GENETIC CONTROL OF PRIMARY INTERACTIONS DURING INFECTION OF WHEAT BY ERYSIPHE GRAMINIS F. SP. TRITICI

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ABSTRACT

GENETIC CONTROL OF PRIMARY INTERACTIONS
DURING INFECTION OF WHEAT BY
ERYSIPHE GRAMINIS F. SP. TRITICI

By

Ronald Stephen Slesinski

The objective of this research was to study powdery mildew development during the initial stages of the host-parasite interaction. Two aspects of fungus development were examined: 1) the effects of the environment upon the initiation of secondary infections by the parasite population, and 2) the effect of specific genes in the plant and pathogen upon host-parasite compatibility.

Formation of secondary appressoria (SA) and the initiation of secondary infections by Erysiphe graminis f. sp. tritici was found to be influenced by environmental conditions. High light (2600 ft-c), temperature (27C), or photoperiod (240 ft-c during 20-30 hr after inoculation followed by 0 ft-c during 30-40 hr) each affected the percentage of parasite units which produced SA. Exposure of inoculated plants to a combination of 27C and 2600 ft-c beginning at 26 hr; or 27C beginning at 26 hr and 0 ft-c beginning at 30 hr; or 2600 ft-c beginning at 26 hr and 0 ft-c beginning at 30 hr, allowed more SA to form than with either the control (22C, 240 ft-c, 20-40 hr photoperiod) or with each condition singly. The optimum environmental

conditions for SA production appeared to be a combination of 27C and 2600 ft-c starting at 26 hr after inoculation with 22C and 0 ft-c starting at 30 hr.

The genetic control of the host-parasite relationship was studied with thirteen near-isogenic wheat lines each of which contained a single dominant Pm gene conditioning reaction to mildew development. These lines represented genes at a minimum of four different loci as well as genes at the same locus but derived from different sources. Chancellor and Little Club, which contain no known Pm genes for incompatibility to mildew development, were used as the standards to which mildew development on the various lines was compared. Production of elongating secondary hyphae (ESH) by the parasite population was used to quantitate the percentage of applied conidia which established a successful parasitic relationship.

Mildew development was similar on all host lines during the stages of germination and formation of appressoria. Differential compatibility was first observed at or near the time of penetration of the host cell and was reflected in the percentage of ESH produced by the parasite population. With the P1/Pm1 and P4/Pm4 parasite/host genotypes, incompatibility was expressed at or near the time of penetration. The lowest percentages of ESH were produced with these combinations. The P3/Pm3 genotypes (except P3c/Pm3c) inhibited only 60-70% of the parasite units from forming ESH. The remaining

30-40% of the parasite population developed as in compatible combinations. The P2/Pm2 genotype did not affect mildew development during primary infection. Incompatibility with this genotype was expressed as a chlorosis and necrosis of host cells 3-4 days after inoculation. Development of ESH with the four possible parasite/host genotypes involving the Pm1 locus (P1/Pm1, P1/pm1, p1/Pm1, p1/pm1) was altered only with the incompatible (P1/Pm1) combination.

^{35}S transfer was used as a criterion of the movement of materials from the wheat leaf to the external mycelium of E. graminis f. sp. tritici. Transfer of ^{35}S was studied with four different corresponding gene pairs (P1/Pm1, P2/Pm2, P3a/Pm3a, and P4/Pm4) conditioning incompatibility to mildew development with the compatible combination MS-1/Chancellor as a basis for comparisons. The amount of ^{35}S transfer with the four incompatible genotypes was found to reflect the inhibition of fungus development previously measured by the reduction in the percentage of ESH. In comparison with the MS-1/Chancellor combination, ^{35}S transfer was slightly greater with P2/Pm2, intermediate with P3a/Pm3a, and lowest with P1/Pm1 and P4/Pm4.

With the four genotypes involving the Pm1 locus, the least quantity of ^{35}S was transferred with P1/Pm1, an intermediate amount with p1/Pm1, and the greatest amounts were transferred with the P1/pm1 and p1/pm1 genotypes. Although morphological development

of the fungus was similar in each of these latter three compatible combinations, ^{35}S transfer with pl/Pml was lower than the other two (pl/pml, Pl/pml) compatible genotypes.

GENETIC CONTROL OF PRIMARY INTERACTIONS DURING INFECTION OF WHEAT BY ERYSIPHE GRAMINIS F. SP. TRITICI

By

Ronald Stephen Slesinski

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TO

MY FAMILY

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INTRODUCTION

The powdery mildew disease of wheat results from the infection of a host plant (Triticum sp.) by the obligately parasitic fungus Erysiphe graminis f. sp. tritici. The environment plays an essential role in determining the progress of disease development and it affects the host, the pathogen, and the combination of the two organisms, the 'aegricorpus' (34). The effects of the environment upon disease development have been studied for the last 70 years or more and the results are numerous and often conflicting. Methods of controlling disease development have also received extensive study. Cultivars 'resistant' to mildew development are available and represent the primary and least expensive control measure. Studies of the inheritance and expression of disease 'resistance', pathogenic variability, and the physiology of 'resistance' are voluminous. Many of these data are confusing because researchers have not always recognized the necessity of defined environmental conditions, inocula, and host plants.

A defined system for examining the early infection processes of E. graminis on wheat and barley has been reported (39, 47, 48).

The development of the fungus was shown to consist of a number of developmental stages which can be distinguished by changes in morphology and differential sensitivity to various environmental conditions. With the appropriate environmental conditions, the synchrony of morphogenesis can be increased and the percentage of successful infections can be maximized. The effect of additional variables in the system can be measured by the alteration of the infection process.

The objectives of this study were the following: 1) to determine the environmental conditions necessary for increasing the synchrony of formation of secondary appressoria by the parasite, 2) to study the interaction of specific genes in the host and parasite which alter development of the relationship, and 3) to characterize the transfer of materials from the host to the fungus in various compatible and incompatible parasite/host combinations utilizing $^{35}SO_4^{=}$ as a radioactive tracer.

LITERATURE REVIEW

Powdery mildew of cereals is an important but frequently underestimated agricultural problem in the United States. The disease is conservatively estimated to produce a 1-2% reduction in yield of wheat (32, 33). This reduction is very significant on a nation-wide basis if we consider the total monetary value of the wheat crop (1.8 billion dollars in 1966) (65). Studies of the disease have been aimed at understanding both the biology of the relationship and in developing effective control measures. The physiological, genetic, and biochemical aspects of disease development have been studied extensively. I will attempt to summarize some of the important developments in this review.

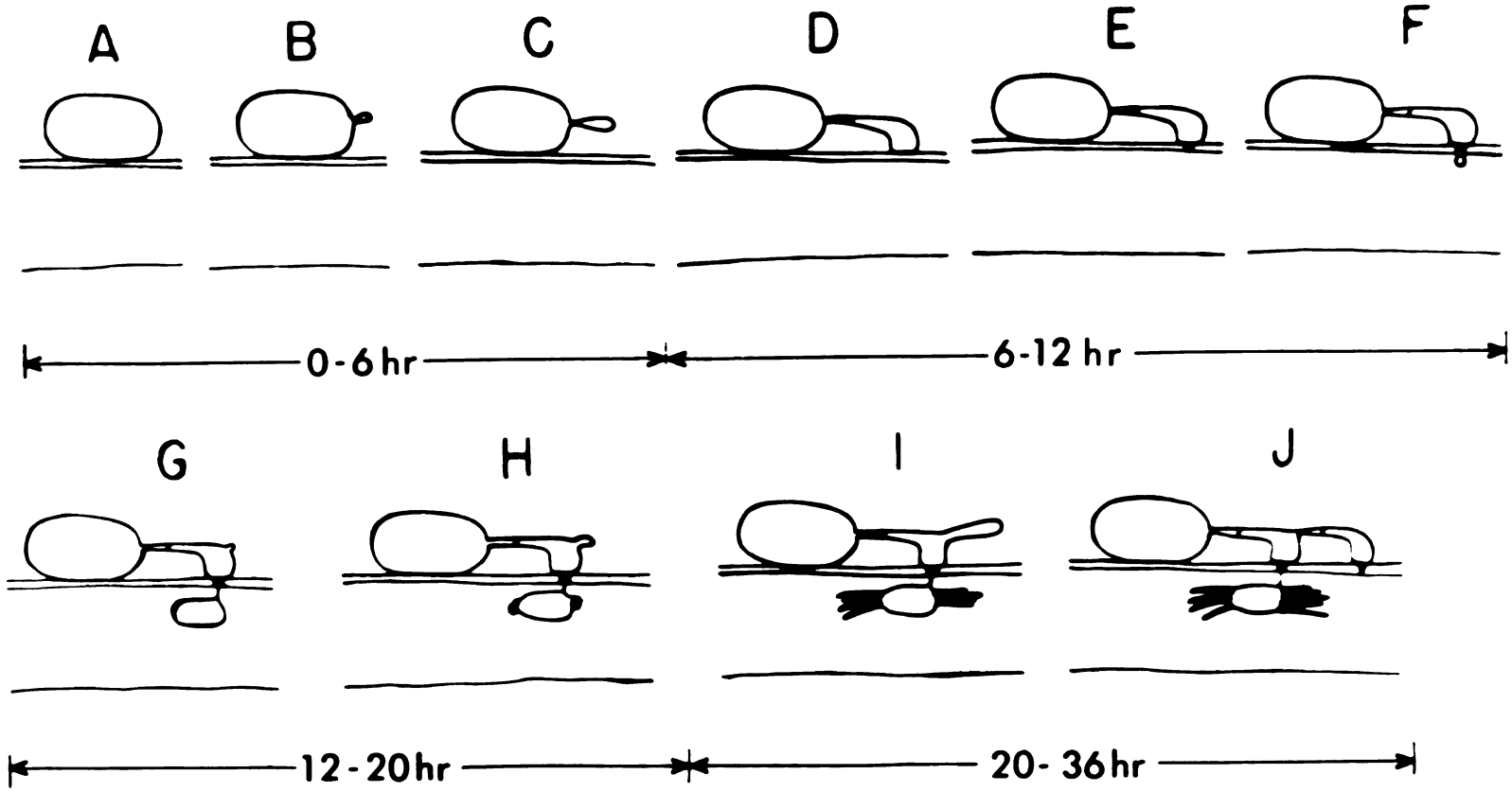
Powdery mildew disease development is affected by environmental conditions and has been correlated with differences in climate. The disease is more prevalent in humid regions such as the Great Lakes states and the U. S. Pacific Northwest (68). Light intensity, light period, relative humidity, temperature, and the genetic constitution of the host and pathogen are some of the variables known to affect disease development (11, 12, 58, 72). Germination and subsequent development of conidia occur over a wide range of temperatures and

relative humidities. Several different optimal conditions for mildew development have been reported (11, 25, 48, 58). There are several excellent reviews concerning the effects of various environmental factors on disease development (39, 47, 58, 72) and I will not attempt to duplicate these efforts. One of the points made in each review, however, is the variability of the present data and the necessity of considering the role of the environment in altering disease development.

Growth of E. graminis on the plant surface can be divided into a number of developmental stages: 1) germination, 2) production of 'club-shaped' appressorial initials, 3) formation of 'mature' appressoria, 4) penetration of the cuticle and epidermal cells, 5) formation of haustoria, 6) development of elongating secondary hyphae (ESH), 7) initiation of additional infections, and 8) sporulation. This sequential development, depicted graphically on Figure 1, has been described by previous workers (11, 25, 37, 72).

Each stage of the infection process differs in the requirement for particular temperature, RH, and light conditions (37, 48). When the optimum conditions for each stage are used, a high percentage of the parasite population undergoes these various developmental stages with an increased synchrony at each stage (37, 47, 48). The term 'infection efficiency' will be used to mean the percentage of the applied conidia which develop on the plant surface and form a functional (17) relationship with the host.

Figure 1. Development of Erysiphe graminis during the stages of primary infection. (A) ungerminated spore, (B) germinated spore, (C) appressorial initial, (D) mature appressorium, (E) penetration peg, (F) haustorial body, (G) enlarged haustorial body and secondary hyphal initial, (H) elongating secondary hypha and developing haustorial appendages, (I) elongating secondary hypha and mature haustorium, (J) secondary appressorium.



TIME AFTER INOCULATION

Increased synchrony and efficiency of infection can be obtained by using the optimum conditions for fungus development during primary infection (37, 47, 48). The production of elongating secondary hyphae (ESH) by the parasite apparently indicates that the fungus is obtaining nutrients from the host which permit the continued growth of the parasite (38, 40). Conidia on non-host plants will germinate, form appressoria, and attempt to penetrate the epidermal cells, but do not form either haustoria or ESH (39, 70). Masri (38) observed that the number of ESH formed on 'resistant' lines of barley corresponded to the number of mature (lobed) haustoria in the host epidermal cells. The ability of the fungus to continue development after the formation of an appressorium appears to depend on the formation of a functional haustorium within the host.

Haustoria are thought to be specialized fungal structures for the uptake and transport of nutrients from the plant to the external mycelium (10, 11, 72). Fungus development on the leaf surface ceases after the destruction of the haustorium (10). Exogenously supplied nutrients, however, supported fungal development for a brief period in the absence of a haustorium. This may indicate an ability of the mycelium to directly absorb a limited amount of nutrients.

Electron microscopic studies have shown that the haustorial wall of various pathogens is separated from the host cytoplasm by a sac-like membrane (4, 16, 42, 51). The cytoplasm of the host and parasite

appear never to come into direct contact and materials transported from the host to the developing fungus must cross both the sac-like haustorial sheath and the haustorial membrane. Very little is known concerning the transfer of materials from the host cell to the haustorium. Recently developed techniques have made possible: 1) the isolation of intact haustoria of E. graminis f. sp. hordei from barley (14), and 2) the removal of the host cytoplasm from an infected cell and replacement with nutrient solutions (10). These techniques may prove useful for studies of the movement of metabolites into the haustorium.

The effects of various experimental treatments upon mildew development have been studied by observing final infection types. Quantitating the effects of various treatments during primary infection has the advantage of precisely measuring the alteration of parasite development shortly after the treatment. The effect of ultraviolet (UV) light upon fungus development has been studied by measuring the percentage of the parasite population inhibited during primary infection. Inhibition of mildew development by UV light depended upon both UV intensity and the time after inoculation that the treatment was given (44, 45). The fungus was more sensitive to inhibition by UV during 6-12 hr after inoculation than during the 14-20 hr period. The difference was correlated with the appearance of a nucleus in the haustorium where it presumably was

more protected from the UV radiation. Similar studies of the effects of various other environmental treatments were possible by observing the development of ESH as a measure of the effects of the treatments on parasite development (46, 48).

The effects of the powdery mildew disease on the host plant appear to be diverse and numerous. Changes in respiratory rates, alteration of the enzyme levels in the plant tissues, and the appearance of new isozymes have been reported (2, 29, 59). The activity of many enzymes normally present in the healthy plant were altered as a result of infection by powdery mildew (36). One criticism of many of these studies is that the analyses in most cases were made as late as 7-12 days after inoculation. The data may simply reflect death or physical disruption of the host cells. This criticism appears to be valid since similar changes can be observed in non-infected senescing wheat leaves (18). More data are needed concerning the physiological changes occurring during early infection, when compatibility or incompatibility is determined.

The use of radioactive tracers in studying host-parasite relationships has made possible the detection of very small changes during early disease development. Mount (46) used $^{32}PO_4$ and $^{35}SO_4$ to study the transfer of materials from wheat to E. graminis f. sp. tritici during primary infection. The amount of ^{32}P and ^{35}S transfered to the fungus was correlated with the morphological

development of haustoria in the host cells.

Radioautography of bean leaves inoculated with either Uromyces phaseoli, Erysiphe polygoni, or Puccinia helianthi demonstrated that ^{35}S derived from $H_2{}^{35}S$ preferentially accumulated around the sites of infection (71). Shaw (60) observed similar accumulations of ^{32}P-phosphate and ^{14}C-sugars around the infection sites of Puccinia graminis and Erysiphe graminis on wheat and barley. These accumulations appeared to be in the mesophyll cells and not in the fungus mycelium. Less $^{14}CO_2$ fixation was observed around the parasitized areas of the leaf. Other workers (23, 28, 30, 66) have shown that radioactive labels, applied to an uninoculated portion of the leaf, preferentially accumulate in infected areas even before macroscopic fungus development is evident. It appears that one of the immediate effects of disease development is the alteration of metabolite distribution within the plant.

The nature of the systems which determine 'resistance' or 'susceptibility' to the powdery mildew disease is a matter of conjecture. Biochemical studies are difficult since they involve the metabolic systems of two inseparable organisms which are both changing continuously. Many changes in infected plants can be observed by utilizing various chemical or biochemical assays, but the cause and effect relationship of these alterations as determinants of disease development is unclear. There are a few diseases for

which causal factors are known. The 'blight' of oats is incited by Helminthosporium victoriae which produces a host-specific toxin necessary for disease production (52). The toxin affects only varieties of oats possessing the Vb gene which is thought to code for a receptor site for the toxin molecule (57, 69). 'Resistance' to the 'onion smudge' disease has been determined to be due to the presence of toxic compounds in the outer scales of colored onion varieties which inhibit the development of Colletotricum circinans, the incitant of the disease (67). 'Resistance' to powdery mildew has been studied physiologically and biochemically, but no clear cut determinants to 'resistance' of the plant or 'pathogenicity' of the fungus have been found.

Aqueous extracts of E. graminis conidia have been observed to produce disease-like symptoms (9). However, no specificity of these extracts was demonstrated and other substances, such as yeast extract, were shown to produce similar results. Many toxic compounds have been isolated from 'resistant' plants (64), but again they lack sufficient specificity and have not been shown to segregate with the specific genes conditioning incompatibility.

