Approximately 6% of the parasite units had haustoria 35μ or longer at this time; 18% of the haustoria were distorted, and 10% had collapse of the ectoparasitic portion of the parasite unit. As early as 14 hr after inoculation, groups of mesophyll cells had collapsed beneath or near the penetrated cell, and usually the highest percentage of collapsed cells adjoining the infected cell was observed 28 hr after inoculation.

Discussion

In this study the time at which different genotypes affected the ontogeny of the host-parasite interactions during primary infection was observed. The data with the compatible genotype, Px/pmx, (Figure 6-A), suggest both development of the haustoria and development of the ectoparasitic portion of the parasite are reasonably synchronized (26, 27, 28, 29).

With the compatible Px/pmx genotype, penetration by the parasite unit occurs at approximately 8-10 hr after inoculation. By 18 hr after inoculation, appendages are present on the primary haustorial body and relatively few of the ectoparasitic structures of the parasite have collapsed. The fact that the haustoria are not distorted and no detectable aniline blue has infiltrated the host cells indicates that the infected cell is still viable. By 30 hr after inoculation, 86% of the conidia applied have produced primary haustoria, and this figure corresponds to the percent of ESH reported at that time (48).

The P1a/Pm1a genotype was observed to affect infection efficiency (5) and the development of haustoria as well as the uptake of aniline blue by host cells during primary infection. The kinetics of haustorial development for P1a/Pm1a show that 33% of the parasite units had infected the host cell and formed haustoria 5μ or less in length by 30 hr after inoculation. Twenty-six percent of the parasitic units were distorted and their host cells

stained with aniline blue. Nineteen percent of the parasitic units formed normal haustoria, a figure comparable to formation of ESH > 10μ long by 26 hr after inoculation (48). It appears, therefore, that only haustoria that develop normally support the production of ESH.

Published studies have not demonstrated an effect of the P2a/Pm2a gene interaction during primary infection (19). Most of the parasite units, 77%, eventually produce ESH > 10μ (48). The kinetics of haustorial development in this research show that by 30 hr after inoculation, 19% of the parasite units had formed haustoria 5μ or less in length, while only 66% had haustoria 35-55μ in length. Four percent of the parasite units had distorted haustoria and host cells which picked up the dye. While the percent of ESH does not show an effect of P2a/Pm2a in primary infection, a study of haustorial development does indicate that P2a/Pm2a genotype significantly differs from the compatible interaction during primary infection.

The interactions of P3a/Pm3a were similar to that of P1a/Pm1a and P4a/Pm4a (Figure 6-B, D, E). The kinetics of haustorial development for P3a/Pm3a are not in complete agreement with the percent of parasite units that have formed ESH more than 10μ long. Thirty percent of the parasite units have haustorial development supporting growth of ESH > 10μ by 26 hr after inoculation. In order for the P3a/Pm3a data to be in

agreement with both parameters, it was necessary to include haustoria 25-35μ, as well as those 35μ or longer in length. Apparently, smaller than normal haustoria are supporting the development of ESH.

The data also showed that haustorial development in the incompatible interactions is not a clear-cut phenomenon (Figure 6-B, C, D, E). In some cases, the host cells were penetrated and subsequent development of the parasite unit was rapidly halted. In other infected cells, the parasite unit appeared to develop normally for a few hours, then development stopped, leaving a rudimentary haustorium (Figure 9). The uptake of the aniline blue by the infected host cell in these interactions led to the assumption that a large percentage of the infected cells were nonfunctional. Only 18% of the infections with the incompatible P3a/Pm3a genotype were normal 30 hr after inoculation.

P4a/Pm4a showed a reduction in the percentage of the parasite units that produced primary haustoria as well as a discoloration of the host cells adjacent to the infected cell (46, 47). Sixty percent of the parasite units penetrated the host cells and stopped growing. Eighteen percent of the parasite units were distorted; rudimentary haustoria and stained host cells were observed. Ten percent of the total number of parasite units applied showed collapse of the ectoparasitic portion, and 3% had haustoria 35-55μ in length.

Figure 9. Drawing of the incompatible interaction, P4a/Pm4a, showing rudimentary haustoria and mesophyll collapse.

