UPTAKE OF ^{32}P BY TRITICUM AESTIVUM AND GENETIC CONTROL OF ^{32}P TRANSFER TO ERYSIPHE GRAMINIS DURING PRIMARY INFECTION

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ABSTRACT

UPTAKE OF ^{32}P BY TRITICUM AESTIVUM AND GENETIC CONTROL OF ^{32}P TRANSFER TO ERYSIPHE GRAMINIS DURING PRIMARY INFECTION

By

Terry Joe Martin

Environmental conditions necessary for synchronous development of Erysiphe graminis f. sp. tritici Em. Marchal during primary infection of wheat were shown to affect the amount of ^{32}P taken up and translocated to the epidermis of excised leaves placed in a ^{32}P solution. Light increased transpiration, which in turn increased the amount of ^{32}P taken up by the leaves and the amount of ^{32}P transferred to the parasite.

Rates of ^{32}P transfer from host to parasite were determined for compatible and incompatible parasite-host interactions during primary infection. Transfer rate reflected the relative compatibility of the host-parasite interaction studied. Interactions with little fungal development had lower rates of transfer, while fully compatible interactions had high rates of transfer.

The four possible parasite/host genotypes (the quadratic check) for each interacting gene pair were evaluated for their effects on final infection type seven days after inoculation, on ^{32}P transfer from host to parasite, and on elongating secondary hyphae. With all gene pairs the final infection type of the three compatible interactions were similar. Only the incompatible interaction (Px/Pmx) resulted in a low infection type. The compatible interactions involving P1 and Pm1, P2 and Pm2, P3b and Pm3b had similar rates of ^{32}P transfer, but this was not true for the compatible interactions involving P4 and Pm4. The p4/Pm4 genotype had a reduced rate of transfer when compared to p4/pm4 and P4/pm4. The production of elongating secondary hyphae was also delayed with p4/Pm4. Two other isolates containing p4 resulted in a reduction in the percent of parasites that produced elongating secondary hyphae. Thus the mutations from P4 to p4 in the parasite did not completely negate the change in the host from pm4 to Pm4. This indicates that specificity of gene-for-gene interactions is for incompatibility, not compatibility. It also demonstrated how the gene-for-gene hypothesis might play a role in general or horizontal resistance.

Germinating conidia of E. graminis were shown to reduce transpiration rates of the host before penetration occurred. This was a result of stomate closure induced by the germinating conidia.

Extracts of germinating conidia did not reduce transpiration rates and abscisic acid was not detected in the conidia. The reduction in transpiration continued through primary infection and was shown to be responsible for less ^{32}P uptake by inoculated plants than noninoculated plants during primary infection.

There were no indications for the presence of metabolic sinks induced by E. graminis, which concentrated ^{32}P at infection sites in the epidermis of inoculated plants during primary infection. Accumulation of ^{32}P in the epidermis was apparently due to increased cuticular transpiration caused by disruption of the cuticle by the developing fungus.

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By

Terry Joe Martin

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DEDICATED

to

Lorna, my wife

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TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (cont.)

LIST OF FIGURES (cont.)

LIST OF FIGURES (cont.)

INTRODUCTION

Many attempts have been made to correlate disease development and physiological or biochemical changes that occur in resistant or susceptible host tissue. Unfortunately most of these correlations have been made during late stages of disease development. Compatibility or incompatibility of an interaction is usually established during early stages of the infection process. The purpose of this study was to examine the interactions between host and parasite during the initial stages of the infection process.

Powdery mildew of wheat is caused by the obligate parasite Erysiphe graminis f. sp. tritici Em. Marchal. The disease has resulted in significant reductions in grain yields in some areas of the world (24, 53). The most practical means of controlling this disease is the development and use of resistant cultivars. Selection for disease resistance has made available many host cultivars with varying degrees of resistance to powdery mildew. The inheritance of resistance to E. graminis has been studied (6) and near-isogenic wheat lines which differ by only single genes for resistance have been developed (7). A

large number of isolates of the fungus that differ in respect to virulence are also available.

A well defined system for studying the primary infection process of E. graminis on wheat has been described (27, 32, 36). The process was divided into distinct stages, based on morphological development of the fungus. Each stage required specific environmental conditions for the parasite population to attain a high degree of synchrony. Synchrony of the parasite population allowed correlation of physiological and biochemical changes to morphological development of the parasite.

Slesinski (49) measured the rates of ^{35}S transfer from wheat leaves to the hyphae of E. graminis during primary infection. Stuckey (50) demonstrated the effect of the environment on the rates of ^{35}S transfer and also put the data on a quantitative basis by determining the amount of ^{35}S transferred to each parasite unit. He also took into consideration the amount of ^{35}S theoretically available for transfer. The rates of transfer were correlated with the morphological development of the parasite and were shown to be dependent on parasite/host genotype.

The objectives of my research were: 1) to examine the uptake and translocation of ^{32}P by the host under the environmental conditions used for synchronous parasite development; 2) to determine the rates of ^{32}P transfer from host to parasite by considering the amount

of label theoretically available in the epidermis for transfer to the parasite, and to correlate these with the morphological stage of development of the parasite with both compatible and incompatible parasite/host genotypes; 3) to use the criteria of ^{32}P transfer, infection efficiency (production of elongating secondary hyphae), and final infection type to determine if the three compatible parasite/host genotypes (Px/pmx, px/Pmx, and px/pmx) involving a single pair of corresponding genes in the parasite and host are identical; and 4) to determine why less ^{32}P is taken up by inoculated excised wheat leaves than by noninoculated excised wheat leaves.

LITERATURE REVIEW

In the following review I will attempt to summarize the important developments in the physiology and genetics of disease development that bear on the results reported herein.

The primary infection process of E. graminis on wheat has been divided into distinct morphological 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, and 6) development of elongating secondary hyphae. Each stage differed in its requirement for temperature, relative humidity, and light (28, 36). Under optimal conditions over 75% of the parasite population moved through each stage with a high degree of synchrony (28, 32, 36). The production of elongating secondary hyphae (ESH) has been used as the criterion for the establishment of a functional relationship between host and parasite (29). For each elongating secondary hyphae (ESH) that formed on the host surface a haustorium was produced in the epidermal cell (29). Conidia on non-host plants germinated, formed appressoria, and attempted to penetrate epidermal cells, but did not form either

haustoria or ESH (27, 54). The presence of the haustorium and ESH indicated the transfer of nutrients and other essential materials from host to parasite, thus the establishment of functional relationships. The percentage of applied conidia that produced ESH was defined as infection efficiency (14, 15).

Flor (18) found that the ability of Melampsora lini to grow and produce symptoms on flax lines containing certain genes was determined by specific corresonding 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 (19, 38). The gene-for-gene hypothesis states that for every (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 in the host to determine incompatibility (low infection type). Incompatibility results only when a (P) gene in the parasite interacts with its specific (R) gene in the host (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 host (R and r) and two at a corresponding locus in a parasite (P or p), there are four possible interactions (Figure 1). This basic scheme was proposed (42) as a biological test to study physiological and biochemical effects of disease development. By the use of different host genotypes in combination with various pathogen genotypes, a four way or

Fig. 1.--The four possible parasite/host genotypes involving a single gene pair governing compatibility of host and parasite. Rx and rx are alternate alleles in the host. Px and px are alternate alleles in the parasite. Px/Rx specifies incompatibility while Px/rx, px/Rx, and px/rx specify compatibility.

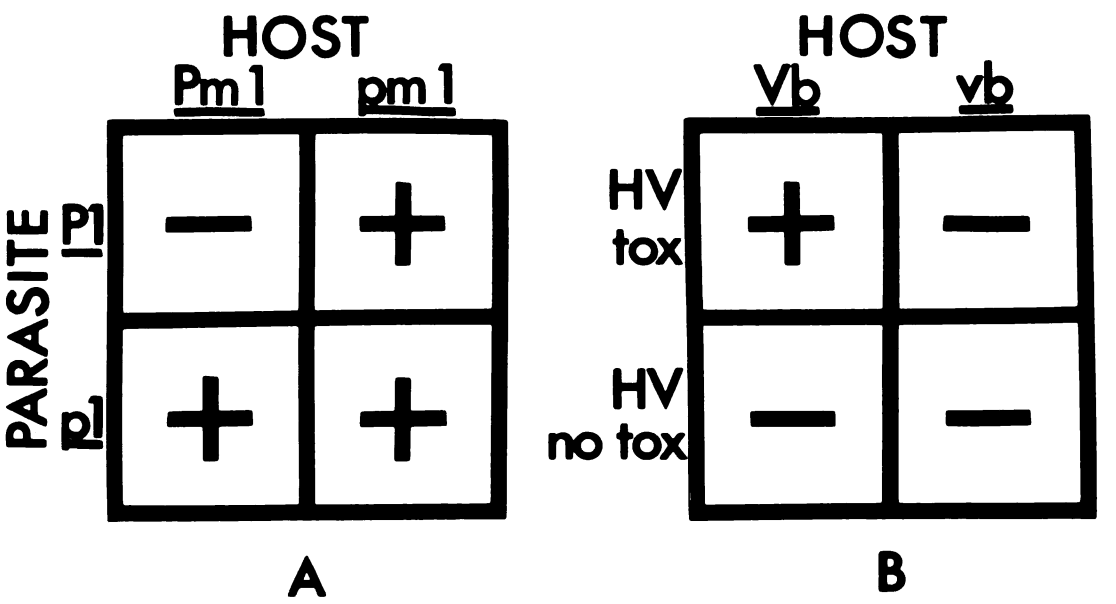

Figure 1

"quadratic check" is developed with which the observed phenomena can be associated. This test is useful in studying disease development, especially with the powdery mildew disease which followed the gene-for-gene relationship (30, 39). The gene-for-gene relationship has been demonstrated between plants and parasitic fungi, bacteria (8, 37), viruses (10), nematodes (23), and insects (20).

The effects of genes that condition incompatibility between parasite and host have been studied for both wheat and barley mildews (29, 47). None of the parasite/host genotypes for incompatibility (Px/Pmx for wheat mildew or Px/M&x for barley mildew) affected the germination of conidia or the formation of mature appressoria. Three of the four gene pairs affecting wheat mildew and all four of the gene pairs affecting barley mildew affected the percentage of parasite units which produced ESH (29) (one gene pair, P2/Pm2, apparently did not affect primary infection of wheat). The fate of parasite units that did form ESH with incompatible genotypes was also determined by the gene pairs (29).

Mount and Ellingboe (34) studied the transfer of ^{32}P and ^{35}S from wheat to E. graminis and were able to detect very small changes during primary infection. The amount of ^{32}P and ^{35}S transferred to the fungus was correlated with the development of haustoria. Slesinski and Ellingboe (49) demonstrated differences in ^{35}S transfer rates between compatible and incompatible parasite/host genotypes.

Environment was shown to affect ^{35}S transfer during primary infection (50). The increased rate of ^{35}S transfer following 20 hours after inoculation reported earlier (34, 49) was largely attributed to increased amounts of ^{35}S being available for transfer at these times rather than to an actual increase in ^{35}S transfer rate (50). The time of the highest rate of transfer was found to be earlier than previously reported but the same differences in ^{35}S transfer rates were found between compatible and incompatible parasite/host genotypes.

Using the criteria of final infection type, ^{35}S transfer, and percent ESH, the quadratic check was completed with the Pl/Pml gene pair (48). The three compatible interactions Pl/pml, pl/Pml, and pl/pml were indistinguishable by the criteria of percent ESH and final infection type, but a reduced rate of ^{35}S transfer was observed with pl/Pml (48, 49). The differences observed may have been due to other gene differences between the two near-isogenic host lines or between the non-isogenic strains of the parasite. The quadratic check was completed for Pg/M&g using the same criteria (23). Identical final infection types, ^{35}S transfer rates, and percent ESH were reported for all compatible combinations of this gene pair, i.e., Pg/m&g, pg/M&g, and pg/m&g. These quadratic checks are considered very important because they bear on the question of where the specificity of the gene-for-gene interactions occurs. Is it with the incompatible or compatible interactions?

The simplest hypothesis is that specific host-parasite interactions occur to give incompatible relationships. If this hypothesis is true, no differences between the two compatible genotypes Px/pmx and px/pmx, would be expected. The third compatible genotype px/Pmx, might be expected to be distinct from the other compatible genotypes Px/pmx and px/pmx because there are no reasons to believe that the transition in evolution from Pl to pl in the parasite would completely negate the transition in evolution from pml to Pml in the host. The pl/Pml interaction has only to have a selective advantage over the incompatible interaction Pl/Pml, not the other two genotypes for compatible interactions Pl/pml and pl/pml. Thus if pl/Pml resulted in a less compatible interaction than pl/pml or Pl/pml it would indicate that the pl product is an altered product of the Pl gene with an altered specificity for the Pml product.

Differences have been observed between the three genotypes for compatibility with one gene pair (48), but not with a second pair (21). Completion of the quadratic check with many gene pairs and with the use of many strains of host and parasite is needed to decide which is more universal.

The multiple alleles in barley at the Ml*a* locus (31) that determine reaction to E. graminis also demonstrates that the simplest explanation is that specific recognition occurs for incompatibility. The alleles in barley were shown to be distinct by reactions to

different strains of E. graminis f. sp. hordei. The corresponding genes in the parasite are not allelic. The pattern of interactions between the two alternate alleles in the parasite with each of six alleles in the host is shown in Figure 2 (16). Only when Pal interacted with M&al did an incompatible interaction result. Pal gave a compatible interaction with hosts with the other M&a alleles. If specific recognition for compatibility occurs this would mean that eleven different specific interactions or parasite/host genotypes can give the same compatible relationship while only one cannot.

If specific genetic recognition occurs between host and parasite to give an incompatible interaction, then intracistron recombination between two host alleles should yield a product that is not recognized by either of the corresponding parasite P genes. Two classes of compatible interactions should result from recombination between the two alleles. The means to distinguish between recombination between tightly linked genes and intracistronic recombination has been developed (Figure 3) (16). If recombination occurs between two tightly linked genes R1 and R2, only one class of recombinants, r1 r2, would give a compatible interaction with a pathogen that possesses the corresponding P1 and P2 genes for R1 and R2. If the two genes were alleles with different specificities, for example X3 + and + X8, and recognition occurred for incompatible relationships, there would be two classes of recombinants that would give compatible

Fig. 2.--The pattern of interaction with a multiple allelic series in the host. (-) = incompatibility, (+) = compatibility.

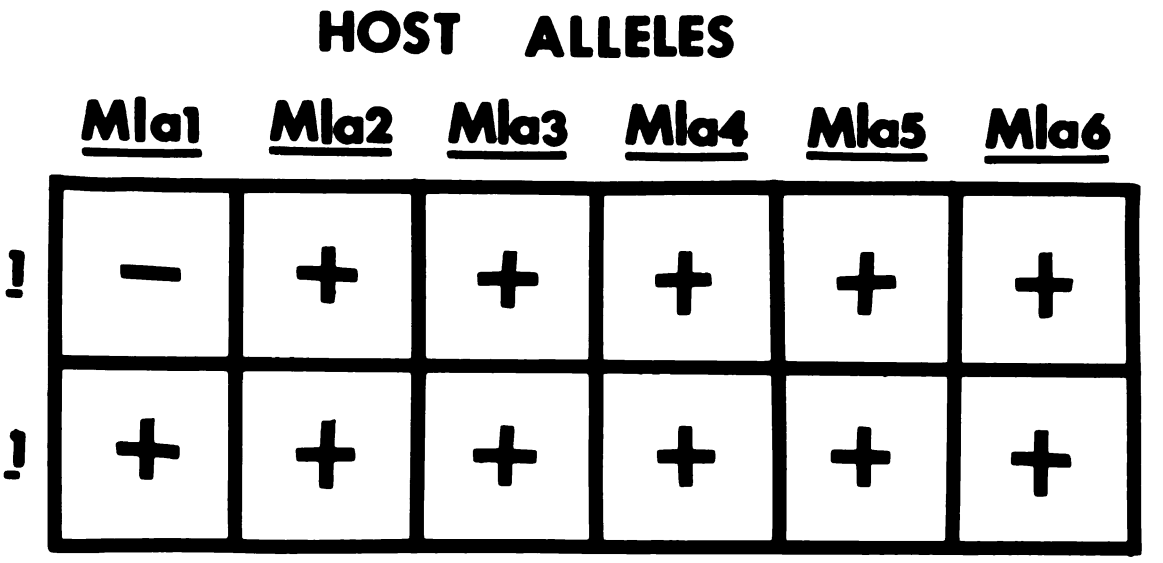

Figure 2

Fig. 3.--The two types of gene products following crossing over between two closely linked genes or within one cistron.

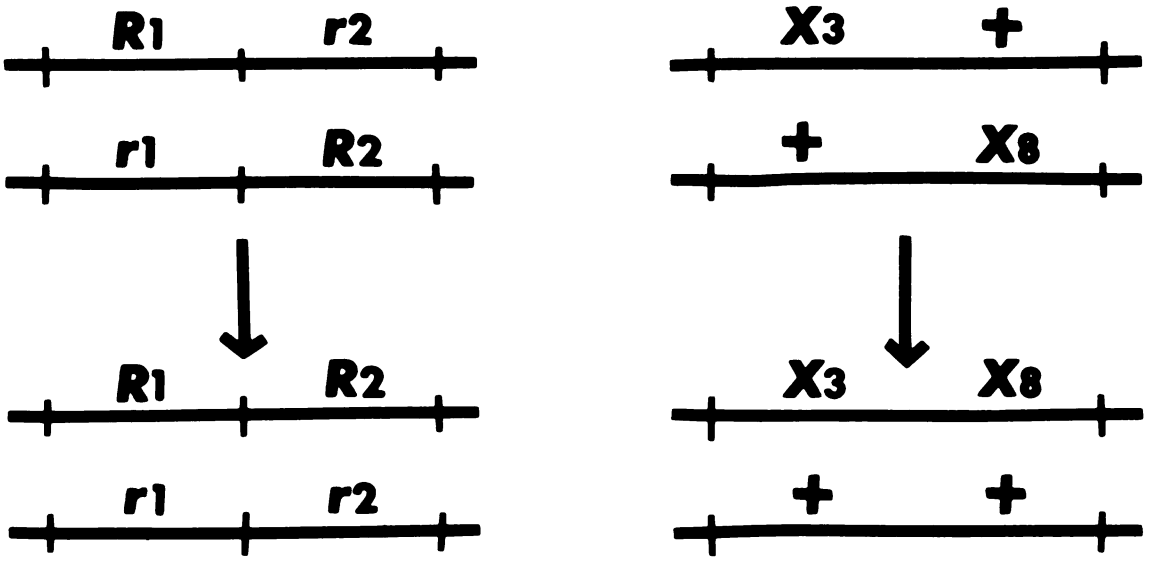

Figure 3

interactions with pathogens possessing the corresponding P genes for the host parental alleles. Crosses involving the *l2* and *l10* alleles at the *l* locus in flax did yield two classes of recombinants which gave compatible interactions with two cultures of *Melampsora lini* which recognize the *l2* and *l10* alleles (45). These results give additional evidence that the specificity in recognition between host and parasite is for an incompatible relationship.

Temperature sensitivity has played an important part in understanding some of the basic processes in life (13, 22, 51). Temperature sensitive mutations are believed to be missense mutations that lead to changes in primary structure of proteins that affect the stability of the tertiary structure of the protein. The tertiary structure is especially affected at high temperatures. Normal or near normal enzymatic activity of the protein may occur at low temperatures but be greatly reduced or absent at high temperatures.

Temperature sensitive genes affecting stem rust of wheat have been reported (25). The interaction between the *Sr6* gene and its corresponding *P6* gene in *Puccinia graminis* f. sp. *tritici* was temperature sensitive. Of the four possible parasite/host interactions, *P6/Sr6*, *P6/sr6*, *p6/Sr6*, and *p6/sr6*, only *P6/Sr6* was temperature sensitive. At the low temperature, the *P6/Sr6* interaction resulted in incompatibility. At the high temperature compatibility ensued. The simplest explanation of this is that the low temperature allows for

a specific interaction between host and parasite and that the high temperature did not allow specific interaction for incompatibility.

It is 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 compatible or incompatible relationships between host and parasite. A number of physiological changes are known to occur following inoculation of wheat with E. graminis. Respiration (1, 40), photosynthesis (43), and translocation patterns of organic molecules (44) have been reported to change two to 10 days after inoculation. However, previous studies (47) have shown that the establishment of compatibility or incompatibility often occurs before 24 hours after inoculation. It is important, therefore, to determine the very earliest interactions between a host and parasite.

MATERIALS AND METHODS

Production of Inoculum

All strains of *Erysiphe graminis* f. sp. *tritici* were maintained in different growth chambers on *Triticum aestivum* L. 'Little Club.' Each strain was purified by isolating and increasing single pustules from Little Club wheat. Each strain was periodically checked for purity by scoring infection types on a set of differential host lines (Table 1).

Wheat seedlings grown in the greenhouse for six days were dusted with conidia from plants inoculated seven days earlier. The cultures were kept under the following conditions.

1. Light--700 to 800 ft-c (650-750 ft-c from white VHO-fluorescent tubes and 50 ft-c from 25 watt incandescent bulbs), 16 hour photoperiod.

2. Temperature--18 ± 1 C during the day and 17 ± 1 C during the night.

TABLE 1.--Infection type produced seven days after inoculation of five near-isogenic wheat lines with six cultures of E. graminis f. sp. tritici.

