ELONGATION OF SECONDARY HYPHAE AND TRANSLOCATION OF ^{35}S AND ^{3}H FROM HOST TO PARASITE DURING PRIMARY INFECTION OF WHEAT BY ERYSIPHE GRAMINIS F. SP. TRITICI

Dissertation for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY
RICHARD E. STUCKEY

1973

THESIS

This is to certify that the

thesis entitled
ELONGATION OF SECONDARY HYPHAE AND TRANSLOCATION OF ^{35}S AND ^{3}H FROM HOST TO PARASITE DURING PRIMARY INFECTION OF WHEAT BY ERYSIPHE GRAMINIS F. SP. TRITICI

presented by

RICHARD E. STUCKEY

has been accepted towards fulfillment
of the requirements for

PH.D. degree in *PLANT PATHOLOGY*

Dr. Albert H. Ellingboe (R.E.S.)
Major professor

Date *August 17, 1973*

O-7639

ABSTRACT

ELONGATION OF SECONDARY HYPHAE AND TRANSLOCATION OF ^{35}S and ^{3}H FROM HOST TO PARASITE DURING PRIMARY INFECTION OF WHEAT BY ERYSIPHE GRAMINIS F. SP. TRITICI

By

Richard E. Stuckey

Development of secondary hyphae >10u long is evidence of successful infection of wheat by Erysiphe graminis f. sp. tritici. The lengths of secondary hyphae at 20 to 30 hours after inoculation varied with the parasite/host genotype. There were differences in the kinetics of secondary hyphal elongation on plants of compatible and incompatible genotypes. Differences between incompatible genotypes suggest that the single gene differences for incompatibility are operating via different mechanisms rather than via altered specificity of the same mechanism.

Rates of ^{35}S transfer from wheat to E. graminis f. sp. tritici differed with the parasite/host genotype, the stage of parasite development and the environmental conditions. Inoculated primary leaves cut at the base and given $^{35}SO_4^{2-}$ for five hour periods in the light had more than twice the radioactivity of inoculated primary leaves given $^{35}SO_4^{2-}$ in the dark. After considering the amount of

label translocated to the epidermis as a result of the environmental conditions used, greatest rates of ^{35}S transfer were found to occur at 18 to 20 hours rather than at 26 hours after inoculation. The amount of ^{35}S uptake and translocation to the epidermis was greater in noninoculated plants than in inoculated plants from 6 to 24 hours after inoculation. Reduction of ^{35}S activity in inoculated plants was independent of spore concentration, parasite/host genotype and species of Erysiphe used for inoculation.

Little is known about the form in which label is transferred from wheat to E. graminis f. sp. tritici. The data suggest that transfer of both methionine and sulfate can occur. The fungus was able to incorporate SO_4^{2-} into a TCA-insoluble form. Inoculated plants given ^{3}H-methionine transferred ^{3}H to the parasite. Five times the number of molecules appear to be transferred when inoculated plants were given ^{3}H-methionine than when given $^{35}SO_4^{2-}$. When inoculated plants were given both ^{3}H-methionine and $^{35}SO_4^{2-}$, the presence of ^{3}H-methionine inhibited ^{35}S transfer to the parasite by ca. 60 percent. Whether this inhibition occurred at the plant cell membrane, the fungal membrane, or both is not known.

The percent of label incorporated into the TCA-insoluble fraction in the parasite and in the epidermis of the host was much less than the percent of label incorporated into the TCA-insoluble fraction in the leaf tissue when cut seedlings were given $^{35}SO_4^{2-}$. A higher percentage of incorporation into the TCA-insoluble fraction occurred when

leaves were given ^{3}H-methionine or $^{35}SO_4^{2-}$ at one to six hours after inoculation than when leaves were given ^{3}H-methionine or $^{35}SO_4^{2-}$ at 21 to 26 hours after inoculation. Environmental conditions prior to labeling and age of leaf affected the increase in the TCA-insoluble fraction at one to six hours after inoculation. Leaves given ^{3}H-methionine at 21 to 26 hours after inoculation had a higher percentage of radioactivity in the TCA-insoluble fraction than did leaves given $^{35}SO_4^{2-}$ for the same time period.

Thin layer chromatography of extracts from the epidermis and leaves showed a higher percentage of radioactivity corresponding to methionine when plants were given ^{3}H-methionine than when plants were given $^{35}SO_4^{2-}$. Cut seedlings given ^{3}H-methionine had nearly 38 percent of the radioactivity in the epidermis corresponding to a value of methionine on TLC plates. Only four percent of the radioactivity in the epidermis corresponded to a value of methionine when cut seedlings were given $^{35}SO_4^{2-}$.

Rates of ^{35}S transfer from host to parasite were greatly reduced when plants were labeled at 4 C instead of 22 C. An active transport system from host to parasite is suggested.

ELONGATION OF SECONDARY HYPHAE AND TRANSLOCATION OF ^{35}S AND ^{3}H FROM HOST TO PARASITE DURING PRIMARY INFECTION OF WHEAT BY ERYSIPHE GRAMINIS F. SP. TRITICI

By

Richard E. Stuckey

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1973

DEDICATED

to

My Wife, Judy

and

Son, Jeffrey James

ACKNOWLEDGMENTS

Sincere appreciation is due to Dr. Albert H. Ellingboe, my major professor, for his interest, guidance and encouragement during this investigation and in the preparation of this manuscript.

Special thanks are also due to my graduate committee, Dr. R. P. Scheffer, Dr. W. G. Fields, and Dr. J. A. Boezi for serving on my committee and their assistance in the preparation of this manuscript.

I am indebted to Mr. Joseph L. Clayton for his technical assistance and to Dr. M. Jost for making his equipment available to me.

I am deeply grateful to my wife, Judy, for her understanding and assistance during the course of this investigation.

Sincere appreciation is due to the many graduate students and faculty members for meaningful discussions of my research and to Dr. W. B. Drew for allowing me the opportunity to study at Michigan State University.

Financial assistance for this investigation was obtained from the National Institute of Health for which I am indebted.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Erysiphe graminis f. sp. *tritici*, the cause of powdery mildew of wheat, is an obligate parasite found in most of the wheat growing areas of the world. This disease causes a significant reduction in grain yield in some areas of the world (47, 93).

The best and least expensive method of controlling powdery mildew has been the development and use of resistant cultivars. As a result of the breeding programs, there are many host genotypes with different degrees of resistance to powdery mildew.

Much attention has been given to studies on the comparative physiology and biochemistry of diseased and healthy tissues in the search for understanding of disease resistance (16, 72, 82, 91). Most of these workers used tissues several days after inoculation. There are conflicting data and interpretations. The probable reasons for conflicting results are differences in procedures for production of inoculum and plants, differences in times of observation, and differences in environmental conditions.

A well defined system for studying the early infection process of *E. graminis* f. sp. *tritici* on wheat has been extablished (53, 58, 62). Several distinct morphological

stages in fungal development were described. The stages required different environmental conditions for optimum development. The fungus population was synchronized in morphological development by manipulating the environmental conditions, and a maximum number of successful infections was achieved.

The objectives of this research were as follows: (1) to collect quantitative data on the kinetics of elongation of secondary hyphae as a function of time, and (2) to determine if the secondary hyphae which do begin to elongate with incompatible genotypes develop similarly to the development of secondary hyphae with compatible genotypes; (3) to examine the uptake and translocation of ^{35}S in the host under the environmental conditions used for synchronous parasite development, (4) to determine the rate of transfer from host to parasite by considering the amount of label theoretically available in the epidermis to the parasite, and correlate rates of transfer with the morphological stage of development of the parasite; (5) to examine the effect of inoculation on ^{35}S uptake by wheat leaves, and (6) to determine the form in which labeled materials may be transferred from host to parasite. Objectives one through four are the objectives of two papers (chapters 1 and 2) which have been submitted for publication.

LITERATURE REVIEW

The genetics, physiological and biochemical aspects of disease development have been studied extensively, often with contradictory conclusions. The powdery mildews are not an exception. In this review, I will attempt to summarize some of the important genetic, physiological and biochemical developments that have been reported and that are related to the results presented in this thesis.

It is clear from many investigations (32, 33, 57, 65) that the interactions between host and parasite are controlled by complementary genes possessed by both host and parasite. Flor (30) found that the ability of Melampsora lini to grow and produce disease symptoms on flax lines containing genes for resistance 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 (31, 67). The gene-for-gene concept is simply that for each R gene in the plant there is a corresponding P gene in the pathogen, and the interaction of these two genes determines disease development (29, 30, 67). With most diseases studied to date, resistance (R) and avirulence (P) are dominant and susceptibility (r) and virulence (p) are recessive (29, 51, 66).

The terms "compatibility" and "incompatibility" have been used to describe the interactions between host and parasite and infection types with particular parasite/host

genotypes (25). An incompatible reaction, a low infection type, (resistance when viewed as a characteristic of the host, avirulence when viewed as a characteristic of the parasite) is established when at least one P gene in the pathogen and one corresponding R gene in the host are present (Px/Rx). This terminology takes into consideration the genes in both the host and pathogen that affect disease development.

Person et al. (67) has suggested that the complementary relationships between host and pathogen have evolved from specific selection pressures which initially favored host plants which could escape disease and, secondly, selection of 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 adaption to newly introduced host lines possessing R genes (68, 83, 84). The discovery of the gene-for-gene relationship controlling parasite/host compatibility has recently been extended to insect-plant relationships also (40).

Development of the powdery mildew disease is greatly affected by environmental conditions, i.e. light intensity, photoperiod, relative humidity, temperature, and so forth (12, 13, 34, 73, 97). Germination and subsequent development of conidia occur over a wide range of temperatures and relative humidities. Several reviews concerning the effects of various environmental factors on disease development (53, 58, 73, 97) have been published, each stressing the

variability of the data and the necessity of considering the role of the environment in altering disease development. Several different optimal conditions for mildew development have been reported (12, 37, 62, 73).

The development of E. graminis spores on the plant surface can be divided into several morphologically distinguishable stages: (1) germination, (2) production of appressorial initials, (3) formation of mature appressoria, (4) penetration of the host, (5) formation of haustoria, (6) development of secondary hyphae, (7) initiation of secondary, tertiary, etc., infections, and (8) sporulation (12, 37, 55, 97). Each stage in primary infection has certain temperature, relative humidity, and light condition requirements for synchronous development of the parasite (54, 62). When optimum environmental conditions for each stage are used, over 75 percent of the parasite population proceeds through the various developmental stages with a high degree of synchrony (53, 54, 55, 56). The production of elongating secondary hyphae (ESH) by the parasite suggests that the fungus is securing nutrients and other essential materials from the host to permit continued fungal growth (55, 56). Conidia on non-host plants will germinate, form appressoria, and attempt to penetrate epidermal cells, but do not form either haustoria or ESH (53, 95). The number of ESH formed on the host surface corresponds to the number of haustoria in the host epidermal cells (55). The percentage of applied conidia that develop ESH and, therefore,

form a functional relationship with the host is defined as the infection efficiency (25, 26).

The fungal haustorium is defined as "a specialized organ which is formed inside a living host cell as a branch of an extracellular (or intercellular) hypha or thallus, which terminates in that host cell, and which probably has a role in the interchange of substances between host and fungus" (9). Bushnell and co-workers (11) have shown that fungus development on the leaf surface ceases after haustorial destruction. Nutrients supplied exogenously have supported fungal development for a brief time in the absence of a haustorium. This may indicate an ability of the mycelium to absorb a limited amount of nutrients.

The E.M. has increased our knowledge of structural changes resulting from infection. Some workers believe that infection pegs of fungi penetrate the walls of host cells with the help of enzymatic digestion (22). Regardless of the degree of dissolution of cuticle and wall, some mechanical force probably is required for penetration by the infection peg of E. graminis (85). Small portions of the cuticle have been observed to be pushed inward in the region immediately beside the infection peg (85). Akai and co-workers (1) observed a crack in the host wall in the E.M. section intersecting the infection peg of E. graminis Edwards and Allen (22) conclude that penetration consists of (1) the enzymatic digestion of the cuticle and cellulose portion of the epidermal wall by enzymes apparently secreted

by the developing mildew infection peg, and (2) a mechanical pushing of the infection peg through a layer of material, called a papilla, which has been deposited by the host on the underside of the epidermal wall. The papilla is believed to be formed after the appressorium attaches to the outer surface of the host epidermal cell wall, but prior to the formation of the infection peg (22).

The host may deposit materials, besides papillae, that form a sheath around the neck and body of the haustorium. However, papillae and sheaths are so commonly found that they can be regarded as a normal response to haustorial fungi and to other plant pathogens if the host cell remains alive for a short time after penetration (9). Thus these reactions are probably nonspecific responses to wounding on the part of the host.

The development of a functional haustorium is required for continued disease development (9, 25, 55). The extrahaustorial membrane (the boundary between the host cytoplasm and the haustorial apparatus), closely resembles the plasma membrane of the host from which it is thought to be derived (9). In powdery mildew fungi, small irregular invaginations extend from the extrahaustorial membrane into the extrahaustorial matrix (24). These invaginations may be involved in movement of materials across the membrane from the host cytoplasm to the extrahaustorial matrix, or in the opposite directions (8, 9). The volume of the extrahaustorial matrix is composed of (1) an aqueous

solution which contains small amounts of uniformly suspended particulates and (2) components that do not intermix freely with the aqueous solution. Hirata and Kojima (42) found a sac-like membrane (analogous to extrahaustorial membrane) that began to separate from the haustorial walls as the haustorium matured. The separation occurred earlier if the mycelium attachment to the haustorium had developed poorly. From these observations he concluded that the haustorium is more functional before, than after, the extrahaustorial membrane separates from the haustorial wall, and that the separation serves to protect the surviving host cell or haustorium when one or the other degenerates.

Transfer of materials from host to parasite (44, 58, 71, 80, 87) and from parasite to host (24, 46) has been shown. The actual form in which the radioactive labeled materials are moving across the membranes in the powdery mildew host-parasite system is not known. If the exchange of nutrients from host to parasite, and vice versa, are through the extrahaustorial matrix, both the host and fungal membrane may act semi-independent of one another. For active transport of an ion across a membrane three distinct steps occur (49, 64), namely, (1) formation of the ion-carrier complex in the membrane surface, (2) translocation of the complex to the opposite surface and (3) release of the ion into solution. The translocation of the complex across the membrane is described as a transport over an activation

energy barrier and is often the rate-determining step in ion transport (49).

The use of radioactive tracers in studying host-parasite relationships has made possible the detection of very small changes during early disease development. Mount and Ellingboe (59) studied the transfer of $^{32}PO_4^{3-}$ and $^{35}SO_4^{2-}$ from wheat to E. graminis f. sp. tritici during primary infection. The amount of ^{32}P and ^{35}S transferred to the fungus was correlated with the morphological development of haustoria in the host cells. Slesinski and Ellingboe (80) showed a difference in ^{35}S transfer rates between compatible and incompatible parasite/host genotypes. Transfer of ^{35}S from host to parasite was greatest with the compatible genotype. The incompatible genotypes varied in their rates of transfer. Upon closer examination of three compatible genotypes (Pl/pml, pl/Pml, and pl/pml) involving one pair of corresponding genes, Slesinski and Ellingboe (80) found a reduced rate of ^{35}S transfer with the compatible genotype pl/Pml than with the other two compatible genotypes Pl/pml and pl/pml.

Reisener et al. (71) have presented evidence that certain amino acids and carbohydrates are transferred from wheat tissue to rust spores. They conclude that glutamic acid, tyrosine, and hexose are taken up from the host by the fungus without alteration and that a part of fungal alanine also comes directly from the host. Furthermore, ^{14}C in the fungus was found first in free amino acids and later in

protein. This was interpreted to mean that the amino acids were transported from host to parasite but that the majority of protein in the fungus was synthesized by the fungus.

The following is a brief review of the pathway of solutes in a plant from a cut stem to the haustorium, and of other factors that affect solute translocation. Transport of solutes in the xylem and in leaves has received considerable attention (48). Sulfur transport in the xylem is mainly as the inorganic ion, although methionine transport has been reported (48). The classical concept that, in xylem vessels, solutes move with the same velocity as water (mass flow) no longer holds for Ca^{2+} and probably not for other divalant and trivalent cations (48). Translocation between the xylem and phloem of various ions K+, Na+, SO_4^{2-} and PO_4^{3-} occur possibly by xylem rays (98), but little is known about the mechanism. In the leaves, ions apparently are transported along with water in the leaf free space which appears confined to the cell walls (15). While being conveyed in the free space in the direction of the transpiring water, ions can be transported across the plasma membrane into the inner compartments of the leaf cells by means of carrier mechanisms (81). A certain fraction of the ions may not be exported from the free space but follow the lateral pathway of water to the main regions of water loss, i.e. the stomata and other epidermal cells (15). Some evidence exists that transport of ions through the mesophyll of leaves may utilize both the free space and the symplasm as pathways.