(A) Rudimentary haustoria
(B) Mesophyll collapse

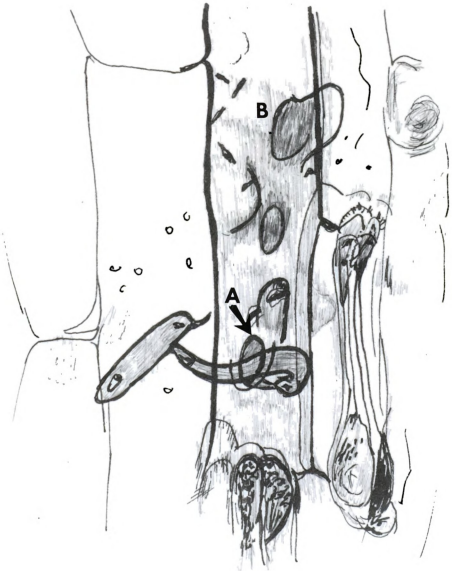


Figure 9

The results presented here provide additional evidence that the different genotypes affect different stages in the ontogeny of interactions between host and parasite and that some of the incompatible interactions affect parasite development earlier than previously reported.

Summary

The stages of haustorial development of Erysiphe graminis f. sp. tritici from 8 to 30 hours after inoculation varies with the genotype of the host and parasite. With the compatible genotype, Px/pmx, haustorial development occurred as was expected compared with the length of ESH formed (Figure 6-A). The incompatible interactions, P1a/Pm1a, P2a/Pm2a, P3a/Pm3a, and P4a/Pm4a, are not as distinctly different as expected. With P1a/Pm1a, the majority of the parasite units that produced haustoria collapsed 26-28 hours after inoculation, but the infected host cells did not appear altered. P2a/Pm2a apparently does have an interaction earlier than previously observed based on the infection efficiency and the development of ESH as modes of comparison. In earlier experiments (48), P3a/Pm3a had 30% ESH longer than 10μ. The present data indicate that with this interaction, the percent of parasite units which produced full-sized haustoria is less than 30%. Therefore, haustoria that are not growing at the normal rate can support the production of ESH.

Results on the relationship between development of haustoria and ESH with P4a/Pm4a are in close agreement with previous findings (19).

CHAPTER II

THE INFLUENCE OF LIGHT PERIODS ON THE PRODUCTION OF SECONDARY HAUSTORIA BY ERYSIPHE GRAMINIS ON SUSCEPTIBLE WHEAT

Introduction

The ontogenesis of haustoria was established for the primary infection processes with both the compatible and incompatible parasite/host genotypes in Chapter 1. The study was possible because conditions for reasonably synchronized primary infection were known. The assumption has been made that, since secondary hyphae longer than 10-15μ are formed only if a haustorium was formed, the formation of ESH was indicative of the establishment of a compatible, functional relationship between host and parasite. It was considered necessary, therefore, to establish that the secondary hyphae formed were capable of initiating secondary infection.

The purpose of this part of the investigation was (1) to define the various compenent stages that make up the process of secondary infection, (2) to attempt to synchronize the parasite

population in the various stages of secondary infection by altering the environment, and (3) to determine useful criteria for the establishment of a compatible host-parasite interaction from 38-58 hr after inoculation.

Materials and Methods

Culture MS-1 of Erysiphe graminis f. sp. tritici was maintained on Triticum aestivum cv. 'Little Club'. The environmental conditions under which these stock cultures are maintained have been described by others (27, 28, 35, 36) and in Chapter 1, as have been the conditions for reasonably synchronous primary infection (34). Some observations had indicated that if standard conditions were used for the first 26 hr after inoculation, the highest percentage of secondary appresoria would form if given 27 C and high light intensity for 4 hr followed by 22 C and darkness (46, 47). No determination was made of the percent of secondary appresoria which produced haustoria (46, 47).

Standard environmental conditions used for the first 26 hr are presented in Table 3. Environmental conditions used beginning 26 hr after inoculation are given in Table 4.

Table 3. Environmental conditions necessary for synchronous development of parasite unit through primary infection

Hour	0-1	1-6	6-20	20-26
Environ-mental Conditions	dark 100% RH 17 C	light 65% RH 22 C	dark 65% RH 22 C	light 65% RH 22 C

Table 4. Experimental alterations of the light periods to obtain synchronous development of the parasite unit through secondary infection

Experiment 1			
Hour	26-30	30-38	38-58
Environ-mental Conditions	light 65% RH 22 C	dark 65% RH 22 C	light 65% RH 22 C
Experiment 2			
Hour	26-36	36-44	44-58
Environ-mental Conditions	light 65% RH 22 C	dark 65% RH 22 C	light 65% RH 22 C

Table 4 (cont'd.)

Experiment 3

Hour	26-30	30-44	44-54	54-58
Environ-mental Conditions	light 65% RH 22 C	dark 65% RH 22 C	light 65% RH 22 C	dark 65% RH 22 C

Experiment 4

Hour	26-36	36-58
Environ-mental Conditions	light 65% RH 22 C	dark 65% RH 22 C

Each set of experiments involving alterations of light periods was repeated four times on four different days with four different sets of inoculated plants. Epidermal strips were removed and stained using the same procedures as in Chapter 1. The data are presented as averages of all replications, and statistical analyses (52) were done using a two-way analysis of variance (Figure 10).