The terms 'resistance' and 'susceptibility' are frequently used loosely, in an agronomic sense, and can be very misleading. A plant pathologist might view the lack of disease development after inoculation as 'resistance' of the plant, while a mycologist might

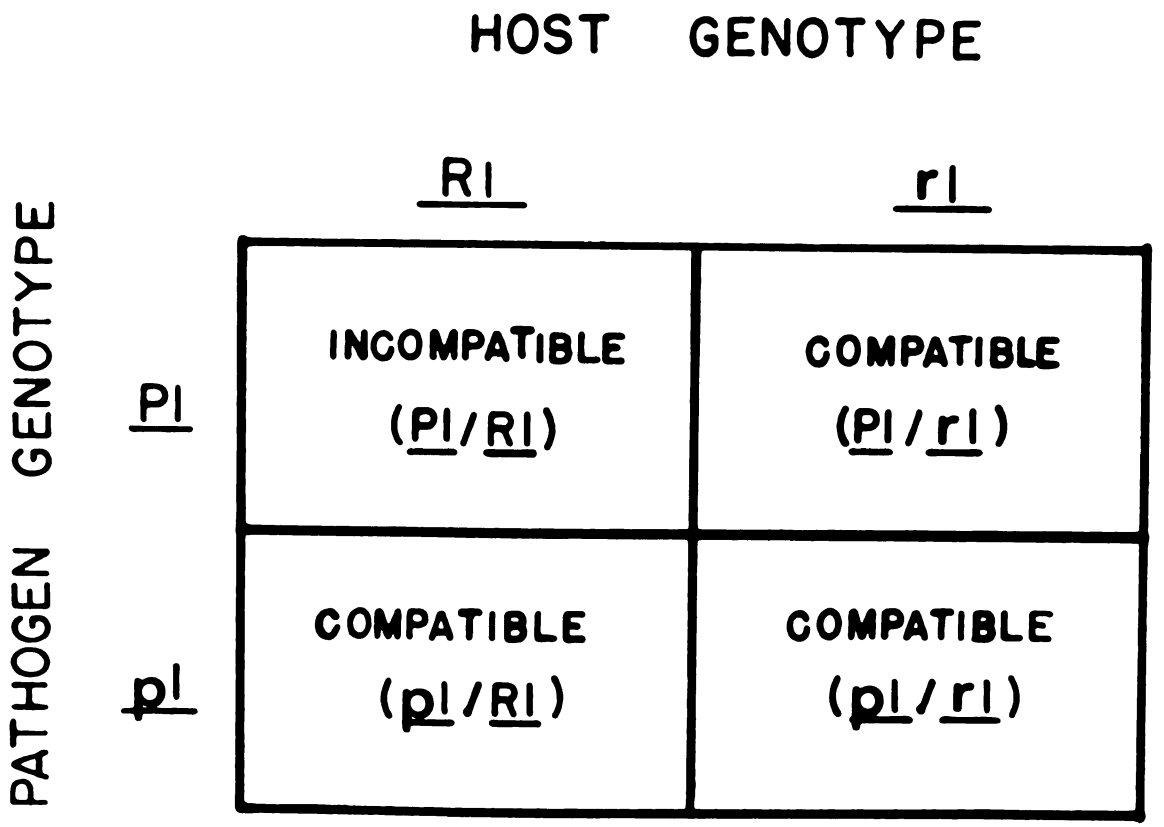
view the same phenomenon as non-pathogenicity or avirulence of the fungus. The production of a disease depends on the genotype of both the host and pathogen; a very specific and defined terminology must be developed to emphasize this fact. The following terminology has been suggested by various people (13, 19, 34). 'Resistance' and 'susceptibility' are used to describe the reaction of a particular cultivar to infection by a particular strain of the pathogen. The terms virulence and pathogenicity are both used to describe the ability of an organism to incite disease; however, pathogenicity is a general attribute of all plant pathogens, while virulence (p) and avirulence (P) defines the compatibility of a particular strain of the pathogen in relation to a particular host genotype. With most diseases studied to date, resistance (R) and avirulence (P) are dominant and virulence (p) and susceptibility (r) are recessive (19, 34).

The combination of host and parasite (the 'aegricorpus' or 'sick body') (34) is the observable manifestation of disease and is described by an infection type scale ranging from low to high mildew development. 'Resistance' and 'susceptibility', therefore, refer solely to alternate genes for reaction in the plant, while compatibility and incompatibility describe the resulting infection type with particular parasite/host combinations. The terms 'compatible' and 'incompatible' have been suggested to describe various parasite/host combinations (17). This terminology takes into consideration the

genes present in both the host and pathogen that are involved in affecting the progress of disease development.

Flor (20) observed that the ability of Melampsora lini to grow and produce disease symptoms on flax lines containing genes for incompatibility was determined by specific corresponding genes in the pathogen. The existence of one gene in the pathogen for each gene in the host led to the development of the gene-for-gene hypothesis (21, 49). The concept has been found to apply to many other host-parasite systems (43, 50, 54). The gene-for-gene concept simply states that each R gene for incompatibility in the plant interacts with a specific corresponding P gene in the pathogen to determine mildew development or parasite/host compatibility (19, 20, 49). The interaction of pairs of corresponding genes as specific pathogen/host genotypes leads to a single phenotypic expression by which the presence or absence of the corresponding gene in either organism may be recognized (19). The interaction of a single corresponding pair of genes in the plant and in the fungus is diagrammed in Figure 2. Incompatibility, or a low infection type, is specified only when the corresponding parasite/host genotype contains at least one P gene in the fungus and the corresponding R gene in the plant (P1/R1). Compatibility or high infection type and unrestricted disease development is specified with the remaining P1/r1, p1/R1, and p1/r1 parasite/host genotypes (34, 56).

Figure 2. The four possible parasite/host genotypes involving a single locus governing compatibility in the plant and the pathogen. R1 and r1 are the alternate alleles in the plant and P1 and p1 are the alternate alleles in the pathogen corresponding to the R1 gene in the plant. P1/R1 genotypes specify incompatibility. P1/r1, p1/R1, and p1/r1 genotypes specify compatibility.


HOST GENOTYPE
R1
r1
PATHOGEN GENOTYPE
P1
p1
INCOMPATIBLE
(P1/R1)
COMPATIBLE
(P1/r1)
COMPATIBLE
(p1/R1)
COMPATIBLE
(p1/r1)

Person (49) has suggested that these complementary relationships have evolved from specific selection pressures which initially favored host plants which could escape disease and secondly, specific pathogen strains which could develop in the presence of the R gene. Studies of pathogenic variability have shown that many pathogens are capable of rapid genetic adaptation to newly introduced host lines possessing R genes for incompatibility (52, 62, 63). The discovery of the gene-for-gene relationship controlling parasite/host compatibility has applicability to defined studies of disease development. Rowell et al (56) have proposed a biological test termed the 'quadratic check' to study the physiological and biochemical effects of disease. The combination of various host genotypes and strains of the pathogen (similar to Figure 2) could be used to give a 4-way (quadratic) check in which the molecular phenomena should parallel the biological phenomena. This test should be valuable for studying many diseases including the powdery mildew disease of cereals which is governed by a gene-for-gene relationship (43, 54).

The study of the bases of parasite/host incompatibility has involved the determination of the types of possible incompatible responses. There is evidence that the different incompatible responses which can be observed are determined by the specific interaction of particular Px/RxRx genotypes. Many workers have observed phenotypically different types of responses to infection by

E. graminis. Parasite development was affected at various stages of disease development (12, 24, 35, 61). A similar wide range of incompatible phenotypes was observed with various rust diseases. Cells of 'resistant' safflower collapsed when invaded by a haustorium (73). No difference in the development of Puccinia sorghi on near-isogenic corn lines was observed with compatible parasite/host combinations. With incompatible genotypes, reduced numbers of haustoria, encapsulation of haustoria in the cells, and fungal lysis were observed (27). The rapidity of cell collapse has been suggested as the basis for resistance to disease development (70). However, cell collapse appears to be only one of the many possible responses which can be observed. The necrotic response of many varieties is absent at elevated temperatures but the compatibility of the parasite/host relationship is not increased (1, 22).

Parasite/host incompatibility has been studied on a quantitative basis during primary infection of wheat and barley by E. graminis. The production of secondary hyphal initials was not affected by major genes which alter mildew development on barley (40), but formation of ESH was affected by these same genes (38). The percentage of the parasite population which established a functional relationship with the host was estimated by the number of parasitic units which produced ESH. The genes in barley affecting mildew development were observed to inhibit the development of only a

proportion of the parasite population during primary infection. The inhibition of final development of mildew (infection type) was similar to the inhibition of the formation of ESH during primary infection (38, 40). The utilization of this defined host-parasite-environment system has allowed the study of fungal morphogenesis and incompatibility responses previously described only qualitatively.

MATERIALS AND METHODS

Production of inoculum--Two strains of Erysiphe graminis f. sp. tritici were used. Michigan strain-1 (MS-1) and Michigan strain-76 (MS-76) were collected in Michigan and maintained on Triticum aestivum L. 'Little Club' wheat in different growth chambers. Each of these strains was twice purified by isolating and increasing single pustules formed on lightly inoculated Little Club wheat. The strains were checked periodically for uniformity by scoring infection type on a set of inoculated differential host lines (Table 1).

Little Club wheat was grown in 4-in. pots in the greenhouse and inoculated with E. graminis f. sp. tritici at 6-7 days after planting. Stock cultures of the mildew fungus were maintained by inoculating each day by dusting with 7-day-old conidia. The stock cultures were 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). Light period, 15 hr/day.
2. Temperature--18±1C during the light period and 17±1C during darkness.
3. Relative humidity--80±5% during the light period and 95±5% during darkness.

Table 1. Near-isogenic lines with single Pm genes for incompatibility to mildew development.

Near-isogenic line	Designation of Gene Involved	Former Gene Symbol	C.I.[1] Number
Axminster X Cc^{8}[2]	Pm1(Axminster)	Ml_t	14114
AsII X Cc^{8}	Pm1(AsII)	Ml_t	14116
Norka X Cc^{8}	Pm1(Norka)	---	14117
C.I. 13836 X Cc^{8}	Pm1(C.I. 13836)	Ml_t	14115
Ulka X Cc^{8}	Pm2(Ulka)	Ml_u	14118
C.I. 12632 X Cc^{8}	Pm2(C.I. 12632)	Ml_x	14119
Asosan X Cc^{8}	Pm3a	Ml_a	14120
Chul X Cc^{8}	Pm3b	Ml_c	14121
Sonora X Cc^{8}	Pm3c	Ml_s	14122
Khapli X Cc^{8}	Pm4(Khapli)	---	14123
Yuma X Cc^{8}	Pm4(Yuma)	---	14124
Unknown X Cc^{8}	Pm(Unknown)	---	-----
Mich. Amber X Cc^{8}	Pm(Mich. Amber)	---	14033
Chancellor(Cc)	pmx	---	12333

(1) Cereal Investigations accession number.
(2) Cc^{8} refers to the 8 backcrosses to the cultivar Chancellor

4. Continuous air circulation.

Conidia produced on the 6th day after inoculation were used in all experiments.

Methods of inoculation--The 'rolling method' of inoculation (48) was used in all experiments studying the development of mildew during primary infection. Conidia were dusted onto a clean glass slide and transferred to a single wheat plant with a cotton swab. Only the lower (abaxial) leaf surface of each plant was inoculated. The progress of the infection process is similar on either side of the leaf (39), but microscopic observations and the removal of the host epidermis are much easier on the lower side of the leaf. A uniform distribution of single conidia ranging from 100-300/cm length of leaf was obtained by this method. Only single separated parasitic units were counted at each observation to eliminate the possibility of inhibition due to crowding (39).

With experiments studying the transfer of ^{35}S-radioisotope from the host to the parasite, plants were inoculated by lightly dusting conidia onto the plants. This method was used because inoculation of many plants by the 'rolling method' required too much time. The development of the fungus during primary infection was the same with both methods, but the efficiency of infection was reduced.

Lines of wheat studied--The cultivars Chancellor and Little Club, which contain no known major genes affecting mildew development,

were used as the standards to which mildew development on other lines was compared. The thirteen backcross-derived lines of wheat (Table 1) which contained Pm genes affecting mildew development were obtained from L. W. Briggle. The data concerning the source of these genes, the results of various genetic tests, and their identification have been published (5, 6, 7, 8, 41, 55). Chancellor, a soft red winter wheat, provided a common genetic background for each of these lines.

The plants for experimental studies were grown in 2-in. pots in the greenhouse. Shortly after emergence (about 3 days after planting), seedlings were transferred to the following standardized conditions:

1. Light--2600 ft-c (2400 ft-c from white VHO-fluorescent tubes and 200 ft-c from incandescent bulbs) over a 15 hour day.
2. Temperature--20±1C during the light period and 18±1C during darkness.
3. Relative humidity, approximately 65%.

The plants grown under these conditions were inoculated approximately 6 days after planting. Plants grown solely at the greenhouse (especially during the hot summer months) were less uniform and possessed tall spindly leaves unsuitable for inoculation and microscopic observation.

Gene symbols and designation of parasite/host genotypes--
The Pm symbols used to designate the R genes conditioning reaction in the host lines (Table 1) follow Briggle's suggested terminology (7, 8).

The genes determined to be at distinct loci have been designated Pm1, Pm2, etc. Genes at the same locus thought to be either allelic or very closely linked are followed by the letters a, b, c, ... when positive evidence for their distinctness is available (eg. Pm3a, Pm3b, Pm3c). The remaining Pm genes at particular loci, derived from different sources, but for which positive evidence of distinctness is not available, are followed by the source of the gene (eg. Pm1(Axminster), Pm1(AsII), etc.). The alternate alleles for each Pm gene will be referred to by their respective recessive designations (pm1, pm2, etc.). The genotypes of the host will be written as Pmx rather than writing the complete genotype of the diploid as Pmx Pmx since we are dealing with reasonably homozygous host lines containing dominant genes.

The symbols for genes in the pathogen conditioning pathogenicity follow the terminology suggested by Loegering (34). The P(Pmx) or p(Pmx) genes for 'avirulence' and 'virulence' in the pathogen, corresponding to the various host loci, will be shortened in this manuscript to Px or px. The symbols for 'avirulence' and 'virulence' will be followed by the corresponding host locus and allele designation where applicable (eg. P(Pm1) = P1, P(Pm3a) = P3a, etc.).

Designation of particular parasite/host combinations studied will utilize the preceeding gene symbolism to refer to the specific genes conditioning the interaction (eg. P1/Pm1 = incompatible, or

pl/Pml = compatible). This abbreviated parasite/host symbolism is possible since each host line contains only a single Pm gene for incompatibility. The remaining host loci, with a Pml line for example, are all for compatibility (ie. Pml pm2 pm3a pm3b pm3c.....).

Environmental conditions--The control of the environmental conditions during experiments was obtained using Sherer-Gillett (Model CEL 512-37 and Model CEL 25-7) growth chambers. The conditions necessary for a high efficiency of infection and synchronous growth of the parasite population have been published and are used in all of the following experiments (37, 47, 48). Briefly, they are the following:

1. From 0 to 1 hr, inoculated plants were maintained in darkness at 18±1C and at approximately 100% relative humidity (RH).
2. From 1 to 6 hr, inoculated plants were maintained in 260 ft-c of light (200 ft-c from white VHO-fluorescent tubes and 60 ft-c from incandescent bulbs), 22±1C, and RH of 65±5%.
3. From 6 to 20 hr, temperature and RH were the same as 2, above, but with darkness.
4. From 20 to 30 hr, conditions were the same as in 2.

In experiments to determine the environmental conditions necessary for increasing the synchrony of formation of secondary appressoria, the environmental conditions in the 20-30 hr period were varied. Utilizing these environmental conditions, 80-90% of the applied conidia

proceed through the various stages of development (ie. germination, formation of appressorial initials, formation of mature appressoria, and production of secondary hyphal initials and elongating secondary hyphae).

Changes in temperature and RH during experiments were monitored with a hygrothermograph calibrated with a sling psychrometer and wet and dry bulb thermometers. Light intensity was measured at the distance of the plants from the lights with a Weston Model 756 light meter.

Examination of fungal structures--Observations of the percentage of the parasite population in each stage of development were performed by direct microscopic counts using the light microscope (160X). At various times after inoculation, the development of the parasitic units on 1 to 2 cm leaf sections (excluding the 1 cm tip section) was examined, the percentage of parasitic units in each stage of development recorded, and the leaf discarded. Approximately 100-125 parasitic units were counted at each observation.

^{35}S labelling of wheat leaves--Wheat leaves were allowed to take up an ^{35}S solution for 5 hr periods starting at different times after inoculation with E. graminis f. sp. tritici. The 5 hr uptake period was used to expose the developing fungus to approximately equal amounts of ^{35}S in the wheat leaf. The amount of transfer to the fungus with the 5 hr 'pulse', therefore, should reflect the development of the

host-parasite relationship and not the exposure to varying amounts of ^{35}S. A 5 hr uptake period provides more reproducible data than shorter labelling times. The initial rapid rate of appearance of radioactivity in the leaf was decreased by 4 hr and the leaves are usually uniformly labelled by 5 hr (47).

Inoculated wheat leaves were cut at the base using a razor blade dipped in water (the water on the cutting edge is necessary to prevent the formation of air bubbles in the xylem tubes of the cut leaf). The leaves were placed in small vials (25 mm x 5 mm I.D.) containing 0.1 ml of a 100 μc/ml solution of sulfur-35 ($H_2{}^{35}SO_4$) in 0.1M phosphate buffer (pH 6.9-7.0). Frequently, 2 or 3 cut leaves were placed in a single vial. Leaves were allowed to take up the radioactive solution for 5 hr periods during the infection process prior to the times that the fungus was removed. Inoculated plants and cut leaves were maintained in growth chambers during the experimental period under the optimum conditions for primary infection described earlier. The darkness period, however, was extended for two hours (6-22 hr) since light during the labeling period resulted in a high percentage of shrivelled leaves after application of parlodion. The 6-22 hr darkness period alters the percentage of ESH production only slightly (47). The shrivelling of leaves at the 24th and 26th hr time of fungus removal was greatly reduced by placing the cut leaves at 100% RH and darkness for

10 minutes at the end of the labeling period prior to parlodion application.

Radioactivity was determined using a Packard Model 3003 Tri-Carb Liquid Scintillation Spectrometer. Scintillation fluid was a mixture of 5 g/liter PPO (2, 5 diphenyloxazole) and 0.1 g/liter POPOP (1, 4-bis-(2-(5-phenyloxazolyl))-benzene) in toluene.

Detection of ^{35}S transferred to E. graminis using parlodion-- The method of using parlodion for removal of ectoparasitic fungi was described by Delp (15) and modified by Mount and Ellingboe (40). A 1.9% solution of parlodion (celloidin) was prepared by dissolving pieces of purified parlodion in a 60:40 v/v mixture of ethyl ether and 95% ethyl alcohol. The inoculated, radioactively labelled wheat leaves were cut, placed lower surface up in plastic petri plates, and the solution applied in a thin layer on the leaf surface with a Pasteur pipette. Care was taken to avoid applying parlodion to either the cut end of the leaf or to the leaf tip. The parlodion dried in minutes and contained the parasitic units embedded in a thin film. The parlodion strips were removed from the plant with forceps. Four strips were placed in each scintillation vial, dissolved in 0.2 ml of 60:40 ether:alcohol, and the vials filled with 15 ml of scintillation fluid. The samples were allowed to stand at least 24 hr at room temperature before counts were taken to permit the parlodion to dissolve.

Sample size and replication of experiments--The experiments characterizing the development of E. graminis f. sp. tritici on the host lines with different Pm genes were repeated a minimum of four times on different weeks. The studies to determine the conditions necessary for increasing the synchrony of formation of secondary appressoria were repeated 4 to 6 times on different days. Data presented in the Figures represent the mean of these observations. The number of parasite units counted at each observation varied from 80-125 depending upon the density of inoculation on each plant. The experiments were repeated on different days using different plants to eliminate small variations in either the plants or the inoculum.

The experiments involving the transfer of sulfur-35 from the host to the parasite were performed on a minimum of 5 different days. The average of at least 15 separate vials (4 parlodion strips/vial or 60 different plants) was plotted. Occasional vials containing parlodion strips which had been contaminated during removal of the parasite from the plant were discarded. Contamination occurs when the parlodion solution flows over the cut end of the leaf and is not observed during removal of the parlodion strip from the plant. Distinguishing these contaminated vials was not a problem since they contained greater than 20 times the mean counts per minute (CPM) for that particular hour.