$^{36}Cl^-$ was located in cell walls and the cytoplasm of the mesophyll cells but not in the vacuoles (99).

Environmental and internal factors undoubtedly affect the complex physiological phenomena of ion transport in leaves. Many of these factors have not been clearly identified, but light has been demonstrated to affect movement of ions in leaves (48). Rains (70) reported that light increased the rate of K+ uptake in corn leaves possibly through energy brought about by synthesis of ATP in cyclic photophosphorylation. Light may cause stomatal opening through the regulation of K+ influx into the guard cells (45). Light not only has a controlling influence on ion transport in leaves by providing the energy for active transport across cellular membranes, but also affects ionic movements through activation of the phytochrome system (48).

Active transport involves an accumulation of materials against a concentration gradient. Cells accumulating solutes should not be viewed only as a system of selective ion pumps because cells operate via solute absorption and secretion in regulating the internal activity of their water. The cell may more accurately be regarded as a complex type of "water pump" manipulating the collective properties of its water (60).

Plant and fungal cells have been shown to take up sulfate by an active process (5, 28, 39, 75). Hart and Filner (39) reported that sulfate uptake in tobacco cells is inhibited by L-methionine, L-cyst(e)ine, and

L-homocyst(e)ine. Bradfield et al. (5) reported that inorganic sulfate entered the mycelia of Aspergillus and Penicillium spp. by a permease transport system that is affected by temperature, energy, pH, ionic strength and substrate concentration. The transport of sulfate was unidirectional into the cell, resulting in an accumulation against a concentration gradient. As in tobacco cells, sulfate accumulation in cells of Aspergillus and Penicillium is depressed in the presence of methionine or cysteine (5, 75). Interpretations given were that either organic sulfur partially or completely suppresses the sulfate transport mechanism, or the organic sulfur is oxidised intracellularly to sulfate causing a dilution of specific activity, or a combination of these mechanisms operates (75). Segel and Johnson (75) favored a combination of several mechanisms because methionine or cysteine suppressed the intracellular concentration of sulfate by 65 to 80 percent and ^{35}S-methionine fed to mycelium had been converted to free ionic sulfate. Puccinia graminis tritici grew in a culture medium with cysteine as the sulfur source; it also grew with other sulfur-containing amino acids, but did not grow with inorganic forms of sulfur (43). Excessive leakage of sulfur amino acids from the mycelium into the medium, rather than insufficient sulfate uptake or inability to convert inorganic sulfur to sulfur amino acids, was thought to account for lack of growth with inorganic sulfur as the sole source of sulfur.

Uptake of L-methionine by mycelium of Penicillium chrysogenum was mediated by two distinctly different, independently regulated, stereospecific membrane transport systems (3). One of the systems is relatively specific for L-methionine and develops under sulfur-starvation conditions. The other is a general (nonspecific) amino acid permease.

The effects of both powdery mildew and rust diseases on host plants appears to be diverse and numerous by several days after inoculation. Durbin (19) stated that substances tend to stay at infection sites instead of being recirculated to growing parts of the plant. Patterns of long distance translocation apparently are altered so that movement of organic substances toward the infection site is favored. Thatcher (89) provided evidence that the permeability of host cells was increased by rust infection thereby facilitating the movement of solutes to cells that contain haustoria. Edwards and Allen (21) have shown that the infection site is the major site of ^{14}C accumulation in plants given $^{14}CO_2$. Transfer of carbon from host to parasite tissue was observed 15 minutes after plants were allowed to take up $^{14}CO_2$. The accumulation of ^{14}C in the parasite occurred after the photosynthetic ability of the infected host tissue was reduced (2, 22, 74). Therefore, ^{14}C must be translocated from healthy to diseased areas of the plant. Edwards (20) stated that ^{14}C given to primary leaves accumulated at the infection site when the fugus

was sporulating. Healthy leaves had ^{14}C distributed uniformly in the leaf. ^{14}C in secondary leaves did not move to the infection sites on primary leaves; only the ^{14}C in primary leaves moved to the infected sites on primary leaves. ^{35}S was found to accumulate at infection sites of two to four day old colonies but not ten day old colonies if ^{35}S was given after colonies were established (96). If ^{35}S was given before inoculation, accumulation of ^{35}S was not detected at infection sites by three days after inoculation, but was detected by ten days (96).

Increased respiration was observed in areas of barley leaves adjacent to the cells invaded by the fungus, but these respiratory increases were not detected until six days after inoculation (2, 10). Respiratory increases begin with the appearance of visible infection flecks (76). Transpiration rate and average stomatal aperature of primary bean leaves were significantly reduced during the fleck stage of rust development (18). Duniway and Durbin (18) reported that from four to six days after inoculation, diseased leaves had less transpiration than healthy leaves. Transpiration at night was slightly less with diseased leaves than healthy leaves until five and one half days after inoculation. Greatly increased transpiration of diseased tissue was observed after five and one half days. The increased transpiration of diseased tissue was considered to be due primarily to excessive cuticular

transpiration caused by the disruption of the cuticle and epidermis by the fungus (18).

When uredospores of Puccinia graminis labeled with ^{14}C were inoculated onto wheat leaves, radioactivity was transferred throughout the leaf by 24 hours after inoculation (23). At the time of fleck reaction, about six days after inoculation, most of the ^{35}S was in the inoculated primary leaves (46). However, some ^{35}S was uniformly distributed in tissues of the second leaves and the roots.

Increases in protein synthesis, especially of particular enzymes, have been reported to occur anywhere from two to ten days after inoculation of resistant tissues (72, 74, 82, 91, 94, 100). The amount of RNA increased parallel to the increase in respiration following infection of susceptible wheat leaves by P. graminis tritici (41).

Changes in the synthesis of soluble proteins were reported as early as six to 18 hours after inoculation of flax with Melampsora lini (92). The rates at which various-sized proteins were synthesized varied for each of six different parasite/host genotypes. In general, the net rate of protein synthesis increased in the four incompatible reactions but did not increase in the two compatible interactions. Increases in enzyme activities have been reported as early as six hours after inoculation (38, 69). Preliminary studies (92) indicate the rate of RNA synthesis likewise is increased within six hours in an incompatible interaction.

The latter was interpreted to suggest changes were occurring at the transcriptional level.

Some of the metabolic alterations observed in diseased tissues are similar to those occurring in detached leaves (27). Thus, many of the parasitically induced metabolic alterations of hosts are highly unspecific (27). One classic example of how easy it is to confuse cause and effect relationships is presented. Increase in peroxidase activity had been attributed to activity of the Sr 6 locus to give resistance in wheat to Puccinia graminis. Daly et al. (17) compared two wheat lines with different genes, Sr 6 and Sr 11, and detected no significant changes in phenolic components with resistant and susceptible plants to race 56 of P. graminis tritici. The increase in total peroxidase activity due to the same isozyme in resistant reactions was similar with Sr 11 and Sr 6. Because the genetic and physiological bases for resistance controlled by Sr 11 and Sr 6 are distinct, it is concluded that increased activity of the same isozyme in both instances is a result of a nonspecific event analogous to wounding (17).

MATERIALS AND METHODS

Maintaining Powdery Mildew

The strain MS-1 of Erysiphe graminis D.C. f. sp. tritici Em. Marchal was maintained on susceptible wheat, (Triticum aestivum L) cv. 'Little Club' for the production of inoculum for all experiments. Thirty to 40 wheat seeds of Little Club were planted in four inch pots daily. Wheat plants were inoculated daily when they were seven days old. Conidia produced seven days after inoculation were dusted onto the leaves of Little Club wheat. Inoculated plants were maintained in a controlled environmental chamber providing adequate air circulation under the following environmental conditions:

1. Light period was 15 hr/day. Total light intensity was 700 to 800 foot candles (650 to 750 foot candles from white VHO flourescent tubes and 50 foot candles from 25 watt incandescent bulbs).

2. Temperature was 18 $\pm$ 1 C during the light period and 17 $\pm$ 1 C during darkness.

3. Relative humidity was 65 $\pm$ five percent during the light period and 95 $\pm$ five percent during darkness. Plants were watered daily.

Mycelial growth was macroscopically evident by three to four days after inoculation. Conidia produced on the sixth day after inoculation were used for experimental purposes in all experiments.

Methods of Inoculation

Controlled Inoculation

Single five-to-six-day-old plants in two inch pots were inoculated by the rolling method (61) in all experiments for study of morphological development of the fungus during primary infection. Conidia from six-day-old inoculated plants were dusted onto clean glass slides and transferred to the abaxial leaf surface of single wheat plants with a cotton swab. Conidia on the glass slide were examined directly with a compound microscope at 125X magnification. If more than ten percent of the conidia were collapsed, the experiment was terminated and fresh conidia collected at a later time. This method of inoculation provided a uniform distribution of single conidia ranging from 100-200/cm length of leaf. Only single, well separated parasite units were examined at each observation to eliminate any effect caused by spore crowding or clumping (53). Infection kinetics are similar on either side of the leaf by microscopic observations and removal of host epidermal strips are easier on the abaxial side (58).

Inoculation by Dusting

Plants were inoculated by gently dusting conidia produced six days after inoculation onto the leaves of five to six-day-old wheat seedlings. Inoculation by dusting resulted in 1200-1500 conidia applied/cm length of leaf. The development of the fungus during primary infection was similar following use of either method, but the efficiency of infection was reduced by dusting.

Environmental Conditions for Experiments

Sherer-Gillett (Model CEL 512-37 and Model CEL 25-7) growth chambers were used for all experiments. The conditions used for high efficiency of infection and synchronous growth of each developmental stage of infection are as follows:

1. From 0 to one hour after inoculation, plants were kept in darkness at 18 $\pm$ 1 C and at approximately 100 percent relative humidity (RH).

2. From one to six hour, inoculated plants were kept in a light intensity of 1.1 by 10^5 ergs $cm^{-2}sec^{-1}$ (0.25 x 10^5 ergs $cm^{-2}sec^{-1}$ from white VHO-fluorescent tubes and 0.9 x 10^5 ergs $cm^{-2}sec^{-1}$ from 25 watt incandescent bulbs), 22 $\pm$ 1C, and RH 65 $\pm$ five percent.

3. From six to twenty hour, plants were kept at the temperature and RH as stated in (2) above, but in darkness.

4. From 20 to 30 hour, conditions were the same as in (2) above.

Changes in temperature and relative humidity during experiments were monitored with wet and dry bulb thermometers and with a recording hygrothermograph calibrated with a sling psychrometer. Light intensity was measured at the distance of the plant from the lights with a YSI Kettering Model 65 Radiometer.

Counting Efficiency of ^{3}H-methionine and $^{35}SO_4^{2-}$

Counting efficiency for ^{3}H-methionine was found to be from 14 to 24 percent depending on preparation of the cocktail. Counting efficiency for $^{35}SO_4^{2-}$ averaged 77 percent.

Other experimental conditions are given with each chapter of results.

CHAPTER I

GENETIC CONTROL OF MORPHOLOGICAL DEVELOPMENT OF E. GRAMINIS ON WHEAT

Introduction

A high percentage of spores of Erysiphe graminis f. sp. tritici on a wheat leaf will germinate, produce appressoria, haustoria, and elongating secondary hyphae if given the appropriate environmental conditions and if the parasite/host genotype specifies compatibility (54, 78). With incompatible genotypes the same percentage of spores germinate and produce appressoria but few of them produce secondary hyphae, but only parasite units that have haustoria appear to be able to produce secondary hyphae longer than 10 u (55, 78). Secondary hyphae less than ca. 10 u have not been observed to produce secondary appressoria and haustoria. Therefore, those elongating hyphae ceasing growth before 10 u in length are considered to be non-functional.

Several of the incompatible parasite/host genotypes affect the percentage of parasite units that produce elongating secondary hyphae (78). A few elongating secondary hyphae are formed with all genotypes, but the percentage that develop is dependent on the particular parasite/host genotype (78).

The objectives of the research reported herein were (1) to collect quantitative data on the kinetics of elongation of secondary hyphae as a function of time after inoculation and (2) to determine if the secondary hyphae which do begin to elongate with incompatible genotypes develop similarly to the development of secondary hyphae with compatible genotypes.

Materials and Methods

Erysiphe graminis f. sp. *tritici*, genotype *Pl P2 P3a P4* (51, 79) (MS-1) was maintained on wheat cultivar Little Club. Environmental conditions for maintenance of cultures, procedures for the production of inocula, and procedures to obtain high infection efficiency and synchronous development of the parasite were described previously (54, 55, 56). Controlled inoculations on the abaxial leaf surface were made by the rolling method (61).

The five homozygous lines of wheat used were designated as follows (6, 7):

$$\frac{pm1\ pm2\ pm3\ pm4}{pm1\ pm2\ pm3\ pm4} = (pmx \text{ or Chancellor}): \quad \frac{Pm1\ pm2\ pm3\ pm4}{Pm1\ pm2\ pm3\ pm4} = Pm1;$$

$$\frac{pm1\ Pm2\ pm3\ pm4}{pm1\ Pm2\ pm3\ pm4} = Pm2; \quad \frac{pm1\ pm2\ Pm3a\ pm4}{pm1\ pm2\ Pm3a\ pm4} = Pm3a;$$

$$\frac{pm1\ pm2\ pm3\ Pm4}{pm1\ pm2\ pm3\ Pm4} = Pm4.$$

Each of the four loci (*Pm1*, *Pm2*, *Pm3a*, and *Pm4*) are known to condition different reactions to powdery mildew.

The host genotype is abbreviated in this study as Pm1 rather than as a diploid Pm1 Pm1, since the wheat lines used are homozygous for these genes. The compatible parasite/host genotype, Px/pmx, was the standard to which all other genotypes were compared. The incompatible genotypes were P1/Pm1, P2/Pm2, P3a/Pm3a and P4/Pm4. Parasite/host combinations are referred to by the corresponding genotypes which specify compatibility or incompatibility of the relationship. The P1/Pm1 genotype specifies incompatibility. The Pm1 gene in the host recognizes only the P1 gene in the pathogen, even though the pathogen possesses many other P genes. A corresponding gene pair which conditions incompatibility is expressed in the presence of any number of other corresponding gene pairs specifying compatibility (80). Only the part of the parasite/host genotypes that specifies incompatibility is given.

The lengths of secondary hyphae were determined at various times after inoculation by direct microscopic measurements of all single, isolated parasite units on leaf sections (1-cm long) from 1-2 cm from the tip portion of the leaf.

The data are presented as percent of the total number of conidia applied to the leaf having secondary hyphae according to length and time of observation. New leaf sections were used for each observation. From 100-150 parasite units were counted on a single leaf for each

observation. Results are the averages of at least five experiments repeated on different days.

Results

Meaningful interpretation of results in relation to work already reported (55, 58, 62, 79) requires a common departure point. The common base for the research reported herein was the ability to reproduce the essential stages of synchronous morphological development of E. graminis f. sp. tritici on wheat during primary infection (55, 58, 62). Nearly identical results were obtained for spore germination, appressorial maturation, and elongation of secondary hyphae as was reported by Slesinski (77) (Figure 1). Thirty-five percent of the spores germinated by one hour after inoculation. By four hours after inoculation, 93 percent of the spores had germinated. Mature appressoria were observed on 35 percent of the spores by six hours after inoculation and 92 percent by eight hours. Initiation of secondary hyphae was observed between 16 and 18 hours after inoculation. Secondary hyphae greater than 10u in length were observed on 17 percent of the spores by 20 hours after inoculation. The percentage of secondary hyphae 10u long increased rapidly between 22 and 24 hours and reached 75 percent by 30 hours after inoculation with a compatible parasite/host genotype.

At 20 hours after inoculation with the genotype Px/pmx, 68 percent of the parasite units had either no secondary hyphae or secondary hyphae 5u or less in length,

Figure 1.--Confirmation of synchronous development of *Erysiphe graminis* f. sp. *tritici* on wheat leaves.

(A) germination,

(B) mature appressoria, and

(C) secondary hyphae >10u long.

(----------) Slesinski (77),

(_____) from results obtained in this study.