Results

The formation of secondary haustoria with the compatible genotype, Px/pmx, is shown in Figure 11. By 30 hr after inoculation, 87% of the parasite units applied formed functional

Figure 10. Formation of secondary haustoria by *Erysiphe graminis* f. sp. *tritici* cultures MS-1 by varying the light periods.

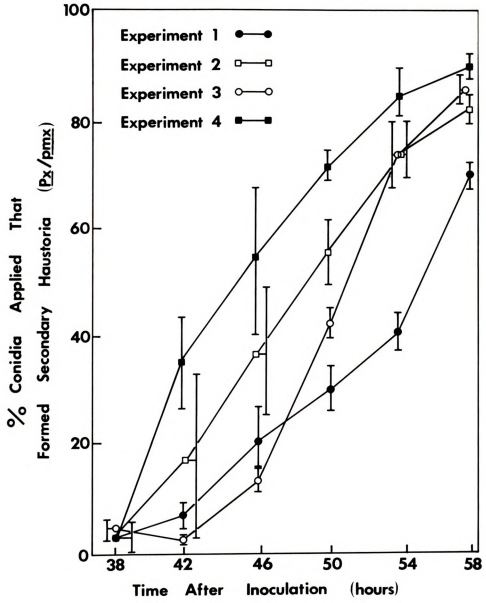

Experiment 1
Experiment 2
Experiment 3
Experiment 4
% Conidia Applied That Formed Secondary Haustoria (Px/pmx)
100
80
60
40
20
0
38
42
46
50
54
58
Time After Inoculation (hours)

Figure 10

Figure 11. Drawing of a primary haustorium and secondary haustoria with a compatible genotype, Px/pmx, at 54 hr after inoculation.

(A) Primary haustorium
(B) Secondary haustoria

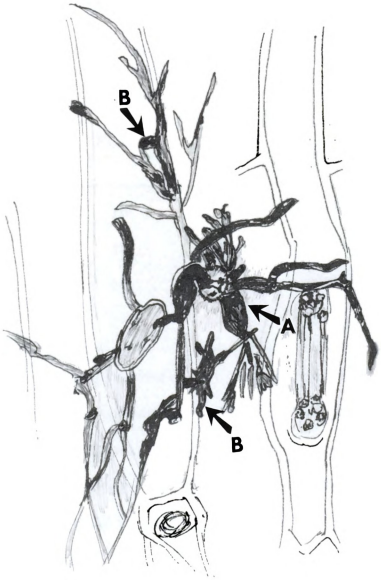


Figure 11

primary haustoria (20). Of this 87%, approximately all of the parasite units developed secondary haustoria given the optimal environmental conditions for secondary infection (Figure 10).

The results of Experiments 1, 2, 3, and 4 (Table 4) may be summarized as follows:

Table 5. Statistical analyses of the percent of parasitic units forming secondary haustoria

Experiment	Figure	Percent of parasitic units forming primary haustoria which continued to form secondary haustoria
1	10	69.00
2	10	80.00
3	10	87.00
4	10	90.00

Even though relatively high percentages of the conidia applied formed secondary haustoria in all the experiments, the synchrony as evidenced by the normal growth curve, failed to approach that observed in Experiment 3. Since synchrony and infection efficiency through secondary haustorial development were equally important considerations, the environmental conditions of Experiment 3 were used in subsequent studies of the development of secondary haustoria.

Discussion

The primary stages of infection of wheat and barley by E. graminis have been studied by employing the optimum environmental conditions necessary for a high infection efficiency and synchrony of the parasite units (17, 22, 23). If a high infection efficiency and synchronized development of the parasite with compatible genotypes had not been attained, the identification of the effects of the genotypes for incompatibility on primary infection would have been essentially impossible.

The percent of elongating secondary hyphae is an accurate indication of the degree of success in establishing infection (46). The data in Chapter 1 demonstrates the extent to which development of primary haustoria is an even finer indicator of the success in establishing an aegricorpus. Both methods delineate a time, unique to each incompatible combination of genotypes, at which the incompatibility is first expressed.

In incompatible interactions, however, a fraction of the parasite units proceed past this critical time. How this occurs is yet unknown. The small fraction that does produce successful primary infections then takes part in the secondary infection. To study this process, a reasonably synchronous population of parasite units was needed. To this end, environmental conditions giving some synchrony of secondary infection in the compatible interactions were empirically developed.