Culture	Infection type[a]				
	Near-isogenic host line				
	(Chancellor) pmx	Pm1	Pm2	Pm3b	Pm4
MS-1	4	0	2	3	0
MS-2	4	0	4	3	4
MS-3	4	4	2	3	4
MS-4	4	4	2	3	0
MS-5	4	0	2	4	0
KhXCc[7]	4	0	2	3	4

[a]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. Relative humidity--80 ± 5% during the day and 95 ± 5% during the night.

4. Continuous air circulation.

Conidia produced on the sixth day after inoculation were used in all experiments. The wheat lines used in experiments were planted

in three inch pots of soil and kept in the greenhouse for five days before being inoculated.

Methods of Inoculation

The rolling technique of inoculation (35) was used for all studies of morphological development of the fungus during primary infection. The conidia were evenly dusted onto clean glass slides. The conidial chains and dead conidia were removed by lightly blowing across the slide. The remaining conidia were then transferred from the slide with a cotton swab to the abaxial side of the leaf. Development of the fungus was similar on both sides of the leaf (27).

Plants used in studies of ^{32}P uptake and transpiration were lightly dusted with conidia since the numbers of inoculated plants required for those experiments were quite high.

Environmental Conditions for Experiments

All experiments were done in Sherer-Gillett (Model CEL 512-37 and Model CEL 25-7) growth chambers. The following conditions were used to obtain high infection efficiency and synchronous growth of the parasite population during primary infection (0-30 hours) (46).

1. Zero to one hour after inoculation, plants were kept in the dark at 18 ± 1 C in 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 ± 5% RH.

3. Six to 20 hours after inoculation, plants were kept at the same temperature and RH as in above, but with no light.

4. Twenty to 30 hours after inoculation, conditions were the same as 2 above.

The above conditions were used for all experiments unless otherwise stated. Light intensity was measured at the leaf tips with a YSI Kettering Model 65 Radiometer. Temperature and RH were monitored during experiments with wet and dry bulb thermometers and a recording hygrothermograph calibrated with a sling psychrometer.

Designation of Genotypes

Briggle's terminology (6, 7) was used to designate the R genes conditioning reaction to E. graminis f. sp. tritici. Genes at a distinct locus have been designated Pm1, Pm2, etc. Genes at the same

locus but thought to be allelic are followed by the letters a, b, c, etc. Alternate alleles for each dominant Pm gene will be referred to by their respective recessive designations (pm1, pm2, etc.). Since the host lines are all homozygous, the genotypes will be written as haploid (Pmx) rather than in the diploid form (Pmx Pmx). The P genes in the parasite that result in incompatibility with host lines with dominate Pm genes will be designated as P1, P2, etc. The number refers to its corresponding Pm gene in the host. The alternate alleles for each P gene will be referred to by their respective recessive designations (p1, p2, etc.).

Parasite/host combinations are referred to by the corresponding genotypes which specify compatibility or incompatibility. For example, P4/Pm4 specifies incompatibility. The Pm4 gene in the host recognizes only the P4 gene in the pathogen, even though the pathogen may possess many other P genes. A corresponding gene pair which conditions incompatibility is expressed in the presence of many gene pairs specifying compatibility (49). Only the genes of the parasite/host genotype that specify incompatibility are given.

Examination of Fungal Structures

Determination of the percentage of the parasite population at each stage of development was made from counts on leaves with a

compound light microscope (160X). The number of parasite units at each stage of development was determined from a two cm length section of leaf at two hour intervals after inoculation. A new leaf section was used for each observation and 80-125 parasite units were counted each time.

Detection of ^{32}P in Leaf Sections, Epidermis, and Parasite

The amount of ^{32}P was determined in a one-cm-long-section of leaf, at least one cm from the leaf tip. Three methods were compared. 1) Leaf sections were placed directly into 15 mℓ of scintillation fluid [5 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-2-(5-phenyloxazolyl) benzene in 1 liter toluene] and radioactivity was determined in a Beckman LS-133 Liquid Scintillation Spectrometer. 2) Four leaf sections were macerated in 5 mℓ of 0.1 M Na-K-PO_4 buffer in a glass tissue grinder. A 0.1 mℓ aliquot was dried in the bottom of a scintillation vial before scintillation fluid was added. Radioactivity was determined as described above. 3) The same procedure for preparation of plant material was used as in 2 above, but a 0.1 mℓ aliquot was dried on a piece of Whatman #1 filter paper which was submerged in scintillation fluid. The data are expressed as CPM in one leaf section. Unless otherwise stated this last method was used in all experiments.

The amount of ^{32}P translocated to the epidermis was determined by stripping approximately 50% of the epidermis from the underside of one leaf section. All green tissue was removed before placing the epidermis tissue in scintillation vials. Scintillation fluid was added and the radioactivity was determined. The data are expressed as the CPM in the abaxial epidermis of a one cm leaf section.

The following procedure was used to separate the ectoparasitic portion of the parasite from the leaf. A 1.9% solution of parlodion in ether:ethanol (40:60) was applied to a one cm section of a leaf (11, 34). The solvent evaporated in minutes and the parlodion film was easily removed with forceps. The ectoparasitic portion of the parasite was embeded within the plastic strip. Four plastic strips were dried and placed into one scintillation vial. The radioactivity was determined as described above.

Uptake of ^{32}P

Twenty hours after inoculation wheat seedlings were cut at the crown with a razor blade (a film of water was present on the cutting edge) and placed into small vials (5 X 25 mm) that contained 0.1 mℓ of a 50 μCi/mℓ solution of $H_3{}^{32}PO_4$ in 0.1 M Na-K-PO_4 buffer (pH 6.9). One set of plants was kept in the dark and another set was kept in the light. Temperature and relative humidity were constant. After one, two,

three, four, five, six, eight, and 10 hours in ^{32}P solution the radioactivity in one cm length leaf sections was measured.

The amount of ^{32}P taken up by the leaf and translocated to the epidermis was also determined for five hour uptake periods beginning at various hours after inoculation. Different plants were used for each five hour uptake period. Inoculated wheat seedlings were cut and placed in the ^{32}P solution. Leaf sections and epidermal strips were taken from different plants every two hours beginning at six and ending at 30 hours after inoculation. Thus, the amount of ^{32}P in a leaf section at 10 hours after inoculation was the result of uptake from five to 10 hours after inoculation.

^{32}P Transfer from Host to Parasite

Rates of ^{32}P transfer from host to parasite were determined. Four parlodion strips were taken from inoculated plants which had taken up ^{32}P for five hours preceding the application of the parlodion. These were placed in a single vial and the amount of radioactivity determined. The results were plotted as radioactivity per 5,000 spores applied to the leaves. The number of spores applied was determined by making direct counts of parlodion strips made from plants inoculated at the same time but not labeled with ^{32}P. The radioactivity in parlodion strips made from noninoculated leaves given ^{32}P during the

same period as inoculated leaves was subtracted from the radioactivity transferred to the parasites. The rates of ^{32}P transfer during primary infection were determined for one compatible and six incompatible parasite-host interactions. These data were then corrected for the amount of ^{32}P theoretically available to be transferred (CPM/4500 CPM in the epidermis) and the amount transferred per functional parasite unit (47).

Quadratic Checks

The quadratic check includes all four possible parasite/host genotypes involving one corresponding gene pair (Figure 1). The criteria of infection type seven days after inoculation and ^{32}P transfer from host to parasite during primary infection were used to complete the quadratic checks for each of the following gene pairs, P1/Pm1, P2/Pm2, P3b/Pm3b, and P4/Pm4. Culture MS-1 (P1, P2, P3b, P4) was used in all cases as the pathogen with the dominant P gene. Cultures MS-4 and MS-5 were used as the pathogens with p1 and p3b, respectively. Culture MS-2 contained both p2 and p4. Chancellor wheat, the recurring parent of the near-isogenic lines, carries no known (Pm) genes. Chancellor was used in all cases as the host carrying the pmx genes. Four isogenic lines each containing one dominant Pm gene were used as the alternate host lines.

The criteria of final infection type and primary infection efficiency were used to complete the quadratic checks for P2/Pm2 and P4/Pm4. Three cultures, MS-2, MS-3, and KhXCc[7], were used as representatives of parasite cultures with the p4 gene. Culture MS-2 was also used as a representative culture with p2. The infection efficiency of culture MS-2 was also determined on the near-isogenic lines containing Pm1, Pm2, or Pm3b.

Effects of Inoculation on ^{32}P Uptake by Detached Leaves

Chancellor wheat seedlings were inoculated heavily by dusting with conidia, cut, and placed in the ^{32}P solution. Noninoculated seedlings were treated in the same manner. After five hours the amount of ^{32}P taken up and translocated to the epidermis was determined for both inoculated and noninoculated seedlings.

The effect of heavily dusting leaves with Puccinia graminis uredospores, Helminthosporium victoriae conidia, E. graminis f. sp. hordei conidia, chalk dust, and carborundum on ^{32}P uptake was also determined.

The effect of nonviable conidia in eliciting an effect on the host was determined by inoculating seedlings with conidia which had been irradiated with ultraviolet (UV) light. Conidia were dusted onto

glass slides and exposed to UV radiation (1.2×10^5 ergs cm^{-2} sec^{-1}) for 0, 10, 30, 60, 120, and 240 seconds. The percent germination of conidia at each exposure was determined by controlled inoculations (35) of wheat seedlings. The remainder of the conidia on the slides were dusted onto wheat seedlings to be used in uptake studies. The seedlings were cut and placed in ^{32}P solution one hour after inoculation. The amount of ^{32}P in a one cm length leaf section and the percent germination was determined at six hours after inoculation.

Effects of Inoculation on Transpiration

Transpiration rates were determined by placing cut inoculated or noninoculated seedlings in 5 X 25 mm test tubes which contained 0.1 mℓ of Na-K-PO_4 buffer (pH 6.9) one hour after inoculation. The test tubes were sealed with petroleum jelly and weighed on a Mettler balance. At six hours after inoculation the plants were weighed to determine water loss. The surface area of the leaves was obtained by tracing an outline of each leaf and measuring the area with a Paragon Compensating Polar Planimeter (Keuffel and Esser Co.).

Transpiration rates of intact plants were also measured. Chancellor (pmx) and four near-isogenic lines of Chancellor, each containing one known dominant gene Pm1, Pm2, Pm3b, or Pm4, were grown

in (10 X 75 mm) test tubes. Test tubes containing inoculated or non-inoculated five-day-old plants were sealed with petroleum jelly, and weighed on a Mettler balance one, three, and six hours after inoculation. The surface area of each leaf was determined as described above.

Relative Diffusion Resistance

Relative diffusion resistance of stomata to water vapor flow was estimated from measurements of permeability to air for noninoculated and inoculated wheat leaves with a modified Alvin's viscous flow porometer (2). Data were expressed as the cube root of the time required for a standard 10 mm Hg change in pressure (4). Because of the small size of the primary leaf of wheat, the third leaf of two-month-old plants was used.

Extraction of Germinated Conidia

Conidia were heavily dusted onto petri dishes containing 10 mℓ of distilled water. The conidia were removed by filtration after five hours at 22 C in the light. The filtrate was atomized onto intact wheat seedlings. Distilled water was atomized onto control plants. The rates of transpiration were determined for five hours after the filtrates were applied. Triton X-100 (one drop/200 mℓ extract) was added to both filtrate and controls for some experiments.

Aqueous extracts were made from conidia which germinated on distilled water. The extracts were tested for the presence of abscisic acid (ABA). Conidia were allowed to germinate for five hours on distilled water before the spore suspension was sonicated for 15 minutes. The suspension was centrifuged to remove spores. The pH of the supernatant was adjusted to 2.5 with 1 N HCl. The acidic solution was extracted four times with equal volumes of ethyl acetate in a separatory funnel. The ethyl acetate was dried over anhydrous Na_2SO_4 and filtered into a boiling flask and evaporated. The residue was taken up in two mℓ of ethyl acetate. The solution was spotted onto a silica gel GL plate for thin layer chromatography (TLC). ABA was also spotted onto the plate. Ethyl acetate:chloroform:acetic acid (60:40:5) was used as the solvent. The areas corresponding to the fluorescent spots of ABA when exposed to ultraviolet light were scraped from the plate and extracted with ethyl acetate. This extract was then methylated with diazomethane (3). The methylated extract was placed on a TLC plate with methylated ABA. Hexane:ethyl acetate (1:1) was used as the solvent. Ultraviolet light was used to determine the methylated ABA regions on the plates.

Effects of E. graminis on Uptake and Translocation of ^{32}P during Primary Infection of Wheat

The amount of ^{32}P taken up and translocated to the epidermis of inoculated and noninoculated plants during five hour uptake periods from one to 30 hours after inoculation was determined. (The ^{32}P in the leaf at 10 hours after inoculation was the result of exposing plants to ^{32}P from five to 10 hours after inoculation, ^{32}P in the leaves at 30 hours was the result of uptake from 25 to 30 hours after inoculation, etc.)

Inoculated and noninoculated plants were cut and placed in the ^{32}P solution in 100% RH 25 hours after inoculation. One set of plants were also pre-treated from 22 to 25 hours after inoculation in 100% RH before cutting. After cutting, the plants were allowed to take up ^{32}P from 25 to 30 hours after inoculation in 100% RH. Radioactivity in the epidermis was determined.

Transpiration Rates of Intact Inoculated and Noninoculated Plants during Primary Infection

Transpiration rates were determined by measuring the water loss from intact plants grown in 10 X 75 mm test tubes. The tubes

were sealed with petroleum jelly and weighed on a Mettler balance at the following times after inoculation: one, three, six, 10, 15, 20, 22, 24, 26, 28, 30, and 34 hours. This allowed the determination of rates of transpiration during various periods of primary infection. Transpiration rates were determined for inoculated and noninoculated Chancellor (pmx) wheat and four near-isogenic lines of wheat each with one of the following genes Pm1, Pm2, Pm3b, or Pm4.

Replication and Statistics

Experiments involving ^{32}P uptake, translocation to the epidermis, and transfer to the parasite were repeated at least six times on six different days with two replications daily. Experiments involving fungal morphological development and transpiration rates were repeated on three or four different days. The data are presented as averages of all replications. Statistical analyses were done using a two-way analysis of variance, using an F-test at the 5% level of significance.

RESULTS

Replication of Previous Work

The development of E. graminis f. sp. tritici during primary infection in a highly synchronous manner has been described (29, 32, 36). It was considered essential that this work be repeated because meaningful interpretation of results reported herein is based on the reproducibility of the earlier work. The optimum environmental conditions for germination, formation of appressorial initials, formation of mature appressoria, production of secondary hyphal initials, and the production of elongating secondary hyphae were used as previously reported (51). The development of the parasite population is presented in Figure 4. It is in close agreement with previous findings.

^{32}P Uptake and Translocation to the Epidermis

The most efficient method of determining the radioactivity in the leaves was to macerate the tissue in buffer and then dry an aliquot on filter paper (Table 2). It appeared that drying the

Fig. 4.--Development of Erysiphe graminis f. sp. tritici during primary infection of wheat leaves. (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; (----------) from Slesinski (54), (_____) results obtained in this study.

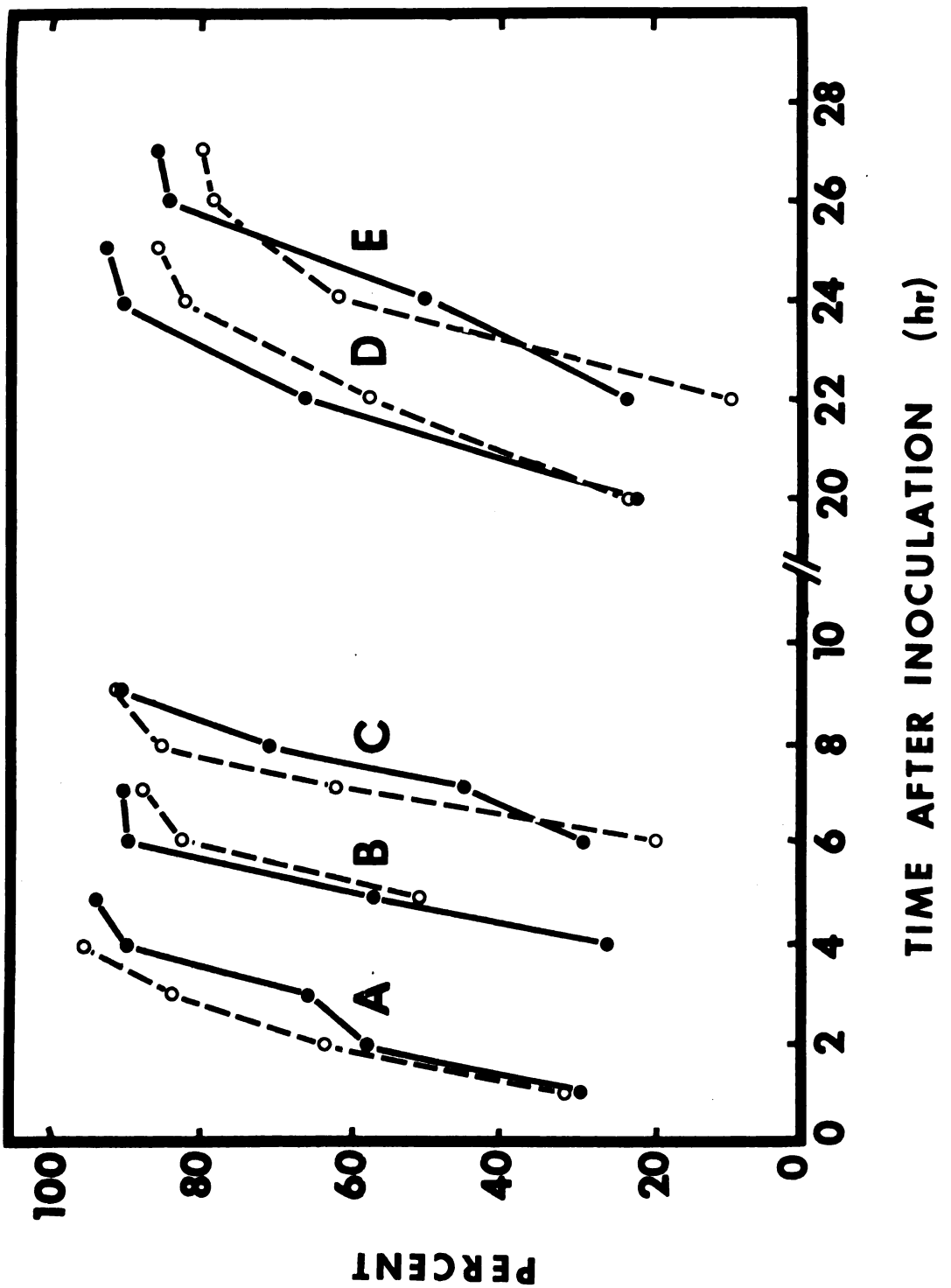
PERCENT
100
80
60
40
20
0
A
B
C
D
E
0
2
4
6
8
10
20
22
24
26
28
TIME AFTER INOCULATION (hr)

Figure 4

TABLE 2.--The amount of ^{32}P taken up by one cm leaf sections (1.0 X 0.5 cm) as determined by three methods of preparing the sample.

Time after Inoculation (hr)	Radioactivity (CPM)		
	Direct Leaf Section Count	Aliquot Dried In Scintillation Vial	Aliquot Dried On Filter Paper
20	39,292	38,350	53,000
28	--	131,200	167,600
30	--	116,176	149,500

aliquot in the bottom of a scintillation vial was no better than placing the whole leaf section in the vials. Samples were dried on filter paper throughout the remaining experiments.

The effect of light on ^{32}P uptake by inoculated leaves was determined (Figure 5). There was a very definite effect of light on ^{32}P uptake. After five hours in ^{32}P the leaves kept in the dark were only 50% as radioactive as plants kept in the light. The rate of ^{32}P uptake during this 10 hour period appeared to be reasonably constant. This was true for plants in the light or the dark.

The effects of the environment during primary infection on uptake of ^{32}P and translocation to the epidermis were determined (Figure 6). The environmental conditions necessary for synchronous parasite development are indicated across the top of the figure.

Fig. 5.--The effect of light on ^{32}P uptake in inoculated wheat leaves one through 10 hours after being placed in ^{32}P solution (^{32}P CPM X 10^{-5} cm^{-1} leaf section).