RESULTS

Fungus Development During Primary Infection

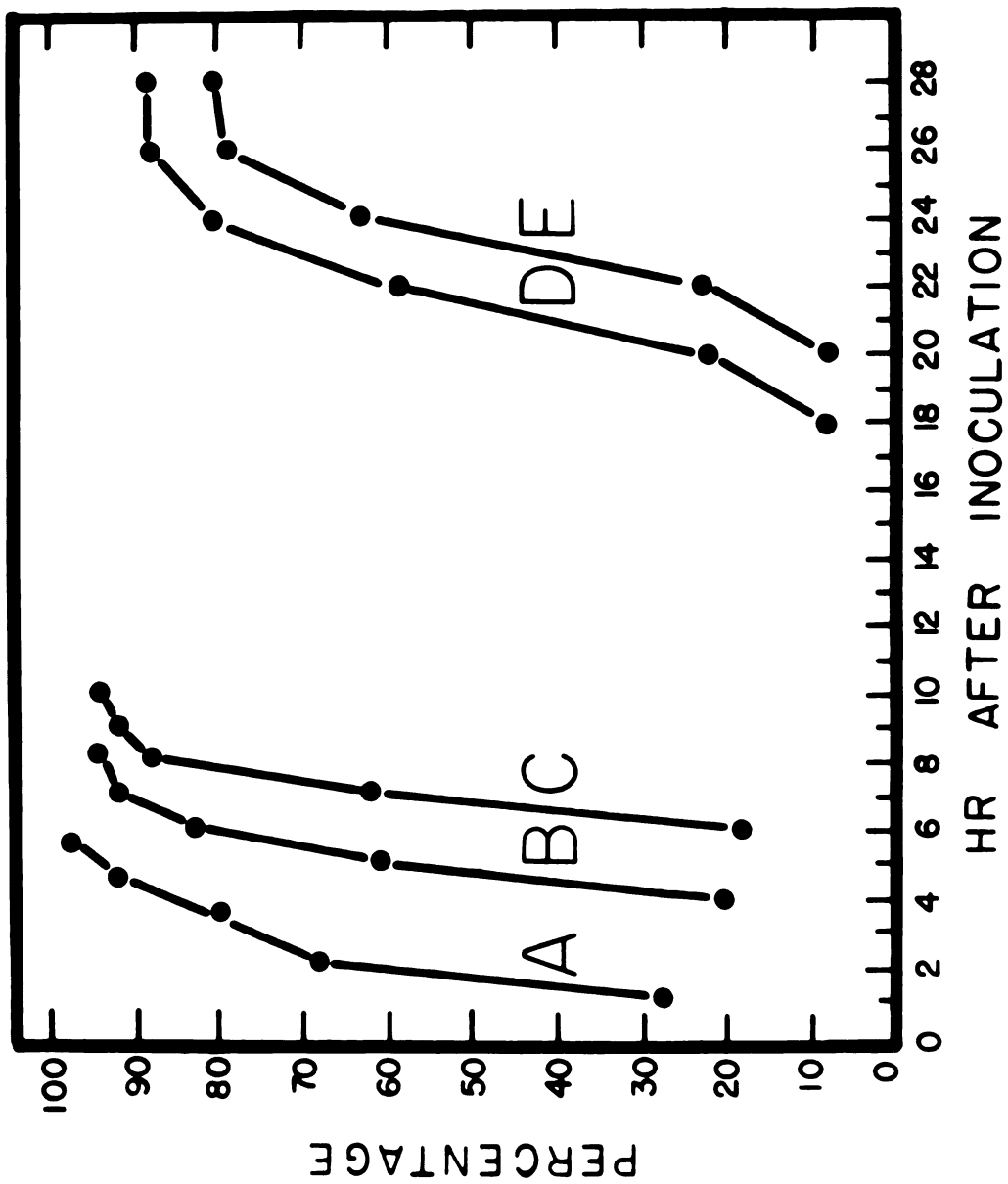
Replication of Previous Work

The development of E. graminis f. sp. tritici during germination, formation of appressorial initials, formation of mature appressoria, and the production of elongating secondary hyphae, previously studied by other workers (39, 47, 48), was re-examined. The optimum environmental conditions for these stages of development have been reported and the development of the parasite population presented in Figure 3 is in close agreement to the earlier data. With the optimum environmental conditions utilized, approximately 80-85% of the applied conidia produced elongating secondary hyphae (ESH) by 26 hr after inoculation. The attainment of this stage of development by the fungus reflects production of mature, lobed haustoria in the host cells and was used in later experiments as the criterion for the formation of a functional parasitic relationship (38, 39, 40).

Effect of the Environment on the Formation of Secondary Appressoria

Environmental conditions are known to be important for the development of ESH (47), but little was known concerning the environmental conditions necessary for secondary infection. I was interested

Figure 3. Development of Erysiphe graminis f. sp. tritici during primary infection. (A) germination, (B) formation of appressorial initials, (C) formation of mature appressoria, (D) formation of secondary hyphal initials, and (E) formation of elongating secondary hyphae (ESH).


PERCENTAGE
100
90
80
70
60
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
HR AFTER INOCULATION
A
B
C
D
E

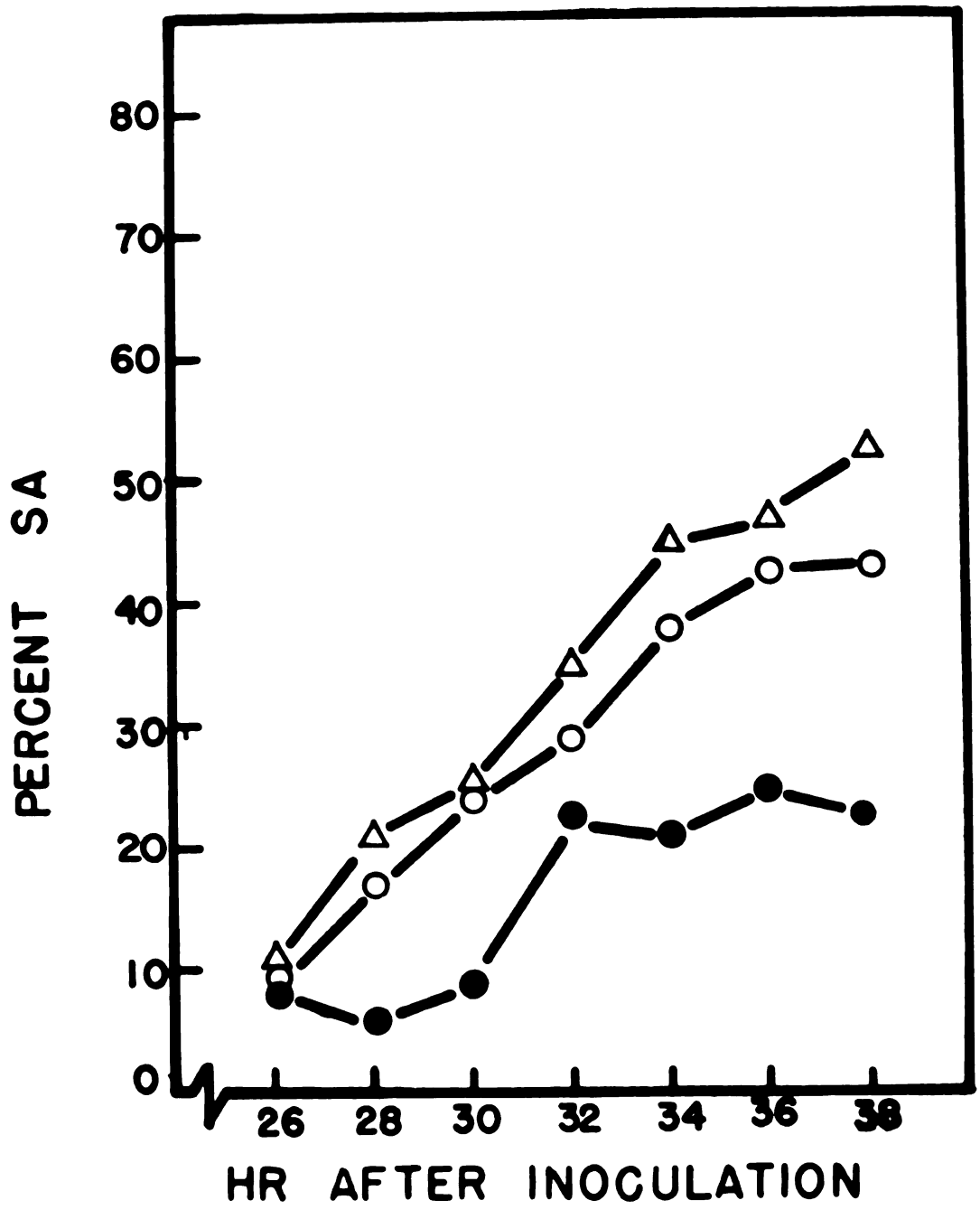
to learn whether a high percentage of the parasite units with ESH were capable of initiating secondary infections. Secondary appressoria (SA) form by the differentiation of the elongating secondary hyphae and represent the infection of additional host cells by each parasitic unit. The effects of the environment upon the development of secondary appressoria were examined.

Production of secondary appressoria with the optimum environmental conditions determined for formation of ESH at 22C is shown in Figure 4. With these conditions (240 ft-c, 22C, 65% RH), approximately 40% of the applied conidia formed secondary appressoria and initiated secondary infections by 38 hr after inoculation.

Effect of temperature on SA formation--Temperatures of 17C and 27C starting at 26 hr after inoculation were compared for their effect on SA formation (Figure 4). The 26th hr was chosen for changing the temperature from 22C since by this time 80-85% of the parasite population had formed ESH. More SA formed at 27C than at 22C and less SA formed at 17C than at 22C or 27C. Other temperatures above 27C and below 17C decreased the percentage of SA formed and were not studied further. The slight increase of SA formation at 27C was the first indication that the efficiency of secondary infections could be increased by environmental treatments.

The time that the exposure to 27C was started also affected the efficiency of SA production. A greater percentage of the population

Figure 4. Effect of various temperatures, beginning 26 hr after inoculation, upon the formation of secondary appressoria (SA). 22C standard temperature (control) (○——○), 17C (●——●), and 27C (△——△).


80
70
60
50
40
30
20
10
0
PERCENT SA
26
28
30
32
34
36
38
HR AFTER INOCULATION

formed SA when 27C was started at 26 hr, than at either 20 or 30 hr (Figure 5). The 26th hr chosen for changing the environmental conditions in the initial experiments appeared to be a good choice.

Effect of high light on SA formation--High light (2600 ft-c) is known to have an inhibitory effect upon the production of ESH (47), but the effect on secondary infections were unknown. The percentage of SA production increased following exposure of inoculated plants to high light during the formation of SA (Figure 6). The increase was greatest when 2600 ft-c was started at 26 hr, less when started at 30 hr, and no effect was observed when started at 20 hr in comparison to the standard ESH conditions (22C, 240 ft-c, 20-40 hr). It appeared that a change in either the temperature or light conditions at 26 hr could be used to increase the production of SA. The lack of a stimulation of SA formation with 2600 ft-c starting at 30 hr was taken as possible indication of a light sensitive portion of the secondary infection process.

Effects of light period on SA formation--The possibility of an increased sensitivity to light during the formation of SA was studied by varying the light period during the formation of secondary appressoria. A greater percentage of SA formed with 0 ft-c starting at 30 hr than with 0 ft-c starting at 26 hr or with continuous light (240 ft-c) (Figure 7). The formation of SA appeared to be affected by both light and temperature at different times after inoculation.

Figure 5. Formation of secondary appressoria (SA) following 27C temperature treatments starting at various hours after inoculation. Control (22C) (□——□), 20 hr (●——●), 26 hr (○——○), and 30 hr (●- - -●).

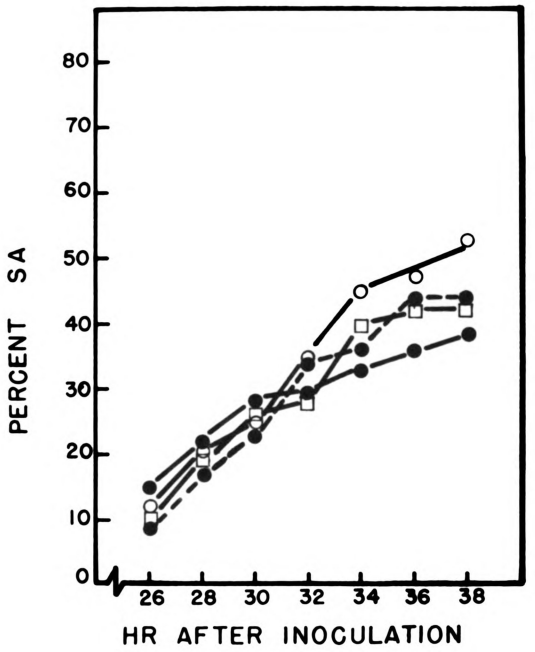

PERCENT SA
80
70
60
50
40
30
20
10
0
26
28
30
32
34
36
38
HR AFTER INOCULATION

Figure 6. Effect of high light intensity (2600 ft-c) beginning 20, 26, or 30 hr after inoculation upon the formation of secondary appressoria (SA). Control (240 ft-c) (●——●), 2600 ft-c 20 hr (○——○), 2600 ft-c 26 hr (△——△), and 2600 ft-c 30 hr (□– – –□).

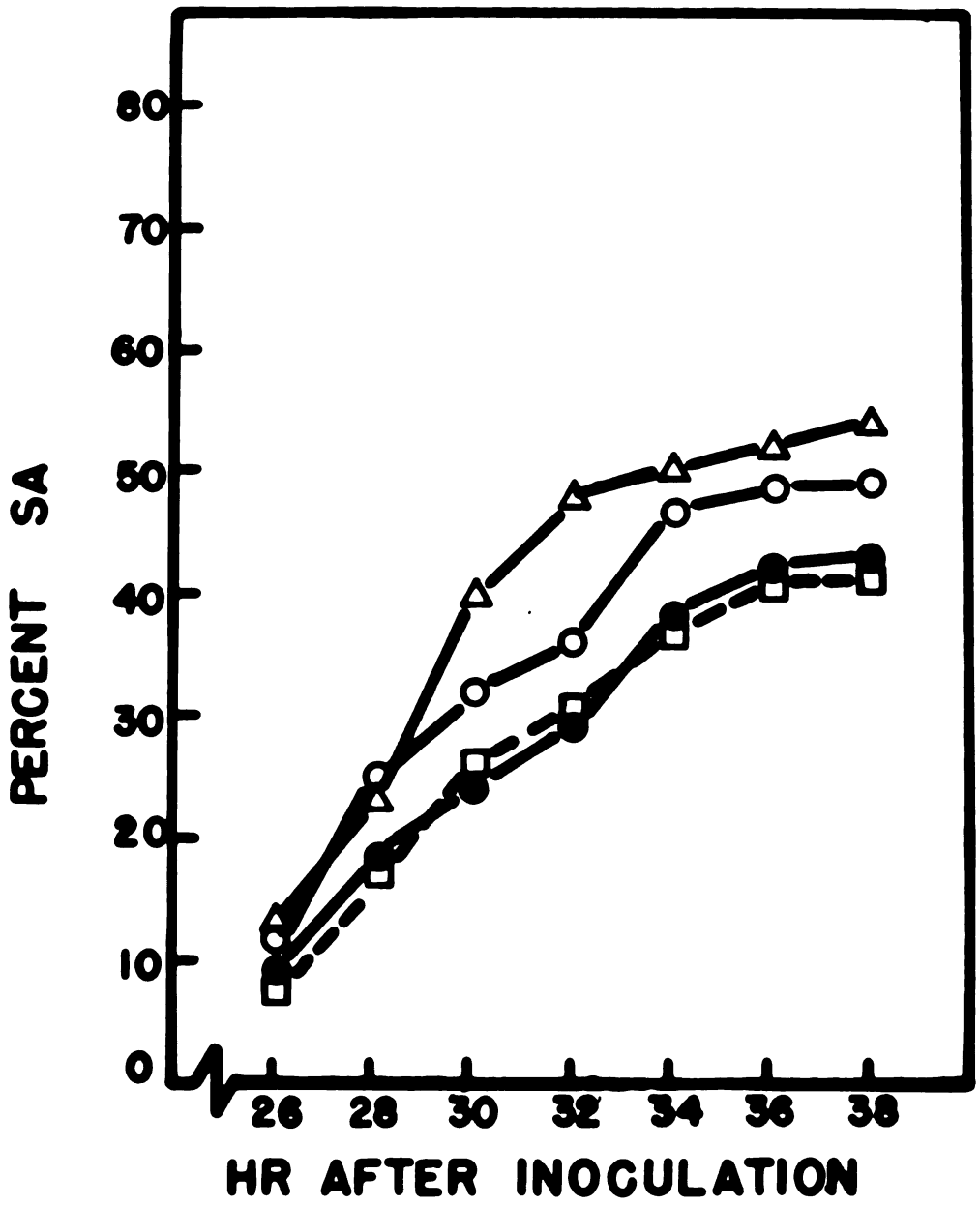
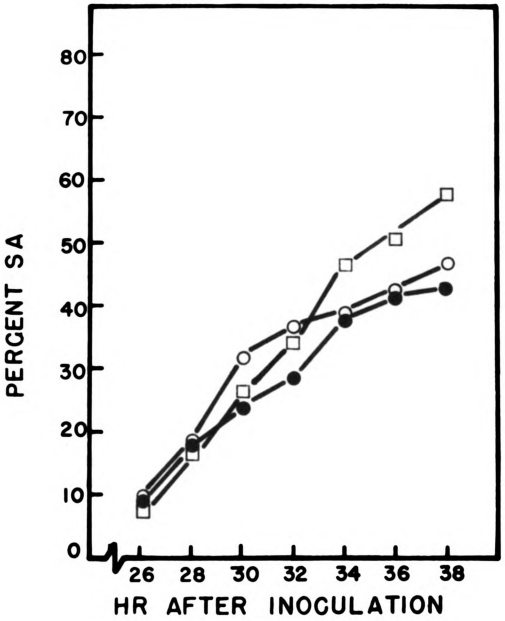

PERCENT SA
80
70
60
50
40
30
20
10
0
26
28
30
32
34
36
38
HR AFTER INOCULATION

Figure 7. Effect of duration of light period (240 ft-c) upon the formation of secondary appressoria (SA). 20-40 hr (●——●), 20-30 hr (□——□), and 20-26 hr (○——○).


PERCENT SA
80
70
60
50
40
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10
0
26
28
30
32
34
36
38
HR AFTER INOCULATION

Effect of combined high light and high temperature on SA formation--Both high light (2600 ft-c) and high temperature (27C) beginning at 26 hr increased the production of SA. Combinations of high light and high temperatures were tested to determine if the effects were additive. Combined high light and high temperature at 26 hr resulted in a greater percentage of SA formation than was obtained with either treatment alone (Figure 8). Similarly, high light at 26 hr with darkness starting at 30 hr was also somewhat additive and resulted in more SA formed in less time than with either condition alone (Figure 9). The combination of all three treatments; 2600 ft-c and 27C starting at 26 hr with 0 ft-c and 22C starting at 30 hr gave the highest efficiency and synchrony of SA production to date (Figure 10). This combination of environmental conditions increased both the efficiency and synchrony of SA production above previous values and should be suitable for additional studies of secondary infections.

Genetic Control of Mildew Development

The development of the powdery mildew disease is known to be controlled by specific genes in the host and pathogen. But only a few studies have attempted to determine when the various gene combinations interact to alter development of the pathogen (3, 27, 38, 61). Various experiments were performed to characterize the alteration

Figure 8. Effect of combined increased light (2600 ft-c) and temperature (27C) treatments, beginning 26 hr after inoculation, upon formation of secondary appressoria (SA). Control (22C, 240 ft-c) (△——△), 2600 ft-c (○——○), 27C (●——●), and 27C with 2600 ft-c (▲- - -▲).