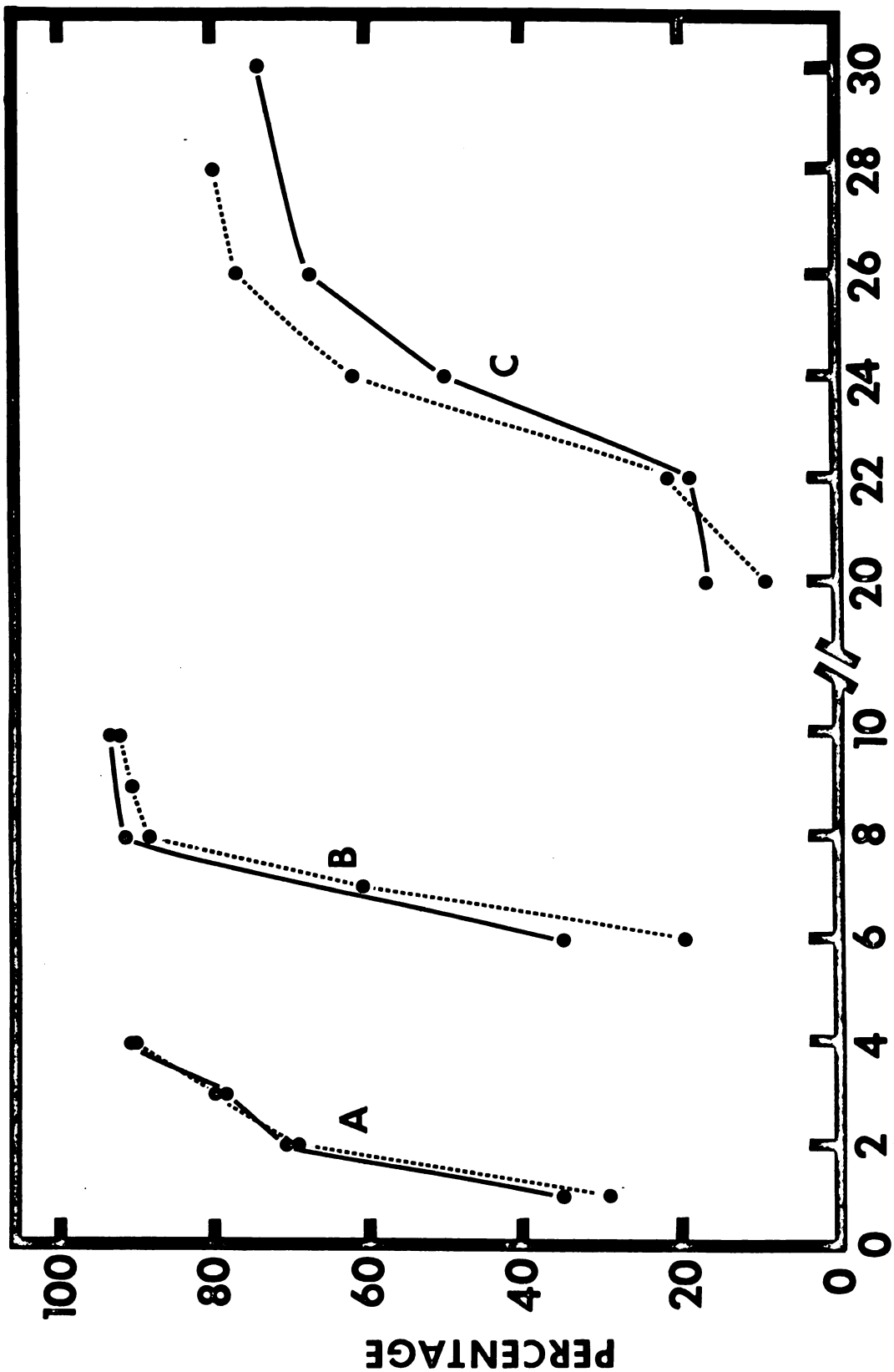

PERCENTAGE
100
80
60
40
20
0
0
2
4
6
8
10
20
22
24
26
28
30
TIME AFTER INOCULATION (hr)
A
B
C

Figure 1.

16 percent were 6-10u, 16 percent were 11-15u, one percent were 16-20u, and no parasite units had secondary hyphae greater than 20u long (Table 1, Figure 2). By 24 hours after inoculation, more parasite units have secondary hyphae 11-15u long (38 percent) than in any of the other classes of lengths. Thirty-three percent were 6-10u long. By 30 hours after inoculation 37 percent secondary hyphae were greater than 20u long. The distribution of lengths of secondary hyphae as a function of time after inoculation as presented in Figure 2A shows that secondary hyphae elongation occurred from 20 hours through 30 hours after inoculation.

The P1/Pm1 genotype was shown to affect infection efficiency (78, 79), and in this experiment 15-20 percent of the secondary hyphae were longer than 10 u (Table 1, Figure 2B). Of those secondary hyphae that did get longer than 10u, only a few elongated to more than 15u. There was no significant change in the distribution of secondary hyphae from 24 to 30 hours after inoculation.

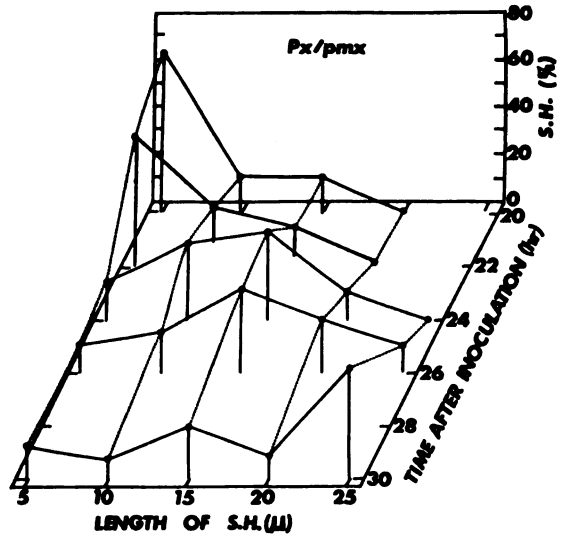
In none of the previous experiments (78) has it been possible to demonstrate an effect of the P2/Pm2 genotype during primary infection. Most of the parasite units (77 percent) eventually produced secondary hyphae longer than 10u, and most secondary hyphae became much longer than 10u (Table 1, Figure 2C).

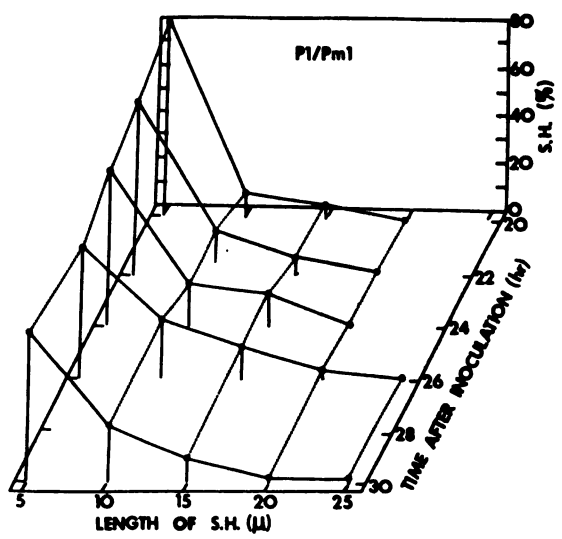
The P3a/Pm3a genotype reduced secondary hyphae that got longer than 10u to ca. 30 percent (Table 1, Figure 2D).

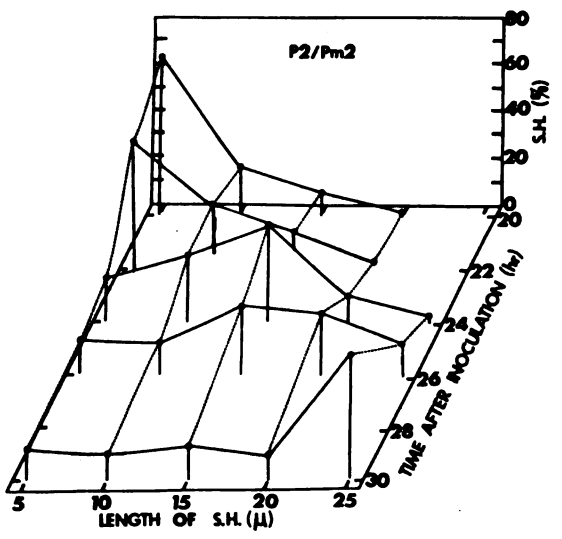
TABLE 1--Effect of genotype of host and pathogen on development of secondary hyphae.

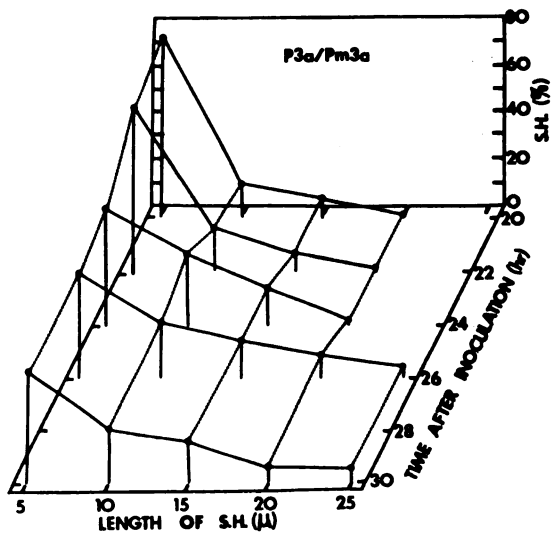
Genotypes (parasite/ host)	Hour after Inoculation	% of parasite units with secondary hyphae of a given length					Functional secondary hyphae (%)
		0-5u	6-10u	11-15u	16-20u	>20u	(>10u in length)
Px/pmx	20	68	16	16	1	0	17
	22	56	26	17	2	0	19
	24	17	33	38	12	0	50
	26	14	20	35	21	10	66
	30	16	12	25	11	37	73
P1/Pm1	20	83	10	6	0	0	6
	22	74	19	7	1	0	8
	24	66	18	14	1	0	15
	26	56	25	14	4	1	19
	30	63	23	10	2	2	14
P2/Pm2	20	67	21	10	1	0	11
	22	54	28	16	3	0	19
	24	18	28	41	11	2	54
	26	14	14	30	27	14	71
	30	12	11	14	10	53	77
P3a/Pm3a	20	77	14	8	1	0	9
	22	71	19	8	1	0	9
	24	50	31	16	2	0	18
	26	45	24	16	10	4	30
	30	47	23	17	6	6	29
P4/Pm4	20	85	11	4	0	0	4
	22	79	14	6	1	0	7
	24	77	17	4	1	0	5
	26	76	17	5	2	0	7
	30	75	18	5	1	0	6

Figure 2.--Effect of five different parasite/host genotypes of the development of secondary hyphae of E. graminis f. sp. tritici. (A) genotype Px/pmx; (B) genotype P1/Pm1; (C) genotype P2/Pm2; (D) genotype P3a/Pm3a; and (E) genotype P4/Pm4. Those secondary hyphae grouped in class 25 refer to all secondary hyphae greater than 20u long.


Px/pmx
S.H. (%)
0
20
40
60
80
TIME AFTER INOCULATION (hr)
20
22
24
26
28
30
LENGTH OF S.H. (μ)
5
10
15
20
25


P1/Pm1
S.H. (%)
0
20
40
60
80
TIME AFTER INOCULATION (hr)
20
22
24
26
28
30
LENGTH OF S.H. (μ)
5
10
15
20
25


P2/Pm2
S.H. (%)
0
20
40
60
80
TIME AFTER INOCULATION (hr)
20
22
24
26
28
30
LENGTH OF S.H. (μ)
5
10
15
20
25


P3a/Pm3a
S.H. (%)
0
20
40
60
80
TIME AFTER INOCULATION (hr)
20
22
24
26
28
30
LENGTH OF S.H. (μ)
5
10
15
20
25

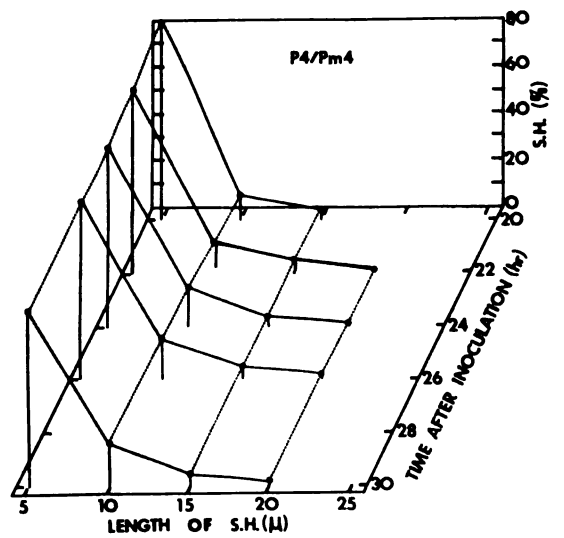

P4/Pm4
S.H. (%)
0
20
40
60
80
TIME AFTER INOCULATION (hr)
20
22
24
26
28
30
LENGTH OF S.H. (μ)
5
10
15
20
25

Figure 2.

The secondary hyphae on some of the parasite units became more than 20 u long.

The P4/Pm4 genotype reduced secondary hyphae that were longer than 10 u to ca. 5 percent (Table 1, Figure 2E). Few secondary hyphae were more than 15 u long and none were longer than 20 u. The maximum percentage of secondary hyphae longer than 10 u was reached by 22 hours after inoculation.

Discussion

The data suggest that it is possible to observe, with time, the distribution of a parasite population having no, or predominantly very short, secondary hyphae to a distribution of a parasite population having predominantly secondary hyphae greater than 10 u. The data with the compatible genotype Px/pmx (Figure 2A), or with the incompatible genotype P2/Pm2 which has not been shown to affect primary infection (Figure 2C), suggest that, once the formation of secondary hyphae is initiated, secondary hyphae elongate at the rate of about 5 u every two hours. This is a much slower rate of elongation than observed with E. graminis f. sp. hordei (44). The observation that some secondary hyphae do not elongate more than 20 u is considered due to the initiation of secondary appressoria. Formation of secondary appressoria are believed to begin about 26 hours after inoculation with the environmental conditions used.

Three genotypes have been shown to affect the percentage of secondary hyphae that attain a length >10 u (78)(Figure 2B, 2D, 2E). P4/Pm4 not only causes a reduction in the percentage of parasite units that produce haustoria and elongating secondary hyphae, but also causes a collapse of most parasite units that produce haustoria and a discoloration of the host cell adjacent to the haustorium (77). The collapse of the parasite units that have produced haustoria was observed at about 21 to 22 hours after inoculation (77). The data presented here suggest that there is no detectable increase in the lengths of secondary hyphae subsequent to 22 hours after inoculation (Table 1, Figure 2D).

P1/Pm1 was also observed to affect the percentage of parasite units that produce haustoria and, therefore, capable of producing secondary hyphae longer than 10 u (77). More than half of the parasite units that produce haustoria collapse about 26 to 28 hours after inoculation. The data presented herein suggest that cessation of elongation of secondary hyphae longer than 10 u occurred by 26 hours after inoculation, or earlier.

P3a/Pm3a was also shown to affect the percentage of parasite units that produce haustoria and elongating secondary hyphae (77, 78). The data presented herein suggest that ca. 30 percent of the parasite units that do produce haustoria also produce secondary hyphae that are longer than 20 u. The relative proportions of secondary hyphae in the 11 to 15,

16 to 20, and >20 u length ranges is approximately the same as with the compatible genotype Px/pmx through 26 hours after inoculation. The parasite units which are successful in producing haustoria have secondary hyphae which appear to elongate to the same extent as with a compatible genotype the first 26 hours after inoculation.

We interpret results presented herein to provide additional supporting evidence that the different genotypes affect different stages in the ontogeny of interactions between host and parasite (25).

Summary

The lengths of secondary hyphae of E. graminis f. sp. tritici at 20 to 30 hours after inoculation varies with the genotype of host and parasite. With a compatible parasite/host genotype (Px/pmx), or the incompatible genotype (P2/Pm2) which does not affect primary infection, secondary hyphae elongate at about two to three u/hours. The secondary hyphae that do become longer than 10 u with the incompatible genotype, P3a/Pm3a, appear to continue to elongate the first 26 hours at the same rate as do hyphae with the compatible genotype. The secondary hyphae which begin to elongate on the incompatible genotypes P1/Pm1 and P4/Pm4, cease elongation at about 26 and 22 hours after inoculation, respectively.

CHAPTER II

^{35}S UPTAKE BY WHEAT AND TRANSFER TO E. GRAMINIS F. SP. TRITICI AS AFFECTED BY ENVIRONMENTAL CONDITIONS

Introduction

Compatible and different incompatible parasite/host genotypes have shown altered rates of nutrient transfer from host to parasite during primary infection (80). Much emphasis has been placed on developing environmental conditions that favor a high degree of synchrony of the parasite during the distinct morphological stages of parasite development that occur during primary infection (55, 58, 62). A synchronous parasite population allows the association of a particular morphological stage of the parasite with the time of greatest rates of nutrient transfer from the host to the parasite. Unfortunately, little attention has been given to the effect of the fungus on the host and to the effect on the host of the environmental conditions used for synchronous parasite development. Do the environmental conditions used affect the amount of label (^{35}S) in the host that would be available to the parasite? If so, would the apparent time of greatest rates of ^{35}S transfer from host to parasite be altered? The remainder of this chapter gives insight into these questions and has been submitted for publication.