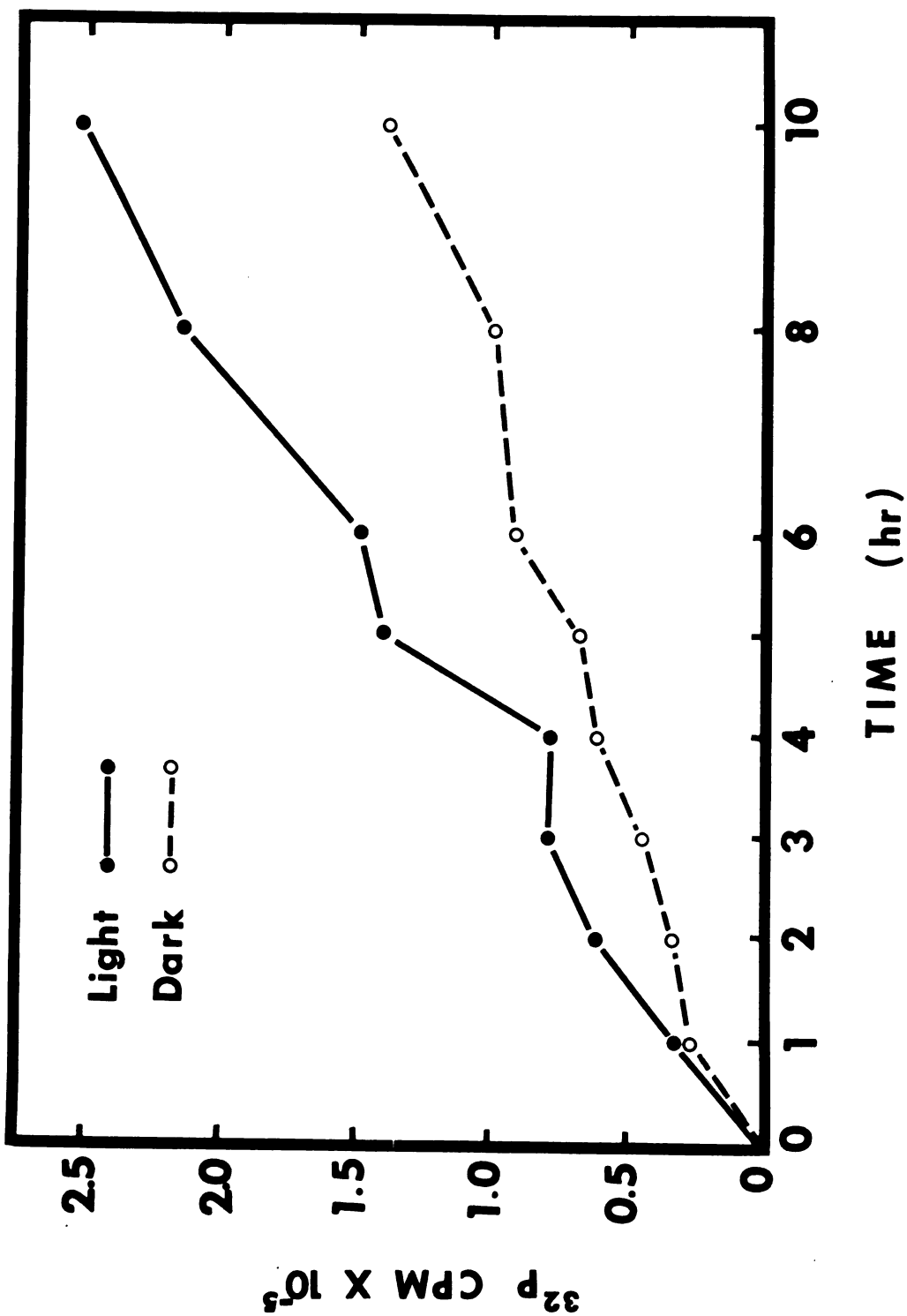

Figure 5

Fig. 6.--The effect of the environmental conditions used for synchronous parasite development during primary infection on ^{32}P uptake during five hour uptake periods by wheat leaves and translocation to the epidermis in wheat leaves inoculated with Erysiphe graminis f. sp. tritici; Leaf sections (o_____o), Epidermis (●---------●).

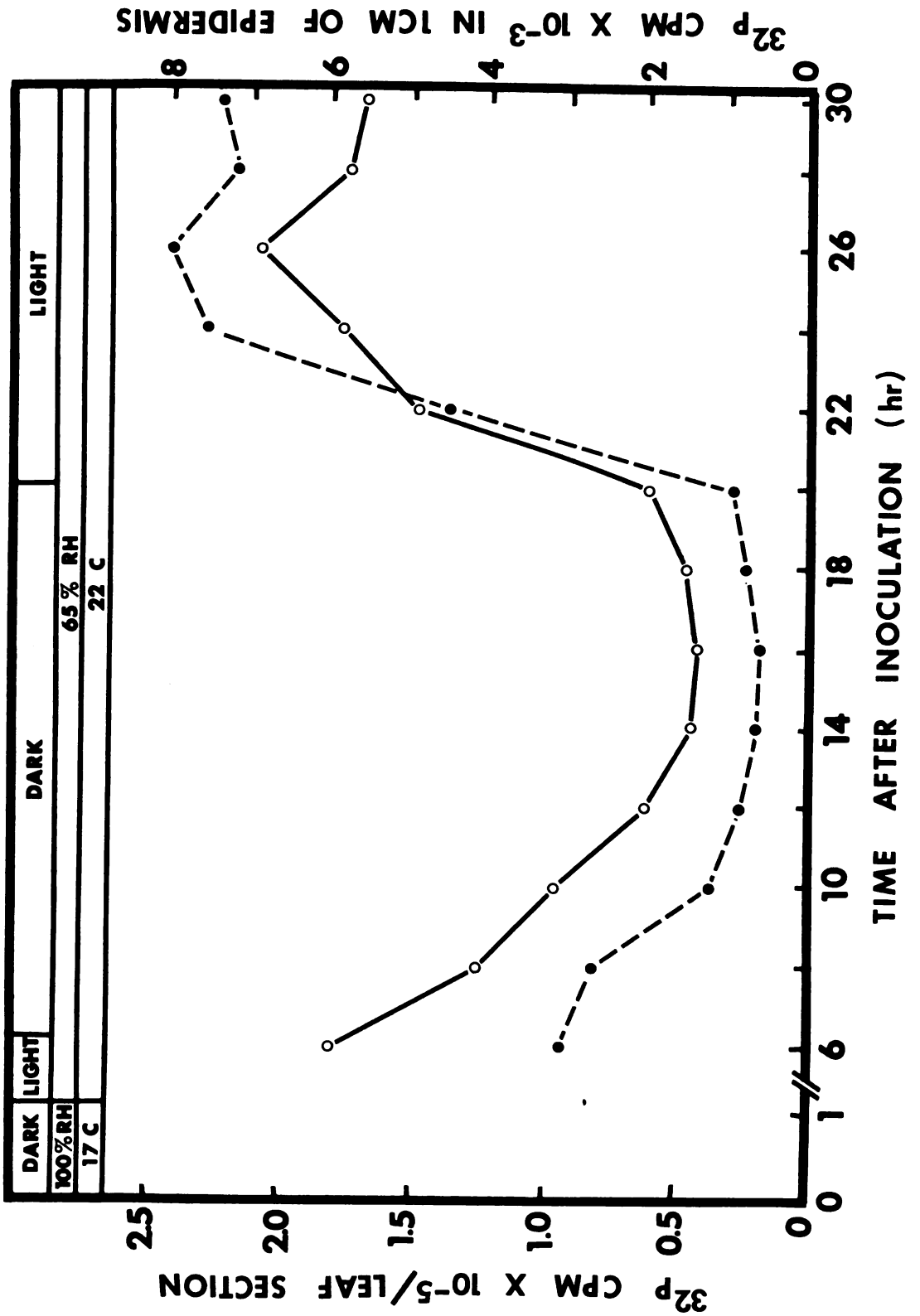
DARK
LIGHT
100% RH
17 C
DARK
LIGHT
65 % RH
22 C
32P CPM X 10-5/LEAF SECTION
32P CPM X 10-3 IN 1CM OF EPIDERMIS
TIME AFTER INOCULATION (hr)
0
0.5
1.0
1.5
2.0
2.5
0
2
4
6
8
1
6
10
14
18
22
26
30

Figure 6

Both curves show a major effect of light on ^{32}P uptake or translocation. The radioactivity in the epidermis of plants taking up ^{32}P from 21 to 26 hours after inoculation was eight times greater than in the epidermis of those plants that took up ^{32}P from 15 to 20 hours. Radioactivity in the leaf sections shows a fourfold increase at the two different uptake times. The ^{32}P in the epidermis would be theoretically available for transfer to the parasite. So there were large differences in the amount available to be transferred to the parasite, depending on the time after inoculation when the ^{32}P was taken up.

Transfer of ^{32}P to the Parasite

The rates of ^{32}P transfer from wheat leaves to E. graminis was determined, using near-isogenic wheat lines (Figure 7). Host lines Pm1, Pm2, Pm3b, and Pm4 are incompatible with culture MS-1 (Px) while pmx is compatible with culture MS-1 (Px). The radioactivity in parlodion strips from noninoculated plants which had taken up ^{32}P during the same time was subtracted from the amount of ^{32}P in parlodion strips from inoculated plants. This control averaged 53 to 81 CPM.

^{32}P transfer from all wheat lines to the fungus started at 10 hours after inoculation and increased very slowly until 20 hours after inoculation. There were no differences between near-isogenic

Fig. 7.--Rates of ^{32}P transfer from five near-isogenic wheat lines to Erysiphe graminis f. sp. tritici culture MS-1 (Px). Plants were cut at the base various hours after inoculation and placed in a ^{32}P solution for five hours. The ectoparasitic portion of the fungus was then removed in parloidion strips and the radioactivity determined (^{32}P CPM X 10^{-3}/5,000 spores applied).

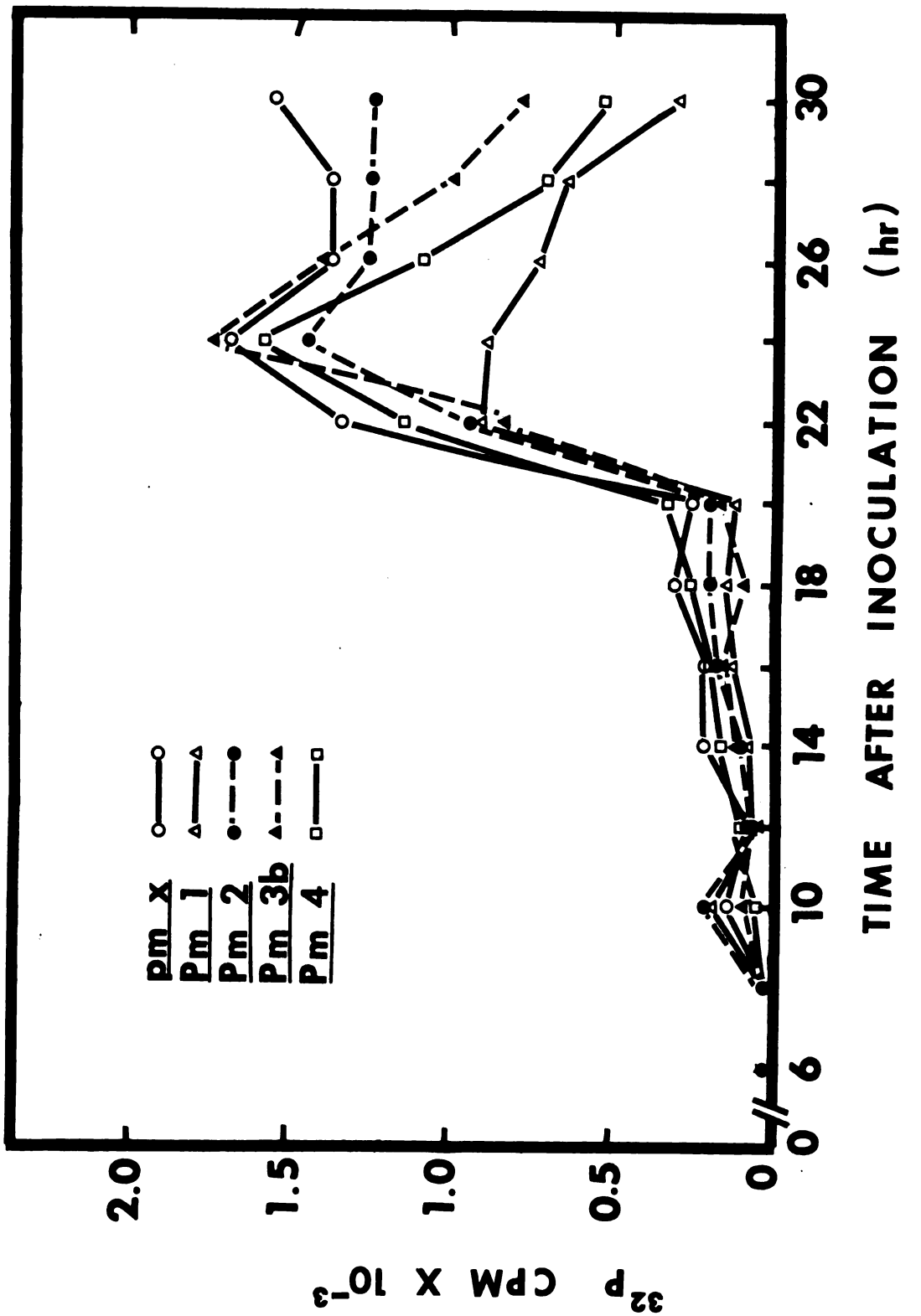
Pm X
Pm 1
Pm 2
Pm 3b
Pm 4
2.0
1.5
1.0
0.5
0
0
6
10
14
18
22
26
30
TIME AFTER INOCULATION (hr)
32P CPM X 10-3

Figure 7

lines during this period. With the compatible genotype (pmx), the rates increased sharply at 22 and 24 hours, then leveled off or decreased slightly from 26 to 30 hours. The rates of transfer from Pm1 were significantly less compared to pmx at 24 hours. Transfer continued to decrease from 22 to 30 hours. The transfer kinetics were the same from Pm2 and pmx. Rates of transfer from Pm3b were not significantly different from pmx until 30 hours after inoculation. Transfer rates from Pm4 were similar to pmx until 28 hours after inoculation, when the rate dropped rapidly.

The kinetics of ^{32}P transfer from pmx and three alleles of the Pm3 locus were determined (Figure 8). Pm3a and Pm3b had similar rates of transfer, while Pm3c did not differ from pmx. Pm3a and Pm3b were both significantly different from pmx and Pm3c 30 hours after inoculation.

The rates of ^{32}P transfer shown in Figure 7 were adjusted for the amount of ^{32}P in the epidermis at each hour after inoculation (Figure 9). The amount of ^{32}P in the epidermis is theoretically the amount available to be transferred from the epidermis to the parasite (Figure 6). The data were plotted as ^{32}P CPM/5000 spores/4500 CPM available in the epidermis at the end of the five hour uptake period. Rates of ^{32}P transfer from the five near-isogenic lines from six to

Fig. 8.--Rates of ^{32}P transfer from near-isogenic wheat lines of the Pm3 allelic series to Erysiphe graminis f. sp. tritici culture MS-1 (Px). Plants were cut at the base various hours after inoculation and placed in a ^{32}P solution for five hours. The ectoparasitic portion of the fungus was removed in parloidion strips and the radioactivity determined (^{32}P CPM X 10^{-3}/5,000 spores applied).

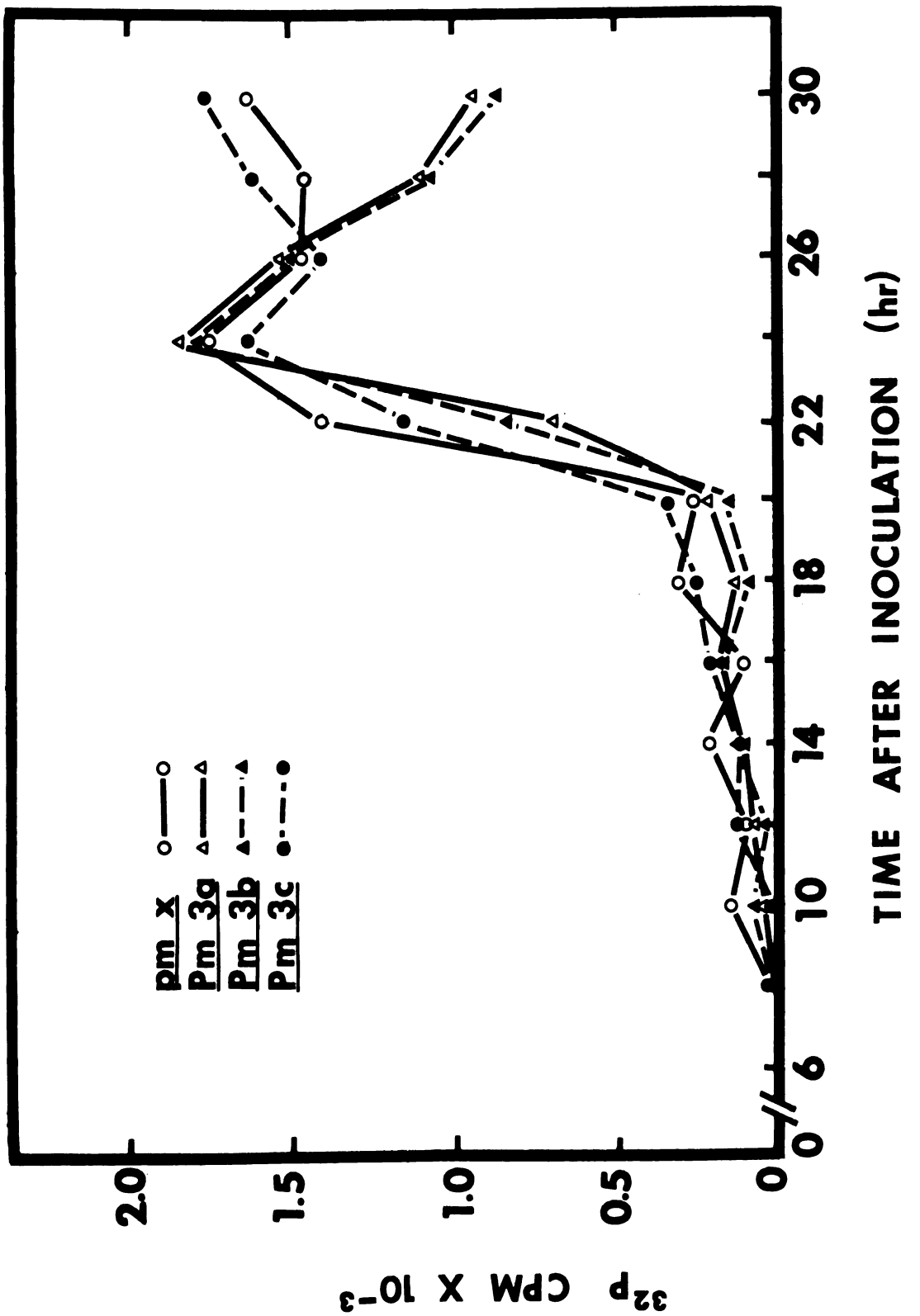
Pm X
Pm 3a
Pm 3b
Pm 3c
2.0
1.5
1.0
0.5
0
0
6
10
14
18
22
26
30
TIME AFTER INOCULATION (hr)
32P CPM X 10-3

Figure 8

Fig. 9.--Rates of ^{32}P transfer from five near-isogenic wheat lines to Erysiphe graminis f. sp. tritici culture MS-1 (Px). Plants were cut at the base various hours after inoculation and placed in a ^{32}P solution for five hours. The ectoparasitic portion of the fungus was then removed in parloidion strips and the radioactivity determined (^{32}P CPM X 10^{-3}/5,000 spores applied/4,500 ^{32}P CPM in the epidermis).

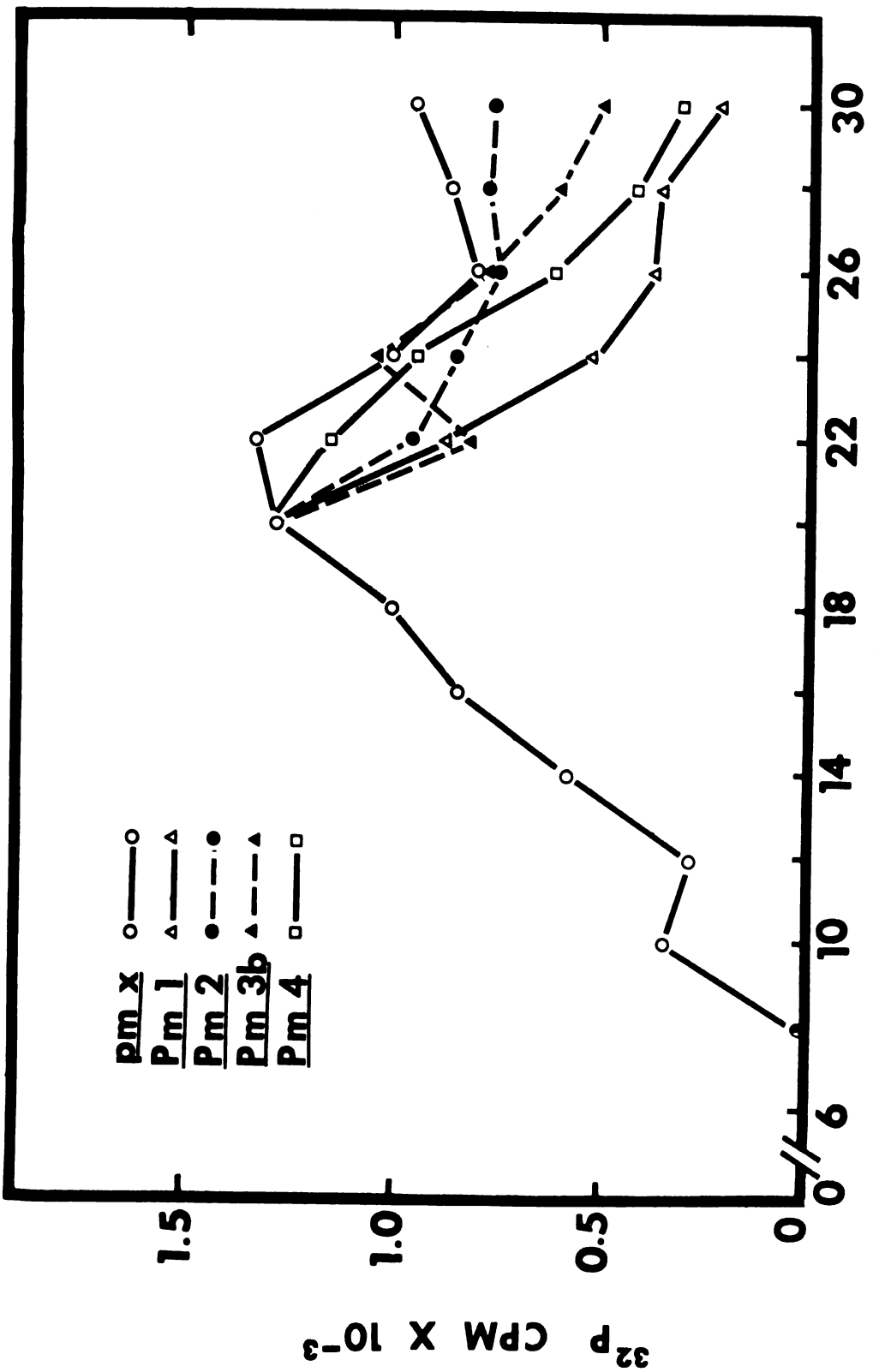
Pm x
Pm 1
Pm 2
Pm 3b
Pm 4
1.5
1.0
0.5
0
0
6
10
14
18
22
26
30
TIME AFTER INOCULATION (hr)
32P CPM X 10-3

Figure 9

20 hours were not significantly different from each other, thus, these data were averaged together and represented by a single line. With the adjustments made (Figure 9), the same differences between host lines which were shown in Figure 7 are seen again, but the maximum rate of transfer from pmx now appears to occur from 20 to 22 hours instead of 24 to 26 hours after inoculation. A steady increase in transfer rate occurred from ten to 22 hours and then a gradual decrease occurred from 22 to 30 hours after inoculation.