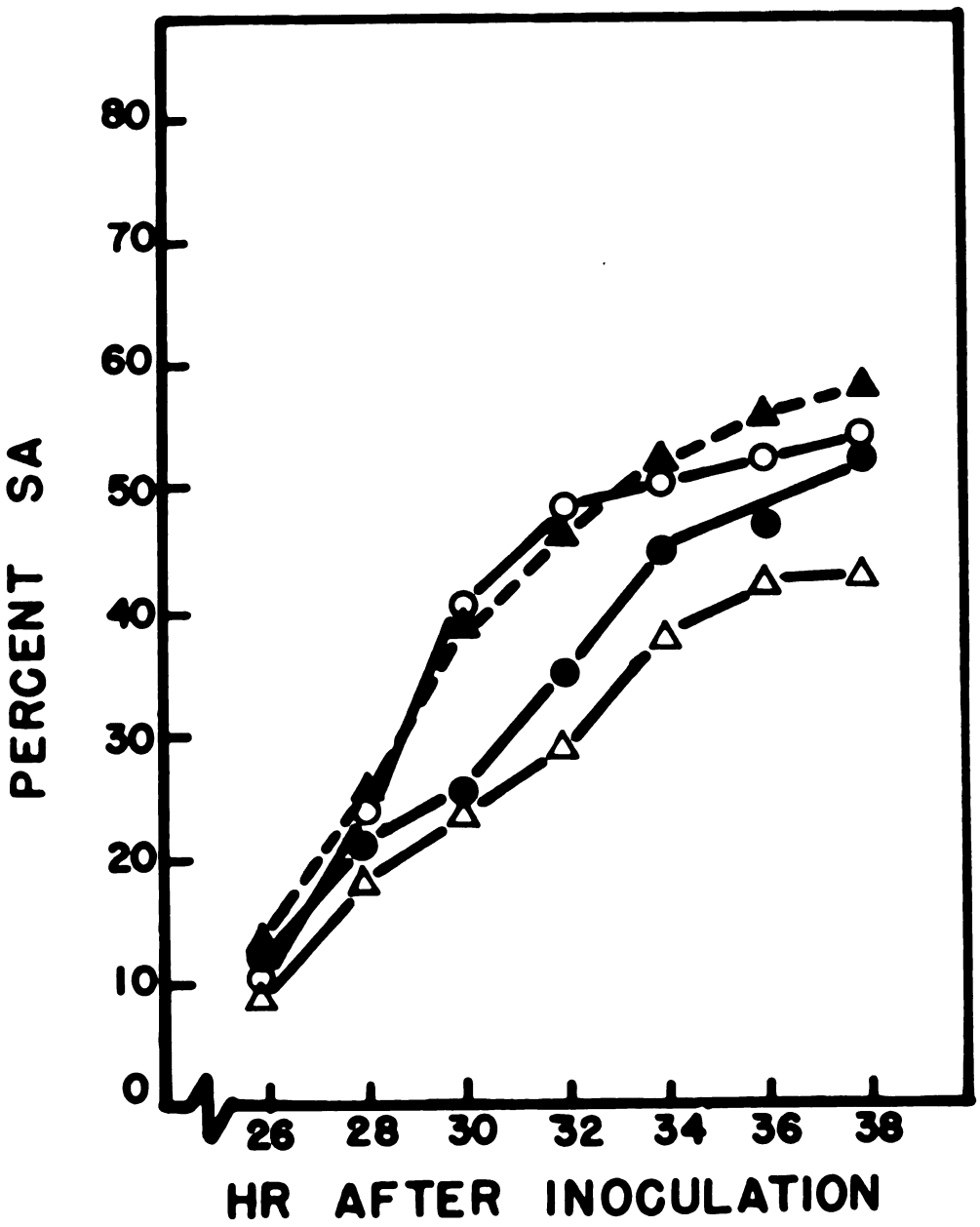

PERCENT SA
80
70
60
50
40
30
20
10
0
26
28
30
32
34
36
38
HR AFTER INOCULATION

Figure 9. Effect of combined treatments of increased light intensity (2600 ft-c) and duration of light period upon formation of secondary appressoria (SA). Control (240 ft-c 20-40 hr) (●——●), 240 ft-c 20-30 hr and darkness 30-40 hr (○——○), 2600 ft-c 26-40 hr (●- - -●), and 2600 ft-c 26-30 hr and darkness 30-40 hr (△——△).

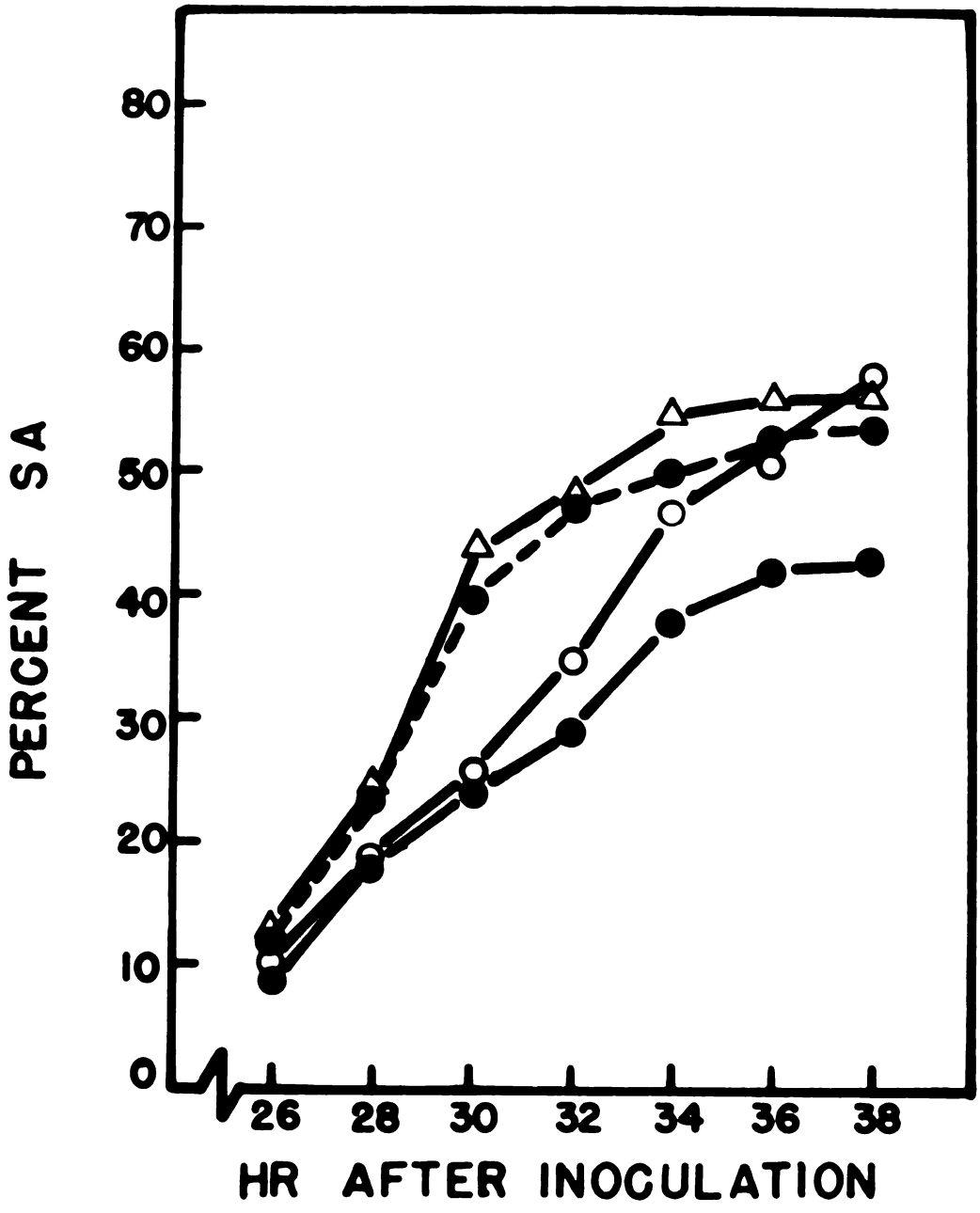
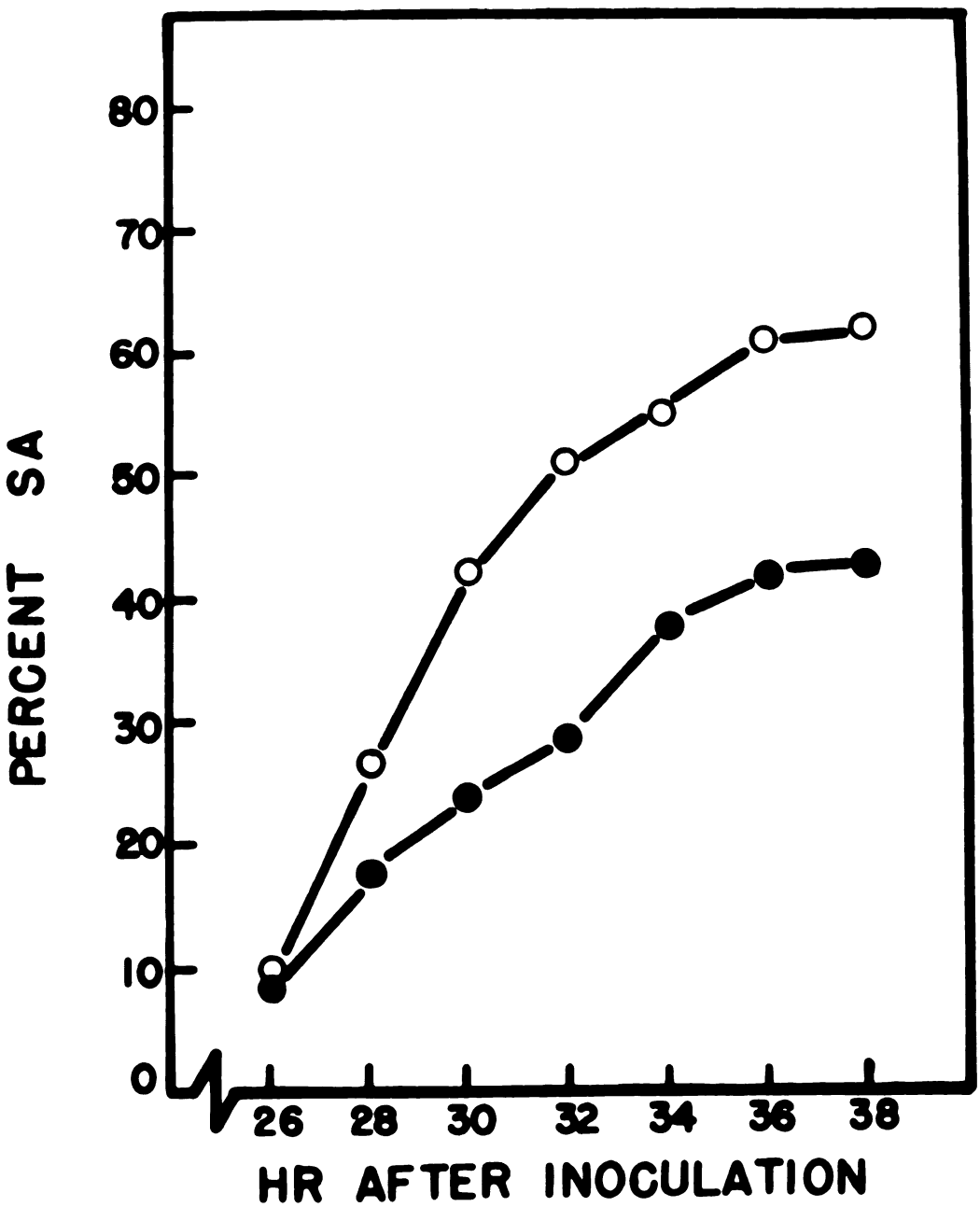

PERCENT SA
80
70
60
50
40
30
20
10
0
26
28
30
32
34
36
38
HR AFTER INOCULATION

Figure 10. Effect of combination of increased light (2600 ft-c), temperature (27C), and duration of light period upon the efficiency of formation of secondary appressoria (SA). Control (22C, 240 ft-c 20-40 hr) (●——●), and 2600 ft-c and 27C 26-30 hr with darkness and 22C 30-40 hr (○——○).


PERCENT SA
80
70
60
50
40
30
20
10
0
26
28
30
32
34
36
38
HR AFTER INOCULATION

of parasite development during primary infection with various incompatible parasite/host gene combinations. The initial studies involved the characterization of the genotype of the pathogen strains to be used in later experiments.

Genotype of Mildew Cultures MS-1 and MS-76

Culture MS-1 was a clonally propagated isolate of E. graminis f. sp. tritici used in the previously described experiments. Culture MS-76 was a second isolate selected because of its ability to develop on the host lines with a Pml gene.

The various host lines were inoculated with cultures MS-1 and MS-76 and mildew development was recorded 7 days later. The infection types produced 7 days after inoculation are presented in Table 2. An infection type lower than that observed on Chancellor was used as evidence that the gene in the fungus corresponding to the Pm gene in the host was for incompatibility (P). Based upon these data the following statements could be made: 1) Isolate MS-1 had the corresponding p(Unknown) and p(Mich. Amber) genes for virulence since this isolate developed as well on host lines with a Pm(Unknown) or Pm(Mich. Amber) gene as on Chancellor. 2) Isolate MS-76 had the corresponding pl and p(Mich. Amber) genes because it developed as well on host lines with a Pml or Pm(Mich. Amber) gene as on Chancellor. The corresponding gene to Pm3c was difficult to characterize since inhibition of mildew development with Pm3c has

Table 2. Infection types produced 7 days after inoculation of near-isogenic wheat lines with cultures MS-1 and MS-76 of *Erysiphe graminis* f. sp. *tritici*.

Near-isogenic line	Infection type[1] MS-1	MS-76
Axminster X Cc^8 (*Pm1*)	0,1	4
AsII X Cc^8 (*Pm1*)	0,1	4
Norka X Cc^8 (*Pm1*)	0,1	4
C.I. 13836 X Cc^8 (*Pm1*)	0,1	4
Ulka X Cc^8 (*Pm2*)	2	2
C.I. 12632 X Cc^8 (*Pm2*)	2	2
Asosan X Cc^8 (*Pm3a*)	3	3
Chul X Cc^8 (*Pm3b*)	3	3
Sonora X Cc^8 (*Pm3c*)	4-	4-
Khapli X Cc^8 (*Pm4*)	0,1	0,1
Yuma X Cc^8 (*Pm4*)	0,1	0,1
Unknown X Cc^8 (?)	3,4	2
Mich. Amber X Cc^8 (?)	4	4
Chancellor (Cc) (*pmx*)	4	4

[1]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 ('compatible').

been reported to be distinguishable only at the third or later leaf stage (5, 7, 55). Both strains MS-1 and MS-76 were assigned the P3c genotype, since in our study there was both a slight but noticeable decrease of mildew development on seedling leaves and a significant reduction of mildew on older leaves. The remaining wheat lines were characterized by decreased mildew development. The corresponding genotypes of the two mildew cultures, based on mildew development on the host lines with various Pm genes, appeared to be the following:

MS-1 Pl P2 P3a P3b P3c P4 p(Unknown) p(Mich. Amber)

MS-76 pl P2 P3a P3b P3c P4 P(Unknown) p(Mich. Amber)

Effect of Different Parasite/Host Genotypes on Mildew Development During Primary Infection

Time of fungus inhibition--The development of appressoria with various incompatible combinations (Px/Pmx) was essentially identical to development in the absence of specific Pm genes (Figure 11). Neither the synchrony of development nor morphogenesis of the parasite was affected prior to the time when penetration of the epidermal cell is thought to occur (39, 45). Later stages of parasite development were altered in a specific manner depending upon the particular gene combinations which interacted.

Compatible parasite/host combinations--The formation of ESH appeared to be essentially identical on the cultivars Little Club and Chancellor (Figure 12). The optimum environmental conditions

Figure 11. Similar development of appressoria during primary infection with either compatible or incompatible parasite/host combinations. MS-1/Chancellor (compatible control) (▲---▲), P1/Pm1(Axminster) (●——●), P2/Pm2(Ulka) (○——○), P3a/Pm3a (△——△), and P4/Pm4(Khapli) (●---●).