The environmental conditions necessary for synchronous development of Erysiphe graminis f. sp. tritici on wheat (54,62) during primary infection have been established. Sixty percent of the spores germinate on seedlings kept in darkness at 100 percent RH and 17 C. Forty percent of the spores applied to a host leaf germinate with 1.1×10^5 erg/cm^2/s of incandescent and fluorescent radiation, 65 percent RH, and 22 C. More than 90 percent of the spores germinate by using the first treatment followed by the second treatment from 0-1 hours and 1-6 hours after inoculation, respectively (25, 54, 61). Appressorial formation and maturation are favored by fluorescent and incandescent radiation totaling 1.1×10^5 erg/cm^2/s with 65 percent RH at 22 C. Penetration takes place in darkness. The next morphologically visible stage on the surface of the leaf is the formation and elongation of secondary hyphae, which is also favored by light at 1.1×10^5 erg/cm^2/s. For the parasite population to develop synchronously for each of the morphologically distinct stages, the following environmental conditions following inoculation are employed: 0-1 h, 17C, 100 percent RH, darkness; 1-6 h, 22C, 65 percent RH, 1.1×10^5 erg/cm^2/s radiation; 6-20 h, 22C, 65 percent RH, darkness; and 20-30 h, 22C, 65 percent RH, 1.1×10^5 erg/cm^2/s radiation (54, 87).

These environmental conditions are known to induce synchronous development of the parasite population on the host but little is known about their effect on the host.

Use of environmental conditions that give parasite synchrony make it possible to test the effects of parasite/host genotypes on transfer of labelled materials to the parasite. Previous workers (80) have reported that rates of ^{35}S transfer from host to parasite are affected by the genotype of both parasite and host. The rates of transfer were assumed to be dependent primarily upon the parasite/host genotype and the stage of morphological development of the parasite. The rate of transfer from host to parasite was determined more accurately by calculating rates of transfer/ 10,000 spores (44, 87).

The objective of this study was to: (1) determine the uptake and translocation of ^{35}S in the host under the environmental conditions used for synchronous parasite development; (2) place the rates of transfer from host to parasite considering the amount of label in the host epidermis that would, theoretically, be available to the parasite; and (3) correlate rates of ^{35}S transfer with the morphological stage of development of the parasite. An abstract describing part of this paper has been published (87).

Materials and Methods

Culture MS-1 of Erysiphe graminis f. sp. tritici was cultured on wheat (Triticum aestivum) cv. 'Little Club' which has no known genes for resistance. The environmental conditions under which these stock cultures were maintained

have been described (54, 62). Wheat plants were uniformly inoculated with mildew by dusting five to six day-old plants with conidia from stock cultures. The five near-isogenic wheat lines were designated as pmx (Chancellor), Pm1, Pm2, Pm3a, and Pm4 (6, 7). The cultivar Chancellor is not known to possess any Pm genes and was the recurrent parent in the establishment of each of the other host lines. Each of the Pm genes is known to be unique and independent for reaction to E. graminis f. sp. tritici. Culture MS-1 possesses the P1 P2 P3a P4 genes conditioning incompatibility with each corresponding gene Pm1, Pm2, Pm3a, or Pm4, respectively, in the host (51, 65).

The uptake and translocation of ^{35}S by wheat seedlings were determined (87) at various times after inoculation. Five day-old wheat seedlings were cut at the crown with a razor blade (with a film of water on the cutting edge) and placed into small vials (25 x 5mm) that contained 0.1 ml of a 100 uc/ml solution of $H_2{}^{35}SO_4$ in 0.1 M Na-K-PO_4 buffer (pH 6.9). After five hours, each leaf was removed from the vial, placed on a paper towel, the tip 1 cm was discarded, and a section (1 to 2 cm long) taken below the tip was coated with a thin film of 1.9 percent parlodion (Mallinckrodt Chemical Works) in 60:40 (v/v) ether: alcohol solution. The parlodion film (containing the ecoparasitic portion of the fungus) was dried, carefully removed, and placed in a scintillation vial for determination of radio-activity by liquid scintillation counting. To determine

leaf activity, 1-cm long leaf sections were taken from the area from which the parlodion was removed and four sections each from different leaves were placed in scintillation vials and dried. Fifteen ml scintillation fluid ([5 g 2, 5-diphenyloxazole and 0.1 g 1, 4-bis-2-(5-phenyloxazolyl) benzene in 1 liter toluene]) were added to each vial. Radioactivity was determined in a Beckman LS-133 Liquid Scintillation spectrometer. Epidermal sections were taken in the same manner. After removal of the parasite from the leaf surface, the epidermis was removed from the leaf with forceps and a razor blade. All chlorophyllous tissue was removed from the epidermal strips and the percent from each 1-cm length of epidermis recovered estimated. The total epidermal strips recovered from four 1-cm sections was generally the equivalent of 2.0-3.0 intact sections. These results were adjusted to 4.0 intact sections to compare the radioactivity in the epidermis with the radioactivity of an equivalent area of the leaf. The epidermal strips were counted in the same manner described for the leaf sections. The results for leaf section activity and epidermal activity are the average of ten replications taken on five or more different days.

To determine the rates of transfer from host to parasite, four parlodion strips were placed in a scintillation vial, dissolved with 0.2 ml 60:40 (v/v) ether : alcohol solution at room temperature overnight, 15 ml scintillation

fluid was added and the radioactivity counted with a Beckman LS-133 Liquid Scintillation spectrometer. The rate of transfer of ^{35}S from host to parasite without regard to level of label in the host was plotted as reported earlier (80, 87). These same results were also plotted as radioactivity per 10,000 spores and per 25,000 CPM in the epidermis (87). Results are the averages of six replications each taken on a different day.

Results

The ^{35}S activity in the leaf sections and in the epidermis after five hour uptake periods during primary infection is presented in Figure 3. Each point represents the radioactivity at the end of a five hour uptake period. For example, the point six hours after inoculation in leaf sections represents the activity in plants that have been allowed to take up ^{35}S from three to eight hours after inoculation, thus having three hours (3-6 hours) in the light and two hours (6-8 hours) in the dark. The points at 12, 14, 16, 18, and 20 hours after inoculation represent uptake of ^{35}S in darkness. Data representing the radioactivity in the leaf at 22, 24, and 26 hours after inoculation have taken up ^{35}S for three hours in dark and two hours in light, one hour in dark and four hours in light, and all five hours in the light, respectively. Results show that plants allowed to take up ^{35}S in the light have more than two times more activity than plants taking up ^{35}S in the dark.

Figure 3.--Effect of light and darkness on uptake and translocation to the epidermis of ^{35}S in cut wheat seedlings inoculated with Erysiphe graminis f. sp. tritici under environmental conditions used for synchronous parasite development in primary infection.

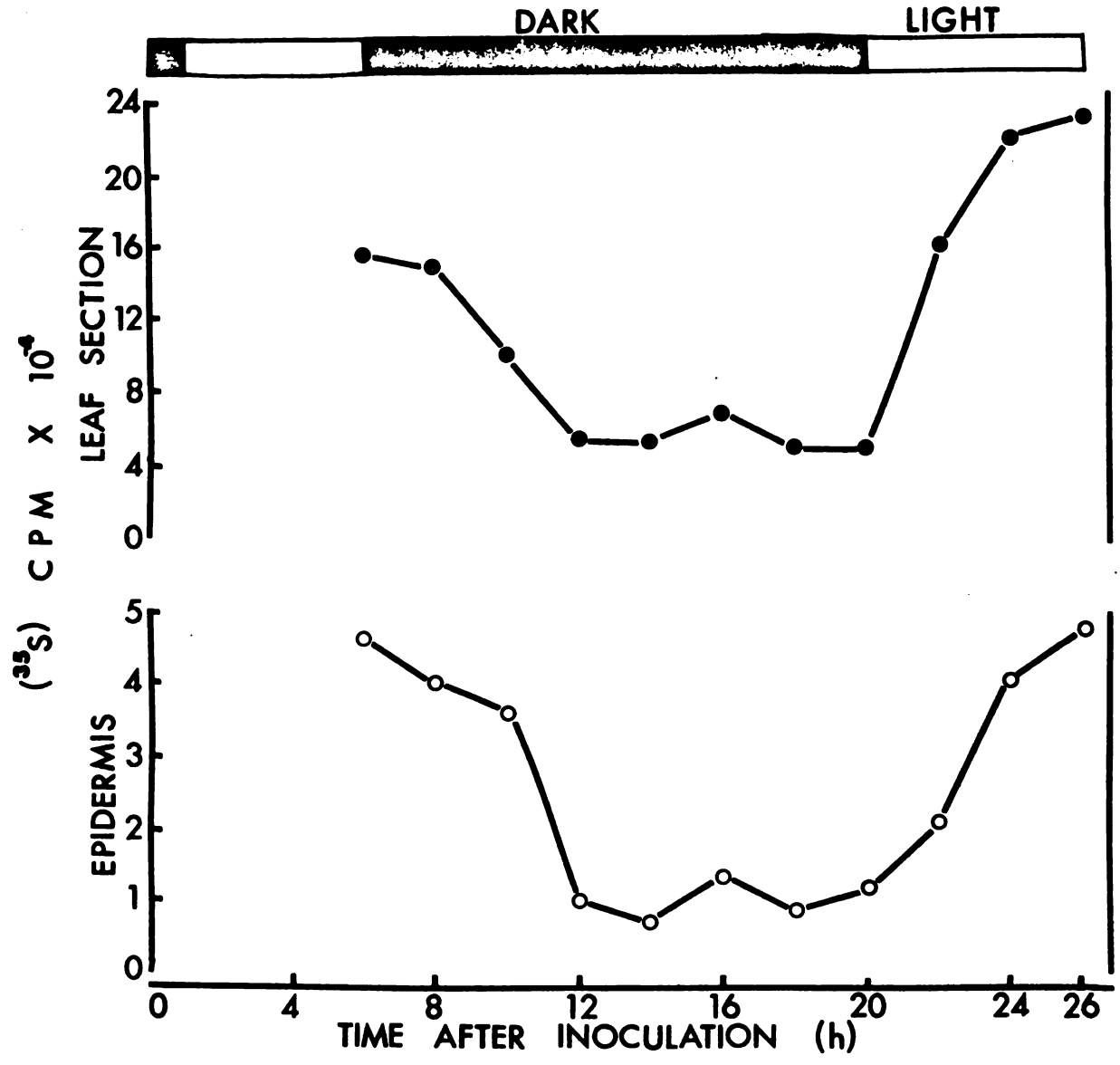

DARK
LIGHT
LEAF SECTION
EPIDERMIS
(^{35}S) CPM X 10^{-4}
TIME AFTER INOCULATION (h)

Figure 3.

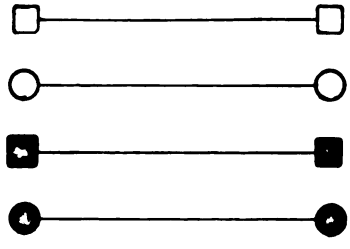
Data for radioactivity in the epidermis show the same relationship. Plants taking up ^{35}S in the light have three to four times more label in the epidermis than do those plants taking up ^{35}S in the dark.

About 20 percent of the label taken up by the leaf in the light appears in the epidermal strip (Figure 3). Leaves that took up ^{35}S in the dark have a smaller percentage of the label in the epidermal strip.

The light has a striking effect on transfer of ^{35}S from host to parasite (Figure 4). When lights remained off from 20 to 26 hours after inoculation, transfer remained low (ca 500 CPM). When lights were on, about 2800 CPM were transferred from Chancellor wheat to E. graminis f. sp. tritici by 26 hours after inoculation. The difference in transfer may be attributed in part to lower uptake and translocation of ^{35}S in the host in the dark. There also appears to be an effect of the light on morphological development of the parasite (Figure 5). The fungus in the dark (Figure 5) failed to produce secondary hyphae at the usual rate (88).

The rates of ^{35}S transfer from the host to parasite of five different parasite/host genotypes at various times after inoculation are shown in Figure 6A. Transfer rates for the three incompatible parasite/host genotypes (P1/Pm1, P3a/Pm3a, P4/Pm4) appear to level off after 18 to 22 hours. The rates for the compatible parasite/host genotype (Px/pmx),

Figure 4.--Effect of light on ^{35}S transfer from Chancellor wheat to E. graminis f. sp. tritici from 20 to 26 hours after inoculation:



inoculated plants in light,

inoculated plants kept in dark,

non-inoculated plants in light, and

non-inoculated plants kept in the dark.

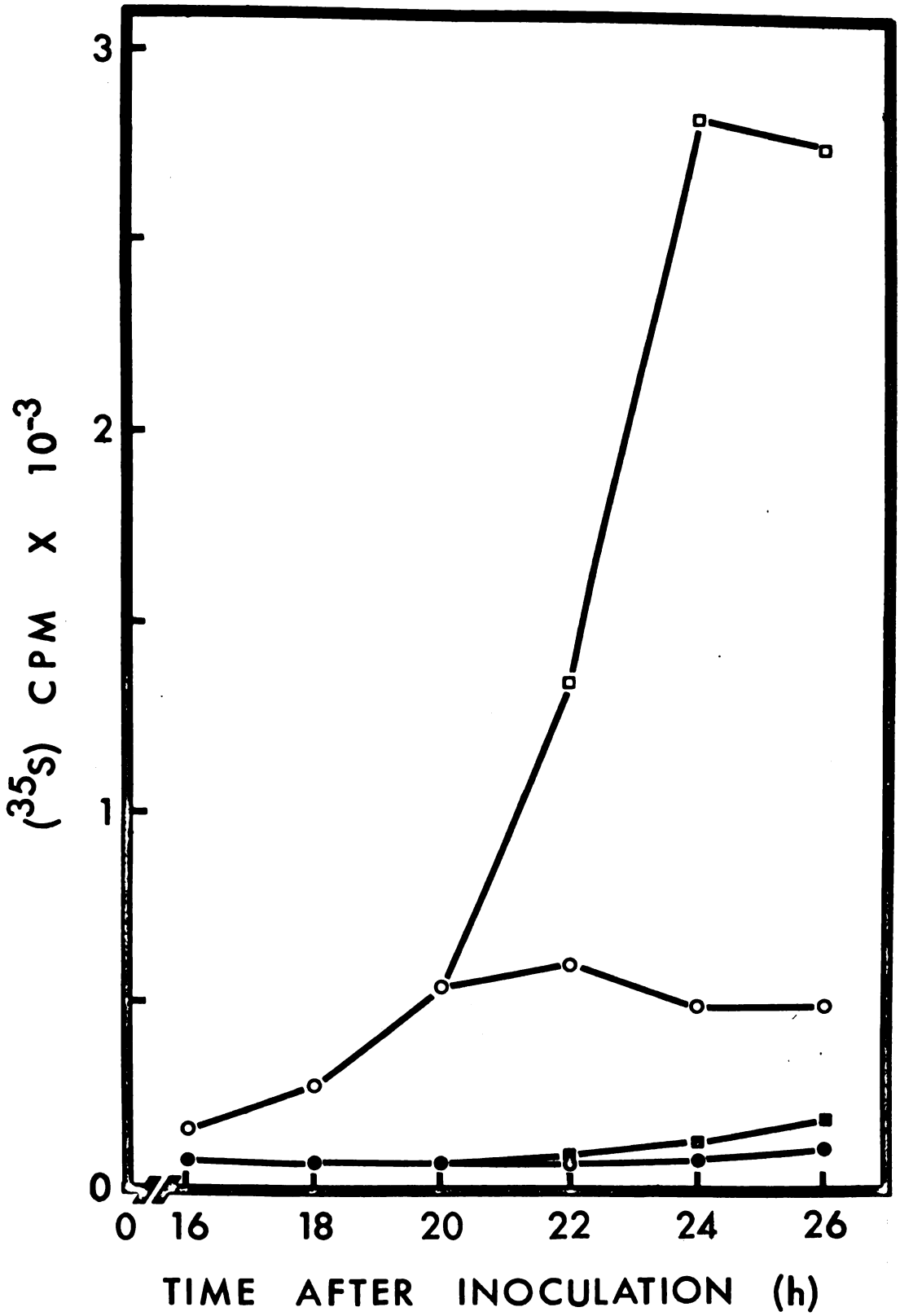


Figure 4.

Figure 5.--Effect of light on the percentage of secondary hyphae (>10 u long) formed by *E. graminis* f. sp. *tritici* on Chancellor wheat 20 to 26 hours after inoculation.