The rates of ^{32}P transfer from pmx in the light and dark from 20 to 30 hours after inoculation were determined (Figure 10). The data were plotted as CPM/5000 spores applied. No correction for the amount of ^{32}P in the epidermis was made. Transfer rates in the light were similar to those reported in Figure 7. Transfer rates in the dark at 23 and 29 hours were not different from the rate of ^{32}P transferred at 20 hours. This gives support to the argument that the adjustment of transfer data to take into consideration the amount of ^{32}P activity in the epidermis is reasonable.

If we assume that only functional parasite units (47), i.e., parasite units with haustoria and ESH, are taking up ^{32}P from the leaf we can further adjust the data in Figure 9 to give the rates of ^{32}P transfer/functional parasite unit/CPM available in the epidermis

Fig. 10.--Effect of light given from 20 to 30 hours after inoculation on ^{32}P transfer from Chancellor wheat (pmx) allowed to take up ^{32}P for five hours to Erysiphe graminis f. sp. tritici culture MS-1 (Px). Dark from six to 20 hours and light from 20 to 30 hours (o_____o), dark from six to 30 hours (●----------●).

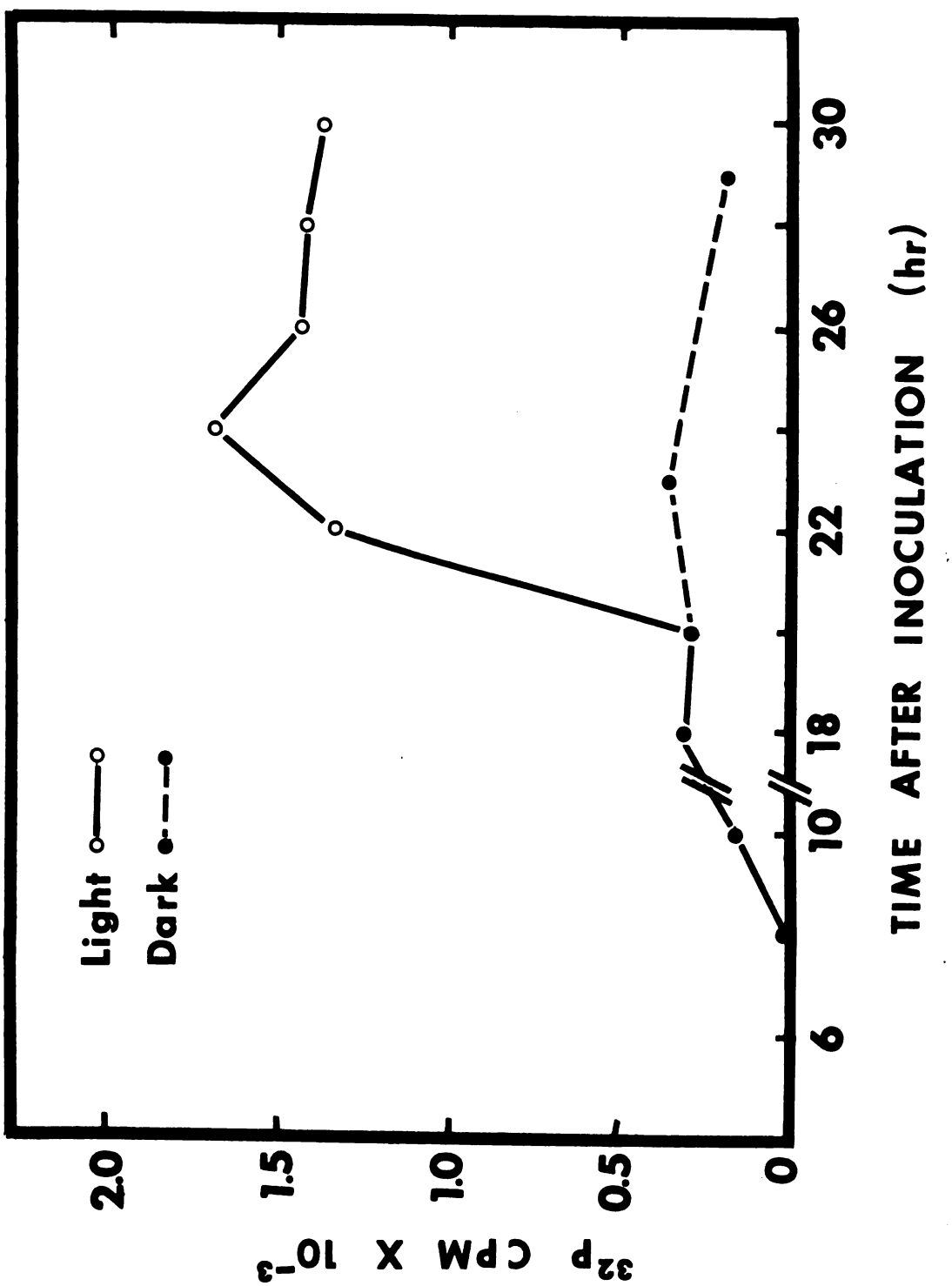
Light
Dark
2.0
1.5
1.0
0.5
0
6
10
18
22
26
30
TIME AFTER INOCULATION (hr)
32P CPM X 10-3

Figure 10

(Figure 11). Infection efficiencies on pmx, Pm1, Pm3b, and Pm4 were 80, 17, 30, and 4%, respectively. The adjustments indicated the rates of ^{32}P transfer/successful primary infection were higher with incompatible genotypes than rates with compatible genotypes. At 30 hours after inoculation the rates of ^{32}P transfer/successful primary infection with the incompatible interactions with Pm1 and Pm3b were the same as the rate for the compatible interaction with pmx. Transfer rates from Pm4 were not the same as from pmx at 30 hours, but the rate was decreasing rapidly. The adjustment was not applied to transfer rates from Pm2 because it gave results similar to transfer from pmx.

Quadratic Checks

The rates of ^{32}P transfer from wheat to E. graminis with the four possible parasite/host genotypes involving P1 and Pm1 were determined (Figure 12). The incompatible interaction P1/Pm1, which gives a low infection type, had a low rate of ^{32}P transfer as shown previously (Figure 7). The three compatible interactions, which all gave the same high infection type, had similar rates of ^{32}P transfer.

The final infection type of the four possible interactions involving P2 and Pm2 followed the usual pattern for the quadratic check, all four genotypes gave the same rates of transfer during

Fig. 11.--Rates of ^{32}P transfer from four near-isogenic wheat lines to Erysiphe graminis f. sp. tritici culture MS-1 (Px). Plants were cut at the base various hours after inoculation and placed in a ^{32}P solution for five hours. The ectoparasitic portion of the fungus was removed in parloidion strips and radioactivity determined (^{32}P CPM X 10^{-3}/5,000 functional parasite units/4,500 ^{32}P CPM in the epidermis).

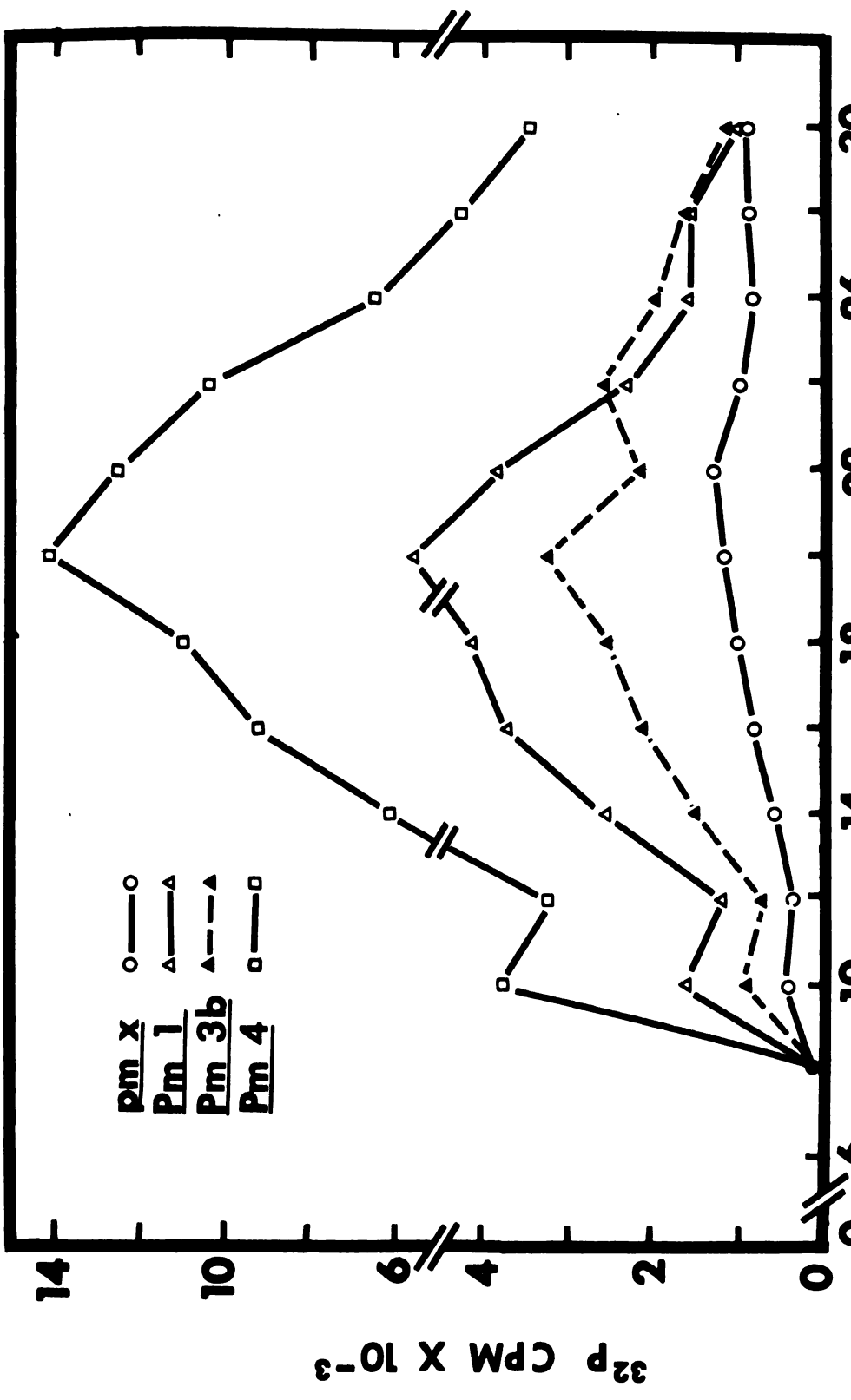
32P CPM X 10-3
0
2
4
6
10
14
0
6
10
14
18
22
26
30
TIME AFTER INOCULATION (hr)
Pm x
Pm 1
Pm 3b
Pm 4

Figure 11

Fig. 12.--Rates of ^{32}P transfer from wheat to Erysiphe graminis f. sp. tritici with the four possible parasite/host genotypes involving Pl and Pml. Plants were cut at the base various hours after inoculation and placed in a ^{32}P solution for five hours. The ectoparasitic portion of the fungus was removed in parloidion strips and radioactivity determined (^{32}P CPM X 10^{-3}/5,000 spores applied).

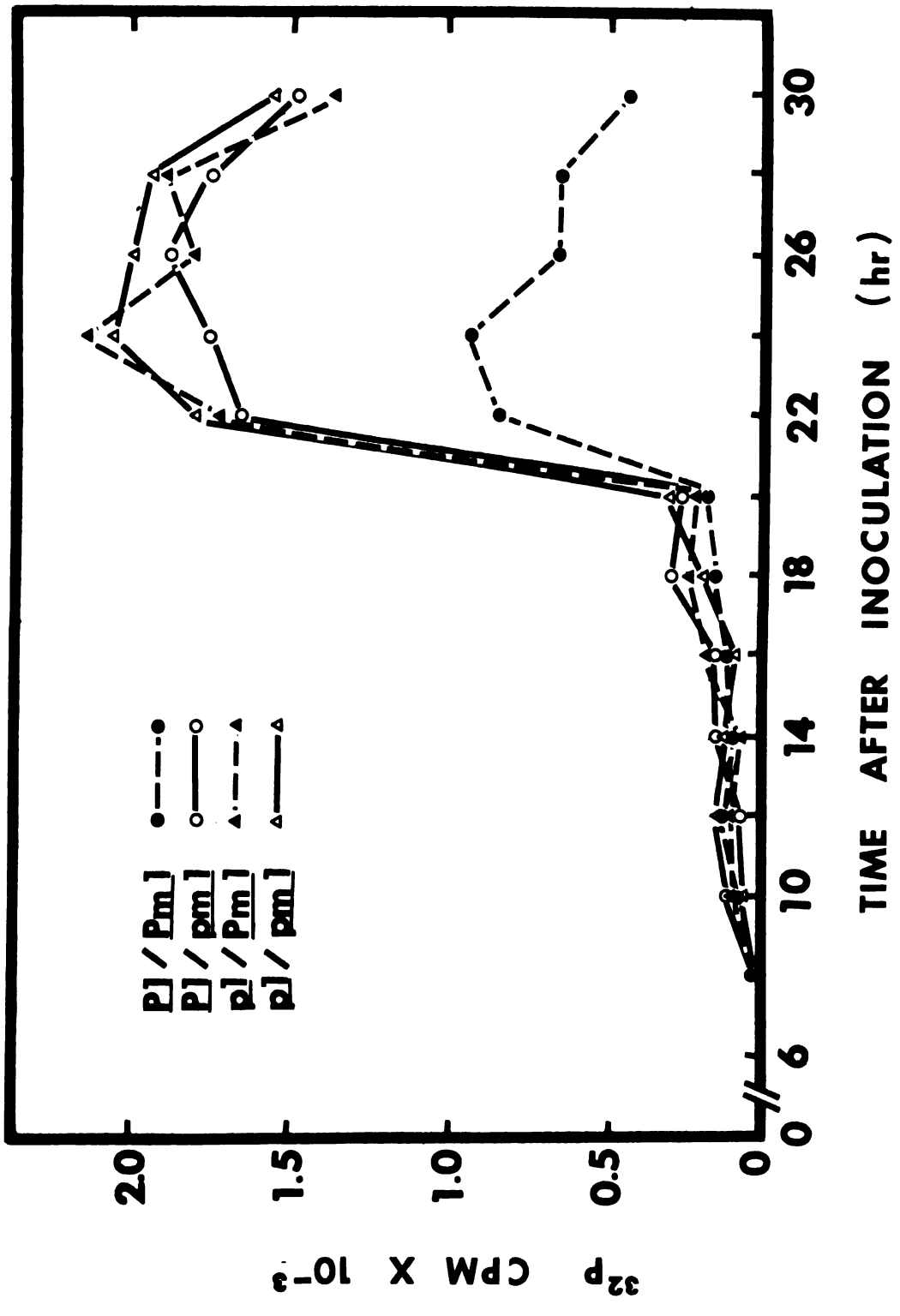
^{32}P CPM X 10^{-3}
2.0
1.5
1.0
0.5
0
0
6
10
14
18
22
26
30
TIME AFTER INOCULATION (hr)
P1/Pm1
P1/pm1
p1/Pm1
p1/pm1

Figure 12

primary infection (Figure 13). P2/Pm2 has not been shown to affect primary infection efficiency (34). The rates of ^{32}P transfer supported that conclusion.

The quadratic check with P3b and Pm3b also resulted in one genotype with low infection type and three genotypes with high infection types. Rates of ^{32}P transfer (Figure 14) were similar with the three compatible genotypes while transfer was lower after 26 hours with the incompatible genotype.

The final infection types of the four possible genotypes involving P4 and Pm4 also followed the expected pattern of the quadratic check, namely, one genotype gave low infection type and three genotypes gave high infection types. The rates of ^{32}P transfer with the incompatible genotype were similar to earlier results (Figure 7). The rates of transfer for the p4/Pm4 genotype were reduced when compared to P4/pm4 and p4/pm4 (Figure 15). The quadratic check using the same cultures was then completed using the criterion of production of elongating secondary hyphae (i.e., infection efficiency). A slower rate of development of elongating secondary hyphae (ESH) with p4/Pm4 than with the other two compatible genotypes was observed (Figure 16). The same infection efficiency was obtained but it was delayed approximately two hours compared to the other compatible genotypes. Standard deviations bracket each point (Figure 16). The behavior of MS-2 cannot be due to nonspecific genes for slow growth because normal kinetics of the formation of ESH were observed on the host line with pm4.

Fig. 13.--Rates of ^{32}P transfer from wheat to Erysiphe graminis f. sp. tritici with the four possible parasite/host genotypes involving P2 and Pm2. Plants were cut at the base various hours after inoculation and placed in a ^{32}P solution for five hours. The ectoparasitic portion of the fungus was removed in parloidion strips and radioactivity determined (^{32}P CPM X 10^{-3}/5,000 spores applied).

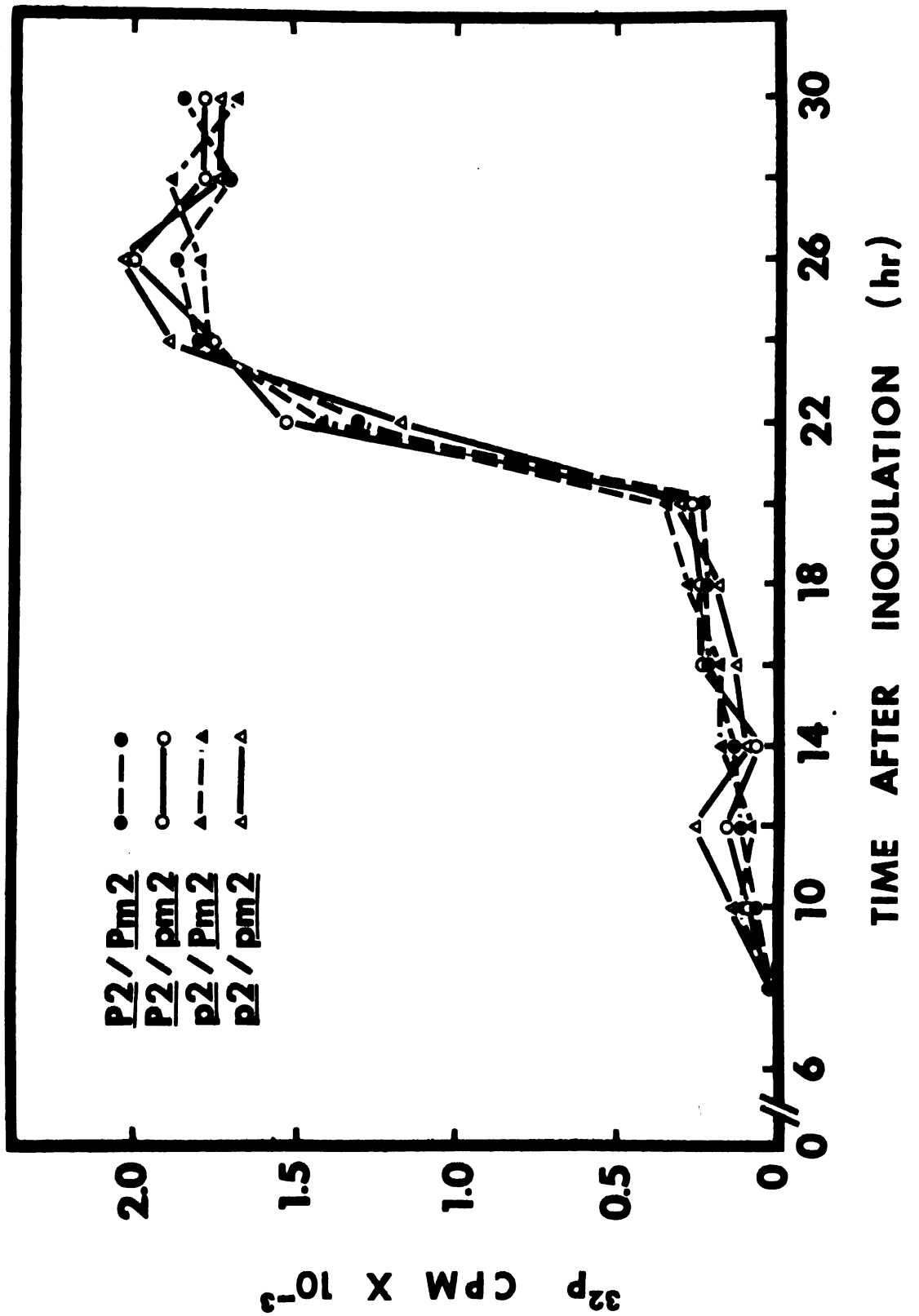
^{32}P CPM X 10^{-3}
2.0
1.5
1.0
0.5
0
0
6
10
14
18
22
26
30
TIME AFTER INOCULATION (hr)
P2/Pm2
P2/pm2
p2/Pm2
p2/pm2

Figure 13

Fig. 14.--Rates of ^{32}P transfer from wheat to Erysiphe graminis f. sp. tritici with the four possible parasite/host genotypes involving P3 and Pm3b. Plants were cut at the base various hours after inoculation and placed in a ^{32}P solution for five hours. The ectoparasitic portion of the fungus was removed in parloidion strips and radioactivity determined (^{32}P CPM X 10^{-3}/5,000 spores applied).