Summary

Light is necessary at specific times during primary and secondary infection for synchronous development of E. graminis on wheat. Synchronous development of E. graminis during secondary infection was increased with changes in the light periods (1.3 ergs $cm^{-2}sec^{-1}$, incandescent and fluorescent) between 26 and 58 hr after inoculation (34). With the optimal environmental conditions (26-30 hr, light; 30-44 hr dark; 44-54 hr light; and 54-58 hr dark, and 65% relative humidity, 22 C for the period 26-58 hr after inoculation) established for the compatible interaction, Px/pmx, approximately all of the parasite units that form primary haustoria from 20-26 hr after inoculation formed secondary haustoria by 58 hr after inoculation. The synchrony of haustorial development was affected if the light schemes were changed. Increased synchrony in development of secondary haustoria on susceptible wheat makes it possible to determine when the different incompatible parasite/host genotypes affect the ontogeny of the host-parasite interactions.

CHAPTER III

GENETIC CONTROL OF SECONDARY HAUSTORIAL DEVELOPMENT OF ERYSIPHE GRAMINIS ON WHEAT

Introduction

A disease caused by a parasite will usually develop so as to reflect the degree to which the parasite maintains growth and reproduction in its interaction with the host.

The kinetics of observable interactions between E. graminis f. sp. tritici and wheat with compatible Px/pmx and incompatible P1a/Pm1a, P2a/Pm2a, P3a/Pm3a, and P4a/Pm4a parasite/host genotypes have been described for the process of primary infection the first 26 hr after inoculation (48).

Chapter 2 has described environmental conditions which give reasonably synchronous development of secondary haustoria of the parasite with compatible parasite/host genotypes during the period from 26-58 hr after inoculation.

The objectives of this investigaion were (1) to determine the rate of development of secondary haustoria of E. graminis on the 5 near-isogenic lines of wheat containing genes for

reaction to E. graminis, and (2) to determine if and when the different incompatible parasite/host genotypes affect secondary infection. The latter should give a more complete understanding of when and how the parasite and host genes interact.

Materials and Methods

The development of the haustorial bodies was observed microscopically using the aniline blue dye technique described in Chapter 1. Every four hours after inoculation, beginning at hour 38, strips of the abaxial epidermis were removed, stained, and examined microscopically as described previously, and the number of haustoria recorded (Table 6). The environmental conditions which gave the most synchronous development of the ectoparasitic portion of the parasite through primary infection (17, 22, 48), and synchronous development of the compatible interaction for secondary infection were used (see Chapter 2).

The data (Table 6) are presented as percent of the total number of parasite units with primary haustoria which formed secondary haustoria of any length. The experiments were repeated on four different days.

Results

Observations of haustorial development with the compatible parasite/host genotype, Px/pmx, (Table 6, Figure 11), showed that, by 38 hr after inoculation, 83% of the parasite units had

Table 6. Effect of parasite/host genotype on development of primary and secondary haustoria

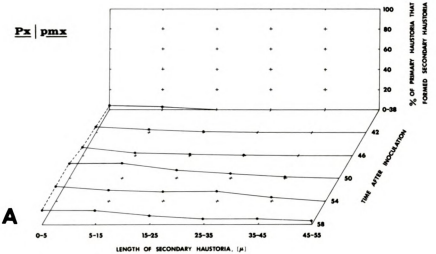
Genotype (parasite/host)	Hours after inoculation	% of conidia applied that had only primary haustoria	% of parasite units with primary haustoria which produced secondary haustoria of a given length (μ)						% parasite units that formed rudimentary primary haustoria	% attempted primary penetrations without haustoria	Total number of parasite units
			0-5	5-15	15-25	25-35	35-45	45-55			
Px/pmx	38	83	4	3					5	6	427
	42	79	6	3	0.5				5	6	408
	46	74	8	3	2	0.4			7	6	386
	50	46	14	14	7	4	1		3	7	341
	54	41	14	11	9	10	4			12	388
	58	36	14	14	8	6	6	4		13	384
P1a/Pm1a											
	38	12							33	48	446
	42	12	7						25	56	264
	46	8	4	2					13	72	405
	50	13	0.9	0.9					8	77	542
	54	12	1	3	2				8	74	460
	58	13	1	2					4	81	423
P2a/Pm2a											
	38	58	1						13	28	482
	42	56	4	1					17	21	429
	46	45	4	8	2	0.6			6	35	340
	50	47	6	5	6	2			3	31	391
	54	39	6	5	8	2			13	24	400
	58	37	7	10	9	2			5	28	286
P3a/Pm3a											
	38	22							37	43	430
	42	19	10	2					29	51	424
	46	11	0.8	0.6					27	61	484
	50	11	2	2	0.4				10	74	457
	54	8	3	3	3				10	71	467
	58	12	2	2	1				7	76	470
P4a/Pm4a											
	38	2							14	83	452
	42	4	0.2						13	82	456
	46	4							9	87	472
	50	4	0.8	0.8					15	79	497
	54	6	0.6						4	90	525
	58	4	0.7						3	93	560