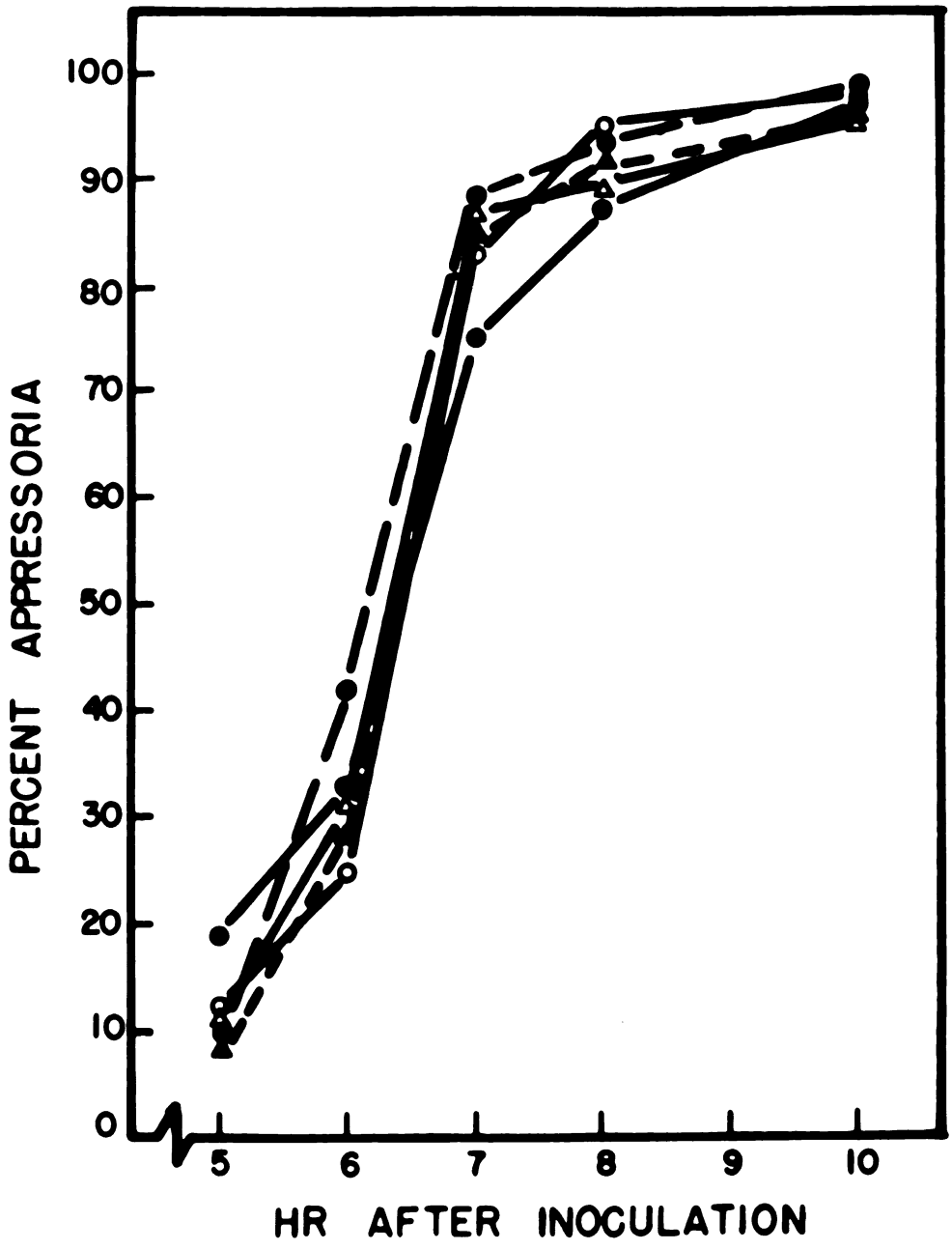
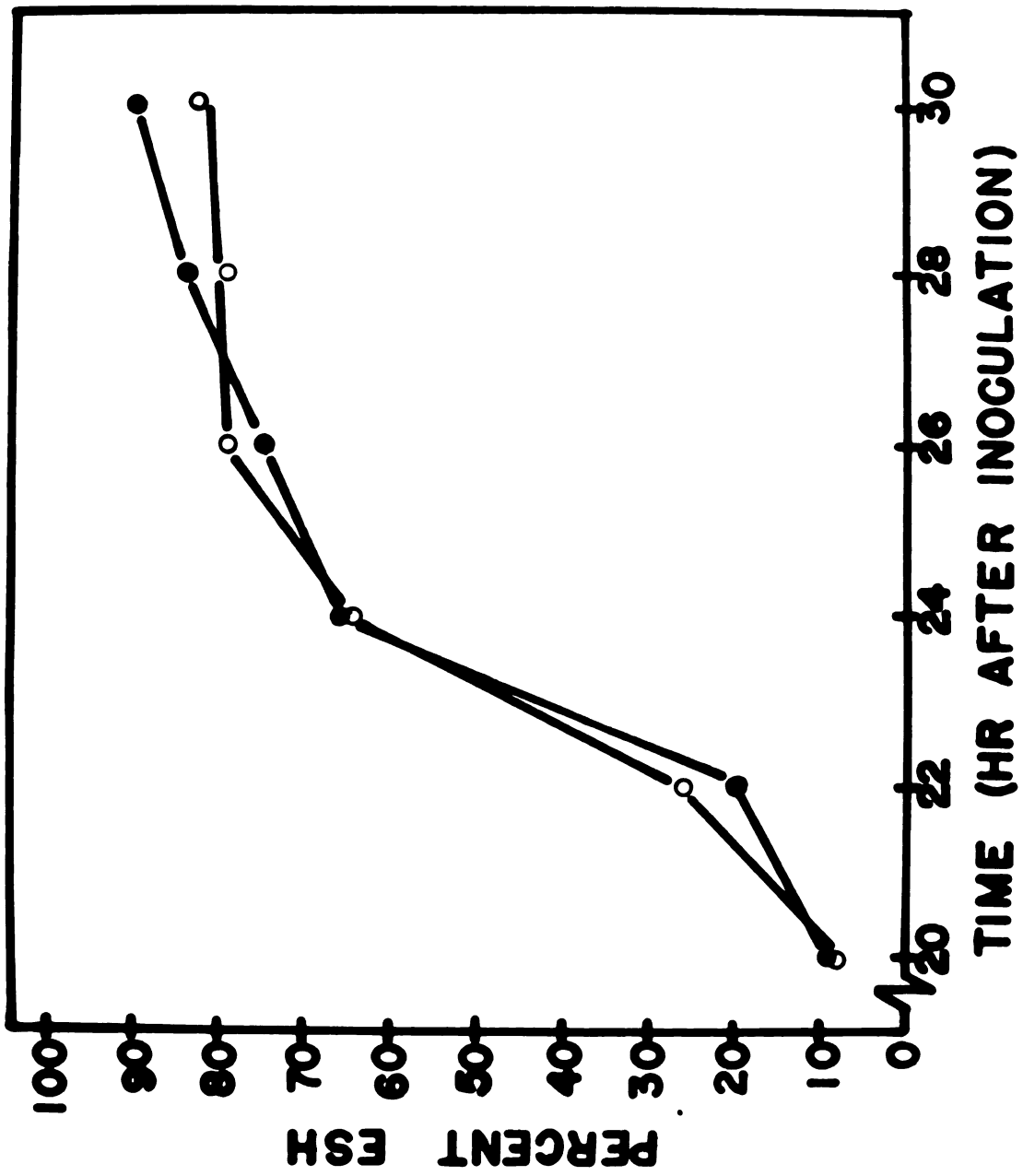

PERCENT APPRESSORIA
100
90
80
70
60
50
40
30
20
10
0
5
6
7
8
9
10
HR AFTER INOCULATION

Figure 12. Formation of elongating secondary hyphae (ESH) by Erysiphe graminis f. sp. tritici (MS-1) on cultivars Chancellor (●——●) and Little Club (○——○).

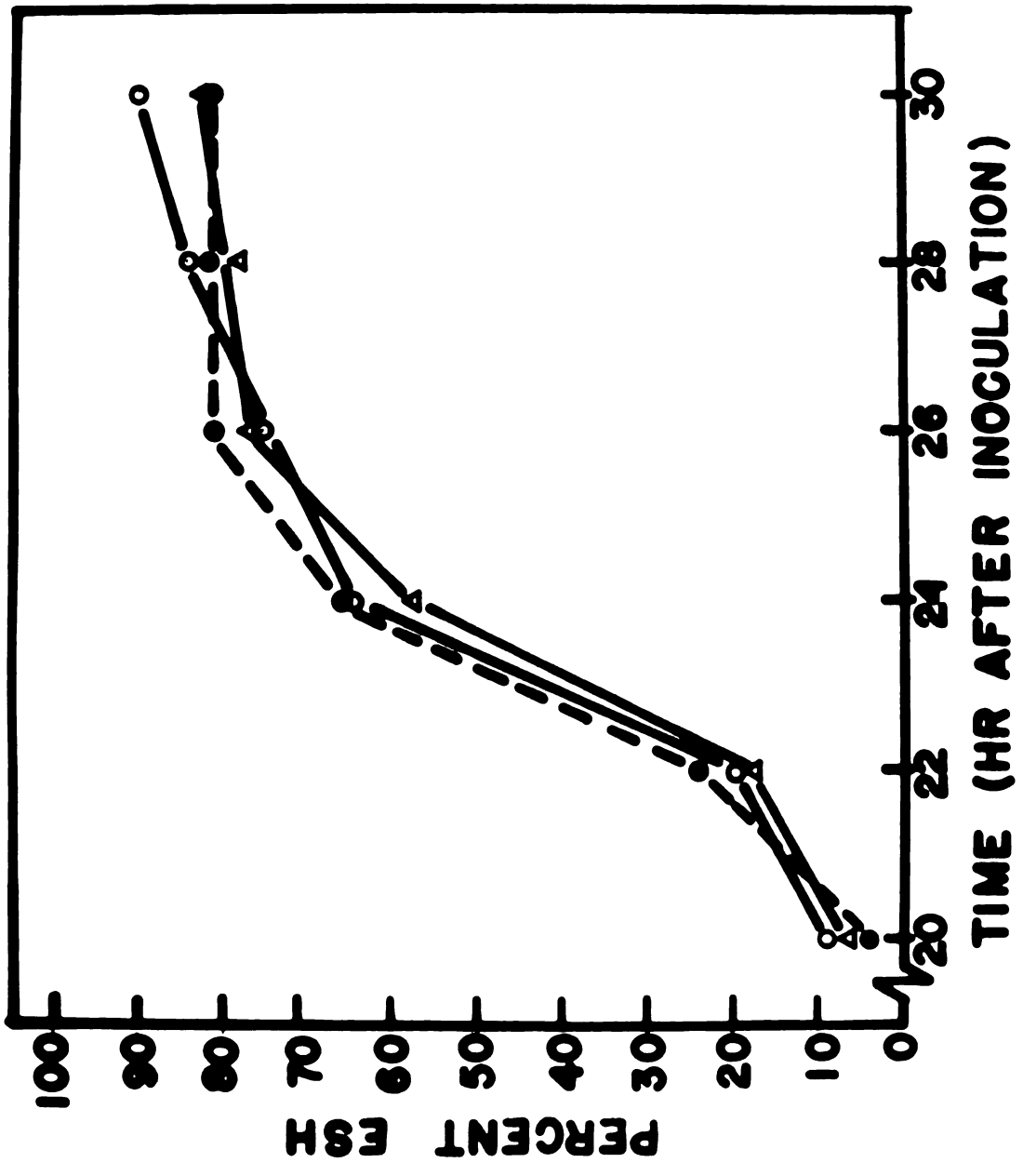

PERCENT ESH
100
90
80
70
60
50
40
30
20
10
0
20
22
24
26
28
30
TIME (HR AFTER INOCULATION)

determined for parasite development with the MS-1/Little Club compatible combination (37, 47, 48) appeared to be satisfactory when studying other parasite/host combinations which lacked pairs of corresponding genes affecting compatibility.

The production of ESH was essentially identical with the p(Unknown)/Pm(Unknown) and p(Mich. Amber)/Pm(Mich. Amber) genotypes as observed with Chancellor and Little Club (Figures 12, 13). Development of the parasite was similar in combinations with px/RxRx genotypes, or with compatible combinations (Px/rxrx or px/rxrx) in the absence of specific R genes altering compatibility. A much wider variation in effects upon the formation of ESH was observed on the remaining ten wheat lines.

Incompatible parasite/host genotypes involving Pm1--The development of secondary hyphae was strongly inhibited with the P1/Pm1 genotypes (Figure 14). The few secondary hyphae which formed elongated briefly and attained a length of only 15-20 microns. Approximately 24-28 hr after inoculation, elongation ceased and many of the parasitic units collapsed. The host cells surrounding the penetration sites frequently appeared brownish and discolored. A few parasitic units formed what appeared to be small distorted haustorial bodies. Mature haustoria with elongated appendages were not found.

Figure 13. Formation of elongating secondary hyphae (ESH) by Erysiphe graminis f. sp. tritici with the following compatible parasite/host combinations: MS-1/Chancellor (O——O) (control), p(Unknown)/Pm(Unknown) (●- - -●), and p(Mich. Amber)/Pm(Mich. Amber) (Δ——Δ).


PERCENT ESH
100
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80
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60
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40
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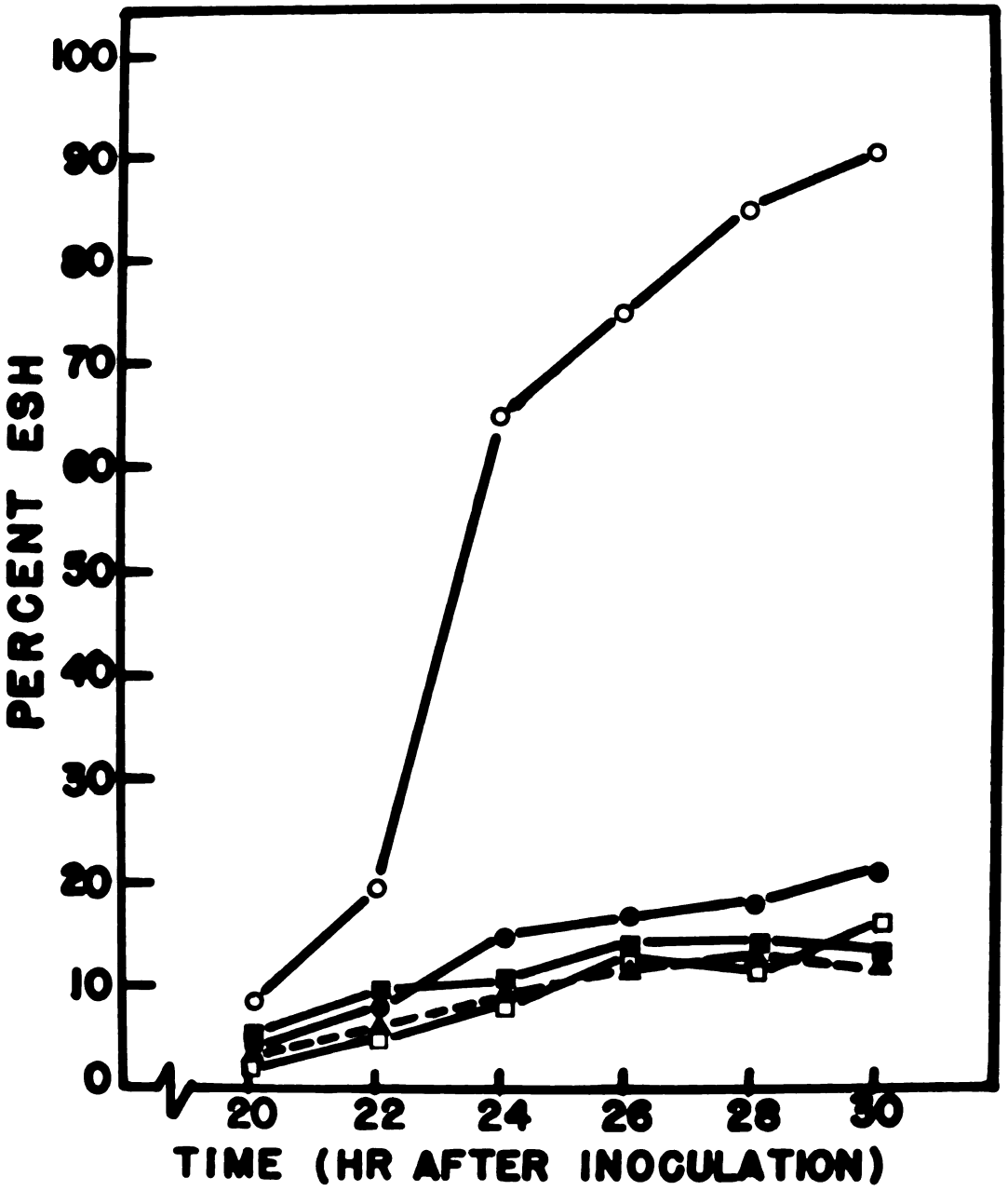
20
10
0
20
22
24
26
28
30
TIME (HR AFTER INOCULATION)

Figure 14. Formation of elongating secondary hyphae (ESH) by Erysiphe graminis f. sp. tritici (MS-1) with the following incompatible parasite/host genotypes: Pl/pml(Chancellor) (compatible control) (○——○), Pl/Pml(Axminster) (●——●), Pl/Pml(AsII) (■——■), Pl/Pml(Norka) (△- - -△), and Pl/Pml(C.I. 13836) (□——□).


PERCENT ESH
100
90
80
70
60
50
40
30
20
10
0
20
22
24
26
28
30
TIME (HR AFTER INOCULATION)

Incompatible parasite/host genotypes involving Pm2--The production of ESH with the Pm2 genotypes (Figure 15) was essentially identical to that observed with Chancellor or with lines with a Pm gene but for which the parasite had the appropriate corresponding p gene for compatibility (Figure 13). Continued development of the ESH at later hours appeared to be similar to the compatible genotypes studied earlier. Haustoria observed in epidermal strips at 20, 26, and 30 hr after inoculation corresponded in number and development to those observed with Chancellor or Little Club. Approximately 3-4 days after inoculation, chlorotic flecking became macroscopically evident on the inoculated leaves. After 2 more days, necrosis of the leaf was more obvious and only a very limited amount of sporulation by the fungus occurred.

Incompatible parasite/host genotypes involving Pm3--Of the three distinct Pm genes available at the Pm3 locus, only two were found to operate during the stages of primary infection at the first leaf stage of plant development. The third, Pm3c, had been reported to be effective only in plants at the third or later leaf stage (5, 7, 55) and no effect upon primary infection was observed. The development of ESH with the P3/Pm3 genotypes is shown in Figure 16. The P3a/Pm3a and P3b/Pm3b genotypes reduced the percentage of ESH which formed. The parasite units which did produce ESH continued development in subsequent hours and limited sporulation was usually

Figure 15. Formation of elongating secondary hyphae (ESH) by Erysiphe graminis f. sp. tritici (MS-1) with the following incompatible parasite/host genotypes: P2/pm2(Chancellor) (compatible control) (○——○), P2/Pm2(Ulka) (●——●), and P2/Pm2(C.I. 12632) (△- - -△).