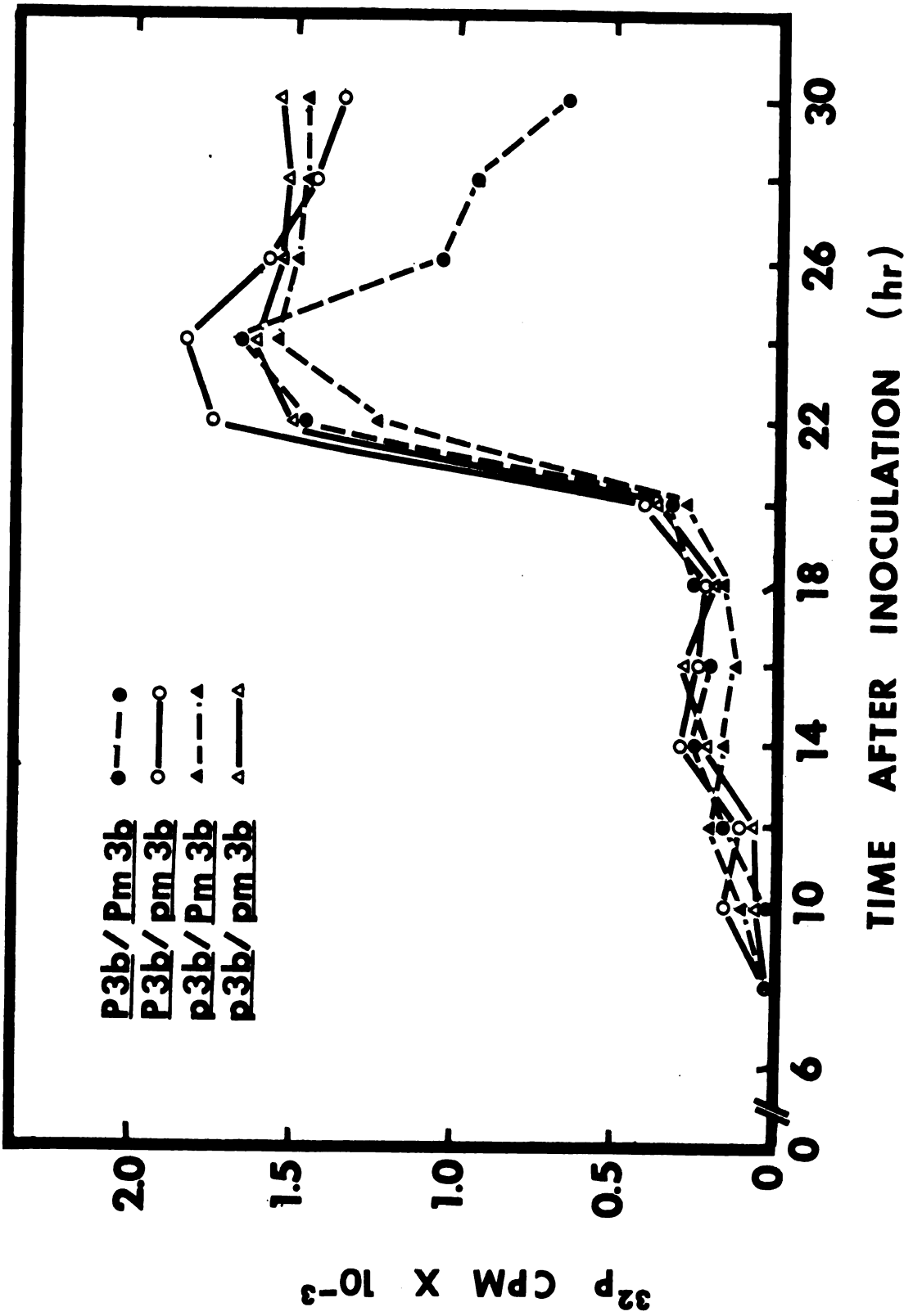
P3b/ Pm 3b
P3b/ pm 3b
p3b/ Pm 3b
p3b/ pm 3b
2.0
1.5
1.0
0.5
0
0
6
10
14
18
22
26
30
TIME AFTER INOCULATION (hr)
32P CPM X 10-3

Figure 14

Fig. 15.--Rates of ^{32}P transfer from wheat to Erysiphe graminis f. sp. tritici culture MS-1 (P4) and MS-2 (p4) with the four possible parasite/host genotypes involving P4 and Pm4. Plants were cut at the base various hours after inoculation and placed in a ^{32}P solution for five hours. The ectoparasitic portion of the fungus was removed in parloidion strips and radioactivity determined (^{32}P CPM X 10^{-3}/5,000 spores applied).

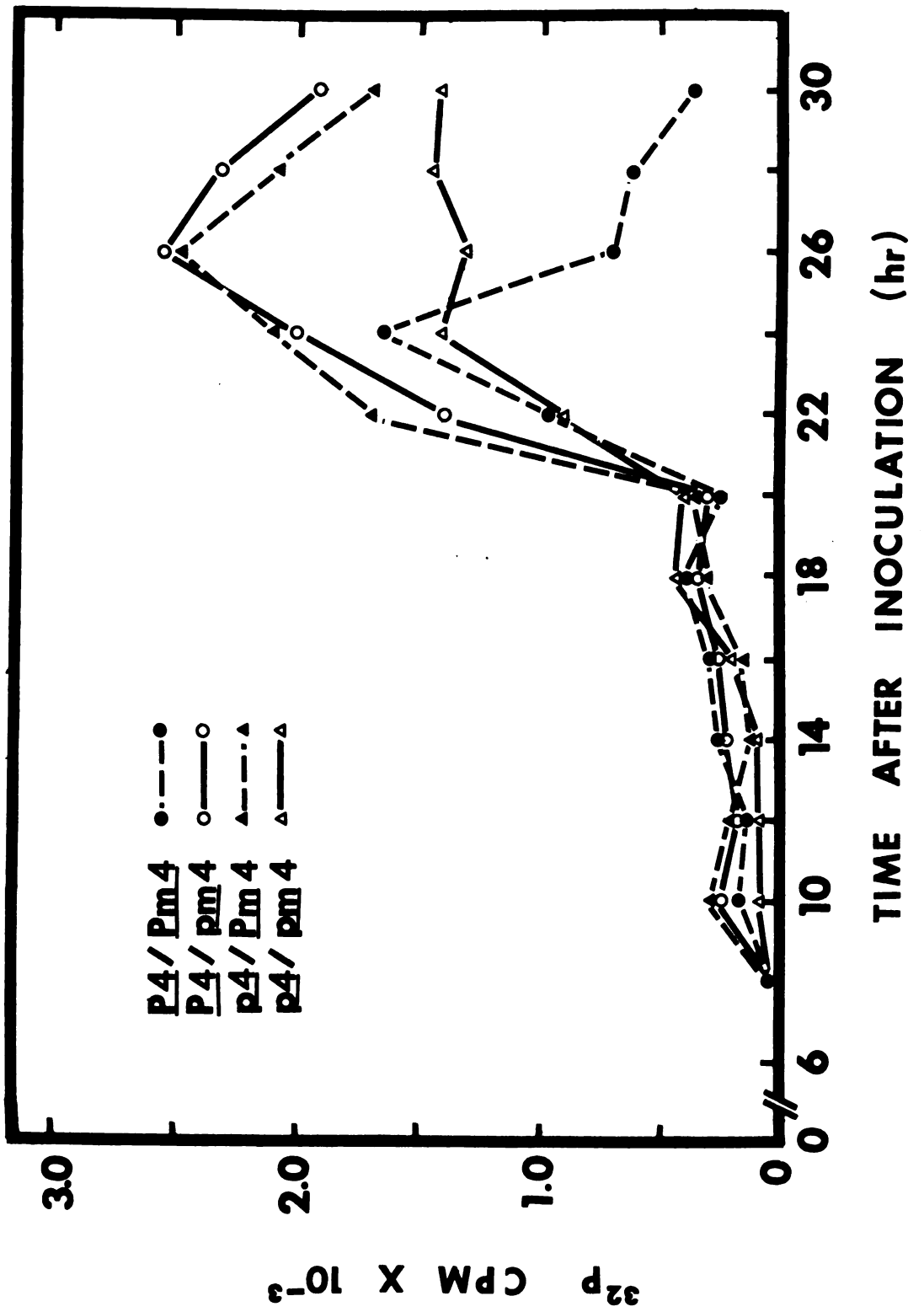
^{32}P CPM X 10^{-3}
3.0
2.0
1.0
0
0
6
10
14
18
22
26
30
TIME AFTER INOCULATION (hr)
P4/Pm4
P4/pm4
p4/Pm4
p4/pm4

Figure 15

Fig. 16.--Formation of elongating secondary hyphae (ESH) by Erysiphe graminis f. sp. tritici cultures MS-1 (P4) and MS-2 (p4) with the four possible parasite/host genotypes involving P4 and Pm4.

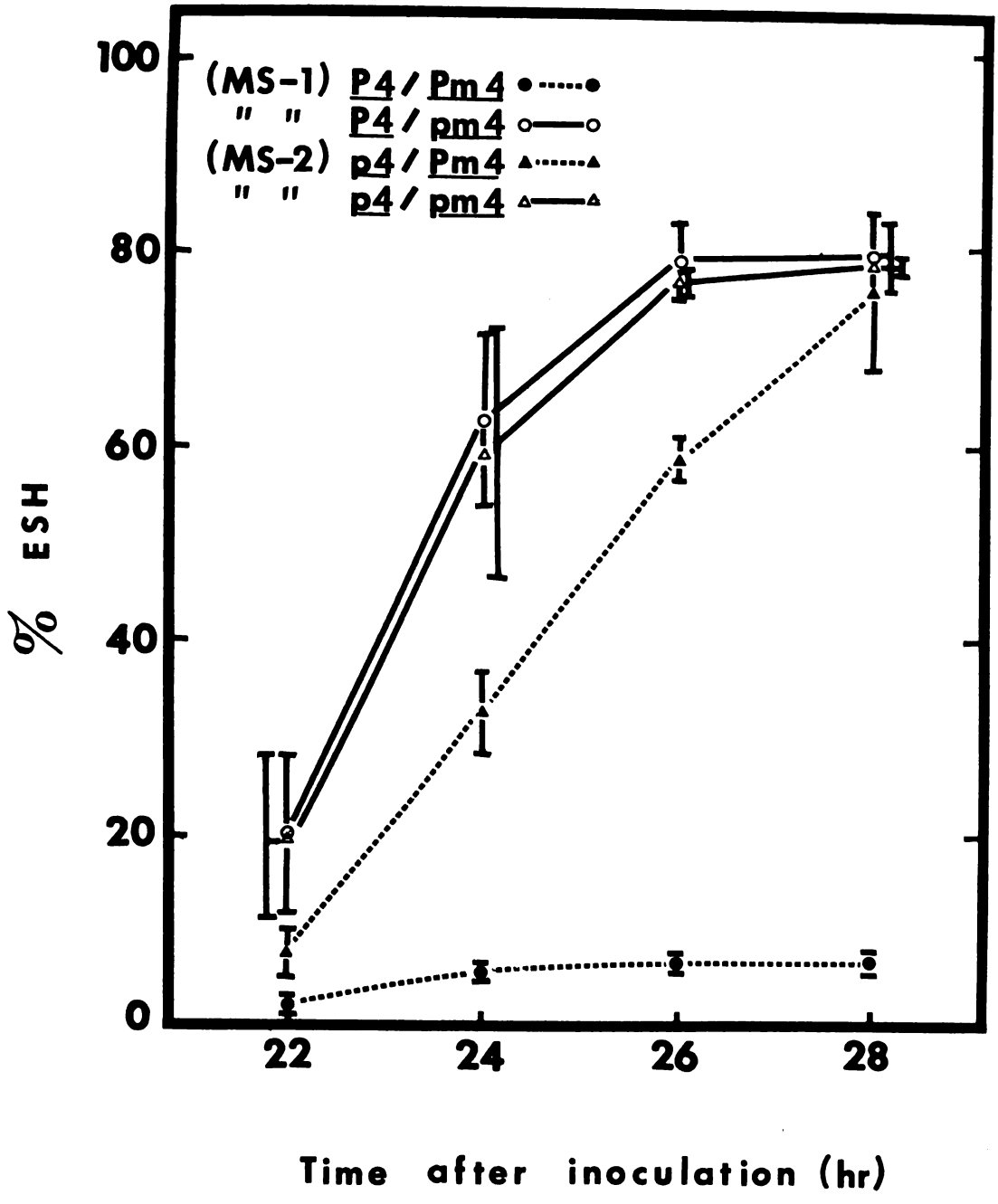
100
80
60
40
20
0
% ESH
(MS-1) P4 / Pm4
" " P4 / pm4
(MS-2) p4 / Pm4
" " p4 / pm4
22
24
26
28
Time after inoculation (hr)

Figure 16

To further substantiate that culture MS-2 did not have genes that merely conditioned slow growth, the infection efficiency of MS-2 was determined on near-isogenic lines containing pmx, Pm1, Pm2, and Pm3b. The production of ESH (Figure 17) was similar to that reported for MS-1 (Px) on the same host lines (47).

The kinetics for production of ESH with the four possible interactions involving Pm2 was determined (Figure 18). Culture MS-2 also contained p2 and was used in this quadratic check to further demonstrate that MS-2 wasn't just a slow growing culture. All four interactions resulted in similar kinetics for the formation of elongating secondary hyphae. This was additional evidence that a slower rate of development was observed only when p4 was interacting with Pm4.

The quadratic check was completed again for P4 and Pm4 but culture KhXCc^{7} was substituted for culture MS-2. KhXCc^{7} carries p4, thus gave a high infection type seven days after inoculation on host plants with Pm4. An infection efficiency of 20% was observed for the p4/Pm4 genotype when KhXCc^{7} was used as the source of p4 (Figure 19). Apparently these 20% of the parasite units that did produce successful primary infections continued to develop and produced a high final infection type. A third culture with p4, culture MS-3, gave an infection efficiency of 33% and a high final infection type when inoculated onto a host line with Pm4 (Figure 20).

Fig. 17.--Formation of elongating secondary hyphae (ESH) by Erysiphe graminis f. sp. tritici culture MS-2 (p4) on four near-isogenic wheat lines three of which contained a dominant Pm gene.

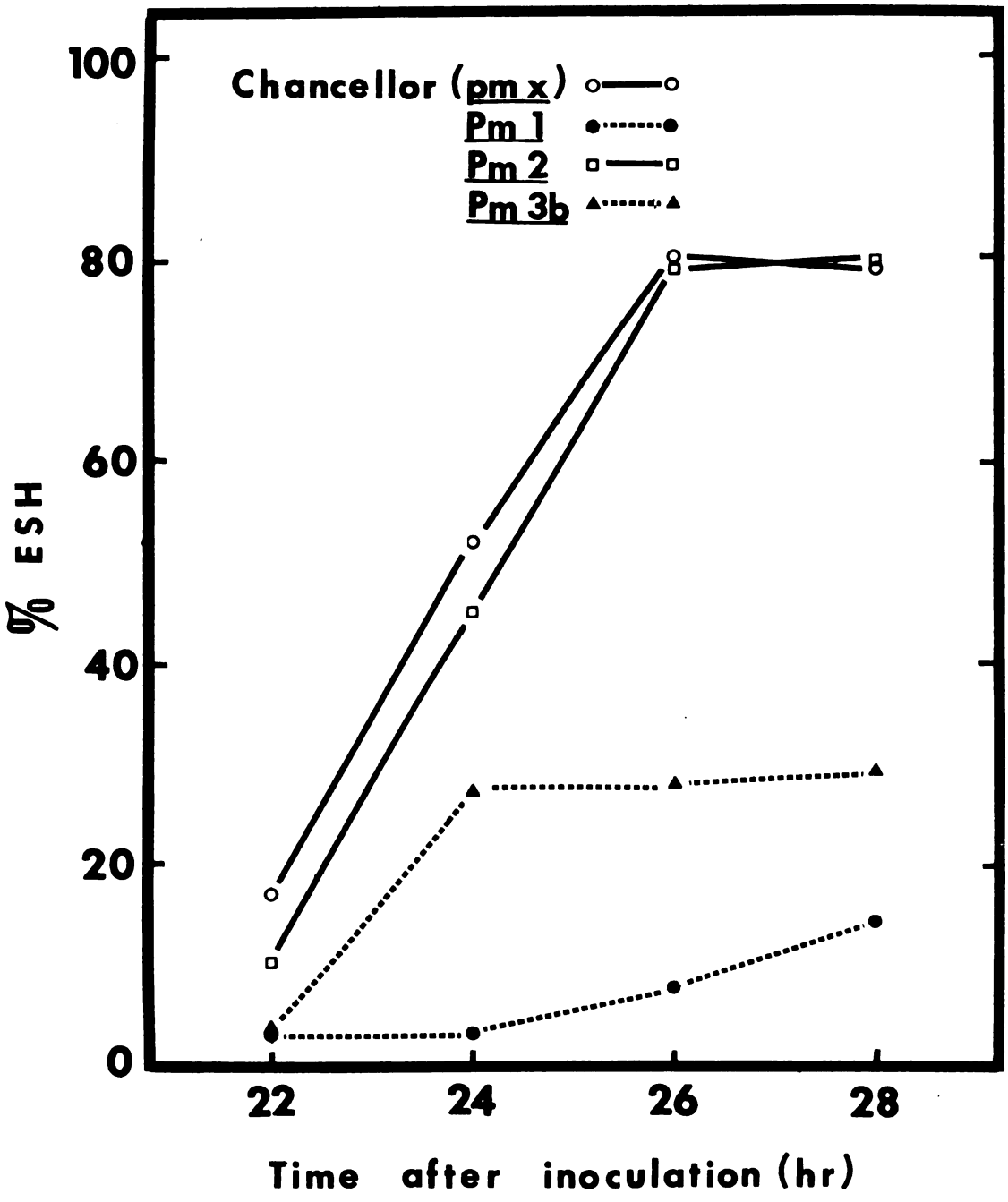
Chancellor (pm x)
Pm 1
Pm 2
Pm 3b
100
80
60
40
20
0
% ESH
22
24
26
28
Time after inoculation (hr)

Figure 17

Fig. 18.--Formation of elongating secondary hyphae (ESH) by Erysiphe graminis f. sp. tritici cultures MS-1 (P2) and MS-2 (p2) with the four possible parasite/host genotypes involving Pm2.