formed primary haustoria only, 4% also had formed secondary haustoria 0-5μ in length, and 3% had formed secondary haustoria 5-15μ in length (Table 6, Figure 12-A). Five percent of the total number of conidia applied had formed rudimentary haustoria (Figure 12-F). Six percent of the total number of conidia applied germinated, penetrated the host cells, and stopped growth. By 58 hr after inoculation, 14% of the parasite units had secondary haustoria 0-5μ, 14% 5-15μ, 8% 15-25μ, 6% 25-35μ, 6% 35-45μ, and 4% 45-55μ in length. Thirteen percent of the parasite units had penetrated the host cell but did not form haustoria. With the compatible genotype some secondary haustoria reached a length greater than 55μ. The mesophyll cells showed no sign of necrosis except for the 13% of the parasite units that had penetrated the host cell but did not form haustoria. The cells with infection pegs but no haustoria took up stain. Progressive development of the parasite unit is depicted by the continued growth of secondary haustoria up to 58 hr after inoculation (Table 6, Figure 12-A).

With the incompatible genotype, P1a/Pm1a, no parasite units that had formed primary haustoria had formed secondary haustoria by 38 hr after inoculation. Thirty-three percent of the parasite units had formed rudimentary primary haustoria while 48% penetrated the host and growth ceased. By 58 hr after inoculation, 1% were 0-5μ in length, 2% 5-15μ, while 4% had

Figure 12. Effect of the five different parasite/host genotypes of the development of secondary haustoria of Erysiphe graminis f. sp. tritici.

(A) Genotype Px/pmx
(B) Genotype P1a/Pm1a
(C) Genotype P2a/Pm2a
(D) Genotype P3a/Pm3a
(E) Genotype P4a/Pm4a
(F) Penetration with rudimentary haustoria and penetration with no haustoria on the compatible and incompatible interactions


Px | pmx
A
100
80
60
40
20
0-38
42
46
50
54
58
% OF PRIMARY HAUSTORIA THAT FORMED SECONDARY HAUSTORIA
TIME AFTER INOCULATION
0-5
5-15
15-25
25-35
35-45
45-55
LENGTH OF SECONDARY HAUSTORIA, (μ)

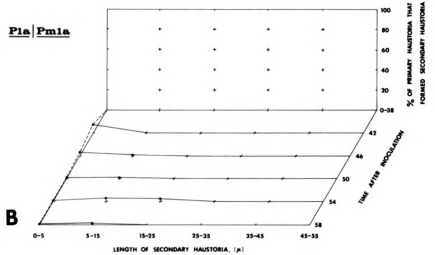
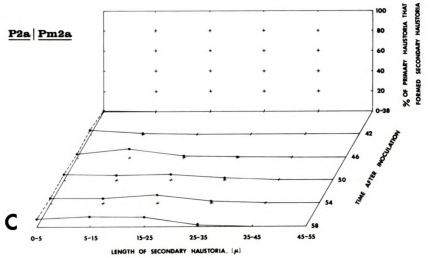

P1a | Pm1a
B
100
80
60
40
20
0-38
42
46
50
54
58
% OF PRIMARY HAUSTORIA THAT FORMED SECONDARY HAUSTORIA
TIME AFTER INOCULATION
0-5
5-15
15-25
25-35
35-45
45-55
LENGTH OF SECONDARY HAUSTORIA, (μ)

Figure 12



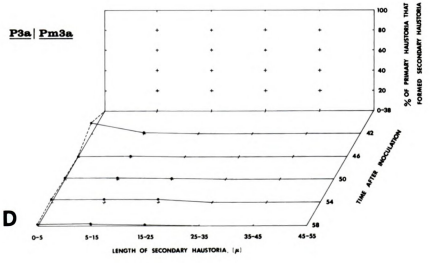
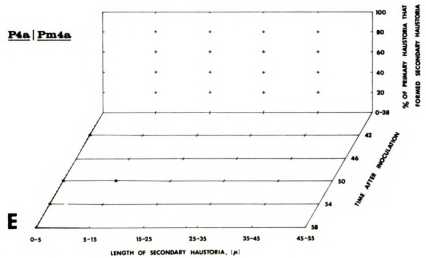


Figure 12 (cont'd.)



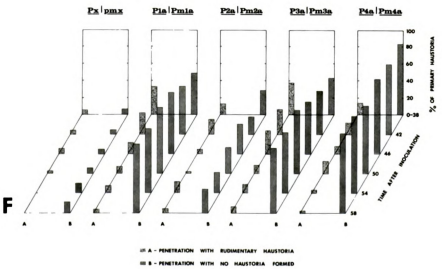


Figure 12 (cont'd.)

formed rudimentary primary haustoria, and 81% had penetrated and stopped development. Secondary haustoria remained relatively constant in size, never exceeding a length greater than 25μ by 58 hr after inoculation (Figure 12-B). Eighty-one percent of the infected cells depicted mesophyll collapse indicating necrogenic protoplasts by 58 hr after inoculation (Figure 12-F).