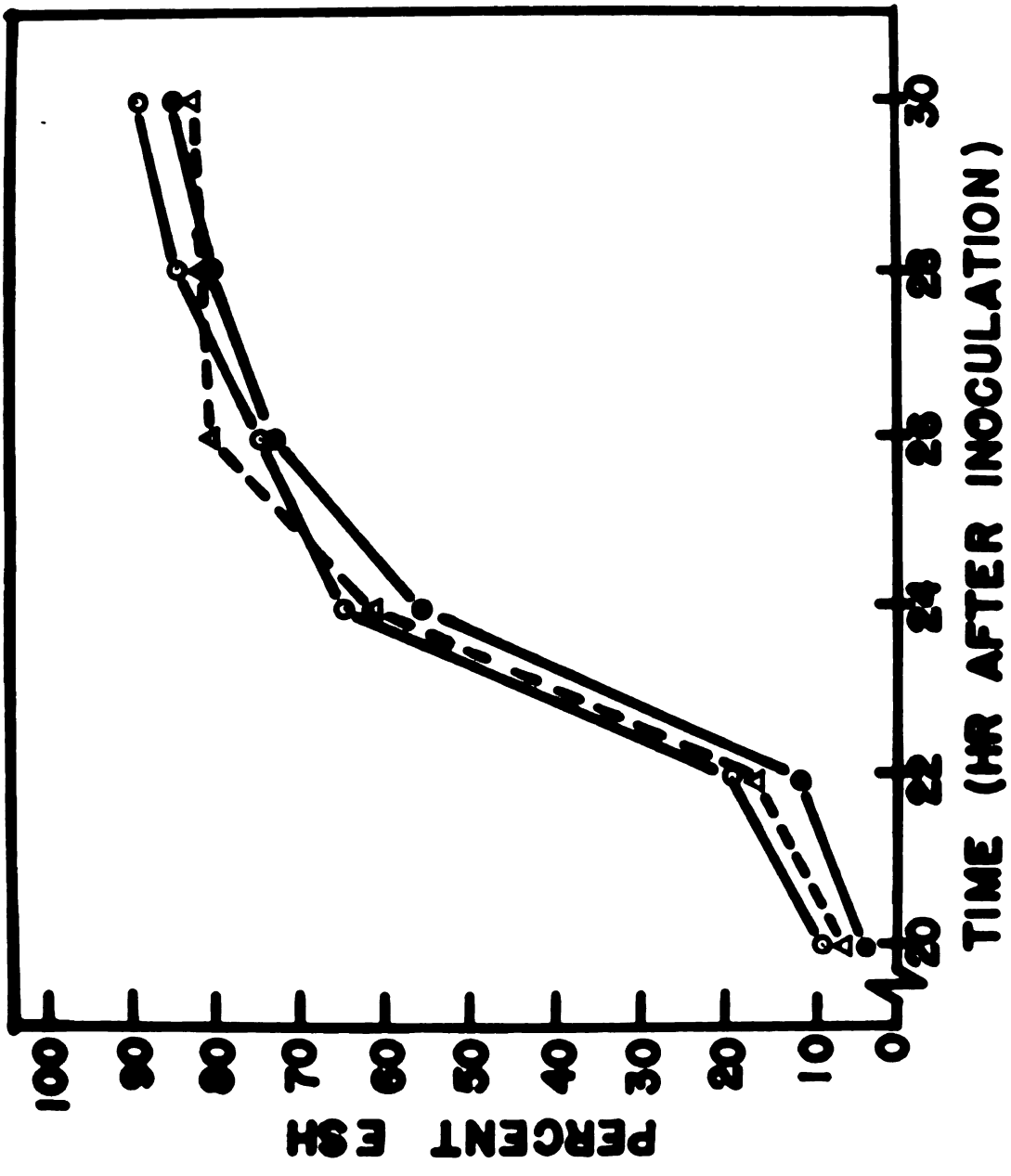
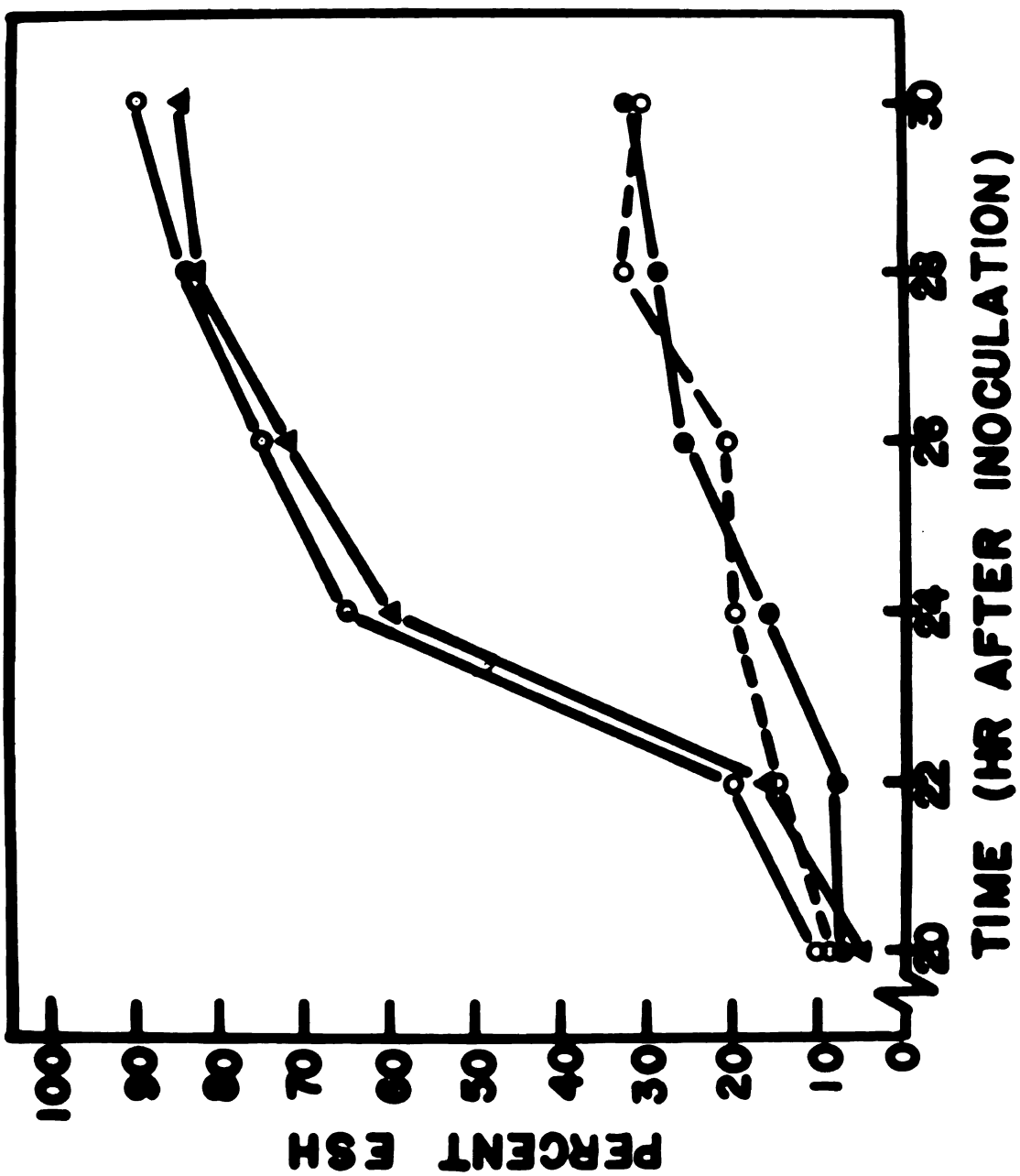

PERCENT ESH
100
90
80
70
60
50
40
30
20
10
0
20
22
24
26
28
30
TIME (HR AFTER INOCULATION)

Figure 16. Formation of elongating secondary hyphae (ESH) by Erysiphe graminis f. sp. tritici (MS-1) with the following incompatible parasite/host genotypes: P3a P3b P3c/pm3(Chancellor) (compatible control) (○——○), P3a/Pm3a (●——●), P3b/Pm3b (○- - -○), and P3c/Pm3c (▲——▲).


PERCENT ESH
100
90
80
70
60
50
40
30
20
10
0
20
22
24
26
28
30
TIME (HR AFTER INOCULATION)

observed 7 days later. However, both infection type and continued development were variable and a strong effect of the environment was evident. With the standardized environmental conditions employed in this study, the P3a/Pm3a and P3b/Pm3b genotypes appeared to lower the efficiency of infection but not to completely inhibit the development of the fungus.

The genetic uniformity of the mildew culture was tested in two experiments. Results obtained with a re-purified isolate of culture MS-1 were essentially identical to those presented in Figure 16. Also, when conidia from mildew which developed on the Pm3 lines were increased on Little Club, ESH production and infection type on the Pm3 host lines were the same as observed using the standard stock culture of MS-1 (Figure 17). It appears that the partial inhibition of development of the fungus with the P3a/Pm3a and P3b/Pm3b genotypes is characteristic of the interaction and is not due to mixed inocula.

Incompatible parasite/host genotypes involving Pm4--The P4/Pm4 genotypes were characterized by rapid and complete inhibition of development of the parasite. The production of ESH on the lines studied is presented in Figure 18. Only a very low percentage of parasitic units produced ESH and those ceased elongating after attaining a length of 15-20 microns. Approximately 20-24 hr after inoculation, most of the parasitic units had collapsed and the host

Figure 17. Formation of elongating secondary hyphae (ESH) with the following incompatible parasite/host combinations: MS-1/Chancellor (compatible control) (▲——▲), P3a(MS-1)/Pm3a (○——○), and P3a(MS-1*)/Pm3a (●——●). MS-1* was obtained from the mildew (MS-1) which sporulated on the Pm3a wheat line.

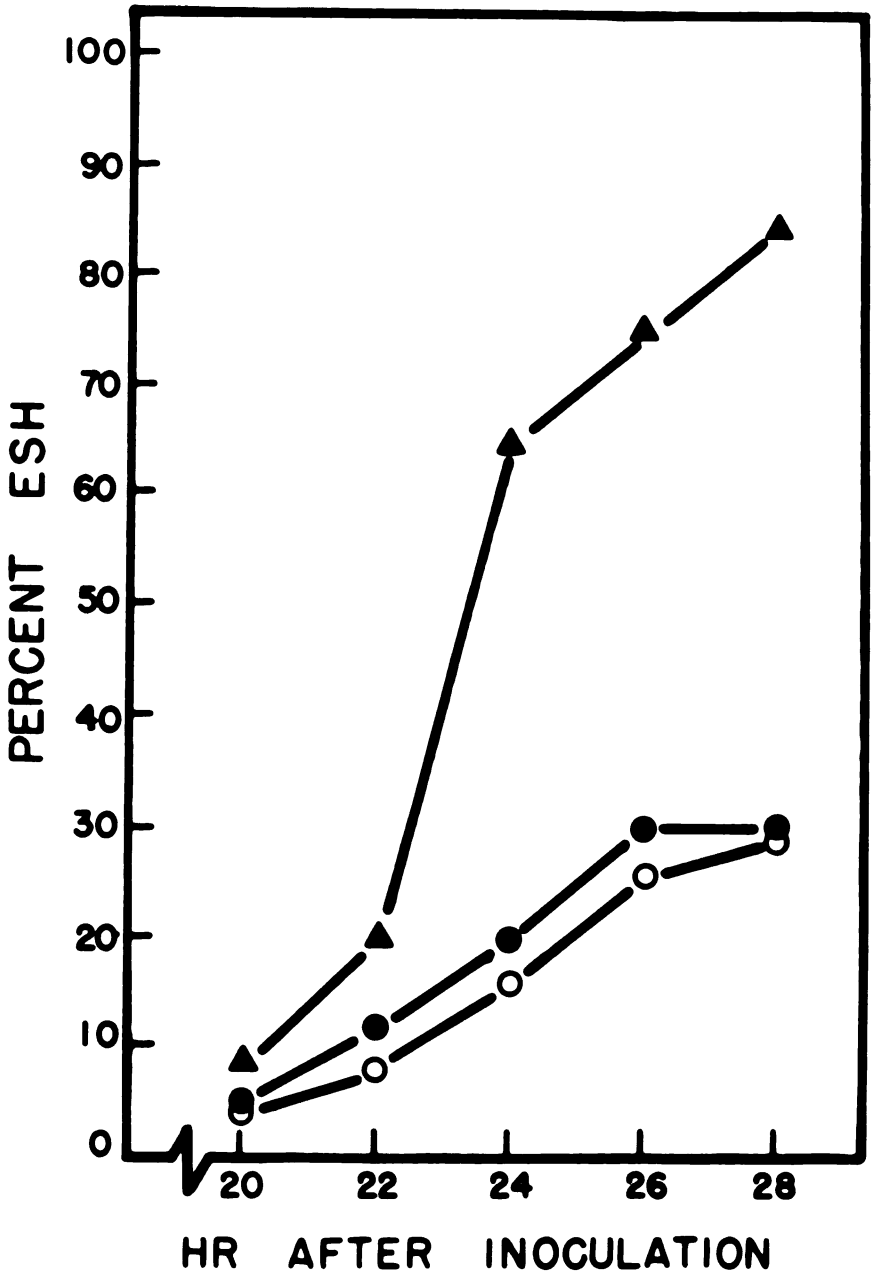
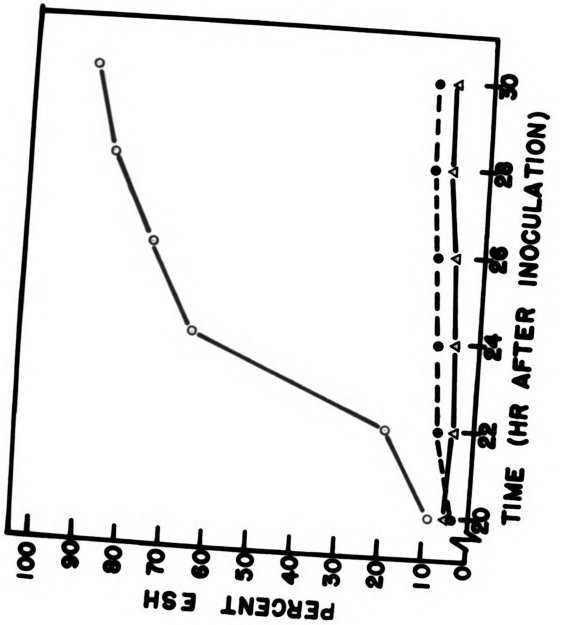

100
90
80
70
60
50
40
30
20
10
0
PERCENT ESH
20
22
24
26
28
HR AFTER INOCULATION

Figure 18. Formation of elongating secondary hyphae (ESH) by Erysiphe graminis f. sp. tritici (MS-1) with the following incompatible parasite/host genotypes: P4/pm4(Chancellor) (compatible control) (○——○), P4/Pm4(Khapli) (●- - -●), and P4/Pm4(Yuma) (△——△).

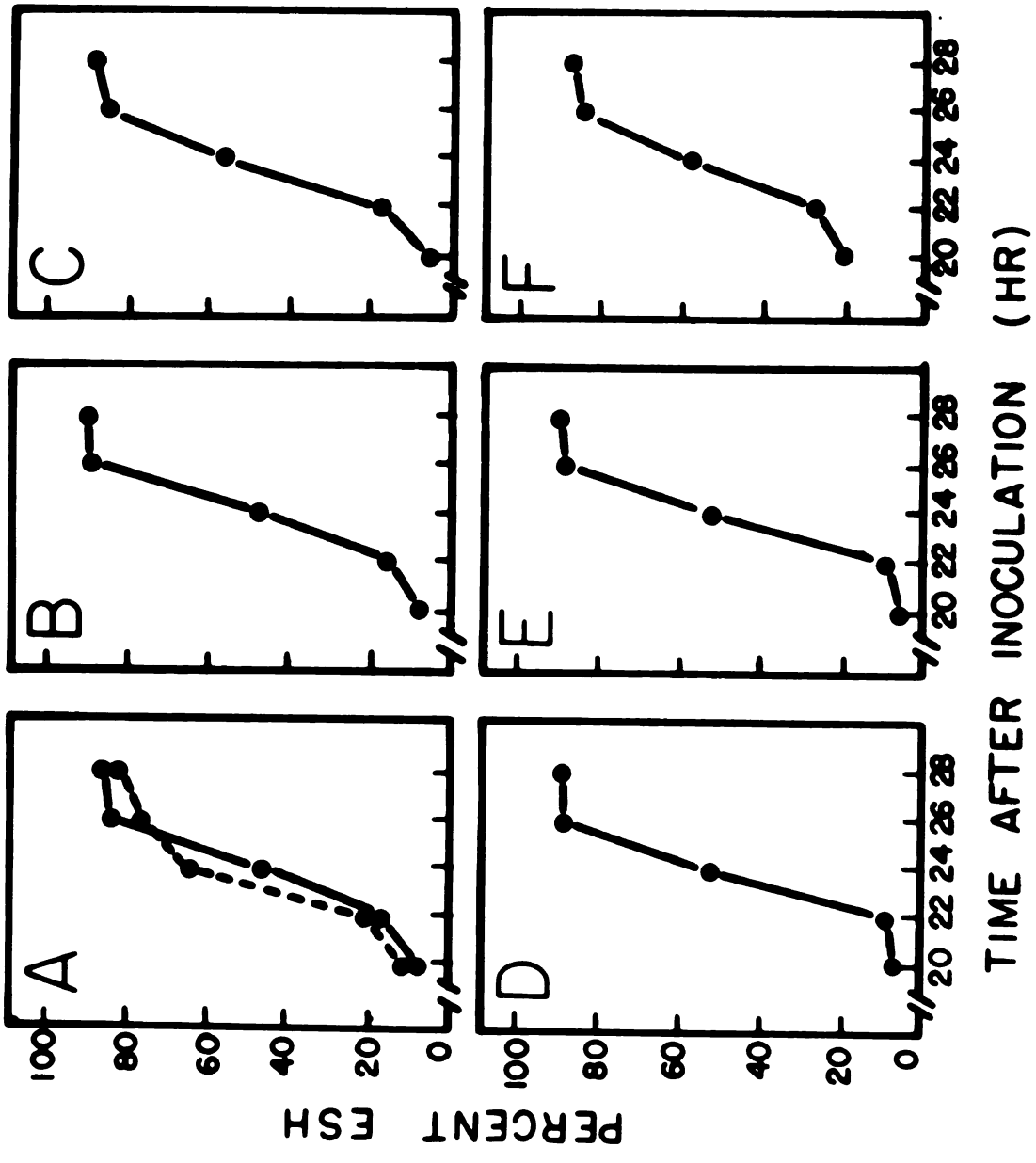

PERCENT ESH
100
90
80
70
60
50
40
30
20
10
0
20
22
24
26
28
30
TIME (HR AFTER INOCULATION)

cells around the sites of penetration were discolored. Examination of epidermal strips revealed that some parasitic units had produced what appeared to be rudimentary distorted haustorial bodies. Mature (lobed) haustoria were not observed. Darker staining areas were frequently observed around the penetration sites during examination of epidermal strips and may indicate a hypersensitive collapse of host cells. Based upon the very low number of ESH observed, it appeared that this gene combination interacted during or soon after penetration of the host cell.

Gene-for-Gene Specificity Expressed During Formation of ESH

Cultures MS-1 and MS-76 which possess the corresponding Pl and pl genes, respectively, were used to study mildew development on wheat lines containing either Pm1 or pm1. Development of ESH with the pl/Pm1 genotype, a compatible combination, was similar to that observed with compatible combinations in the absence of specific Pm genes (pl/pm1 or Pl/pm1) (Figure 19). The formation of ESH was inhibited only with the Pl/Pm1 incompatible combination. The development of ESH with the four genotypes involving Pm1 (Pl/Pm1, Pl/pm1, pl/Pm1, pl/pm1) corresponded to the predictions of the gene-for-gene hypothesis developed using infection type data (20, 21). In addition, the development of ESH during primary infection corresponded to the 'quadratic check' proposed by Rowell et al (56). The 'quadratic check' has been suggested to correlate the biochemical

Figure 19. Similarity of production of elongating secondary hyphae (ESH) with the following compatible parasite/host genotypes involving Pml. (A) Pl(MS-1)/pml(Little Club) (●——●) and Pl(MS-1)/pml(Chancellor) (●- - -●), (B) pl/pml(Chancellor), (C) pl/Pml(Axminster), (D) pl/Pml(AsII), (E) pl/Pml(Norka), and (F) pl/Pml(C.I. 13836).


A
B
C
D
E
F
100
80
60
40
20
0
20 22 24 26 28
PERCENT ESH
TIME AFTER INOCULATION (HR)

and physiological data concerning host-parasite interactions with the gene-for-gene specificity controlling the relationship.

Transfer of ^{35}S from Wheat Leaves to E. graminis f. sp. tritici

^{35}S transfer with incompatible parasite/host combinations--The movement of ^{35}S from radioactively labelled wheat leaves to the external mycelium of the fungus was examined with various incompatible (Px/Pmx) parasite/host combinations. Inoculated wheat leaves were allowed to take up an ^{35}S solution for five hour periods during primary infection. The fungal mycelium on the surface of the leaf was removed after the 5 hr period with a parlodion solution and the radioactivity contained in the parlodion strip was determined. With the P1/Pm1(Axminster) genotype, which affects parasite development at or near the time of penetration, only a small amount of ^{35}S was transferred as compared to the compatible P1/pm1(Chancellor) combination (Figure 20). With the P2/Pm2(Ulka) genotype, which did not inhibit the formation of ESH, the amount of ^{35}S transfer was somewhat greater, but essentially very similar, to transfer with the compatible P2/pm2(Chancellor) combination (Figure 21). The amount of ^{35}S transfer with the P3a/Pm3a combination, in which the development of only a fraction of the parasite population was inhibited, was intermediate to that observed with the P3a/pm3a(Chancellor) compatible combination (Figure 22). With the P4/Pm4(Khapli) combination, in which the formation of ESH was greatly inhibited,

Figure 20. Transfer of ^{35}S from wheat to the external mycelium of Erysiphe graminis f. sp. tritici with the following parasite/host genotypes: Pl/pml(Chancellor) (○—○) and Pl/Pml(Axminster) (●—●).