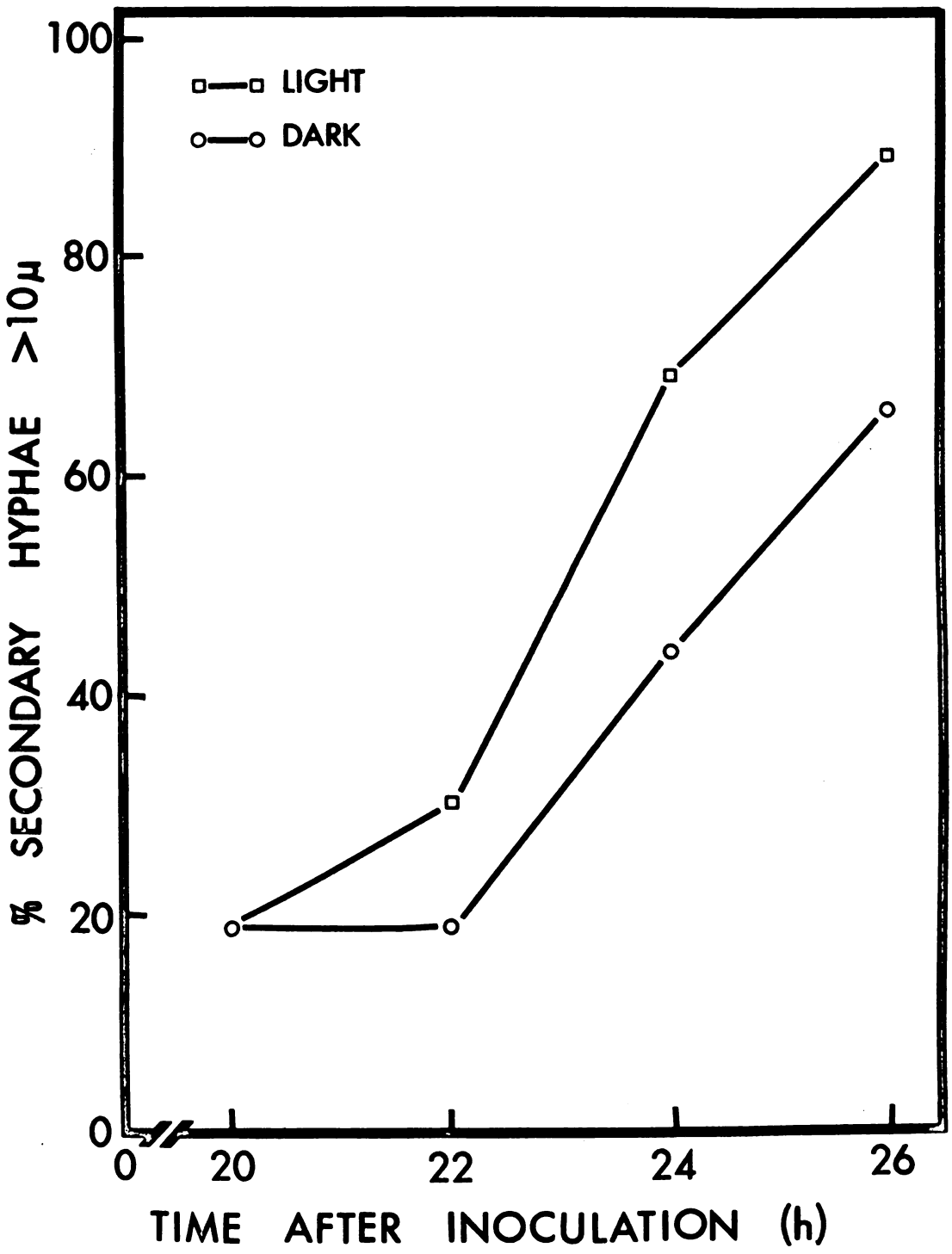

100
80
60
40
20
0
LIGHT
DARK
% SECONDARY HYPHAE >10μ
0
20
22
24
26
TIME AFTER INOCULATION (h)

Figure 5.

Figure 6.--Rates of ^{35}S transfer from five near-isogenic wheat lines to Erysiphe graminis f. sp. tritici.

(A) CPM/10,000 spores.

(B) CPM/10,000 spores/25,000 CPM in the epidermal strips of the host.

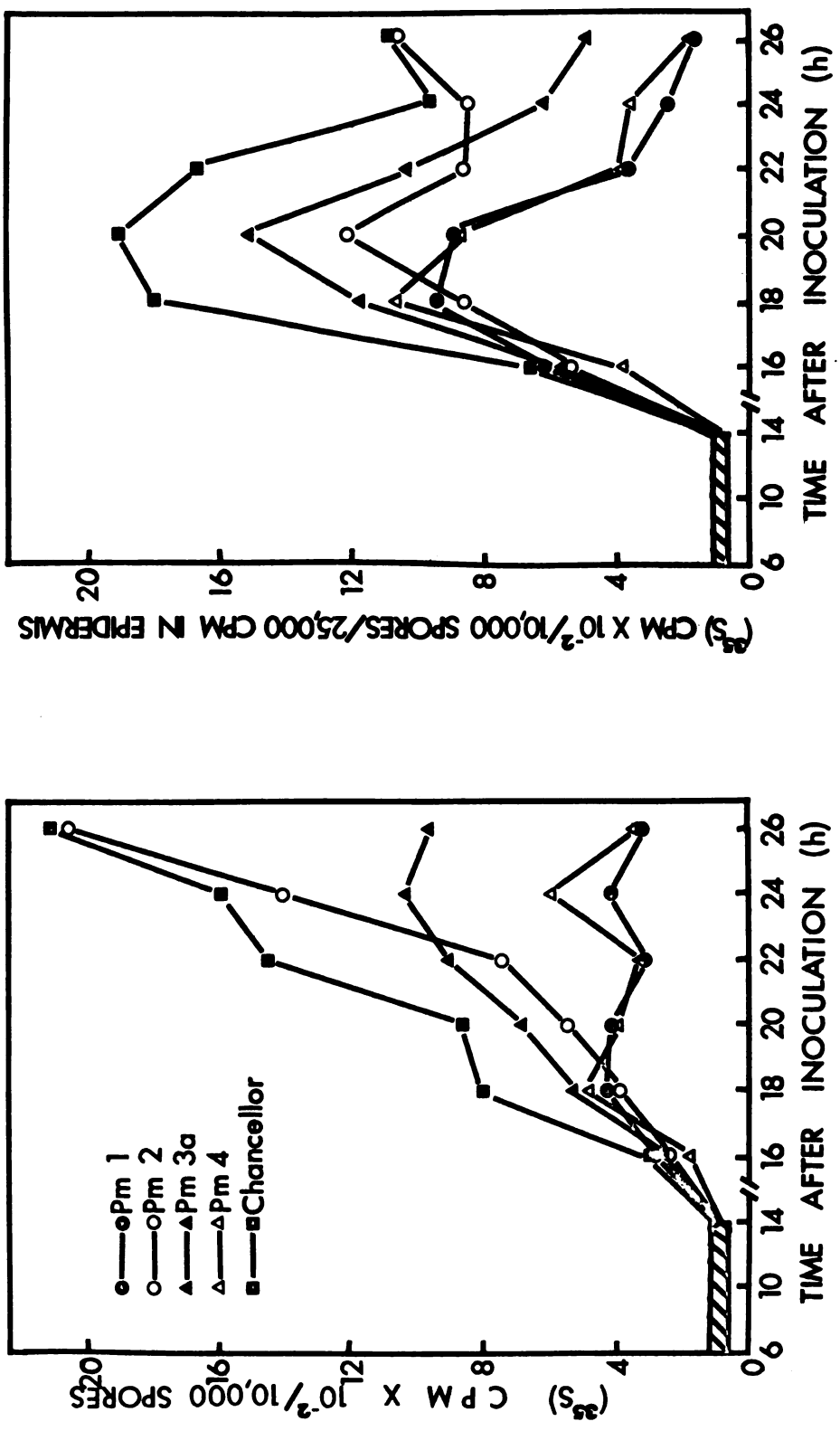

(35S) CPM X 10-2/10,000 SPORES/25,000 CPM IN EPIDERMIS
TIME AFTER INOCULATION (h)
(35S) CPM X 10-2/10,000 SPORES
Pm 1
Pm 2
Pm 3a
Pm 4
Chancellor
TIME AFTER INOCULATION (h)

Figure 6.

and the incompatible genotype P2/Pm2 which does not affect primary infection, continue to increase with time after inoculation.

The same transfer data were plotted as radioactivity per spore considering the amount of label in the epidermal strip (Figure 6B). Genotype differences are still evident but the shapes of the curves are changed. Transfer rates for all genotypes are maximum by 18 to 20 hours after inoculation. The rates of transfer appear to decrease or to level off after 20 hours with the P2/Pm2 and Px/pmx genotypes. Transfer of ^{35}S definitely decreases after 20 hours with the genotypes P1/Pm1, P3a/Pm3a, and P4/Pm4.

Discussion

The effect of several different incompatible parasite/host genotypes on rates of ^{35}S from the host to the parasite were described in a previous report (80). There were also attempts to correlate transfer rates to the morophological stage of parasite development with compatible genotypes. Work described here verifies the differences and correlations observed earlier. In addition, we have shown that environmental conditions, which are varied to obtain high efficiency of infection and synchronized development of the parasite, will greatly affect the amount of ^{35}S taken up by the plant. The amount of label available in the host will affect the observed rate of transfer to the parasite. Light appears to be a major factor in uptake by the plant, and in transfer from plant to fungus.

The amount of ^{35}S collected in the intact leaf and, to a greater extent, in the epidermis is much higher in light than in darkness (Figure 3). This is presumed to be the result of greater transpiration rates by plants in the light. The amount of residual solution left in vials showed that plants labelled in the light had a greater loss than those kept in the dark (unpublished data). Apparently, there is no selective absorption because the amount of ^{35}S remaining in vials was equal on a volume basis for plants that had taken up ^{35}S in the light or in the dark. Approximately 20 percent of the label taken up by the plant under light conditions is in the epidermal strips, whereas only ca 10 percent is found in the epidermal strips of plants labelled in the dark. The light, therefore, affects the amount of ^{35}S taken up by the plant and also the amount translocated to the epidermal strips.

Low rates of transfer from plant to fungus occurred in the dark at 20 to 26 hours after inoculation (Figure 4). However, the fungus did not develop secondary hyphae as rapidly in darkness as in light during this period (Figure 5). Slower morphological development may have accounted for part of the lower transfer activity. However, failure of transfer activity to increase at 24 and 26 hours with increasing percentages of elongating secondary hyphae in the dark lead the authors to believe the absence of light to be the major reason for failure to get increased transfer rates.

Data on the effect of genotypes on transfer from host to fungus confirm earlier results (80), but are believed to be more precise because the data are calculated on a per spore basis (Figure 6A). The two parasite/host genotypes that do not affect primary infection, i.e., P2/Pm2, Px/pmx, give rates of transfer that continue to rise 26 hours after inoculation. The three parasite/host genotypes that do affect primary infection, i.e., P1/Pm1, P3a/Pm3a, and P4/Pm4, give rates of transfer that level off at various hours after inoculation.

The rates of transfer from host to parasite appear quite different when the results are plotted as radioactivity per spore per unit radioactivity in the epidermal strips. At 18 to 20 hours after inoculation the rates of transfer appeared to be low by radioactivity per spore (Figure 6A) but adjustment for activity in epidermal strips shows that this is the time of most rapid transfer in primary infection.

The data (Figure 6B) indicate that the most rapid rates of transfer occur earlier than was previously thought (59, 80). At 18 to 20 hours after inoculation, the period of the most rapid rate of transfer, the haustorial bodies are forming appendages and secondary hyphal growth is initiated (80). It would be interesting to know whether or not the rates increase again after secondary penetration, haustorial formation, and further fungal growth. This question is difficult to answer because we have no

method of maintaining synchrony of fungal development during secondary infection. We are attempting to develop such methods.

These data support previous conclusions that transfer will vary with the morphological stage of fungal development, and with genotypes of both host and fungus (59, 80). Also, it is evident that the rate of transfer is affected by the amount of radioactive label in the host epidermis. This amount will vary with the environmental conditions during primary infection.

Summary

Light is necessary at specific times during primary infection for synchronous development of Erysiphe graminis f. sp. tritici on wheat. The effects of the environmental conditions on the uptake and translocation to the epidermis of ^{35}S and subsequent transfer to the parasite in the inoculated host were examined. Inoculated wheat plants were cut and fed ^{35}S for various five hour periods beginning one to 21 hours after inoculation. Radioactivity was determined in 1-cm leaf sections, epidermal strips, and the fungus on the leaf surface. Leaf sections and epidermal strips incubated in light yielded 20 and 4.5×10^4 CPM, respectively, which is more than twice the CPM obtained for plants incubated in darkness. Radioactivity in the fungus on the leaf surface ranged from background to 2100 CPM. The rates of ^{35}S transfer from host to parasite were affected by the amount

of tracer absorbed by the plant, the stage of development of the parasite during primary infection, and by parasite/host genes for compatibility. There were differences in rates of ^{35}S transfer between four different parasite/host genotypes for incompatibility (P1/Pm1, P2/Pm2, P3a/Pm3a, and P4/Pm4) after considering the amount of label in the host epidermis. Rates of ^{35}S transfer from host to parasite during primary infection are greater at 18 to 20 hours after inoculation than at later times. Haustoria are not fully developed at these times.

CHAPTER III

UPTAKE OF RADIOACTIVE ISOTOPES BY WHEAT LEAVES, THE DISTRIBUTION AND FORM OF RADIOACTIVITY IN WHEAT LEAVES, AND TRANSFER OF RADIOACTIVITY FROM HOST TO PARASITE

Previous research has raised questions about the relationship between morphological development of the parasite and the kinetics of ^{35}S and ^{32}P transfer from host to parasite. Of particular interest has been to determine (1) whether or not there is an interaction between host and parasite prior to penetration of the host cell by the parasite, (2) the chemical form of the radioactive label in the host and the form in which label is transferred from host to parasite, and (3) whether transfer is an active or a passive process.

Effect of Inoculation on ^{35}S Uptake in the Leaf and Translocation to the Epidermis

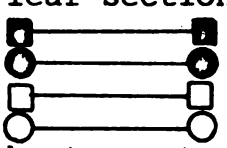
Plants of the five near-isogenic host lines (pmx, Pm1, Pm2, Pm3a, and Pm4) were uniformly inoculated with conidia of culture MS-1 (genotype P1P2P3aP4). The uptake and translocation of ^{35}S into the epidermis of the leaf, and into the remainder of the leaf, was determined during five hour labeling periods at various times after inoculation. The ectoparasitic portion of the fungus was removed in parlodion

from inoculated plants prior to removal of epidermal strips from the leaves.

The ^{35}S activity in the leaf sections and in the epidermal strips of noninoculated and inoculated wheat, genotype P1/Pm1, is presented in Figure 7A.

There was less ^{35}S in the inoculated than noninoculated leaves with genotype P1/Pm1 except for the 26 hour observation (Figure 7A). The epidermal strips of inoculated leaves had less ^{35}S than epidermal strips of noninoculated leaves at all time periods tested. The same general trend of less ^{35}S activity in the leaves and epidermal strips of inoculated than in noninoculated plants was observed with each of the other parasite/host genotypes (P2/Pm2, P3a/Pm3a, P4/Pm4 and Px/pmx) (Figure 7 B-E). No differences between parasite/host genotypes were detected in the level of ^{35}S activity in the epidermal strips of inoculated or noninoculated leaves. The data in Figure 8 are the averages of data shown in Figure 7 A-E. Except for the 26 hour observation, inoculated leaf tissue and epidermal strips had less ^{35}S activity than noninoculated leaf tissue and epidermal strips, respectively.

The relative amount of $^{35}SO_4^{2-}$ removed from solution by inoculated and noninoculated leaves from 16 to 26 hours after inoculation was determined by measuring the decrease in the height of $^{35}SO_4^{2-}$ solution in the vials from the beginning to the end of the labeling period. At most times

Figure 7.--Uptake of ^{35}S and translocation to the epidermis of noninoculated wheat leaves and wheat leaves inoculated 6 to 26 hours previously with E. graminis f. sp. tritici. ^{35}S activity in four leaf sections (each 1 cm long) of:

noninoculated leaves,
inoculated leaves,
noninoculated epidermal strips, and
inoculated epidermal strips. Parasite/host genotypes were: (A) P1/Pm1; (B) P2/Pm2; (C) P3a/Pm3a; (D) P4/Pm4; and (E) Px/pmx. Each point is the average of two replications.