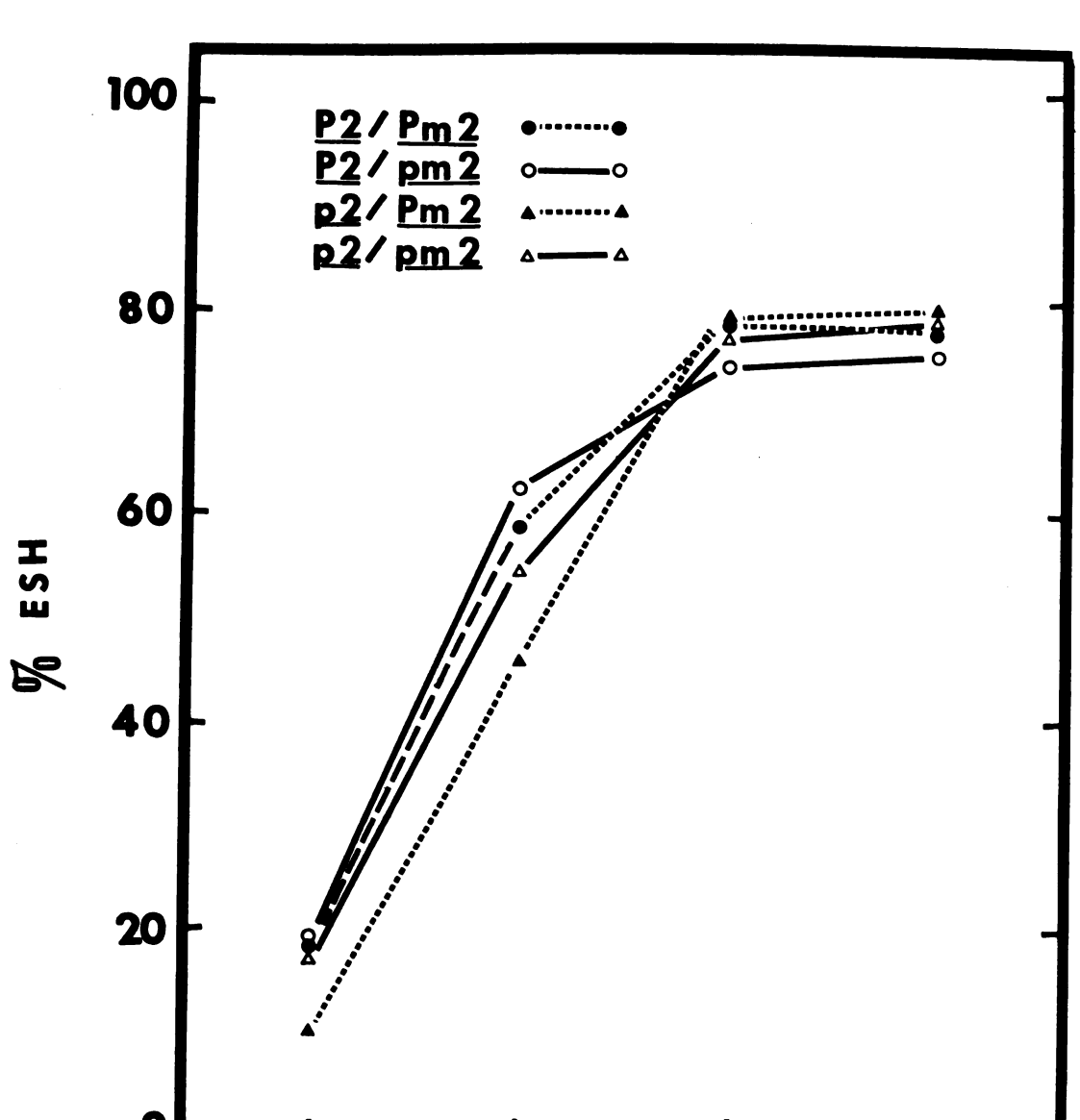
P2 / Pm2
P2 / pm2
p2 / Pm2
p2 / pm2
100
80
60
40
20
0
% ESH
22
24
26
28
Time after inoculation (hr)

Figure 18

Fig. 19.--Formation of elongating secondary hyphae (ESH) by Erysiphe graminis f. sp. tritici cultures MS-1 (P4) and KhXCc7 (p4) with the four possible parasite/host genotypes involving P4 and Pm4.

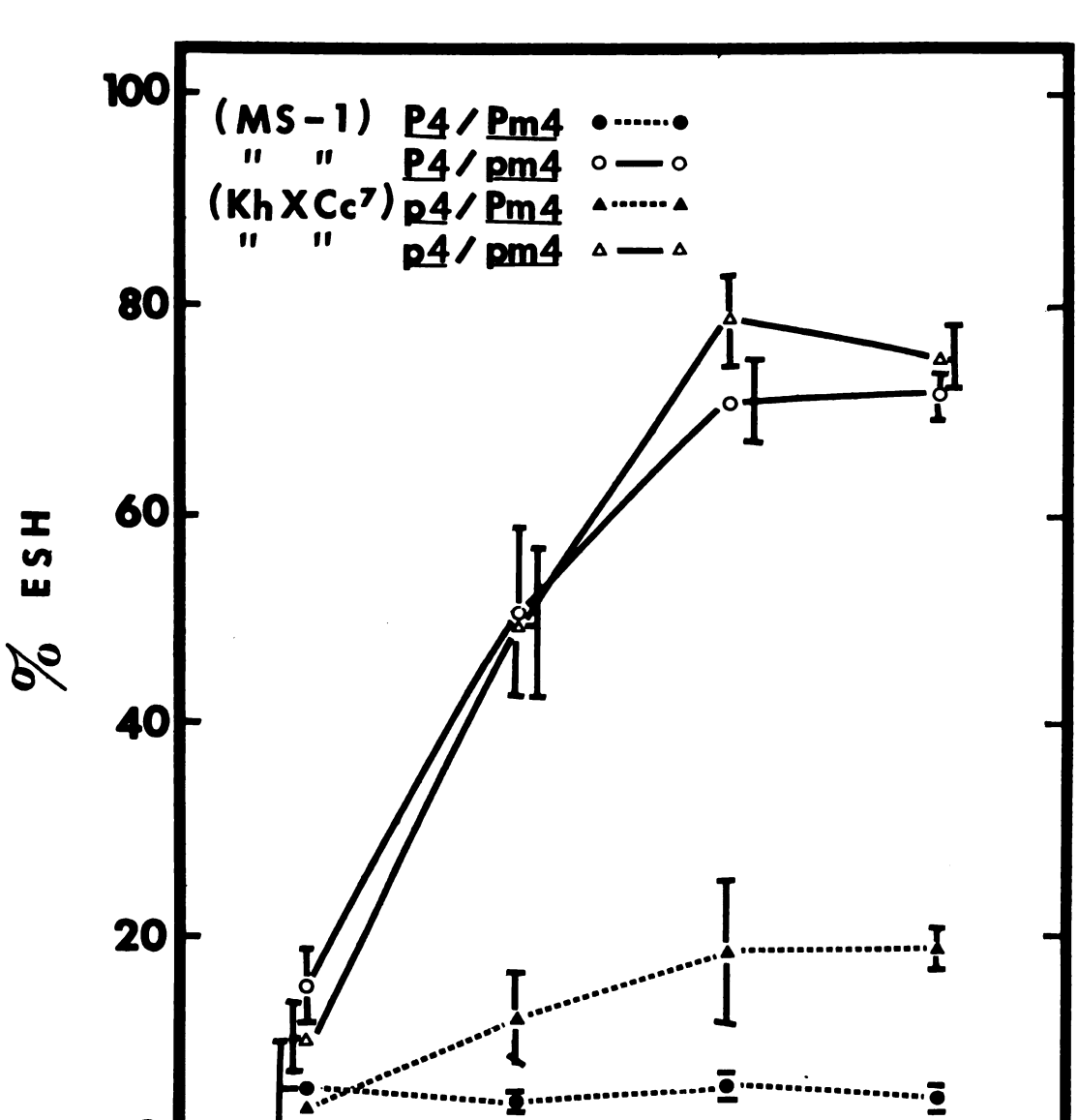
100
80
60
40
20
0
% ESH
(MS-1) P4/Pm4
" " P4/pm4
(Kh X Cc⁷) p4/Pm4
" " p4/pm4
22
24
26
28
Time after inoculation (hr)

Figure 19

Fig. 20.--Formation of elongating secondary hyphae (ESH) by Erysiphe graminis f. sp. tritici cultures MS-1 (P4) and MS-3 (p4) with the four possible parasite/host genotypes involving P4 and Pm4.

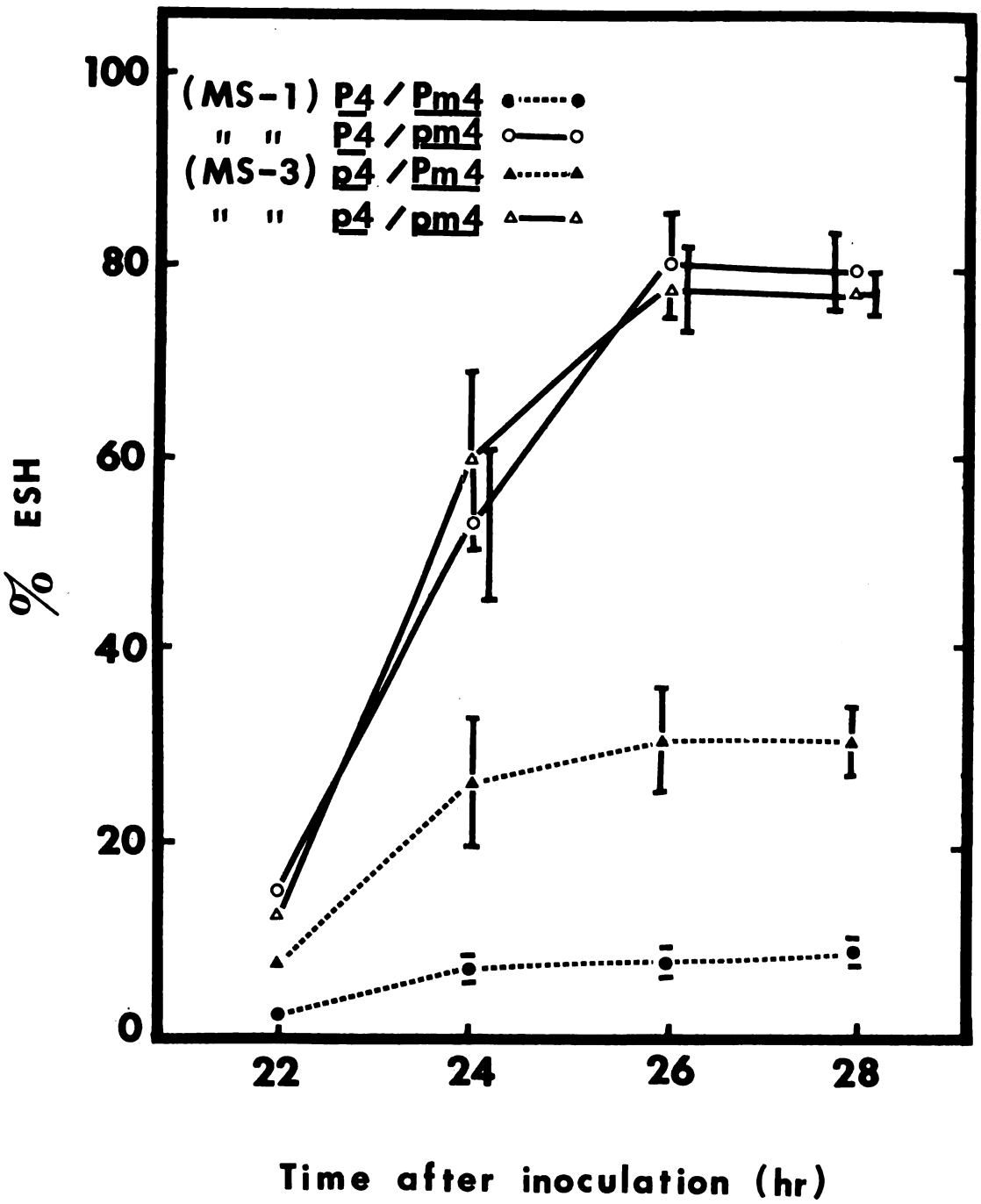
100
80
60
40
20
0
% ESH
(MS-1) P4 / Pm4
" " P4 / pm4
(MS-3) p4 / Pm4
" " p4 / pm4
22
24
26
28
Time after inoculation (hr)

Figure 20

Effects of Inoculation on ^{32}P Uptake by Detached Leaves

The average radioactivity in noninoculated and inoculated leaf sections six hours after inoculation with E. graminis was 2.4 X 10^5 CPM and 1.8 X 10^5 CPM, respectively. Twenty-five percent less ^{32}P was taken up by inoculated plants than by noninoculated plants. This effect was observed prior to attempted penetration of the plant by the fungus. Epidermal strips from noninoculated and inoculated leaf sections contained 6.5 X 10^3 CPM and 3.2 X 10^3 CPM, respectively. There was 51% less ^{32}P taken up into the epidermis of inoculated plants than noninoculated plants.

Puccinia graminis uredospores, Helminthosporium victoriae conidia, chalk dust, or carborundum heavily dusted onto leaves did not reduce ^{32}P uptake. Uredospores of P. graminis and H. victoriae conidia did not germinate under the conditions used. H. victoriae conidia which were atomized onto the leaves in distilled water germinated but did not reduce the ^{32}P uptake when compared to leaves inoculated with distilled water only.

The effect of UV-irradiated spores on ^{32}P uptake was determined (Figure 21). As the UV dose increased the percentage germination decreased. Uptake of ^{32}P increased with the decrease in germination. The uptake of ^{32}P by plants inoculated with conidia that

Fig. 21.--The uptake of ^{32}P in one cm leaf sections of wheat seedlings one to six hours after inoculation and the percent germination of conidia of Erysiphe graminis f. sp. tritici exposed to various doses of ultraviolet irradiation.

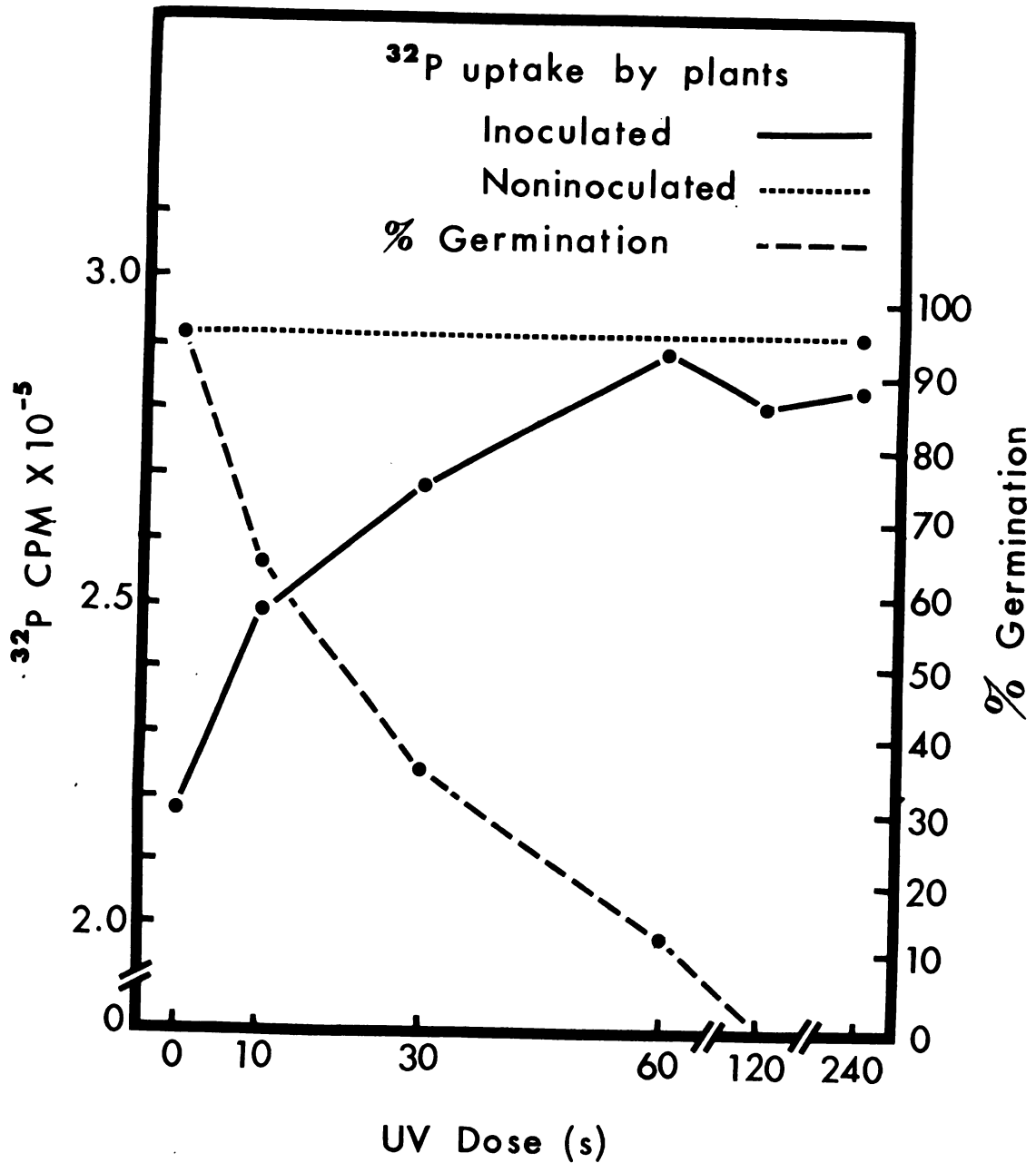
^{32}P uptake by plants
Inoculated
Noninoculated
% Germination
^{32}P CPM X 10^{-5}
3.0
2.5
2.0
0
100
90
80
70
60
50
40
30
20
10
0
% Germination
0
10
30
60
120
240
UV Dose (s)

Figure 21

received a 10 second exposure of UV radiation was significantly different at the 5% level from both the noninoculated and the inoculated with no UV exposure. The 30, 60, 120, 240 second, and noninoculated treatments were not different from each other. All were different at the 5% level from the plants inoculated with conidia that had no UV treatment. Calculation of germination percentage was based on the production of germ tubes by six hours. The germ tubes produced following 30 second and 60 second UV treatments were usually malformed.

Transpiration Rates of Detached Leaves

The average transpiration rates of noninoculated and inoculated seedlings one to six hours after inoculation were 1.25 and 0.93 mg cm^{-2} hr^{-1}, respectively. This represented a 25% decrease in the transpiration rate of the inoculated seedlings. The difference was significant at the 5% level.

Relative Diffusion Resistance

The time required for a standard 10 mm Hg change in pressure was 48 seconds for noninoculated and 367 seconds for inoculated wheat leaves at six hours after inoculation. The relative diffusion

resistances of stomata were estimated to be 3.6 for noninoculated and 7.1 for inoculated leaves. This amounted to approcimately a 100% increase for inoculated plants.

Interaction Specificity

The relationship of compatibility between host and parasite and the reduction in transpiration rate was determined by observing the rates of transpiration of inoculated and noninoculated near-isogenic wheat lines which contain different Pm genes (Table 3).

TABLE 3.--Transpiration rates of intact noninoculated and inoculated near-isogenic wheat seedlings three and six hours after inoculation with Erysiphe graminis f. sp. tritici (MS-1).

		Transpiration rate ($mg\ cm^{-2}\ hr^{-1}$)		
		Noninoculated	Inoculated	% decrease from noninoculated
pmx	3 hr	1.11	0.85	24
	6 hr	1.13	0.76	33
Pm1	3 hr	1.11	0.90	19
	6 hr	1.18	0.73	38
Pm2	3 hr	0.98	0.72	27
	6 hr	1.35	0.87	36
Pm3	3 hr	1.00	0.87	13
	6 hr	1.18	0.95	20
Pm4	3 hr	0.90	0.61	32
	6 hr	1.26	0.86	32

Intact plants were used to show that no artifacts were being introduced by cutting the leaves. All inoculated lines showed a lower transpiration rate than noninoculated plants after three hours. The decrease in transpiration at six hours was comparable to that observed for cut leaves. In all cases, except with Pm4, the effect increased from the third to the sixth hour after inoculation. The effect of inoculation was the same at three and six hours for Pm4.

The uptake of ^{32}P by wheat seedlings inoculated with E. graminis f. sp. hordei was reduced by 25%. This fungus was not pathogenic on wheat, but did germinate and produce appressoria.

Extracts of Germinated Conidia

Aqueous extracts of germinated conidia did not affect transpiration rates of wheat. Efforts to isolate ABA from germinating spores or spore filtrates were not successful.

Effects of E. graminis on Uptake and Translocation of ^{32}P During Primary Infection

The amount of ^{32}P taken up by wheat leaves and translocated to the epidermis of inoculated and noninoculated leaves during primary infection was determined (Figure 22). The amount of ^{32}P taken up by inoculated leaf sections six through 20 hours was

Fig. 22.--Uptake and translocation of ^{32}P to the epidermis of wheat given ^{32}P for five hours during primary infection. Inoculated leaf sections (■-----■), noninoculated leaf section (□——□), inoculated epidermis (▼--------▼), and noninoculated (Δ_____Δ).