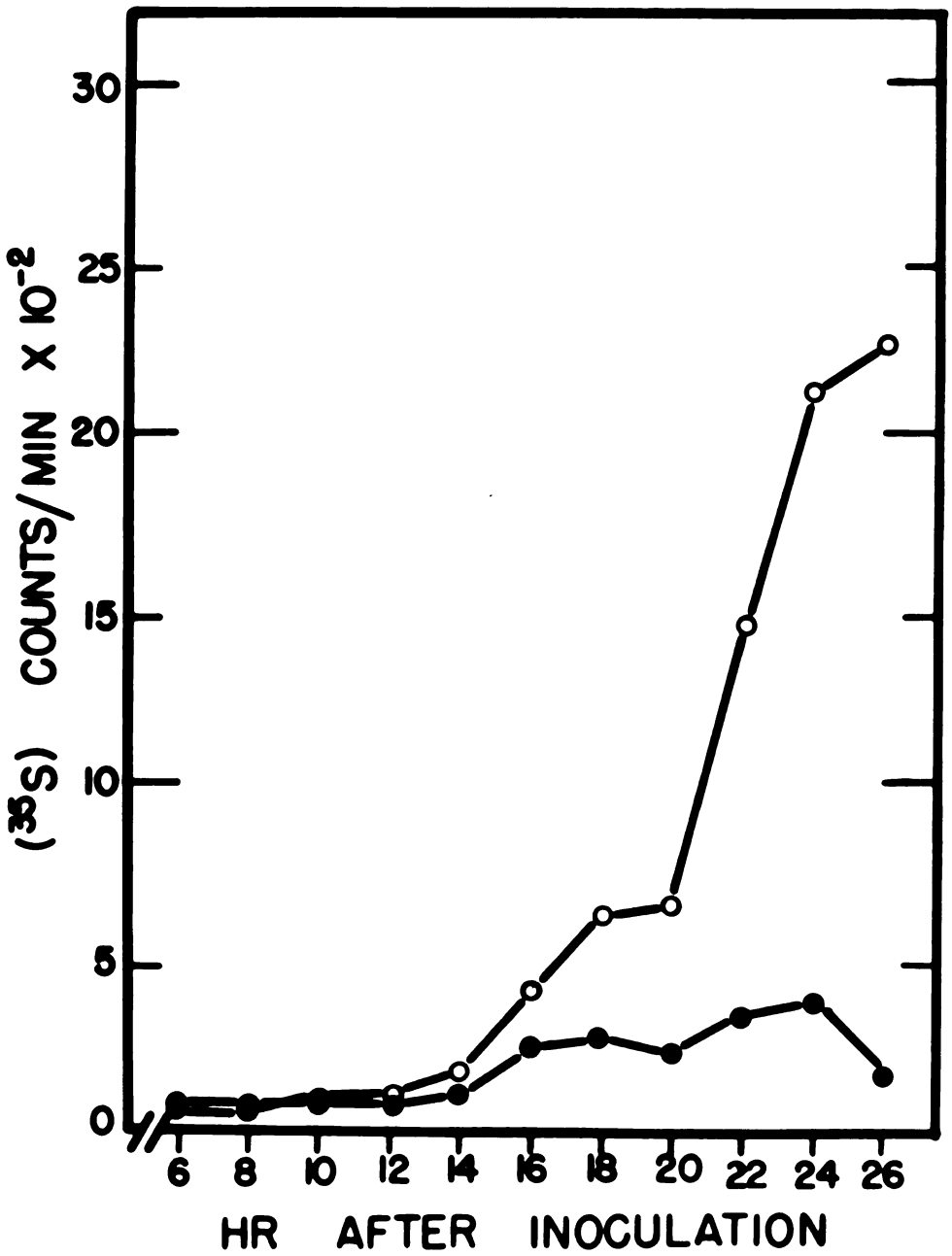

30
25
20
15
10
5
0
(^{35}S) COUNTS/MIN X 10^{-2}
6
8
10
12
14
16
18
20
22
24
26
HR AFTER INOCULATION

Figure 21. Transfer of ^{35}S from wheat to the external mycelium of Erysiphe graminis f. sp. tritici with the following parasite/host genotypes: P2/pm2(Chancellor) (○——○) and P2/Pm2(Ulka) (●——●).

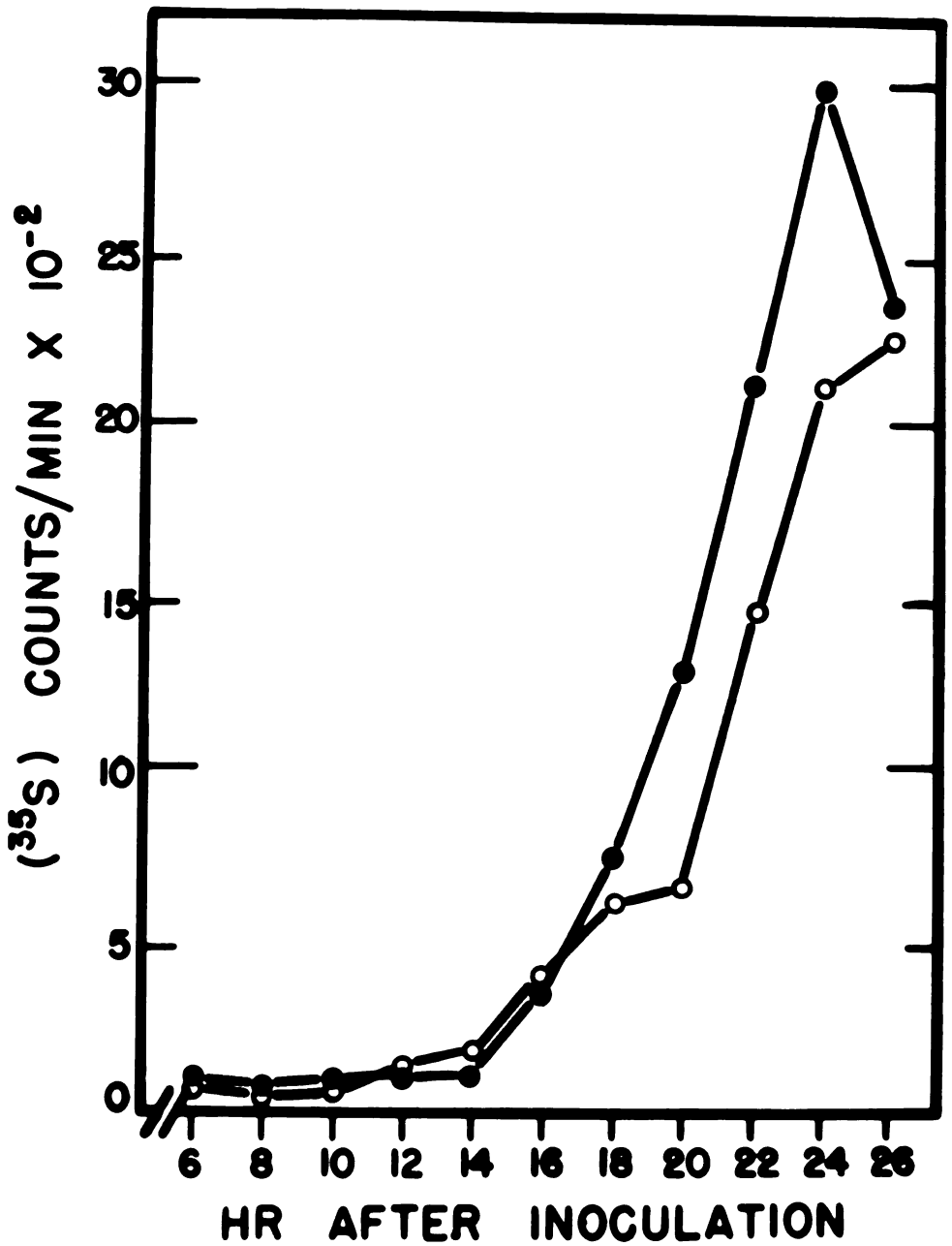
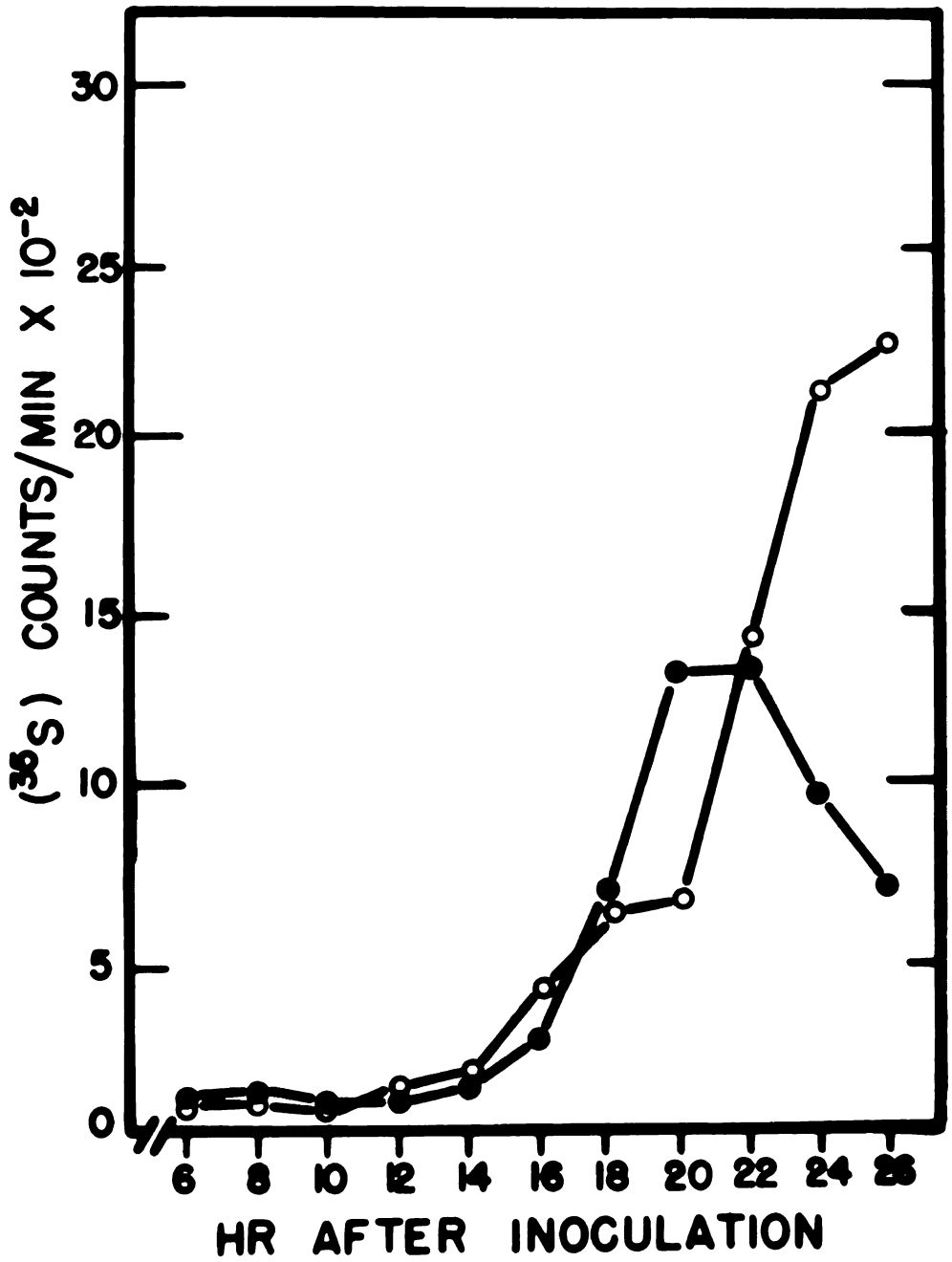

30
25
20
15
10
5
0
(^{35}S) COUNTS/MIN X 10^{-2}
6
8
10
12
14
16
18
20
22
24
26
HR AFTER INOCULATION

Figure 22. Transfer of ^{35}S from wheat to the external mycelium of Erysiphe graminis f. sp. tritici with the following parasite/host genotypes: P3a P3b P3c/pm3(Chancellor) (○——○) and P3a/Pm3a (●——●).


30
25
20
15
10
5
0
(^{35}S) COUNTS/MIN X 10^{-2}
6
8
10
12
14
16
18
20
22
24
26
HR AFTER INOCULATION

only a small amount of ^{35}S transfer was detected (Figure 23). The amount of ^{35}S transfer was essentially identical to the transfer with Pl/Pml (Figure 20). The transfer of ^{35}S with the various genetically controlled combinations appeared to be a very good indicator of the inhibition of morphological development of the parasite on the surface of the host.

Transfer of ^{35}S with the four genotypes involving Pml--The movement of ^{35}S from the wheat plant to the external mycelium was studied with compatible and incompatible genotypes involving the Pml locus. Three of these combinations (Pl/pml(Chancellor), pl/pml(Chancellor), and pl/Pml(Axminster)) resulted in a compatible relationship indistinguishable by either infection type or by alteration of the development of ESH (Table 2 and Figure 19). The remaining incompatible Pl/Pml(Axminster) genotype inhibited parasite development and allowed only a low number of ESH to form (Figure 14). The transfer of ^{35}S, studied earlier with the Pl/Pml(Axminster) and Pl/pml(Chancellor) combinations (Figure 20), was found to reflect the inhibition of development of the parasite during primary infection. Culture MS-76 was used to study the remaining two compatible pl/Pml(Axminster) and pl/pml(Chancellor) genotypes.

The transfer of ^{35}S with all four genotypes is shown in Figure 24. The least quantity of ^{35}S was transferred with the incompatible Pl/Pml genotype, an intermediate amount was transferred with the pl/Pml

Figure 23. Transfer of ^{35}S from wheat to the external mycelium of Erysiphe graminis f. sp. tritici with the following parasite/host genotypes: P4/pm4(Chancellor) (○——○) and P4/Pm4(Khapli) (●——●).

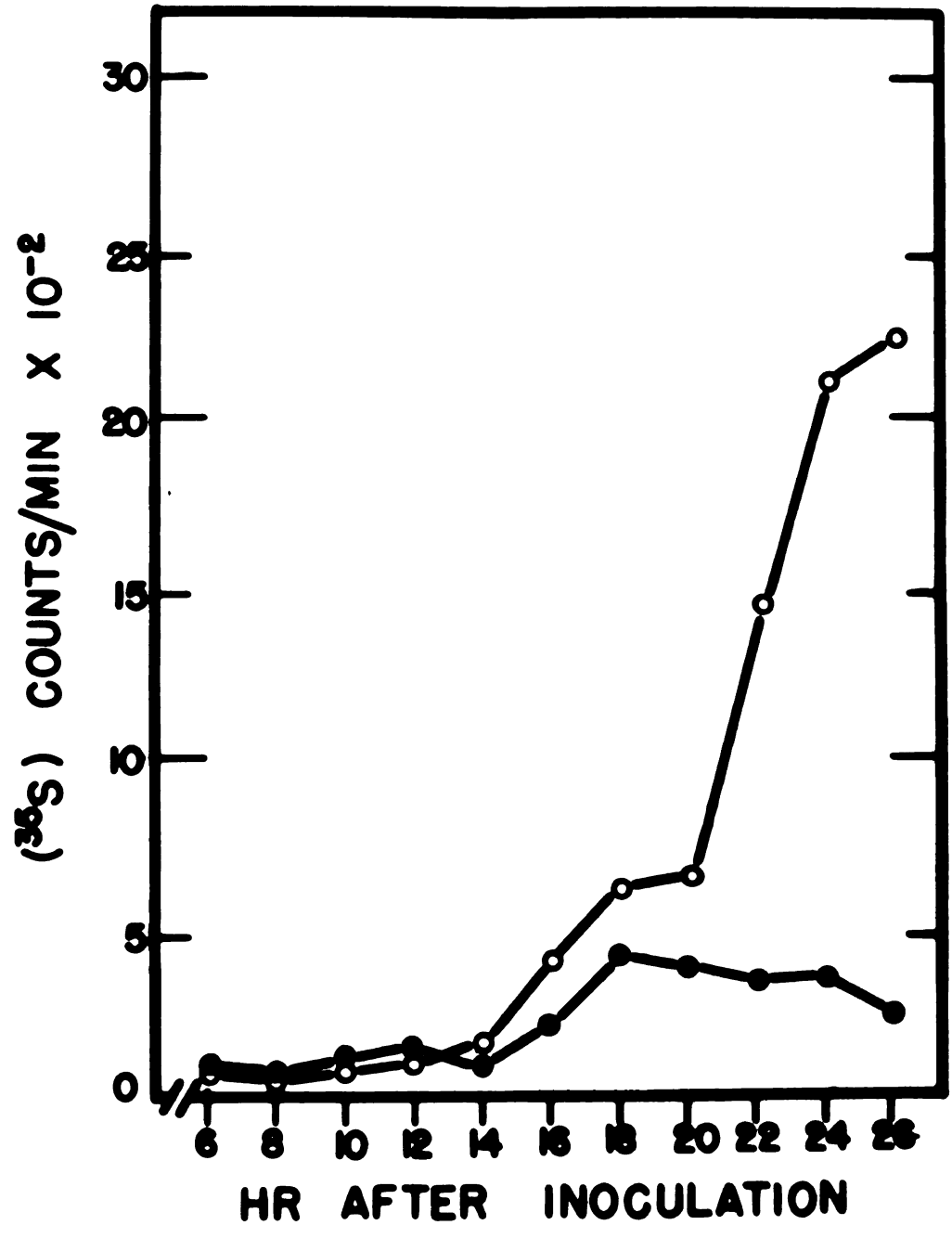
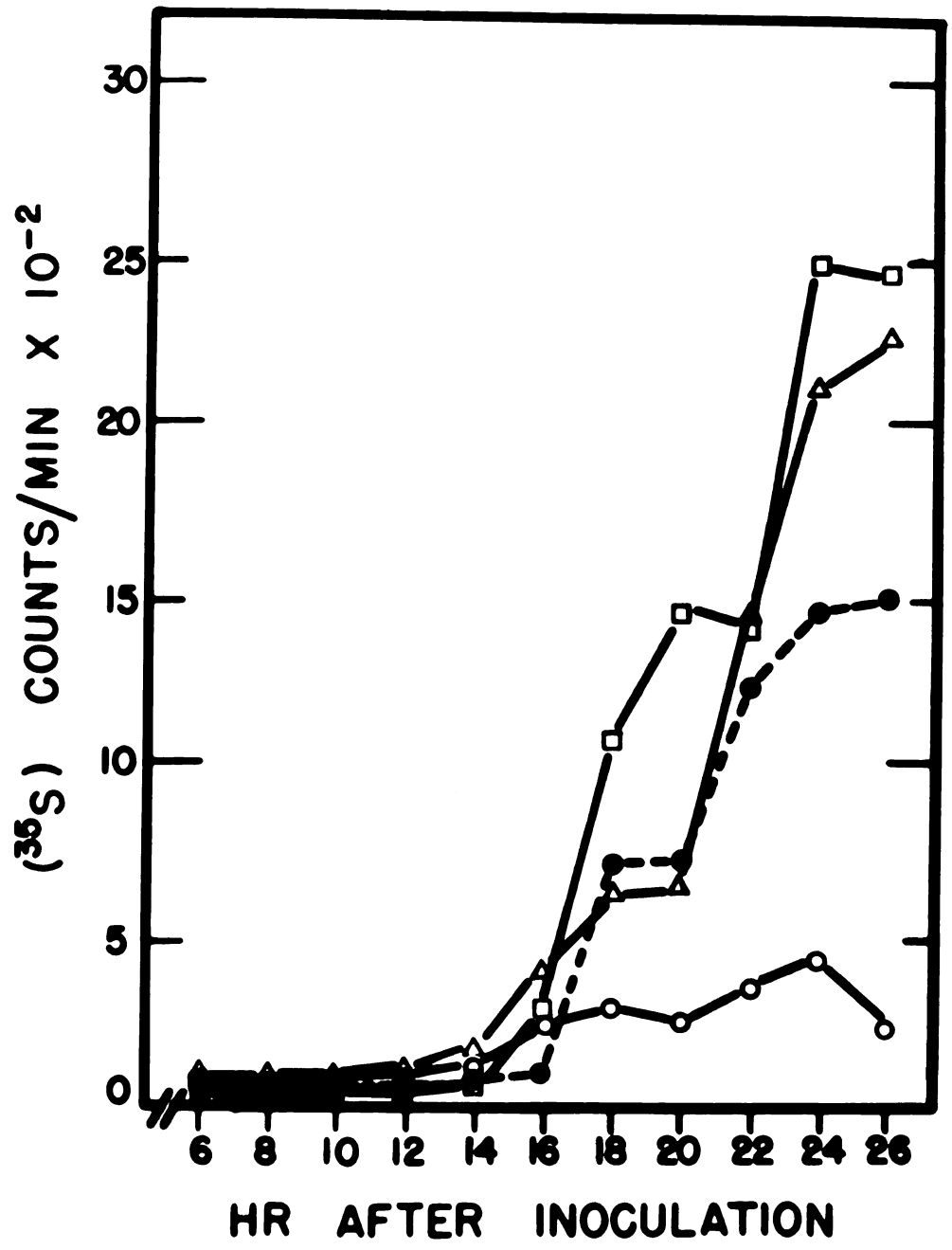