With the incompatible genotype, P2a/Pm2a, by 38 hr after inoculation, 1% of the parasite units had formed haustoria 0-5μ in length. Thirteen percent of the parasite units had formed rudimentary haustoria and 28% had penetrated the host cell. By 58 hr after inoculation, 7% had secondary haustoira 0-5μ in length, 10% 5-15μ, 9% 15-25μ, and 2% 25-35μ. Secondary haustorial development never exceeded 35μ in length (Figure 12-C). Five percent formed rudimentary haustoria and 28% of the parasite units showed mesophyll collapse and uptake of dye by 58 hr after inoculation (Figure 12-F).

In the incompatible aegricorpus, P3a/Pm3a, no measurable number of the parasite units that had formed primary haustoria formed secondary haustoria by 38 hr post inoculation. However, some 37% of the parasite units that had formed primary haustoria formed secondary haustoria, and some 43% had simply penetrated the host before growth ceased. By 58 hr after inoculation, only 2% of the parasite units had formed secondary haustoria 0-5μ, 2% 5-15μ, and 1% 15-25μ in length (Figure 12-D).

Seven percent of the parasite units had formed rudimentary haustoria and 76% penetrated the host cell and picked up the aniline blue (Figure 12-F).

With the incompatible P4a/Pm4a genotype, very few of the parasite units had secondary haustoria 0-5μ in length as late as 58 hr after inoculation (Figure 12-E). Three percent of the parasite units had formed rudimentary primary haustoria, and 93% had simply penetrated the host cell. Mesophyll collapse was evident not only in the infected cells but in the adjoining cells (Figure 12-F).

Discussion

Microscopic observations of haustorial development in the early stages of infection were expected to elucidate the mechansim underlying resistance and susceptibility (10). In this study, the time at which different genotypes affect the ontogeny of the host/parasite interactions was established.

The data from the compatible genotype, Px/pmx, (Figure 12-A) suggest that haustorial development is still relatively well synchronized and that the parasite units will continue to undergo normal development and to reach sporulation. By 58 hr after inoculation, 52% of the parasite units have formed secondary haustoria. Apparently cells undergoing normal development do not absorb aniline blue as the ectoparasitic

structures appear normal presenting no sign of imminent collapse.

The kinetics of secondary haustorial development for P1a/Pm1a indicate that approximately 6% of the parasite units may reach sporulation. In primary infection, 81% of the parasite units penetrated but failed to develop haustoria. Similarly, of those 19% producing primary haustoria, 81% failed to produce secondary haustoria.

While 28% of the parasite units in the P2a/Pm2a interaction had formed functional secondary haustoria by 58 hr after inoculation, the compatible interaction, Px/pmx, at the same hour had 52% of the parasite units with secondary haustoria. The P1a/Pm1a, P3a/Pm3a, and P4a/Pm4a showed only 6, 5, and 1% respectively of the parasite units with secondary haustoria at this time.

The incompatibility conditioned by P2a/Pm2a seems to be intermediate between the extreme incompatibility of P1a/Pm1a, P3a/Pm3a, and P4a/Pm4a and the compatibility of Px/pmx.

The observations of P3a/Pm3a development were essentially identical to those of P1a/Pm1a. The kinetics of haustorial development for P3a/Pm3a demonstrate very slow formation of secondary haustoria; 6% at 58 hr was shown previously for development of secondary haustoria. Some 76% of the parasite units are stopped at the stage of secondary penetration, and an

additional 7% are stopped at the formation of a rudimentary haustorium.

A greater reduction in the percentage of the parasite units that produce secondary haustoria and of the infected and adjacent cells that stained with aniline blue was observed in P4a/Pm4a. Ninety-three percent of the applied conidia penetrated, but failed to form haustoria. Approximately 1% of the total number of conidia applied continued to develop. P4a/Pm4a had the lowest secondary infection efficiency of all the interactions studied.

Summary

The rate of development of secondary haustoria of Erysiphe graminis f. sp. tritici on 5 near-isogenic lines of wheat containing single genes for reaction to E. graminis (MS-1) were observed microscopically after staining with aniline blue. Haustorial measurements, taken from 38-58 hr after inoculation under environmental conditions which ensured synchronous development of the parasite, showed that 52% of the parasite units on the compatible host, Px/pmx, had formed secondary haustoria up to 55μ in length. The percent of secondary haustoria formed with the incompatible parasite/host genotypes P1a/Pm1a, P2a/Pm2a, P3a/Pm3a, and P4a/Pm4a were 6, 28, 5, and 1% respectively. More than 75% of the parasite units

were associated with necrotic host tissue as implied by uptake of aniline blue. These findings provide additional evidence that different genotypes affect different stages in the ontogeny of the host/parasite interactions as well as indicating that the incompatible interactions can be demonstrated much earlier than previously reported.