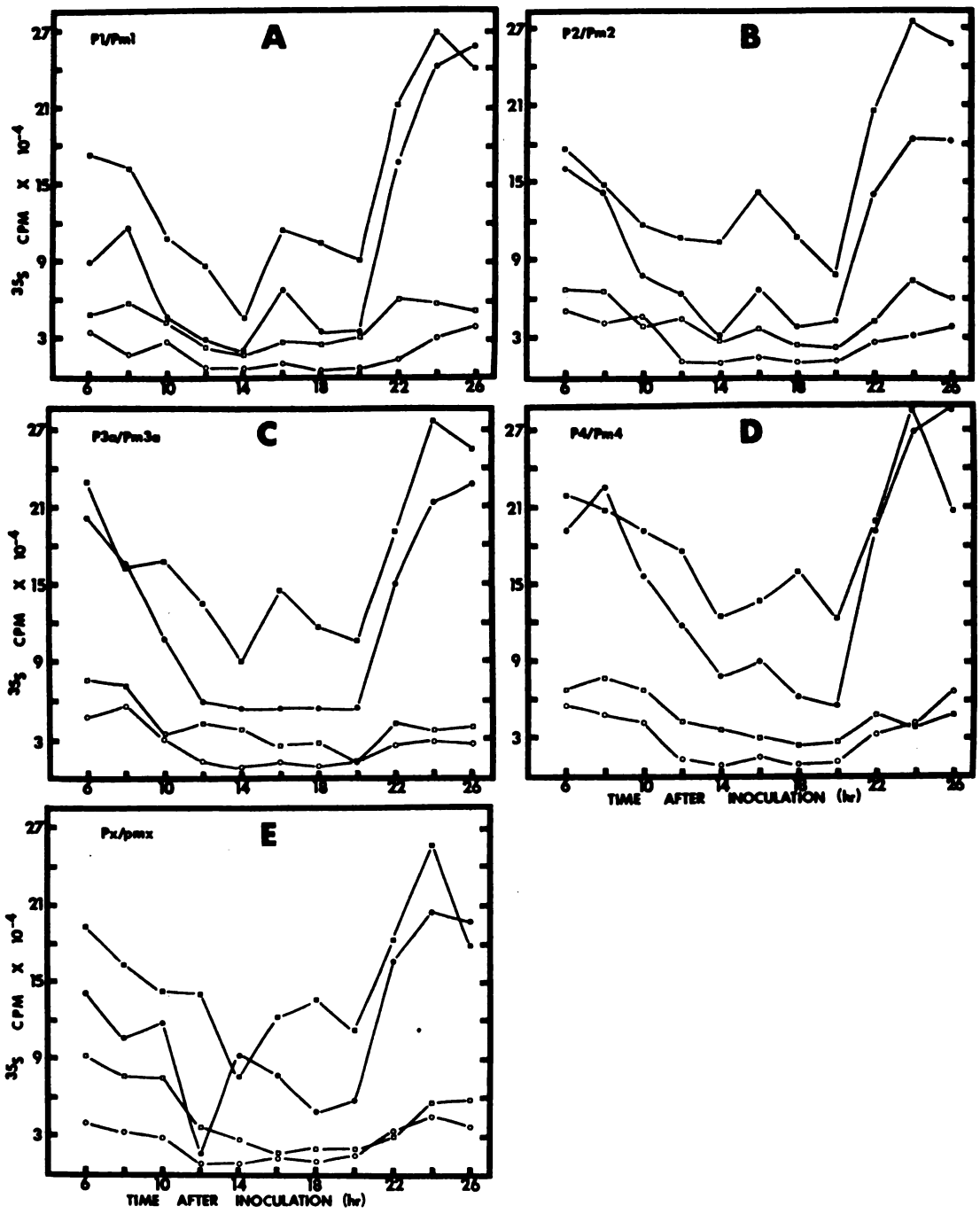

A
P1/Pm1
B
P2/Pm2
C
P3a/Pm3a
D
P4/Pm4
E
Px/pmx
35S CPM X 10-4
TIME AFTER INOCULATION (hr)

Figure 7.

Figure 8.--Uptake of ^{35}S and translocation to the epidermis in cut noninoculated wheat seedlings and wheat seedlings inoculated 6 to 26 hours previously with *E. graminis* f. sp. *tritici*. See Figure 7. The data are from averaging data in Gigure 7 A-E.

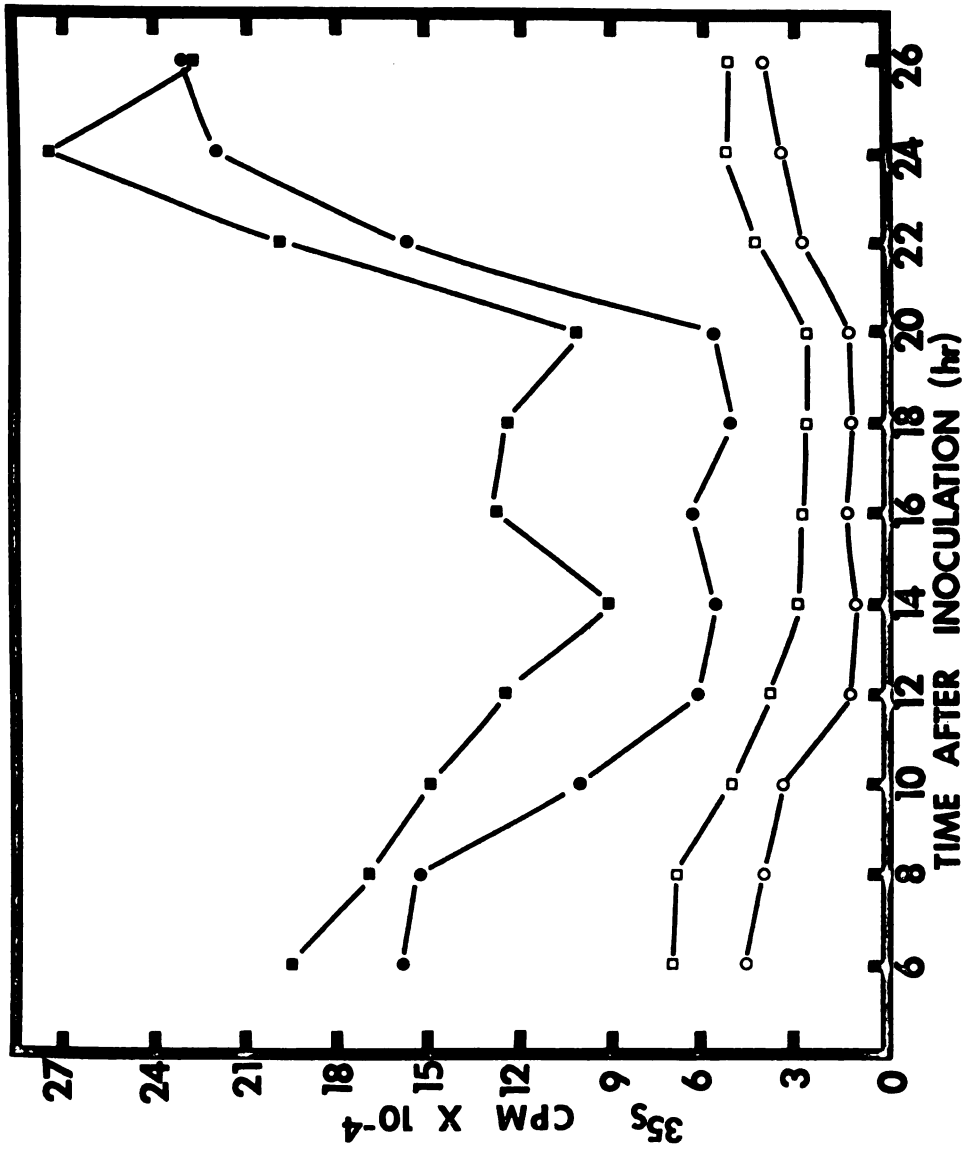

35S CPM X 10-4
0
3
6
9
12
15
18
21
24
27
6
8
10
12
14
16
18
20
22
24
26
TIME AFTER INOCULATION (hr)

Figure 8.

after inoculation the noninoculated leaves absorb more $^{35}SO_4^{2-}$ solution than did inoculated leaves (Table 2). There was a high positive correlation ($r = 0.88$) between loss of $^{35}SO_4^{2-}$ in the ambient solution in the vials and the radioactivity translocated to leaves (Figure 9).

TABLE 2.--Removal of $^{35}SO_4^{2-}$ solution from vials by inoculated and noninoculated seedlings at various times after inoculation.

Time after Inoculation (HR)	Loss of $^{35}SO_4^{2-}$=Solution (mm)	
	Inoculated	Noninoculated
16	5.88[a]	6.71
18	4.98	5.46
20	5.15	4.94
22	6.55	7.13
24	8.91	8.42
26	8.15	7.02

[a]Values are averages for four seedlings per replication, 26 replications.

The cut seedlings took up much more $^{35}SO_4^{2-}$ solution in the light than in the dark (Figure 10). Greater uptake of $^{35}SO_4^{2-}$ solutions by plants in the light correlated well with increased ^{35}S activity found in such plants.

The effect of inoculation density (2,500 vs 7,500 spores/cm length of leaf on four leaves) on ^{35}S radioactivity in inoculated wheat leaves is shown in Table 3. Leaves

Figure 9.--The relationship between ^{35}S activity in wheat leaves and the loss of $^{35}SO_4^{2-}$ solution from vials.

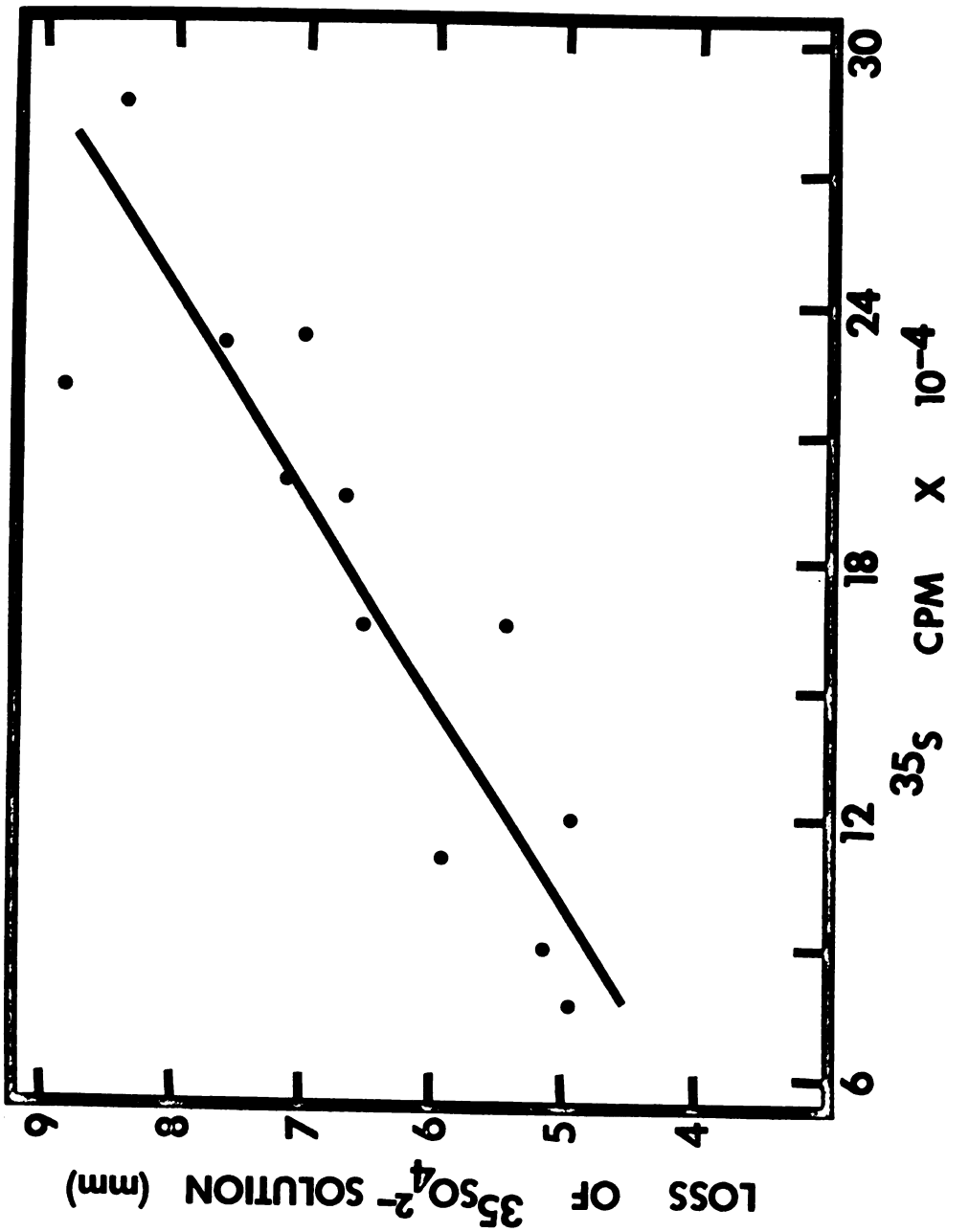


Figure 9.

Figure 10.--The amount of $^{35}SO_4^{2-}$ solution (mm) taken up by four inoculated wheat seedlings from vials (0.5 mm diameter) during the dark period (16, 18, 20 hours) and during the light period (24, 26 hours).

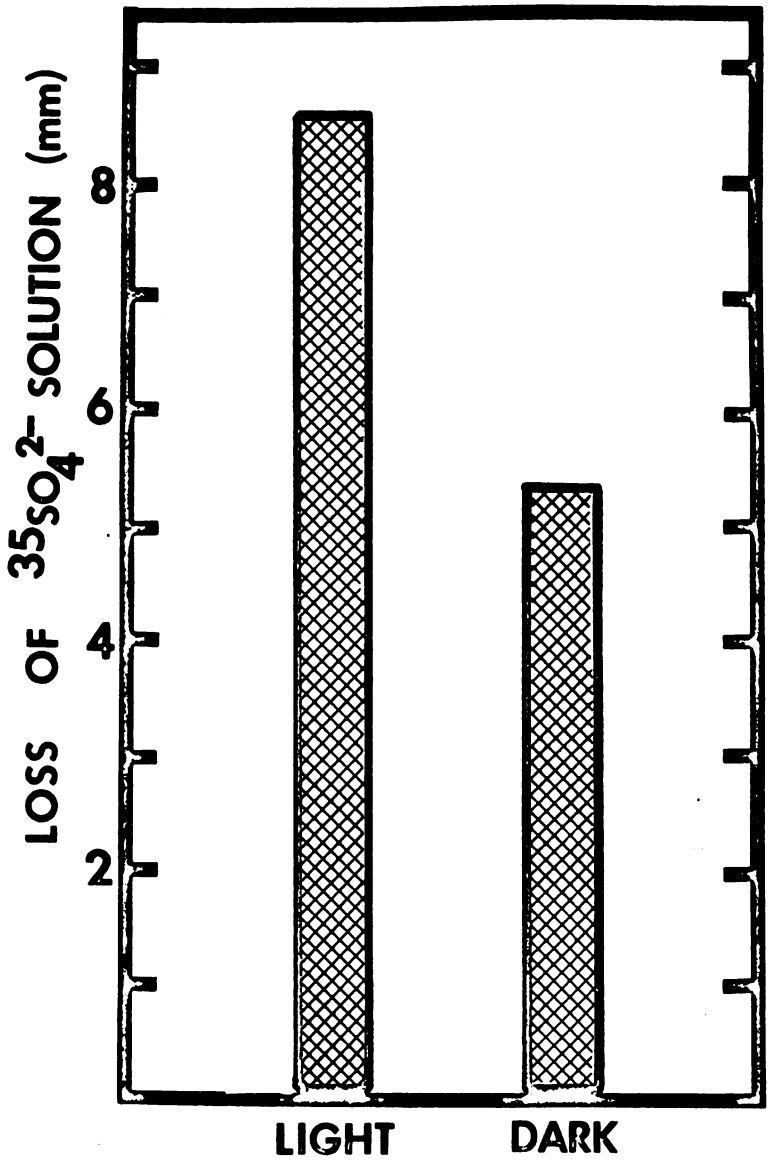


Figure 10.

inoculated with spores at both inoculation densities had lower ^{35}S activity than did the control in the epidermal strips at 6, 18 and 26 hours after inoculation. Furthermore, leaves inoculated with 2,500 spores had as great a reduction of ^{35}S activity in the epidermis as did leaves with 7,500 spores. Total activity in leaf tissue in most cases appeared also to be reduced equally well by both inoculation densities.

TABLE 3.--Effect of inoculation density of *E. graminis tritici* on ^{35}S radioactivity in inoculated wheat leaves.

No. of Spores on Four Leaves	Rep I		Rep II	
	6 hr[a]	18 hr.	6 hr.	26 hr.
1. 0				
a. epidermis	83.0[b]	34.2		67.6
b. leaf	138.1	103.4	373.7 325.7	320.3 267.4
2. 2,500				
a. epidermis	54.9	8.2		55.2
b. leaf	158.0	64.0	307.5 248.5	239.8 287.7
3. 7,500				
a. epidermis	63.1	7.4		51.3
b. leaf	116.0	30.6	340.0 238.7	184.5 286.2

[a]hour after inoculation when 5 hour uptake time was discontinued.

[b]radioactivity in CPM x 10^{-3}.

Wheat leaves were inoculated with E. graminis f. sp. tritici or with nonpathogenic E. graminis f. sp. poae, and were c ompared for the effects on uptake and translocation of ^{35}S to the epidermis (Table 4). There was no evident difference between the fungi in their effects on ^{35}S uptake and translocation to the epidermis.