30
25
20
15
10
5
0
(^{35}S) COUNTS/MIN X 10^{-2}
6
8
10
12
14
16
18
20
22
24
26
HR AFTER INOCULATION

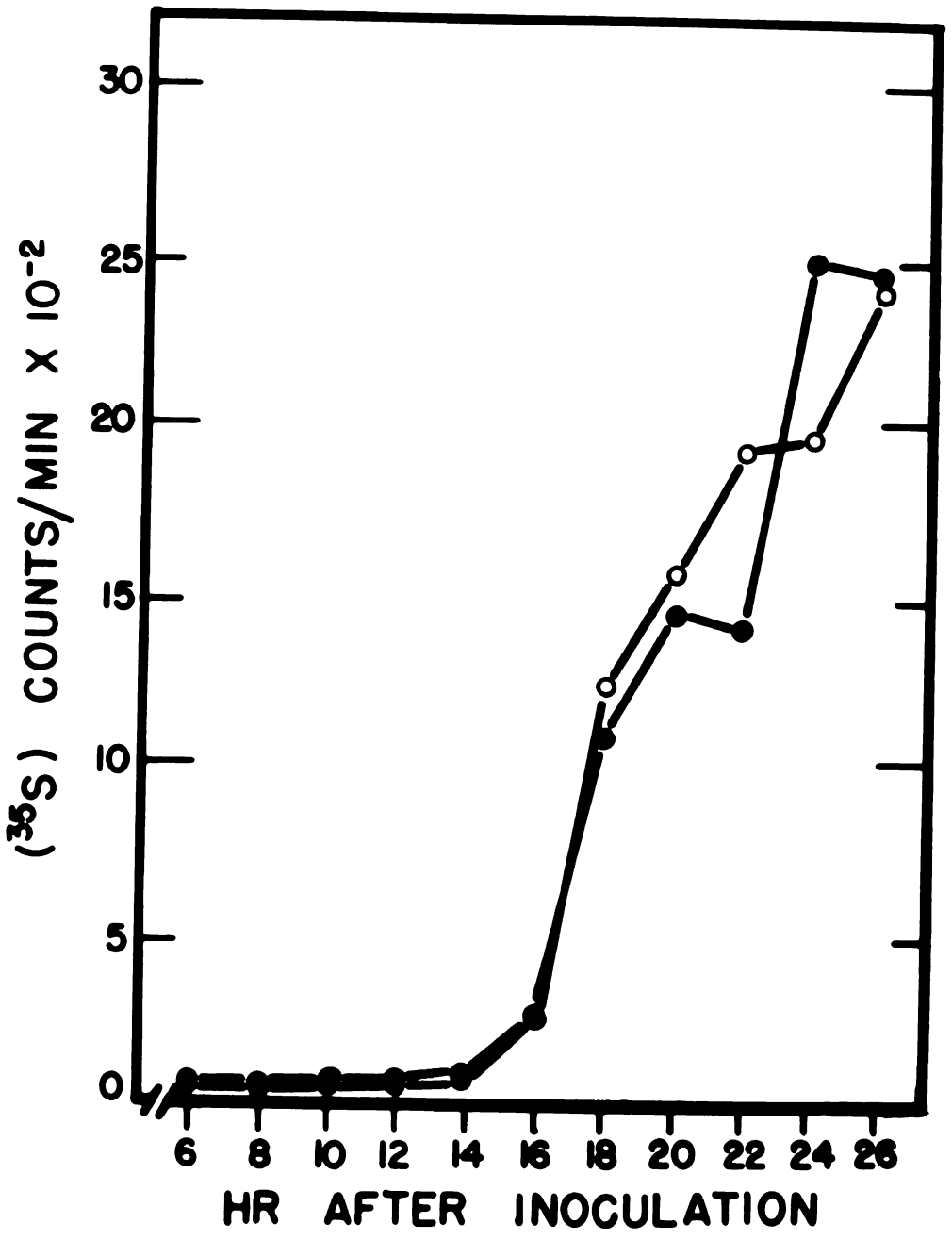
Figure 24. Transfer of ^{35}S from wheat to the external mycelium of Erysiphe graminis f. sp. tritici with the four possible genotypes involving the Pml locus. Pl/pml(Chancellor) (△——△), pl/pml(Chancellor) (□——□), pl/Pml(Axminster) (●——●), and Pl/Pml(Axminster) (○——○).


30
25
20
15
10
5
0
(^{35}S) COUNTS/MIN X 10^{-2}
6
8
10
12
14
16
18
20
22
24
26
HR AFTER INOCULATION

genotype, and the greatest and similar amounts were transferred with the Pl/pml and pl/pml genotypes. The transfer of ^{35}S in the compatible pl/Pml genotype was lower than the other two compatible combinations. The reduced transfer of ^{35}S with pl/Pml is not explainable by variations in either environmental conditions, plants, or the inoculation densities since the plants for both combinations were grown together, inoculated at the same time, and maintained in the same growth chamber each time the experiment was performed. Furthermore, the difference in transfer is not due to inhibition of parasite development, since the development of ESH by the fungus was essentially identical with all the compatible genotypes.

The shape of the transfer curves in these compatible combinations appeared to reflect a two component process involved in the movement of ^{35}S from the plant to the fungus. There may be two explanations for this result: 1) the 6-22 hr after inoculation are in darkness and turning the lights on at the 22 hr may have an effect on transfer, or 2) the host-parasite relationship is in a state of rapid development during this period of the infection process and the steps in the curve may reflect either the morphogenesis of fungal haustoria or a lag period preceeding the initiation of secondary hyphae. The first alternative was tested by leaving the lights off for the 22-26 hr period to see if a step was still obtained. The results in Figure 25 demonstrate that a step still exists in continuous darkness, but the

Figure 25. Effect of light upon the transfer of ^{35}S from wheat to Erysiphe graminis f. sp. tritici. Darkness 6-22 hr with 240 ft-c 22-30 hr (●——●), and darkness 6-26 hr with 240 ft-c 26-30 hr (○——○).


30
25
20
15
10
5
0
(^{35}S) COUNTS/MIN X 10^{-2}
6
8
10
12
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16
18
20
22
24
26
HR AFTER INOCULATION

step was delayed two hours. This suggests that the step is a reflection of parasite morphogenesis and treatments which alter the synchrony of morphogenesis also affect the transfer data.

DISCUSSION

The primary stages of infection of wheat and barley by Erysiphe graminis have been studied by employing the optimum environmental conditions necessary for a high infection efficiency and synchrony of parasite development (37, 47, 48). This defined system has allowed studies of the initial host-parasite interactions during primary infection and could be used in determining the effects of environmental or other treatments upon the establishment of a successful infection. The production of elongating secondary hyphae by the parasite population was used as the criterion of a functioning infection and was believed to result from a dynamic interaction between the host and parasite which allowed continued fungal development. This latter assumption was tested in later experiments by measuring the transfer of ^{35}S from host to parasite.

Initial studies were concerned with the development of ESH which eventually form SA and initiate secondary infection sites on the plant. The production of SA was found to be affected by both light and temperature during the 20-30 hr period after inoculation. An increase in the production of SA following environmental changes indicated that the formation of SA had particular environmental

requirements for maximum efficiency. High light or high temperature beginning at the 26 hr was found to increase SA production. Light period also appeared to have a definite effect. Increased percentages of SA were formed if the lights were turned off at 30 hr after inoculation. This inhibitory effect of light upon SA is similar to the inhibition of the formation of primary appressoria which require an initial 4 hr period of light (240 ft-c) followed by darkness for maximum efficiency (48).

Combinations of various environmental conditions were employed to further study the effects of light, darkness, and temperature upon SA formation. Combination of high light (2600 ft-c) and high temperature (27C) beginning at 26 hr after inoculation or high light at 26 hr followed by darkness at 30 hr resulted in a greater percentage of SA formed than with any of the conditions tested alone. Combination of all three conditions (2600 ft-c and 27C beginning at 26 hr with 0 ft-c and 22C at 30 hr) was also somewhat additive and resulted in the highest percentage of SA formation attained. This additive effect of combining single environmental conditions each known to increase infection efficiency is similar to that observed in studies of germination of E. graminis conidia (48). In those studies the optimum conditions for germination required a combination of certain light, humidity, and temperature conditions during 0-6 hr after inoculation. The possibility exists that the combination of environmental conditions

utilized for increased SA production were additively affecting physiologically distinct portions of the parasite population. This possibility must be studied further since physiological non-uniformity has been demonstrated during germination with an otherwise genetically uniform clonal population of E. graminis conidia (48).

The inhibition of E. graminis development during the stages of primary infection was examined with various near-isogenic wheat lines containing single Pm genes altering compatibility to mildew development. The phenotypic expression of various parasite/host combinations indicated that the gene-for-gene specificity governing the relationship was also reflected in the mode of interaction of the particular genotypes studied during primary infection. The effects of this interaction with each parasite/host genotype studied could be observed at a specific stage of pathogenesis. The earliest effects were observed during or soon after the penetration of the host epidermal cell and are reflected in the low number of parasitic units which produced ESH in combinations of either P1/Pm1 or P4/Pm4. The morphological similarity of the responses conditioned by these two incompatible combinations may indicate that a similar basis for the reaction is involved. The P4/Pm4 genotype was thought to interact at slightly earlier stages of the infection process since a lower percentage of ESH formed with P4/Pm4 than with P1/Pm1.

The development of the parasite with the P2/Pm2 genotype was

especially interesting because compatibility was not altered during primary infection. The effects of the incompatible interaction were first observed 72-96 hr after inoculation, long after a functional host-parasite relationship had formed. These results are somewhat similar to delayed hypersensitive responses observed by other workers (12, 27, 35, 38), but unique in that no inhibition of parasite development could be observed during primary infection, even by using a very sensitive system of measurement. The $\underline{P_k}/\underline{Ml_k}$ genotype with barley mildew specifies a delayed hypersensitive reaction 3-4 days after inoculation with E. graminis f. sp. hordei, but it also inhibits the development of 60-70% of the parasite population during primary infection (38). The P3c/Pm3c combinations are somewhat similar to P2/Pm2 genotypes, but the expression of the P3c/Pm3c interaction is a function of host maturity. Pm3c appears to inhibit mildew development of pathogen strains with the corresponding P3c gene only at the third or later leaf stage of plant development. The determination of the bases for these delayed responses should be possible. The use of a defined host-parasite-environmental system together with biochemical and genetic studies may provide some insight concerning the systems operating to alter parasite/host compatibility.

The results with the P3a/Pm3a and P3b/Pm3b genotypes are somewhat confusing since only a proportion of the parasite population

is inhibited during primary infection. Continued development of the 30% of the parasite units which do form ESH appears to be strongly affected by later environmental conditions. Additional studies of these genotypes at later developmental stages are necessary to clarify this preliminary observation.

The inhibition of fungal development with the various incompatible gene combinations was tested further by observing the transfer of ^{35}S from the plant to the fungus. These methods were developed by Mount (46) with the MS-1/Little Club compatible combination. The amount of ^{35}S transfer to the fungus was found to parallel the development of the fungal haustoria in the host cells. The transfer of ^{35}S possible with incompatible combinations, therefore, should reflect the time that fungal development and the host-parasite relationship was altered.

The amount of ^{35}S transfer was found to correspond to the morphological development of the fungus with the various incompatible genotypes studied. ^{35}S transfer was greatest with genotypes which appeared fully compatible, as evaluated by the production of ESH during primary infection. The reduction of transfer of ^{35}S to the fungus with the P1/Pm1, P2/Pm2, P3a/Pm3a, and P4/Pm4 incompatible combinations was similar to the inhibition of the percentage of ESH with each combination. The correspondence of the amount of ^{35}S transfer and the relative production of ESH during primary

infection suggests that the development of ESH is closely related to the transfer of materials from the plant to the fungus. This movement of materials and nutrients is presumed to allow continued fungal development after the production of appressoria.

The correspondence between fungal development and ^{35}S transfer appeared useful to further characterize the four Pml genotypes studied during primary infection. The characterization of these genotypes would complete the 'quadratic check' on three levels: 1) by final mildew development (infection type), 2) by parasite development during primary infection, and 3) by the amount of ^{35}S transfer with each gene combination. The data on ^{35}S transfer demonstrated, however, that not all of the three compatible combinations were the same, and that the pl/Pml genotype was distinct from Pl/pml and pl/pml. This difference is considered to be extremely interesting since it indicated a unique interaction with the pl/Pml genotype. The difference appeared to be real, since the pl/Pml and pl/pml combinations were investigated on the same days, with simultaneous inoculations, and identical environmental conditions in the same growth chamber. Furthermore, the results were not subjectively biased since the experiment was initially expected to demonstrate the similarity of the compatible combinations. The significance of demonstrating the pl/Pml genotype to be unique lies in the applicability to model systems attempting to describe the basis of

host-parasite compatibility. The systems specified by the Pml gene involved in altering compatibility are apparently overcome or by-passed by strains of the fungus with the corresponding pl gene. The pl/Pml combination is apparently fully compatible on a morphological basis but can be shown to be different with respect to ^{35}S transfer. This difference offers many suggestions for future studies to determine the basis for the altered transfer which does not appear to be due to inhibition of parasite development. Additional parasite/host genotypes and radioactive tracers must be investigated to corroborate these observations. Further experiments will eliminate the possibility that the inhibition of ^{35}S transfer is due to modifier genes with only a slight effect on fungus development.

The nature of the systems operating to alter compatibility remains a matter of conjecture. Several people have speculated that the induction of specific enzyme systems is involved in the incompatible response (26, 31). The inducer is thought to be produced by pathogenic strains possessing a Px gene which activates the corresponding Rx system in the host. Induction does not occur in the absence of an R gene and the inducer is either altered or absent in the pathogenic strain containing the px gene. This possibility is interesting but unfortunately it has been recently applied to the production of 'phytoalexins' for which genetic specificity has not been demonstrated (26). Furthermore, the possibility overlooked by this type of

model is that the P locus in the pathogen is involved in altering sensitivity of the fungus to a substance produced by the R locus in the plant. Sensitivity may be controlled at the membrane level as has been proposed for the mechanism of toxic action of H. victoriae toxin on oat tissue (57), or in the breakdown of an inhibitory substance in the plant by the developing pathogen. The ability to produce the inhibitor would be controlled by the R genes in the plant and sensitivity to the toxic effects would be determined by the P gene in the fungus.

The specificity in the operation of the gene-for-gene interactions and the large number of possible R and P loci involved demand a corresponding specificity in the operation of the incompatibility mechanisms. The presence or absence of a toxic substance would neither explain the specificity observed nor the various effects upon parasite development with the various possible parasite/host genotypes. Ellingboe (17) has suggested that if the incompatibility system is operating via the DNA-RNA-protein pathway, then alleles at a particular R locus should operate similarly since the structure or function of the same protein would be involved. This suggestion is consistent with the incompatible responses observed in this study for the P3a/Pm3a and P3b/Pm3b genotypes known to represent distinct alleles at the Pm3 locus in the plant. Whether this result is generally true for other allelic genes at other Pm loci must be studied further.

The genetic determination of compatibility can be used as a

tool for estimating both the number and nature of the systems involved in the establishment of a host-parasite relationship. The diversity of the incompatible responses observed in this study suggests that the systems controlling parasite/host compatibility may be based on a number of possible mechanisms. The range of different incompatible responses and phenotypes observed should serve as a guide for selection of particular genotypes for biochemical studies. The eventual biochemical characterization of the particular systems involved in the incompatible response, however, must be shown to segregate with the genes governing compatibility and to operate at the time of the earliest observed effects of the incompatible interactions.

SUMMARY

The infection process of Erysiphe graminis on wheat consists of a number of morphological stages of development (39, 47, 48). Experiments demonstrated that the formation of secondary appressoria (SA) could be altered by various environmental conditions. The efficiency and synchrony of SA production were increased by environmental alterations. Sixty-five percent of the applied conidia produced SA following exposure of inoculated plants to high light intensity and high temperature (2600 ft-c and 27C, respectively) at 26 hr after inoculation followed by 22C and darkness at 30 hr after inoculation.

Mildew development was studied during primary infection with various compatible and incompatible parasite/host combinations. The development of E. graminis f. sp. tritici on near-isogenic wheat lines containing single genes affecting mildew development was altered in a specific manner with each gene combination. The development of the strain of the pathogen possessing the complementary pl gene(s) on host plants with the Pm1 gene(s) (pl/Pm1) was indistinguishable from compatible gene combinations involving pm1 (Pl/pm1 or pl/pm1). With the four possible parasite/host

genotypes involving the Pml locus (Pl/Pml, Pl/pml, pl/Pml, pl/pml), alteration of both infection type and the development of elongating secondary hyphae (ESH) was observed only with the incompatible Pl/Pml genotype.

^{35}S transfer from the wheat leaf to the developing fungus was less with incompatible genotypes than with compatible genotypes. The amount of ^{35}S transfer was greatest with parasite/host combinations which did not alter the processes of primary infection and was least with combinations which greatly inhibited fungus development. Measurement of ^{35}S transfer with the four Pml genotypes (Pl/Pml, Pl/pml, pl/Pml, pl/pml) revealed that the compatible pl/Pml genotype could be distinguished from the compatible Pl/pml and pl/pml genotypes. The amount of transfer was lowest with Pl/Pml, intermediate with pl/Pml, and greatest with Pl/pml and pl/pml.

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