LITERATURE CITED

LITERATURE CITED

1. ALLEN, P. J. and R. D. GODDARD. 1938. A respiratory study of powdery mildew of wheat. Amer. J. of Bot. 25:613-621.

2. BRIGGLE, L. W. 1966. Three loci in wheat involving resistance to Erysiphe graminis f. sp. tritici. Crop. Sci. 6:461-465.

3. BRIGGLE, L. W. 1966. Transfer of resistance to Erysiphe graminis f. sp. tritici from Khapli emmer and Yuma durum to hexaploid wheat. Crop. Sci. 6:459-461.

4. BRIGGLE, L. W. 1969. Near-isogenic lines of wheat with genes for resistance to Erysiphe graminis f. sp. tritici. Crop. Sci. 9:70-75.

5. BUSHNELL, W. R., J. DUREK, and J. B. ROWELL. 1967. Living haustoria and hyphae of Erysiphe graminis f. sp. hordei with intact and partly dissected host cells of Hordeum vulgare. Can. J. Bot. 45:1719-1732.

6. CHEREWICK, W. J. 1944. Studies on the biology of Erysiphe graminis DC. Can. J. Research. 22:52-86.

7. CLARK, G. 1973. Staining procedures. The Williams and Wilkins Co. Maryland. 418 pp.

8. CORNER, E. J. H. 1935. Observations on resistance to powdery mildews. New Phytologist. 34:180-200.

9. DURBIN, R. D. 1967. Obligate parasites, effect on the movement of solutes and water. In The Dynamic Role of Molecular Constituents in the Plant-Parasite Interaction. ed. C. J. Mirocha, I. Uritani, pp. 80-99. St. Paul: Am. Phytopathol. Soc. 372 pp.

10. ELLINGBOE, A. H. 1968. Inoculum production and infection by foliar pathogens. Ann. Rev. Phytopath. 6:317-330.

11. ELLINGBOE, A. H. 1972. Genetics and physiology of primary infection by Erysiphe graminis. Phytopath. 62:401-406.

12. ELLINGBOE, A. H. 1973. Genetics and Physiology of Plant Parasitism. National Science Foundation Research Proposal. 17 pp.

13. FLOR, H. H. 1946. Genetics of pathogenicity in Melamspora lini. J. Agr. Res. 73:335-337.

14. FLOR, H. H. 1955. Host-parasite interactions in flax-rust---its genetics and other implications. Phytopath. 45:680-685.

15. FLOR, H. H. 1956. The complementary genetic systems in flax rust. Advan. Genet. 8:29-54.

16. FLOR, H. H. 1971. Current status of the gene-for-gene concept. Ann. Rev. Phytopath. 9:275-296.

17. GRAF-MARIN, A. 1934. Studies on the powdery mildew of cereals. Cornell Univ. Agr. Exp. Sta. Mem. 157. 48 pp.

18. HAYAT, M. A. 1970. Principles and Techniques of Electron Microscopy. Van Nostrand Reinhold Co. New York, 412 pp.

19. HAYWOOD, M. J. 1974. Proceedings of the American Phytopath. Soc. Vol. 1: p. 67.

20. HAYWOOD, M. J. 1974. Proceedings of the American Phytopath. Soc. Vol. 1: p. 126.

21. LARGE, E. C. and D. A. DOLING. 1962. The measurements of cereal mildew and its effect on yield. Plant Pathology. 11:47-57.

22. LARGE, E. C. and D. A. DOLING. 1963. Effect of mildew on yield of winter wheat. Plant Pathology. 12:128-130.

23. LACHLI, A. 1972. Translocation of inorganic solute. Ann. Rev. Plant Physiol. 23:197-218.

24. LOEGERING, W. Q. 1966. The relationship between host and pathogen in stem rust of wheat. (Proc. 2nd. Int. Wheat Symp. Lund. 1963) Hereditas, Suppl. Vol. 2:167-177.

25. MARTIN, T. J. 1974. Uptake of ^{32}P by Triticum aestivum and genetic control of ^{32}P transfer to Erysiphe graminis during primary infection. Ph. D. Thesis. Michigan State University, 116 pp.

26. MASRI, S. S. 1965. The development of appresoria, haustoria, and secondary hyphae during primary infection of wheat and barley by Erysiphe graminis. Ph. D. Thesis. Michigan State University. 89 pp.