TABLE 4.--Comparison of uptake and translocation of ^{35}S radioactivity by wheat leaves inoculated with E. graminis tritici and E. graminis poae.

Fungus	Tissue	Rep. I			Rep. II	
		6 hr[a]	11 hr	26 hr	6 hr	26 hr
E. graminis poae	epidermis	23.4[b]	8.9	36.5	--	54.2
	leaf	79.3	37.2	240.1	271.4 359.3	211.8 284.8
E. graminis tritici	epidermis	25.6	10.3	32.6	--	53.3
	leaf	108.7	67.7	97.8	324.2 243.6	212.2 286.9

[a]hr after inoculation at which 5 hr uptake time was discontinued

[b]radioactivity in CPM x 10^{-3}

Uptake of ^{3}H-methionine by Wheat Leaves and Transfer to E. Graminis f. sp. Tritici

The form in which ^{35}S is present in leaves and parasite, and the form in which ^{35}S is transfered to the parasite, is not known. The possibility that the kinetics of uptake, distribution, and transfer of a radioactive label given to the plant in an amino acid might be different was examined.

Inoculated pmx plants were allowed to take up ^{3}H-methionine (in 0.1M phosphate buffer, pH 6.9, specific activity 1.66 C/mM). Four activity levels (50, 100, 200 and 1000 uc/ml) were used for a five hour labeling period of 20 to 25 hours after inoculation. Inoculated wheat seedlings were also allowed to take up ^{3}H-methionine (100 uc/ml) for three, five or seven hour periods corresponding to 22 to 25, 20 to 25, and 20 to 27 hours after inoculation, respectively.

Increasing concentrations of ^{3}H-methionine given to inoculated wheat leaves did not give a corresponding increase in radioactive transfer to the fungus (Table 5). Greatest rates of transfer were achieved using 1000 uc/ml ^{3}H-methionine. With the high specific activity and because of the expense of ^{3}H-methionine, 100 uc/ml was selected to be used for other experiments.

The number of hours wheat leaves were allowed to take up ^{3}H-methionine did not greatly affect the amount of ^{3}H transferred to the parasite (Table 6). However, the amount of ^{3}H in the epidermal strips appears to increase with

TABLE 5.--Effect of different concentrations of ^{3}H-methionine given to inoculated pmx wheat leaves on the amount of transfer to the fungus at 25 hours after inoculation.

Specific Activity (uc/ml)	Radioactivity (CPM)[a]	No. Reps
0	70[b]	5
50	80	7
100	179	29
200	150	5
1000	294	3

[a]CPM in the ectoparasitic portion of the fungus on 1 cm length of leaf on four leaves.

[b]CPM background.

TABLE 6.--Effect of Different Labeling Times of ^{3}H-methionine (100 uc/ml) Given to Inoculated pmx Wheat Leaves on the Amount of Radioactivity (CPM) in the Epidermis and in the Fungus.

Labeling Period		Radioactivity (CPM)			
Hr. after Inoculation	No. of Hours	Transfer[a]	Reps	Epidermis[b]	Reps
25-50	0	70[c]	2	70	2
22-25	3	202	2	714	2
20-25	5	212	6	1049	5
20-27	7	228	2	1648	1

[a]CPM in the ectoparasitic portion of the fungus on 1 cm length of leaf on four leaves.

[b]CPM in the epidermis of 1 cm length of leaf on four leaves.

[c]CPM background.

increasing incubation times. Five hour uptake periods were selected for use in later experiments.

The distribution of radioactivity between the parasite and the host when ^{3}H-methionine (100 uc/ml) was given to inoculated plants from 20 to 25 hours after inoculation is shown in Table 7. Although the CPM of plants labeled with ^{3}H-methionine were much lower than when plants were labeled with $^{35}SO_4^{2-}$, the distribution of radioactivity between fungus, host epidermal strips, and host leaf tissues was very similar. The radioactivity on a concentration per volume basis was much less in the remaining solution after plants were labeled with ^{3}H-methionine. Apparently, selective absorption of ^{3}H-methionine occurred as contrasted to non-selective absorption when plants were labeled with $^{35}SO_4^{2-}$.

TABLE 7.--Comparison of radioactivity (CPM) in the parasite, epidermis and leaf tissue of inoculated pmx wheat seedlings given ^{3}H-methionine (100 uc/ml) at 25 hours after inoculation.

Source	Radioactivity (CPM)	Reps
Background	70	8
Fungus[a]	185	35
Epidermis[b]	1,260	16
Leaf[c]	6,580	6

[a]CPM in the ectoparasitic portion of the fungus on 1 cm length of leaf on four leaves.

[b]CPM in the epidermal strips of 1 cm length of leaf on four leaves.

[c]CPM in the leaf tissue of 1 cm length of leaf on four leaves.

When plants were given 50 uc/ml each of $^{35}SO_4^{2-}$ and ^{3}H-methionine, both ^{35}S and ^{3}H radioactivity were transferred to the parasite. The actual number of ^{35}S and ^{3}H counts in the parasite was computed (Table 8). All of the 285 CPM above background (305-20) in the ^{14}C channel was ^{35}S and this was 75.8 percent of the total ^{35}S counts in the parasite. In the ^{3}H channel, 91 (24.2 percent of ^{35}S activity) of the 180 CPM above background were ^{35}S radioactivity. The remaining 89 CPM above background represent the ^{3}H counts of radioactivity in the parasite. There were a total of 376 CPM above background of ^{35}S and 89 CPM above background of ^{3}H transferred to the parasite (Table 8).

TABLE 8.--Transfer of ^{35}S and ^{3}H grom inoculated pmx wheat leaves to E. graminis tritici at 25 hours after inoculation.

Labeled Source Specific Activity (100 uc/ml)	Reps	Radioactivity (CPM) ^{3}H Channel	Radioactivity (CPM) ^{14}C Channel	% of CPM in ^{14}C Channel
background		20	20	
$^{35}SO_4^{2-}$	4	411	1248	75.8% ± 2.1
^{3}H-methionine	3	180	21	
$^{35}SO_4^{2-}$ + ^{3}H-methionine (50 uc/ml each)	9	200	305	60.1
		corrected for background		
$^{35}SO_4^{2-}$		91	285	75.8
^{3}H-methionine		89	0	

Solubility in TCA of Radioactivity in Leaves, Epidermis and Parasite

Efforts to show differences in proteins synthesized in inoculated and noninoculated plants during the first 30 hours after inoculation have given variable results. Therefore, after determining the distribution of ^{35}S and ^{3}H (given to the plant as $^{35}SO_4^{2-}$ and ^{3}H-methionine) in leaf parts, the proportion of the radioactive label that was TCA-insoluble was examined. The proportion of the radioactivity that is TCA-insoluble might give some basis for evaluating the effect of inoculation, environmental conditions, age of host leaves, etc., on the metabolism of the host and parasite, and possibly the form of the radioactivity available for transfer from host to parasite.

Conidia of E. graminis f. sp. tritici which were germinated on two percent water agar in the presence of $^{35}SO_4^{2-}$, and ^{35}S or ^{3}H labeled leaf sections, epidermal strips, and ectoparasite units on the leaf surface were assayed for percentage of label in the TCA-insoluble fraction. A flow diagram of the extraction procedures is given in Figure 11 (4). The percent of TCA-insoluble radioactivity in samples of two leaf sections (1-cm length), 2.0 epidermal equivalents (see chapter 2) or 12 parlodion strips containing the ectoparasitic portion of the fungus was determined. These materials were each ground with a small amount of acid-washed sea sand in 0.8 ml Tris HCl buffer, pH 7.5, containing 0.1M Tris HCl, 0.25 M sucrose, 10^{-4}M sodium sulfate and 0.01M

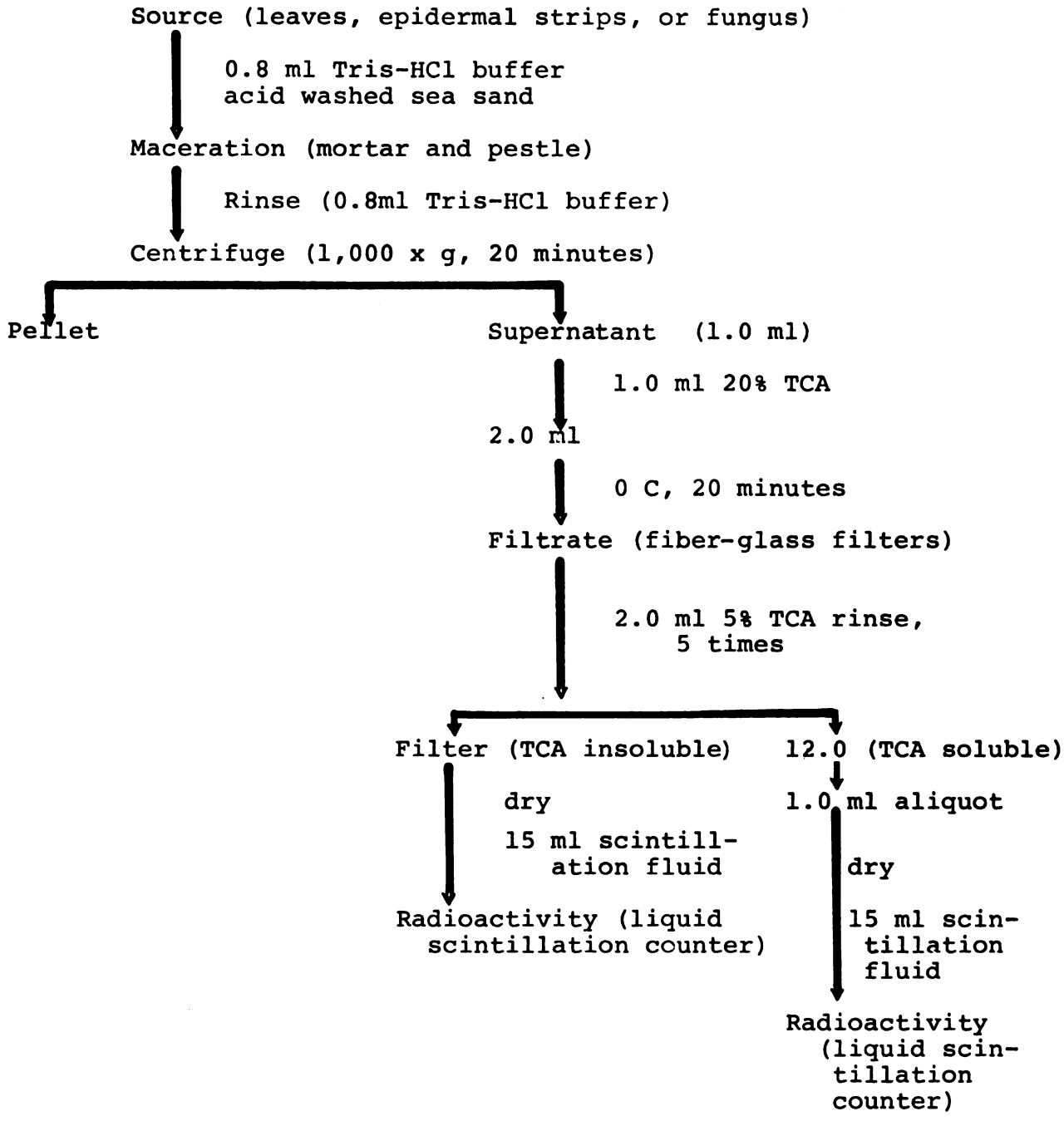


Figure 11--Flow diagram of procedures used to determine the percent of the radioactivity that was TCA-insoluble.

ascorbic acid, with a cold mortar and pestle. After maceration of tissues, the mortar was rinsed with 0.8 ml Tris buffer, both 0.8 ml aliquots combined and centrifuged at 1,000 x g for 20 minutes in a Sorvall SS-1 centrifuge. One ml of the supernatant was collected and an equal volume of 20 percent TCA added to make a 10 percent TCA solution. The 2.0 ml solution was allowed to stand for 20 minutes at 0 C, then filtered with Millipore fiber-glass filters, and washed five times with two ml of 5 percent TCA. The filter was removed, dried, and put in a scintillation vial (TCA-insoluble fraction). A 1.0 ml aliquot was taken from the filtrate, placed on a filter paper in a scintillation vial, and allowed to dry completely. Fifteen ml of scintillation fluid (see chapter 2) was then added to each vial and radioactivity determined in a Beckman LS-133 scintillation spectrometer.

In a separate experiment the distribution of ^{35}S radioactivity of leaf tissue among the pellet, and TCA-soluble and TCA-insoluble supernatant fractions was examined (Table 9). Subsequent to maceration and rinsing of the leaf tissue with a total of 1.6 ml Tris buffer, one 0.8 ml aliquot was assayed directly for radioactivity. The other aliquot was fractionated (Figure 11) and the pellet and TCA-soluble and TCA-insoluble fractions assayed for radioactivity. The amount of radioactivity associated with each fraction (Figure 11) is shown in Table 9. The total radioactivity

in the centrifuged sample closely approximates that of the noncentrifuged sample. Since a sizeable portion of the radioactivity was associated with the pellet, the pellet was repeatedly washed with 5 percent TCA. Thorough rinsing of the pellet with 5 percent TCA washed 90 percent of the activity out of the pellet. Therefore, approximately the same percentage of radioactivity of the pellet was TCA-insoluble as in the supernatant after centrifigation. In subsequent experiments, only the supernatant was examined.

TABLE 9.--Distribution of ^{35}S by TCA Fractionation of Inoculated pmx Wheat Leaves 25 Hours after Inoculation.

Distribution[a]	Radioactivity (CPM)	
	Rep I	Rep II
Noncentrifuged Sample	246,420	175,836
Centrifuged sample		
Pellet	29,925	36,613
Supernatant		
TCA-insoluble	14,330	14,745
TCA-soluble	196,042	116,285
Total	240,297	167,643

[a]See flow diagram Figure 11.

The ability of the parasite to incorporate ^{35}S into TCA-insoluble material was examined. If the parasite cannot utilize $^{35}SO_4^{2-}$, then transfer from host to parasite must be

other than $^{35}SO_4^{2-}$ alone. To test the ability of the parasite to convert $^{35}SO_4^{2-}$ into a TCA-insoluble form, conidia were dusted onto two percent water agar plates and kept under environmental conditions for parasite development (see chapter 1). $^{35}SO_4^{2-}$ was placed onto the agar surface at eight hours after dusting, and the plates were placed in the dark for two hours followed by two hours in the light. Spores were then scraped with a razor blade into cold mortars and extracted as described above. Germinated spores bathed in 0.4 ml 100 uc/ml $^{35}SO_4^{2-}$ for four hours had approximately one percent of the radioactivity in the TCA-insoluble fraction (Table 10). The control, which consisted of germinated spores flooded with 0.4 ml $^{35}SO_4^{2-}$ (100 uc/ml) and harvested immediately, had 0.16 percent in the TCA-insoluble fraction. Spores on the host surface from 20 to 25 hours after inoculation had 1.55 percent radioactivity in the TCA-insoluble fraction. Radioactivity in the epidermal strips and leaf tissue was 0.64 and 6.7 percent, respectively, in the TCA-insoluble fraction.

The percentages of label in the TCA-insoluble fraction in leaf tissue of inoculated and noninoculated leaves labeled with $^{35}SO_4^{2-}$ and ^{3}H-methionine are shown in Table 11. Considerable variability was observed in leaves labeled from one to six hours after inoculation. There does not appear to be a difference between inoculated and noninoculated leaves labeled with either ^{3}H-methionine and $^{35}SO_4^{2-}$. Leaves labeled with ^{3}H-methionine from 21 to 26 hours after

TABLE 10.-- The percent of radioactivity in the TCA-insoluble fraction in conidia germinated on agar in the presence of $^{35}SO_4^{2-}$ and in pmx wheat leaves and in the fungus at 25 hours after inoculation.

	TCA-insoluble (%)	Reps
Germinated Spores 0 hr	0.16	2
Germinated Spores 4 hr	0.98	5
Spores on Wheat 5 hr	1.55	6
Epidermal Strips	0.64	10
Leaf	6.7	13

TABLE 11.--The percent of radioactivity in the TCA-insoluble fraction in inoculated and noninoculated pmx wheat leaves labeled with $^{35}SO_4^{2-}$ or ^{3}H-methionine at various times after inoculation.