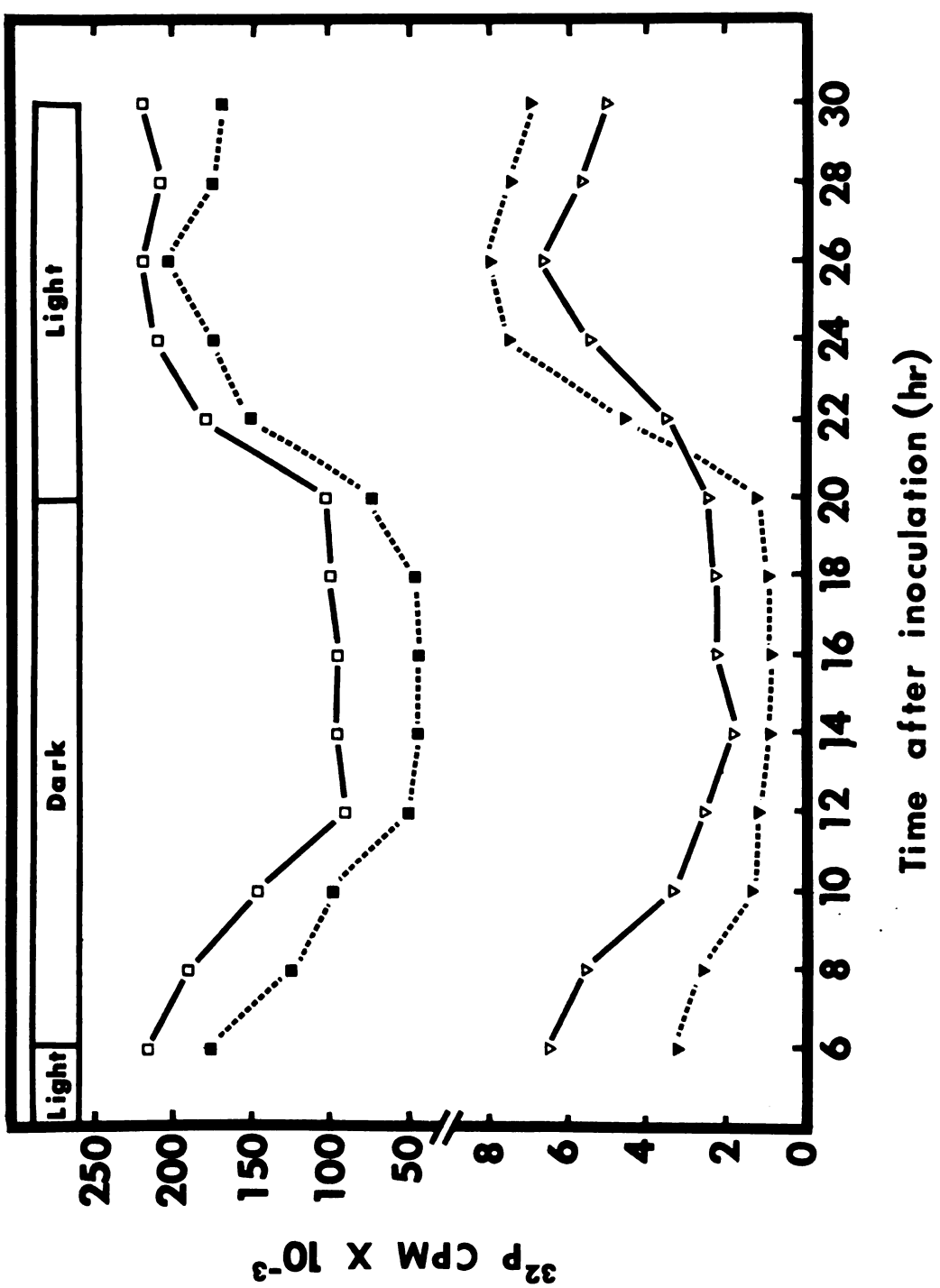
Light
Dark
Light
250
200
150
100
50
8
6
4
2
0
6 8 10 12 14 16 18 20 22 24 26 28 30
Time after inoculation (hr)
32P CPM X 10-3

Figure 22

significantly less than that taken up by noninoculated leaves. At 22, 24, and 26 hours no significant differences existed. At hours 28 and 30 the differences were statistically significant. The ^{32}P translocated to the epidermis of inoculated plants was significantly less than in the epidermis of noninoculated plants at the 5% level six through 20 hours after inoculation. There was no difference at 22 hours but at 24 through 30 hours there was more ^{32}P in the epidermis of inoculated plants than noninoculated plants. The epidermis of inoculated and noninoculated plants plants which took up ^{32}P from 25 to 30 hours in 100% RH in the light contained 1,484 CPM and 3,870 CPM, respectively. Thus, when the transpiration stress was removed, the inoculated epidermis contained 62% less ^{32}P than did the noninoculated epidermis. If the plants were pretreated for two hours in 100% RH before being cut and given ^{32}P from 25 to 30 hours after inoculation the epidermis of the inoculated plants contained 780 CPM and the non-inoculated epidermis contained 1,616 CPM, 51% less ^{32}P in inoculated epidermis. This is approximately the amount of ^{32}P that is taken up in the dark (Figure 22).

Transpiration Rates of Inoculated and Noninoculated Near-isogenic Lines of Wheat During Primary Infection

The average rates of transpiration during primary infection of inoculated and noninoculated near-isogenic wheat lines were measured (Figures 23-27). All lines have curves shaped similar to those seen in Figure 22. Transpiration rates of inoculated plants were lower through 20 hours. However, after 20 hours, the difference between inoculated and noninoculated plants was not similar for all lines. Table 4 gives the average transpiration rates of inoculated and noninoculated wheat lines from 22 to 30 hours after inoculation. It also gives the percent differences between inoculated and noninoculated lines and the P value for those differences. A P value of $< .25$ was shown for the difference between inoculated and noninoculated Chancellor, while Pm1, Pm2, and Pm3b inoculated and noninoculated plants had P values of $< .05$ for their differences. There was no difference between inoculated and noninoculated Pm4.

Fig. 23.--Average transpiration rates of Chancellor (pmx) wheat seedlings at different time periods during primary infection.

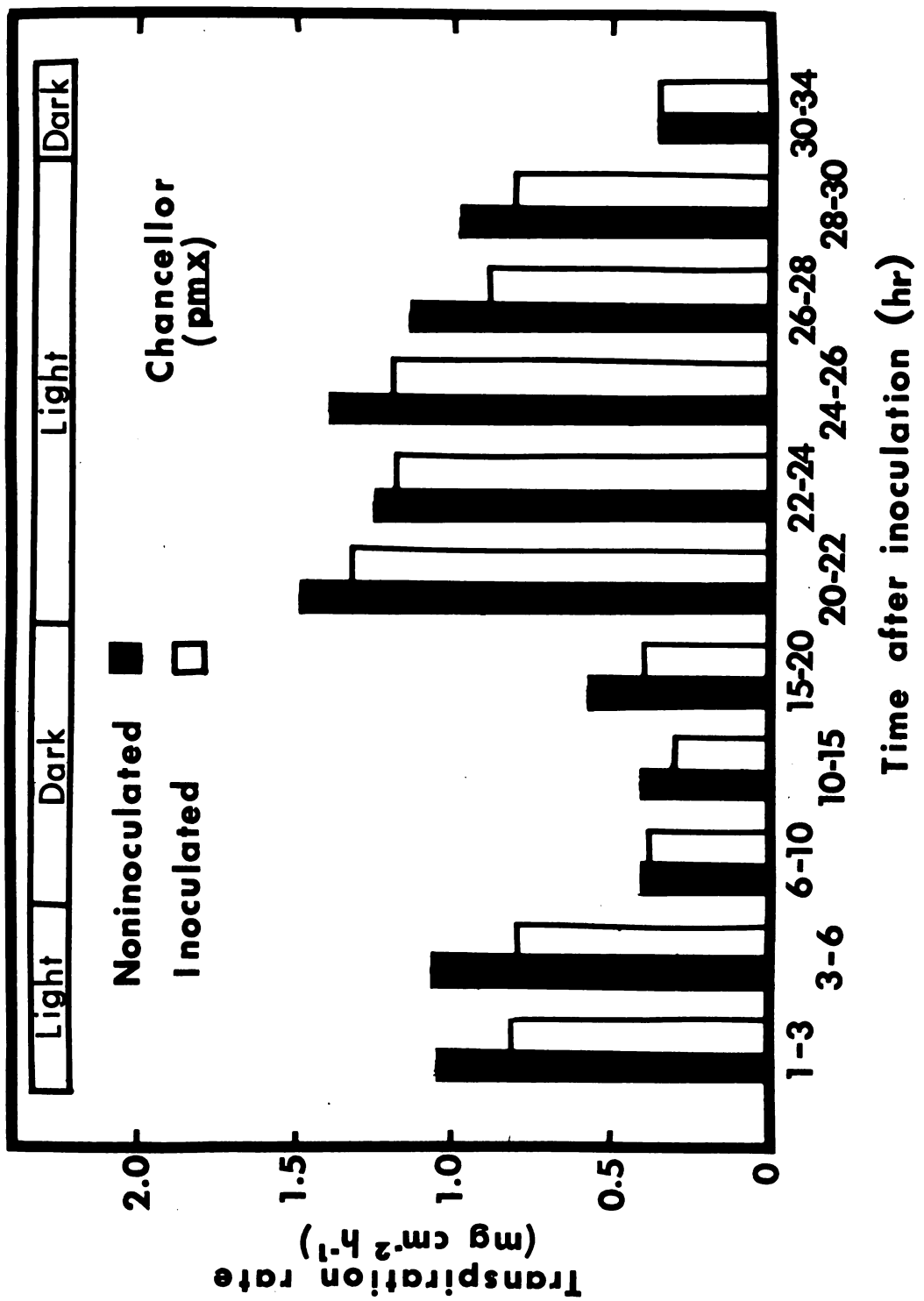
Light
Dark
Light
Dark
Noninoculated
Inoculated
Chancellor
(pmx)
Transpiration rate
(mg cm⁻² h⁻¹)
2.0
1.5
1.0
0.5
0
1-3
3-6
6-10
10-15
15-20
20-22
22-24
24-26
26-28
28-30
30-34
Time after inoculation (hr)

Figure 23

Fig. 24.--Average transpiration rate of Pm1 wheat seedlings at different time periods during primary infection.

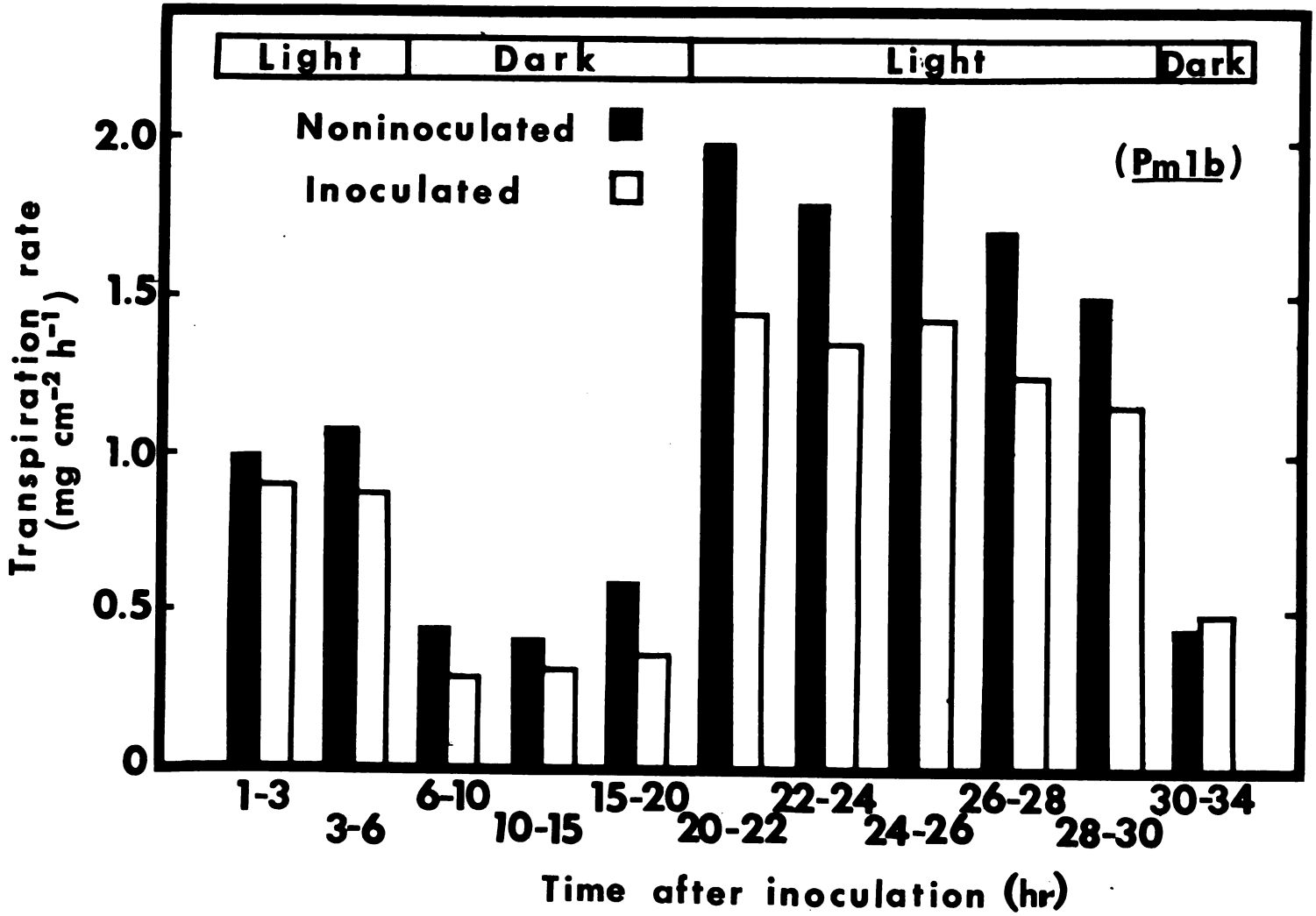
Light
Dark
Light
Dark
Noninoculated
Inoculated
(Pm1b)
Transpiration rate
(mg cm-2 h-1)
2.0
1.5
1.0
0.5
0
1-3
3-6
6-10
10-15
15-20
20-22
22-24
24-26
26-28
28-30
30-34
Time after inoculation (hr)

Figure 24

Fig. 25.--Average transpiration rate of Pm2 wheat seedlings at different time periods during primary infection.

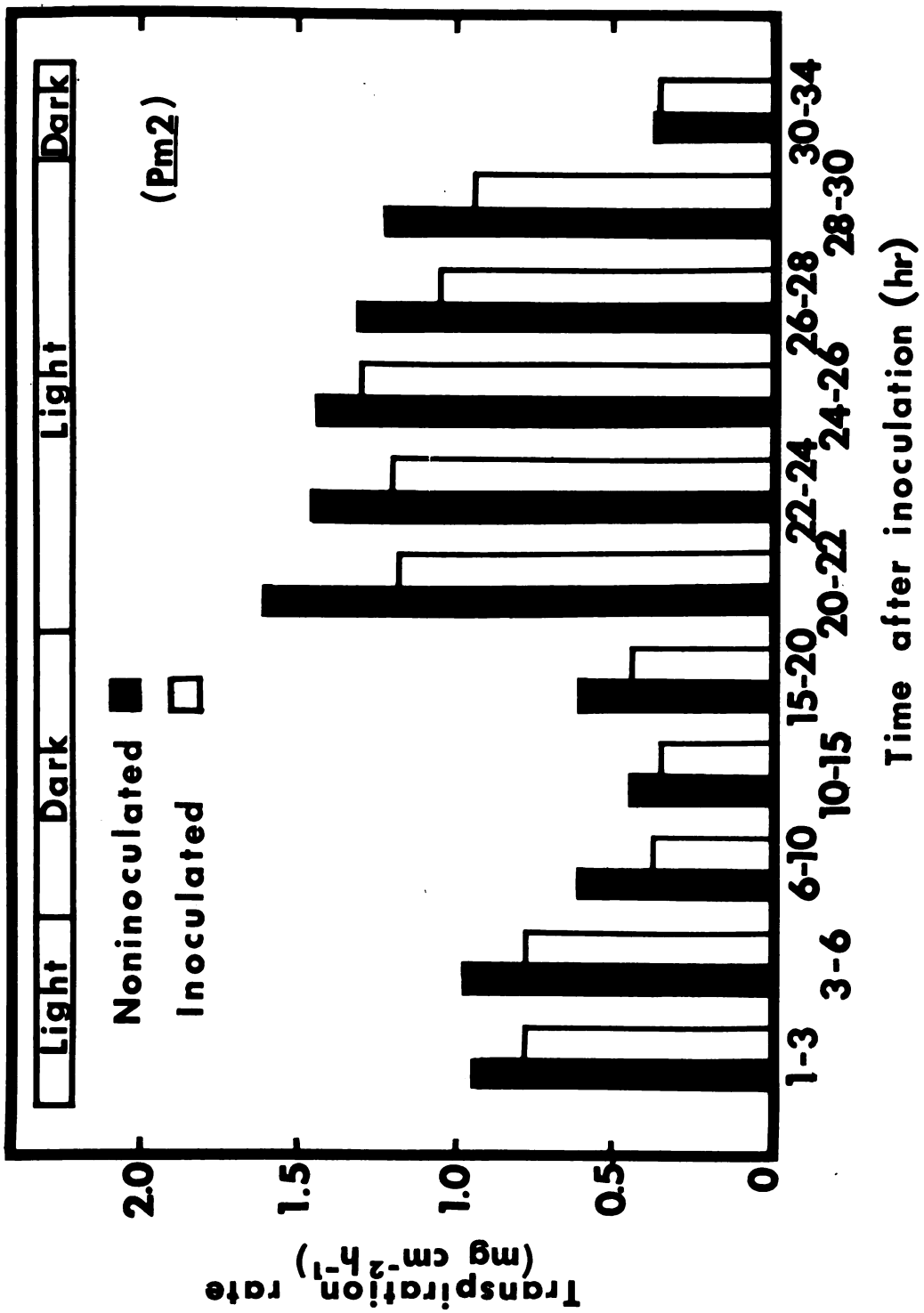
Light
Dark
Light
Dark
(Pm2)
Noninoculated
Inoculated
2.0
1.5
1.0
0.5
0
Transpiration rate
(mg cm-2 h-1)
1-3
3-6
6-10
10-15
15-20
20-22
22-24
24-26
26-28
28-30
30-34
Time after inoculation (hr)

Figure 25

Fig. 26.--Average transpiration rate of Pm3b wheat seedlings at different time periods during primary infection.

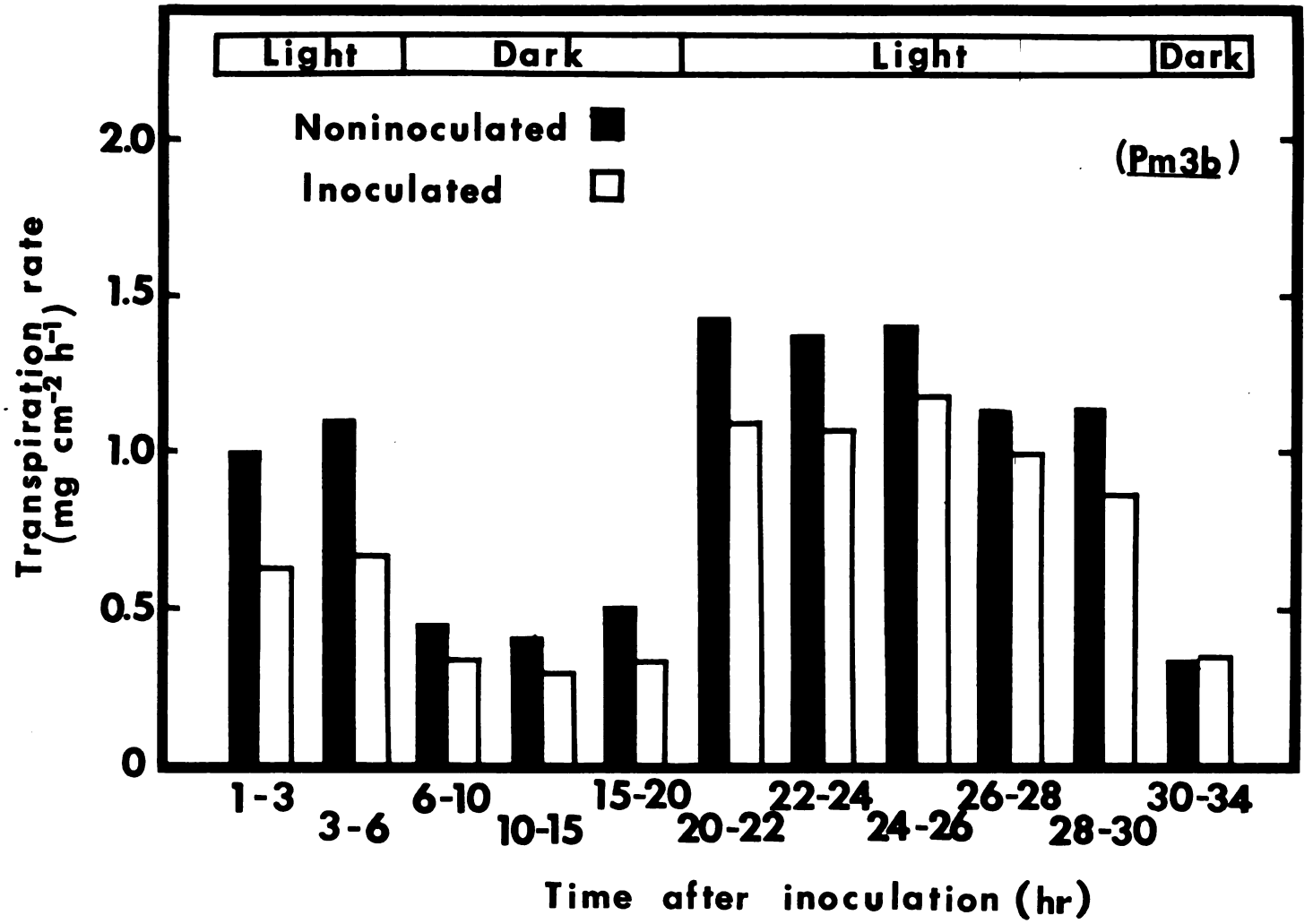
Light
Dark
Light
Dark
Noninoculated
Inoculated
(Pm3b)
Transpiration rate (mg cm-2 h-1)
2.0
1.5
1.0
0.5
0
1-3
3-6
6-10
10-15
15-20
20-22
22-24
24-26
26-28
28-30
30-34
Time after inoculation (hr)

Figure 26

Fig. 27.--Average transpiration rate of Pm4 wheat seedlings at different time periods during primary infection.