27. MASRI, S. S. and A. H. ELLINGBOE. 1966. Primary infection of wheat and barley by Erysiphe graminis. Phytopath. 56:389-395.

28. MASRI, S. S. and A. H. ELLINGBOE. 1966. Germination of conidia and formation of appresoria and secondary hyphae in Erysiphe graminis f. sp. tritici. Phytopath. 56:304-308.

29. McCOY, M. S. and A. H. ELLINGBOE. 1966. Major genes for resistance and the formation of secondary hyphae by Erysiphe graminis f. sp. hordei. Phytopath. 56:683-686.

30. MOLLENHAUER, H. H. 1959. Permanganate fixation of plant cells. J. Biophys. Biochem. Cytol. 6:431.

31. MOLLENHAUER, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. Stain. Technol. 39:111.

32. MOSEMAN, J. G. 1959. Host-pathogen interaction of the genes for resistance on Hordeum vulgare and for pathogenicity in Erysiphe graminis f. sp. hordei. Phytopath. 49:469-472.

33. MOSEMAN, J. G. 1966. Genetics of powdery mildews. Ann. Rev. Phytopath. 4:269-290.

34. MOUNT, M. S. 1968. Environmental effects and transfer events during primary infection of wheat by Erysiphe graminis. Ph. D. Thesis, Michigan State University. 112 pp.

35. NAIR, K. R. S. and A. H. ELLINGBOE. 1962. A method of controlled inoculations with conidiospores of Erysiphe graminis var. tritici. Phytopath. 52:714.

36. NAIR, K. R. S. and A. H. ELLINGBOE. 1965. Germination of conidia of Erysiphe graminis f. sp. tritici. Phytopath. 55:365-368.

37. PERSON, C. 1959. Gene-for-gene relationship in host: parasite systems. Can. J. Bot. 37:1101-1130.

38. PERSON, C., D. J. SAMBORSKI, and R. ROHRINGER. 1962. The gene-for-gene concept. Nature. 194: 561-562.

39. POWERS, H. R. and W. J. DANDO. 1960. Genetic control of the host-parasite relationship in wheat powdery mildew. Phytopath. 50:454-457.

40. PRATT, R. 1938. Respiration of wheat infected with powdery mildew. Sci. 88:62-63.

41. ROWELL, J. R., W. Q. LOEGERING, and H. R. POWERS, JR. 1963. Genetic model for physiologic studies of mechanisms governing development of infection type in wheat stem rust. Phytopath. 53:932-937.

42. SAKO, N. and M. A. STAHMANN. 1972. Multiple molecular forms of enzymes in barley leaves infected with Erysiphe graminis f. sp. hordei. Physiological Plant Path. 2:217-222.

43. SCHNATHORST, W. C. 1965. Environmental relationships in the powdery mildews. Ann. Rev. Phytopath. 3:343-366.

44. SEMPIO, C. 1950. Metabolic resistance to plant diseases. Phytopath. 40:799-819.

45. SHAW, M., S. A. BROWN, and D. R. JONES. 1954. Uptake of radioactive carbon and phosphorus by parasitized leaves. Nature. 173:769-798.

46. SLESINSKI, R. S. 1969. Genetic control of primary interactions during infection of wheat by Erysiphe graminis f. sp. tritici. Ph.D. Thesis, Michigan State University. 107 pp.

47. SLESINSKI, R. S. and A. H. ELLINGBOE. 1969. The genetic control of primary infection of wheat by Erysiphe graminis f. sp. tritici. Phytopath. 59:1833-1837.

48. STUCKEY, R. E. and A. H. ELLINGBOE. 1975. Effect of environmental conditions on ^{35}S uptake by Triticum aestivum and transfer to Erysiphe graminis f. sp. tritici during primary infection. Physiological Plant Pathology. 5:19-26.

49. STUCKEY, R. E. 1973. Elongation of secondary hyphae and translocation of ^{35}S and ^{3}H from host to parasite during primary infection of wheat by Erysiphe graminis f. sp. tritici. Ph. D. Thesis, Michigan State University. 119 pp.

50. WALKER, J. C. 1957. The powdery mildews. In Plant Pathology. pp. 312-318. McGraw-Hill Book Co., New York.

51. WHITE, N. H. and E. P. BAKER. 1954. Host-pathogen relations in powdery mildew of barley. I. Histology of tissue reactions. Phytopath. 44:657-662.

52. WOOLF, C. M. 1968. Principles of Biometry. D. Van Nostrand Co., Inc., New Jersey. 35-45 pp.

53. YARWOOD, C. E. and LOIS JACOBSON. 1955. Accumulation of chemicals in deceased areas of leaves. Phytopath. 45:43-48.