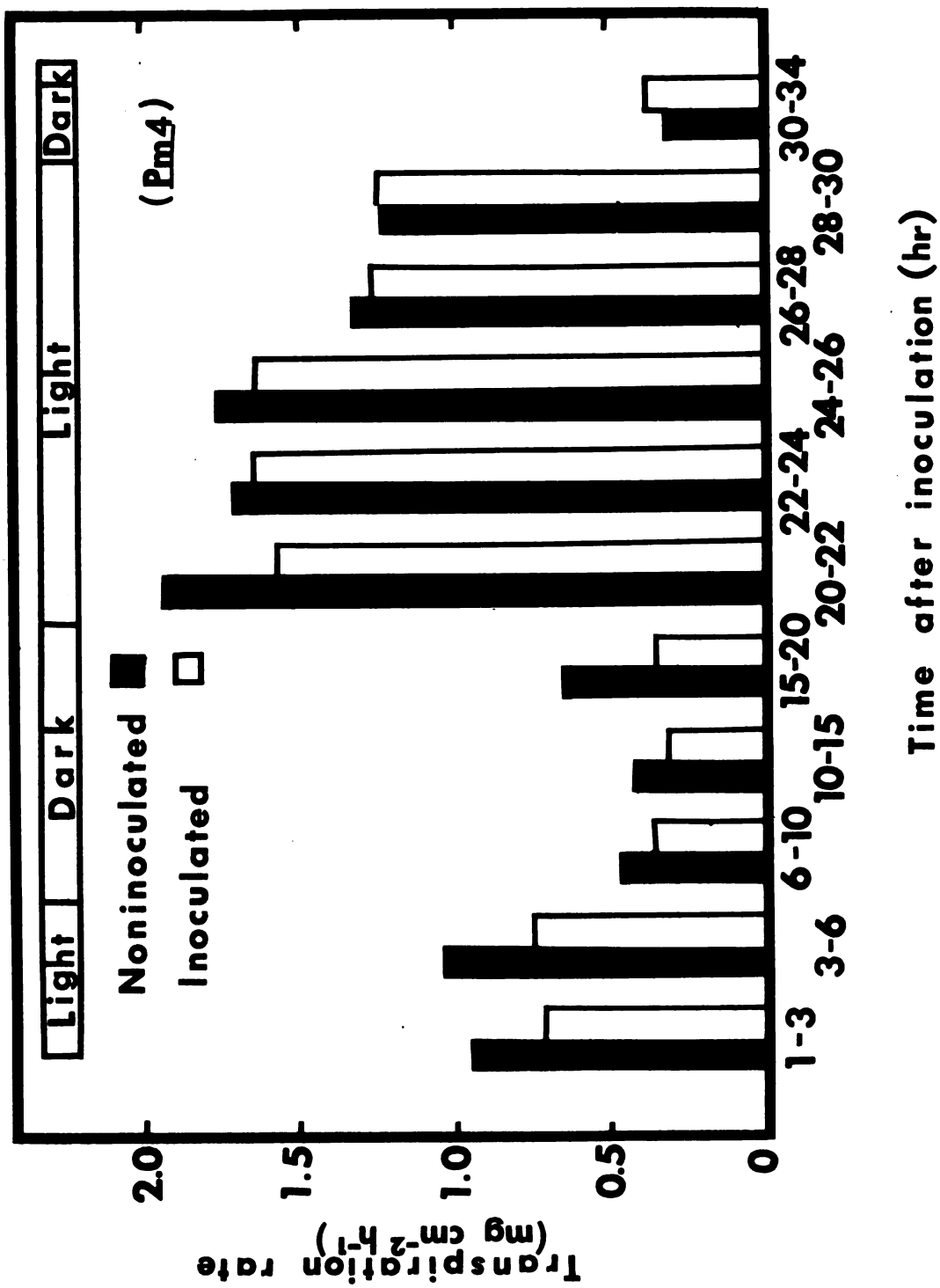
Light
Dark
Light
Dark
(Pm4)
Noninoculated
Inoculated
Transpiration rate
(mg cm-2 h-1)
2.0
1.5
1.0
0.5
0
1-3
3-6
6-10
10-15
15-20
20-22
22-24
24-26
26-28
28-30
30-34
Time after inoculation (hr)

Figure 27

TABLE 4.--The average transpiration rate (mg cm^{-2} hr^{-1}) of near-isogenic wheat lines, the percent decrease, and the P value for the difference between inoculated and noninoculated plants 20-30 hours after inoculation with E. graminis f. sp. tritici.

	Host Genotype				
	(Chancellor) pmx	Pm1	Pm2	Pm3b	Pm4
Noninoculated	1.36	1.03	1.41	1.35	1.06
Inoculated	1.14	1.32	1.08	1.04	1.04
% decrease from noninoculated	17	32	23	23	2
P value	< .25	< .005	< .05	< .05	> .75

DISCUSSION

The original goal of this research was to study the genetic control of ^{32}P transfer from host to parasite. It was hoped that this would give us a more sensitive measure of compatibility or incompatibility between host and parasite. It would also lay the basis for a more detailed biochemical study on how compatibility or incompatibility affects the different metabolic pathways involving phosphorus in both host and parasite during primary infection.

In preliminary experiments, the environmental conditions necessary for synchronous development of E. graminis f. sp. tritici were shown to be quite influential on the uptake of ^{32}P during primary infection (Figure 6). It appears that light is the major factor. The rate of ^{32}P uptake by plants in the dark is only about 50% of the rate of uptake by plants in the light (Figure 5). Earlier work (32) indicated that leaves given ^{32}P for four hours were saturated and additional uptake time would not greatly increase the amount of ^{32}P in the leaf. It was argued that if the leaf was already saturated with ^{32}P, the environment should not affect the amount of ^{32}P in the leaf. The data presented here (Figure 5) conclusively shows that light does

affect ^{32}P uptake and saturation does not occur for at least ten hours. Earlier experiments were done with 100 μCi ^{32}P/mℓ solutions, but the 50 μCi/mℓ increase over what was used in this study should have been negligible because the ^{32}P was in a 0.1 M phosphate buffer in both studies. The differences between earlier studies and the data presented herein may be due to procedures used in this study which minimize the possibility of getting air into the vascular system when leaves were cut at the base and placed in solutions containing ^{32}P.

If ^{35}S and ^{32}P were both transferred from host to parasite by simple diffusion one would expect similar rates of transfer of each isotope. However there are some important differences between the data reported in this thesis for transfer of ^{32}P and the data on ^{35}S transfer for a compatible parasite/host interaction (50). The earliest ^{32}P was detected in the parasite was ten hours after inoculation, which corresponds to the time haustoria can first be seen in the host cells (32). ^{35}S was first detected in the parasite at 16 hours, which is the time that haustoria begin to develop appendages (Personal communication from Mary Joy Haywood). This may indicate that ^{32}P simply diffuses into the parasite as soon as the parasite penetrates the host cell wall, while ^{35}S may require a more complex transport system. The possibility exists that the transport systems for ^{32}P are merely functioning earlier than those for ^{35}S. ^{32}P and ^{35}S transfer were very

similar from 18 hours onward. However the amount of ^{32}P transferred was much higher than the amount of ^{35}S transferred. This could be a function of the ability of ^{32}P to move through the leaf faster than ^{35}S.

When corrections are applied for the amount of ^{32}P available to be transferred we find that the highest rate of transfer occurs from 20 to 22 hours after inoculation. Similar results were obtained for ^{35}S transfer (50). The rates of ^{32}P and ^{35}S transfer dropped off after 22 hours. The validity of correcting transfer rates for the amount of ^{32}P available is supported by the fact that, when the lights were left off from 20 to 30 hours, the same amount of ^{32}P was available for transfer after 20 hours as before, the rates of transfer in the dark did not increase after 20 hours after inoculation. The dark period from 20 to 30 hours does slow down development of ESH but only for two hours (50). Thus slower rates of ESH development would not explain the decreased transfer in the dark.

The amount of ^{32}P transferred from host to parasite was found to correspond to morphological development of the fungus with the various genotypes. The reduction of the percentage of ESH for the incompatible interactions (47), P1/Pm1, P2/Pm2, P3b/Pm3b, and P4/Pm4, was similar to the reduction in ^{32}P transfer, however, the decreases in ^{32}P transfer were later than those observed for ^{35}S transfer.

Decreases in ^{32}P transfer were not seen until after morphological differences were noted. ^{35}S transfer was affected concurrent with or prior to effects of the different incompatible genotypes on morphological development of the fungus (50).

Results from the comparison of ^{32}P transfer from host lines with the allelic series of genes at the Pm3 locus failed to differentiate between Pm3a and Pm3b. If these genes are truely allelic, then one would expect them to affect parasite development in the same way. Pm3c had similar kinetics of transfer as pmx. This was expected since Pm3c does not have an apparent effect in the primary leaf (5, 6, 41).

If only functional parasite units transfer ^{32}P, then the amount of ^{32}P transferred by the functional units in incompatible interactions is greater than the rates per parasite unit in compatible interactions. With the exception of Pm4, the rates of ^{32}P transfer in incompatible interactions eventually decreased to the same level as in the compatible interactions. Rates of ^{32}P transfer from plants with Pm4 were higher at 30 hours, but they were decreasing and it is predicted that the rates may eventually reach the same as in the compatible interactions. An alternate explanation is of course possible. The nonfunctional parasite units could be taking up ^{32}P until 30 hours after inoculation, at which time the process is halted. It is also possible, especially with P4 and Pm4, that after collapse of the parasite, which

occurs approximately 22 hours after inoculation, that there was diffusive flow of ^{32}P into the collapsed parasite. By 30 hours after inoculation this "dead" parasite may be such that ^{32}P did not diffuse into it or the necrotic host cell which the parasite had attempted to penetrate.

The four possible parasite/host genotypes (the quadratic check) for each pair of interacting genes were evaluated for their effect on rates of ^{32}P transfer from host to parasite during primary infection. The incompatible parasite/host genotype P1/Pm1 gave low rates of ^{32}P transfer. The three compatible genotypes P1/pm1, p1/Pm1, and p1/pm1, all gave similar, high rates of transfer. The incompatible genotype P3b/Pm3b gave low rates of transfer while the three compatible genotypes P3b/pm3b, p3b/Pm3b, and p3b/pm3b, gave similar, high rates of ^{32}P transfer from host to parasite during primary infection. The three different compatible genotypes for each gene pair could not be distinguished on the basis of rates of ^{32}P transfer.

There were no differences in the rates of ^{32}P transfer for the four genotypes P2/Pm2, P2/pm2, p2/Pm2, and p2/pm2 during primary infection. This was consistent with earlier observations that P2/Pm2 does not affect primary infection (47).

The genotype P4/Pm4 gave a low final infection type, low rates of ^{32}P transfer from host to parasite during primary infection, and a

low primary infection efficiency. In this respect it was similar to the P1/Pm1 and P3b/Pm3b interactions. The culture MS-2, which by definition has p4 because it gives a high infection type seven days after inoculation on the host line with Pm4, gave a primary infection efficiency similar to that observed on the host line with Pm4 but the formation of elongating secondary hyphae was delayed (Figure 16). The rates of transfer of ^{32}P from host to parasite during primary infection were also lower with this genotype, i.e., p4/Pm4, than with the other two compatible genotypes, P4/pm4 and p4/pm4. If culture MS-2 just had a gene for slower growth it should have developed more slowly on the host line with pm4 also, but it did not. Therefore, the slower development of MS-2 on host lines with Pm4 is very probably related to the presence of Pm4.

When other cultures containing p4 were substituted into the quadratic check, one was found to reduce infection efficiency to 20% and a second to 33% with the p4/Pm4 genotype. The p4/pm4 genotype gave normal infection efficiency in both cases. These results are important because they bear on where the specificity of gene-for-gene interactions occur. They indicate that a unique interaction occurs with the p4/Pm4 genotype. The simplest explanation of where the specificity of gene-for-gene interactions resides is in the incompatible interaction (Px/Pmx). The data indicate that the transition

in evolution from P4 to p4 in the parasite did not completely negate the transition in evolution from pm4 to Pm4 in the host. There are no reasons we should always expect complete restoration of compatibility since the p4/Pm4 genotype only has to have a selective advantage for the parasite over the incompatible genotype P4/Pm4. The p4 product is probably an altered product of P4 with an altered specificity for the Pm4 product.

The results from these efforts to complete the quadratic checks for several gene pairs also demonstrate how the gene-for-gene hypothesis may play a role in nonspecific or general resistance. The first p4 isolate used was found to give a slow rate of ESH development in combination with Pm4. A high infection efficiency was reached but it was delayed two hours. With the second two isolates with p4, a reduced infection efficiency was obtained on host lines with Pm4. These two characteristics, i.e., slow mildew development and the development of fewer normal pustules, have always been associated with "general" resistance. This means that "general" resistance may result from the accumulation of px/Rx interactions. Techniques with high resolution are needed to detect the differences between the compatible genotypes.

All pathogens which carried some form of px have probably been selected and eventually the parasites with Px were lost from the

population. The low frequency, or loss, of Px in a population would explain the difficulty to demonstrate the specific interactions that are probably occurring with "general" resistance. Manchurian barley which was previously thought to contain no (R) genes for resistance to powdery mildew was shown to possess (R) genes when inoculated with isolates from the regions of origin of barley (17). This shows that if the avirulent (P) pathogen is available, the (R) genes could be detected and selected. Varieties of potatoes introduced from Europe in 1833 and maintained in the mountains of Basutoland in Africa under almost no selection pressure from late blight were found to be much more susceptible to blight than the susceptible varieties still grown in Europe (52). This could be explained by the accumulation of px/R interactions between Phytophthora infestans and the European varieties which have been under selection through the years. The mountain-grown varieties would not be expected to possess these particular (R) genes since they were never under selection pressure. The (R) gene in the European varieties could be identified if the avirulent (P) pathogens were still available.

If this hypothesis is true, then it should be possible to identify a few of the known (R) genes which are not completely negated by its corresponding (p) gene, similar to what I have done here. These interactions should also be shown to have an additive effect. Then

with the use of avirulent cultures, which would be readily available, it should be possible to produce new sources of "general" resistance from old sources of specific resistance. This would involve the use of the same principles presently used in breeding for specific resistance and would eliminate the hit and miss techniques presently used to transfer "general" resistance from known sources to new varieties.

The reduction in amounts of ^{32}P taken up by inoculated plants one to six hours after incoulation showed that a pathogen could interact with its host before the pathogen penetrated. Since chalk dust, carborundum, P. graminis uredospores, and H. victoriae conidia did not reduce the amount of ^{32}P taken up by the leaves, the reduction in label uptake was probably not due to the physical presence of particles on the surface of the leaves. As UV radiation decreased the viability of E. graminis conidia there was an increase in the ^{32}P uptake. The uptake of ^{32}P increased to the level of noninoculated plants with 12% germination. However the 12% which germinated with the 60 second dose of UV radiation had malformed germ tubes. Mount and Ellingboe (33) reported lower UV doses given to E. graminis conidia resulted in the formation of no elongating secondary hyphae. Thus, the germinated conidia at the 60 second dose would probably not have penetrated the host. We can clearly see that germinating conidia on the surface of the leaf were responsible for the reduced

amount of label taken up by the leaves one to six hours after inoculation. The decreased uptake of ^{32}P by inoculated plants can be explained by the decreased transpiration rates. The reduced transpiration rates can be attributed to the closure of stonates as determined from measurements with a viscous flow porometer.

The transpiration rates of inoculated and noninoculated near-isogenic wheat lines revealed lower transpiration rates by three hours after inoculation in incompatible as well as compatible parasite/host combinations. Inoculation of wheat with conidia of E. graminis f. sp. hordei also reduced the amount of ^{32}P taken up by the leaves. This form-species on wheat represented an incompatible parasite/host interaction. The spores did germinate and form appressoria on wheat. Thus this effect is not specific for the compatible or incompatible parasite/host genotypes.

Decreased transpiration rates have been reported for barley infected with E. graminis f. sp. hordei one to seven days after inoculation (26). Decreased transpiration rates of bean two days after infection by Uromyces phaseoli have also been reported (12). Our data show a decrease in wheat transpiration as early as three hours after inoculation and before fungus penetration of the host, which is much earlier than previously reported (26). The stomates of wheat were shown to close upon attack by the germ tubes of leaf rust uredospores

which penetrate the host through the stomates (9). The data reported here indicate that the stomates are responding to some stimulis(i) produced by the germinating conidia of E. graminis before there was direct penetration of host cells. The possibility may exist that, in the previous study (9), many of the stomates may have been closing in the near vicinity of the germinating uredospores and not just those stomates being attacked by the pathogen.

Stomate closure induced by E. graminis may have been initiated by diffusable substances produced by the germinating conidia, but abscisic acid was not demonstrated to be present in germinating conidia. None of the extracts made of germinating conidia were shown to reduce transpiration.

Inoculated plants were shown to have reduced transpiration rates throughout the primary infection process. Even though inoculated leaves transpired less, and took up less ^{32}P, the epidermis of inoculated plants contained more ^{32}P than did the epidermis of noninoculated plants by 26 hours after inoculation. This could be the result of a nutrient sink effect created by the developing fungus. A sink effect has been reported during later stages of mildew development (44). When transpiration stress was removed from the leaves by placing them in 100% RH while taking up ^{32}P from 25 to 30 hours after inoculation, the epidermis of inoculated leaves contained

less ^{32}P. The increase in ^{32}P activity in the epidermis was probably due to increased cuticular transpiration around the areas of penetration. This would have the effect of concentrating the ^{32}P at the epidermis. Transpiration effects have been overlooked in most reports of nutrient sinks created by developing parasites. Transpiration changes during primary infection of wheat have explained all the differences in ^{32}P uptake between inoculated and noninoculated plants.

The incompatibility of an interaction seemed to have an effect on transpiration rates, especially at times when incompatibility is being expressed. Transpiration rates of inoculated and noninoculated pmx plants are not significantly different from 22 to 30 hours. This is probably due to increased cuticular transpiration due to fungal development. With P1/Pm1 and P3b/Pm3b, transpiration differences between inoculated and noninoculated plants were highly significant from 22 to 30 hours. This could be due to inhibition of fungal development. Transpiration rates of noninoculated and inoculated Pm4 plants with a culture of P4 were identical from 22 to 30 hours. This might be explained by the nature of the P4/Pm4 incompatible interaction. At approximately 22 hours many of the parasite units collapse concomitant with the browning of the host cell in which it had penetrated. The possibility exists that the dead necrotic cell allows more water loss or causes more disruption of the cuticle than the development of a

compatible interaction. Thus the transpiration rates of inoculated and noninoculated plants would be expected to be similar.

The ability of a pathogen and its host to interact before penetration of the host by the pathogen has clearly been demonstrated. These results emphasize the need for more investigations into the very earliest detectable interactions between parasite and host to determine those interactions that are critical to the establishment of compatible or incompatible parasite/host relationships.

SUMMARY

The environmental conditions necessary for synchronous development of the parasite during primary infection were shown to affect the amount of ^{32}P taken up by the leaves and translocated to the epidermis. The lights increased the amount of ^{32}P in the leaf and the amount transferred to the parasite.

^{32}P transfer rates reflected the relative compatibilities of the interactions studied. Interactions with little fungal development had lower rates of transfer, while fully compatible interactions had high rates of transfer. Rates of ^{32}P transfer did not show differences between compatible and incompatible interactions as early as did ^{35}S transfer rates (50) or production of elongating secondary hyphae (47). Rates of ^{32}P transfer were observed earlier than ^{35}S transfer and the amount of ^{32}P transferred was much higher.

The four possible parasite/host genotypes (the quadratic check) for each interacting gene pair were evaluated for their effect on final infection type seven days after inoculation, on ^{32}P transfer from host to parasite, and on the formation of elongating secondary hyphae (ESH). With all gene pairs the final infection type of the

three compatible interactions were similar. Only the incompatible interaction (Px/Pmx) resulted in a low infection type. The compatible interactions involving P1 and Pm1, P2 and Pm2, P3b and Pm3b had similar rates of ^{32}P transfer. The rates of ^{32}P transfer were not similar for the compatible interactions involving P4 and Pm4. The p4/Pm4 genotype had a reduced rate of transfer when compared to p4/pm4 and P4/pm4. The production of ESH was also delayed with p4/Pm4. Two other isolates containing p4 resulted in a reduction in the percent of parasites that produced ESH. Thus the mutations from P4 to p4 in the parasite have not completely negated the change in the host from pm4 to Pm4. This indicates that specificity of gene-for-gene interactions is for incompatibility, not compatibility. It also demonstrates how the gene-for-gene hypothesis might play a role in general or horizontal resistance.

Germinating conidia of E. graminis were shown to reduce host transpiration rates before penetration occurred. The transpiration reduction was a result of stomate closure induced by the germinating conidia. Extracts of germinating conidia did not reduce transpiration rates and abscisic acid was not detected in the conidia. The reduction in transpiration continued through primary infection and was shown to be responsible for less ^{32}P uptake by inoculated plants than noninoculated plants during primary infection.

Metabolic sinks induced by E. graminis, which concentrated ^{32}P at infection sites in the epidermis of inoculated plants during primary infection, were not demonstrated. Any accumulation of ^{32}P in the epidermis was due to increased cuticular transpiration caused by disruption of the cuticle by the developing fungus.

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