Hr. after Inoculation	TCA-insoluble Fraction (%)[a]			
	Inoc. leaves		Noninoc. leaves	
	$^{35}SO_4^{2-}$	^{3}H-Met	$^{35}SO_4^{2-}$	^{3}H-Met
1-6	23.2± 10.8	27.1± 6.3	18.4± 10.5	26.3± 10.3
21-26	5.5± 1.8	10.8± 3.0	5.0± 1.6	16.7± 5.7

[a]All values are the average of 8 reps. The standard deviations are indicated.

inoculation had a greater percentage of the label as TCA-insoluble than did leaves labeled with $^{35}SO_4^{2-}$ for the same time period. Table 11 shows that the percentage of label in the TCA-insoluble fraction was less when plants were labeled from 21 to 26 hours rather than one to six hours after

inoculation. Both labeling periods, one to six and 21 to 26, are times when plants are in the light (lights are used at those times for synchronous parasite development). Perhaps either the age of the plant or the difference in light and dark periods prior to the labeling period could account for less incorporation of radioactivity into the TCA-insoluble fraction in the leaf tissue at 21 to 26 hours after inoculation.

The effect of host age on the percentage of radioactivity in the TCA-insoluble fraction is presented in Table 12. The percentage of label in the TCA-insoluble fraction is less in six to seven day old plants than five to six day old plants at both one to six and 21 to 26 hours after inoculation. The age of the leaf definitely influences the percentage of label in the TCA-insoluble fraction in the leaf. However, five to six day old plants at 21 to 26 hours after inoculation are nearly the same age as six to seven day old plants at one to six hours after inoculation. Therefore, the values 6.8 and 12.5 (Table 12) should be nearly equal if plant age is the only determining factor in the percent of the radioactivity that was in the TCA-insoluble fraction.

Prior to inoculation, all wheat leaves have at least an eight hour light period. Since lower activity in the TCA-insoluble fraction also occurs in noninoculated leaves (Table 11) noninoculated leaves were used to determine the

TABLE 12.--The effect of host age on the percent of radioactivity in the TCA-insoluble fraction in inoculated pmx wheat seedlings labeled with $^{35}SO_4^{2-}$.

Hr after Inoculation	TCA-insoluble Fraction (%)[a]	
	5 - 6 Day Plants	6 - 7 Day Plants
1 - 6	29.8 ± 14.2	12.5 ± 2.5
21 - 26	6.8 ± 0.4	2.6 ± 0.2

[a]Each value is the average of 2 reps. The standard deviations are indicated.

effect of the lights between hours six to 20. Except for the lights, standard environmental conditions were used. (Inoculated leaves would have introduced another variable, namely, effect of synchronous vs nonsynchronous parasite development.) Plants labeled from 21 to 26 hours with $^{35}SO_4^{2-}$ that were kept in the light prior to labeling had a higher percentage of label in the TCA-Insoluble fraction (10.4%) than those plants kept in the dark (2.5%) preceding labeling (Table 13). Therefore, both the increased age of the leaf and darkness prior to the 21 to 26 hour labeling period contribute to decreased percentages of label in the TCA-insoluble fraction in leaves labeled at 21 to 26 hours after inoculation. The data from Tables 11, 12, and 13 are summarized in Figure 12.

TABLE 13.--The effect of light and dark preceeding labeling of noninoculated wheat seedlings labeled with $^{35}SO_4^{2-}$ on the percent of radioactivity in the TCA-insoluble fraction.

Hr after Inoculation	TCA-insoluble Fraction (%)[a]	
1 - 6	17.0 ± 5.7	
	Plants in dark 6 - 20 hr	Plants in light 6 - 20 hr
21 - 26	2.5 ± 0.4	10.4 ± 2.5

[a]Each value is the average of 2 reps. The standard deviations are indicated.

Figure 12.--The percent of radioactivity in the TCA-insoluble fraction in inoculated (□) and noninoculated (■) pmx wheat leaves given label from 1 to 6 or 21 to 26 hours after inoculation:

(A) leaves given ^{3}H-methionine,

(B) leaves given $^{35}SO_4^{2-}$,

(C) comparison between 5 to 6 day old plants vs 6 to 7 day old plants given $^{35}SO_4^{2-}$ and

(D) comparison between plants given $^{35}SO_4^{2-}$ kept in light from 6 to 20 hours after inoculation and plants kept in darkness from 6 to 20 hours after inoculation.

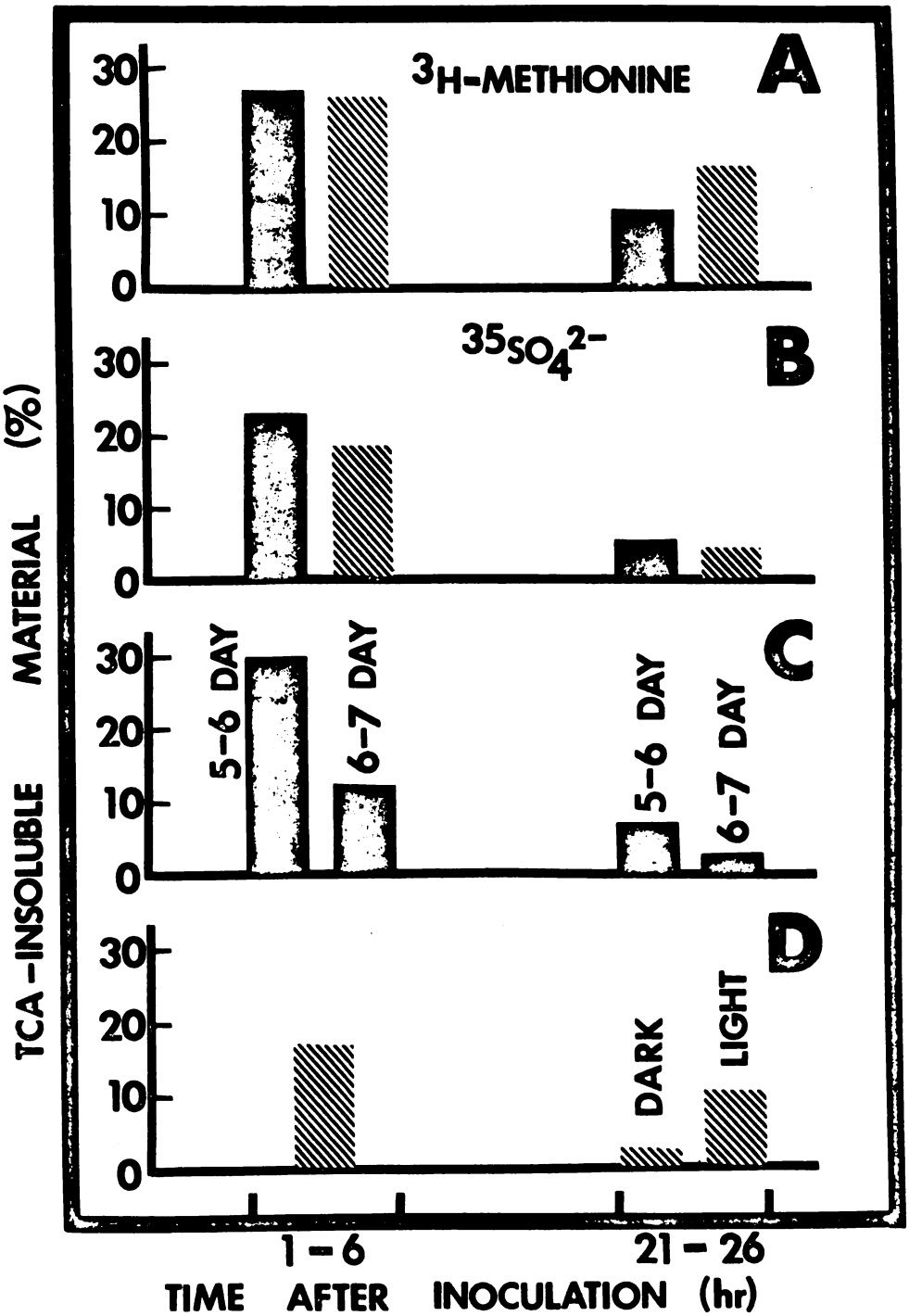

TCA-INSOLUBLE MATERIAL (%)
30
20
10
0
^{3}H-METHIONINE
A
$^{35}SO_4^{2-}$
B
5-6 DAY
6-7 DAY
5-6 DAY
6-7 DAY
C
DARK
LIGHT
D
1-6
21-26
TIME AFTER INOCULATION (hr)

Figure 12.

Thin Layer Chromatography of Radioactivity in Leaves, Epidermis and Parasite

Radioactivity is transferred from host to parasite but the form in which transfer occurs is not known. An attempt was made to determine the form in which the ^{3}H and ^{35}S was present in the host and parasite. If a plant is allowed to take up $^{35}SO_4^{2-}$, what proportion of the radioactivity is in methionine in the plant and parasite? If a plant is allowed to take up ^{3}H-methionine what proportion is still in methionine in host and parasite? Answers to these questions should help to determine the form in which the label is available to be transferred from host to parasite and the extent of turnover of the radioactivity in host and parasite.

Thin layer chromatography (TLC) was used to separate methionine from SO_4^{2-} and other components. Prior to spotting of materials, the thin layer plate was air dried and the glass chamber saturated with the solvent components for at least 30 minutes. Moistened filter papers lined the glass chamber to insure saturation. Leaf sections of $^{35}SO_4^{2-}$ or ^{3}H-methionine-labeled plants, epidermal strips, and dissolved parlodion strips containing the parasite were ground in water:propanol (90:10 v/v) and spotted with 10 ul pipettes two cm from the base of the plate, and dried with a fan. The plate was then inserted into the glass moisture chamber at a slight angle, the lid greased tightly with vacuum grease and the plate allowed to develop. The solvent front moved

ten to twelve cm in a developing time of 3.5 hours. The plate was then removed from the chamber, allowed to dry, sprayed with ninhydrin reagent, and heated to expose amino acid bands. The developed areas (1 cm x 10 cm) of the radioactive TLC plates were divided in ten 1 cm sections. Ten sections were obtained from each sample chromatographed on TLC plates. Each section was scraped into a liquid scintillation counting vial, and 15 ml of scintillation fluid was added. Each vial was counted for at least ten minutes in a Beckman liquid scintillation counter, model LS-133, equipped with an external standard to determine counting efficiency.

Silica gel GL plates and a solvent of ethanol-2-propanol - 1N HCl (2:2:1 v/v/v) were found to be the best thin layer system to separate $^{35}SO_4^{2-}$ and sulfur-containing amino acids (Figure 13A, B). All of the $^{35}SO_4^{2-}$ remained near the origin while methionine had an R_F of about .58, cystine and its residues had R_F's of .46, .35, and .31, and cysteine had an R_F of .33. Inoculated wheat leaves fed a 0.1M phosphate buffer from 20 to 25 hours after inoculation were ground in 0.1 ml water:propanol (90:10) and 0.1 ml 100 uc/ml $^{35}SO_4^{2-}$. The radioactivity on the thin layer plate was very similar to $^{35}SO_4^{2-}$ spotted in that no activity was determined above a R_F of .30 (Figure 13C). Figure 13 C clearly shows that $^{35}SO_4^{2-}$ is not carried along with other compounds to a higher R_F and that any activity found above R_F .30 is not $^{35}SO_4^{2-}$.

Figure 13.--Thin layer chromatography of $^{35}SO_4^{2-}$ and amino acids using a silica gel GF plate[4] and an ethanol-2-propanol-IN HCl (2:2:1 v/v/v) solvent system. Movement of:

(A) sulfur containing amino acids, identified by ninhydrin reagent;

(B) $^{35}SO_4^{2-}$;

(C) inoculated pmx wheat leaves given 0.1M phosphate buffer from 20 to 25 hours after inoculation and ground in $^{35}SO_4^{2-}$.

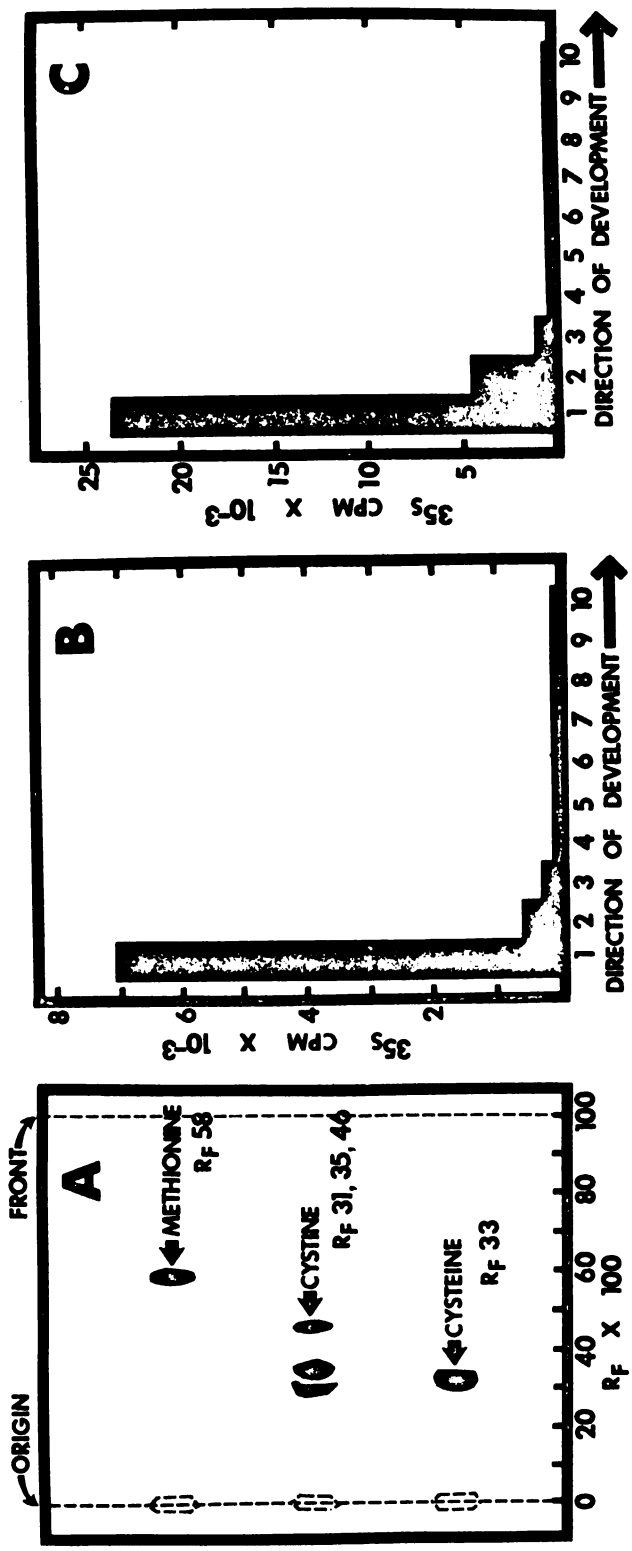

A
FRONT
ORIGIN
METHIONINE
R_F 58
CYSTINE
R_F 31, 35, 44
CYSTEINE
R_F 33
R_F X 100
0 20 40 60 80 100
B
^{35}S CPM X 10^{-3}
2 4 6 8
1 2 3 4 5 6 7 8 9 10
DIRECTION OF DEVELOPMENT
C
^{35}S CPM X 10^{-3}
5 10 15 20 25
1 2 3 4 5 6 7 8 9 10
DIRECTION OF DEVELOPMENT

Figure 13.

Nearly four percent of the radioactivity of inoculated wheat labeled with $^{35}SO_4^{2-}$ was found in an area corresponding to the R_F of methionine (Figure 14A, Section 6). Greater radioactivity occurred regularly at an R_F of .70 to .80 when the experiment was repeated. That spot was not identified. The epidermis of inoculated leaves labeled with $^{35}SO_4^{2-}$ had a slightly higher percentage (4.5 percent) of its activity at an R_F corresponding to methionine (Figure 14 B). The radioactivity from leaf tissues with an R_F from .70 to .80 was not found in the epidermis (Figure 14 B). The fungus on the leaf surface of plants given $^{35}SO_4^{2-}$ from 20 to 25 hours after inoculation had as much as 25 percent of its activity at an R_F corresponding to methionine (Figure 14 C).

A much lower total activity was observed on the chromatogram with inoculated wheat leaves labeled with 100 uc/ml ^{3}H-methionine from 20 to 25 hours after inoculation. The percentage corresponding to methionine, however, was much greater than with $^{35}SO_4^{2-}$ labeled leaves (Figure 15 A). Up to 25 percent of the activity could be found in the area corresponding to methionine. The epidermis of leaves labeled with ^{3}H-methionine contained up to 38 percent of the total activity in the approximate area corresponding to the R_F of methionine (Figure 15 B). The results for methionine in the epidermis were somewhat variable from day to day.

Inoculated wheat leaves labeled with either ^{3}H-methionine or $^{35}SO_4^{2-}$ showed similar amino acid development with the use of ninhydrin spray reagent. Four purple (two heavy) bands developed with R_F ranging from .43 to .64.

Figure 14.--Thin layer chromatography from inoculated pmx wheat leaves labeled with $^{35}SO_4^{2-}$ from 20 to 25 hours after inoculation.

(A) leaf tissue,

(B) epidermal strips,

(C) ectoparasitic portion of E. graminis f. sp. tritici.

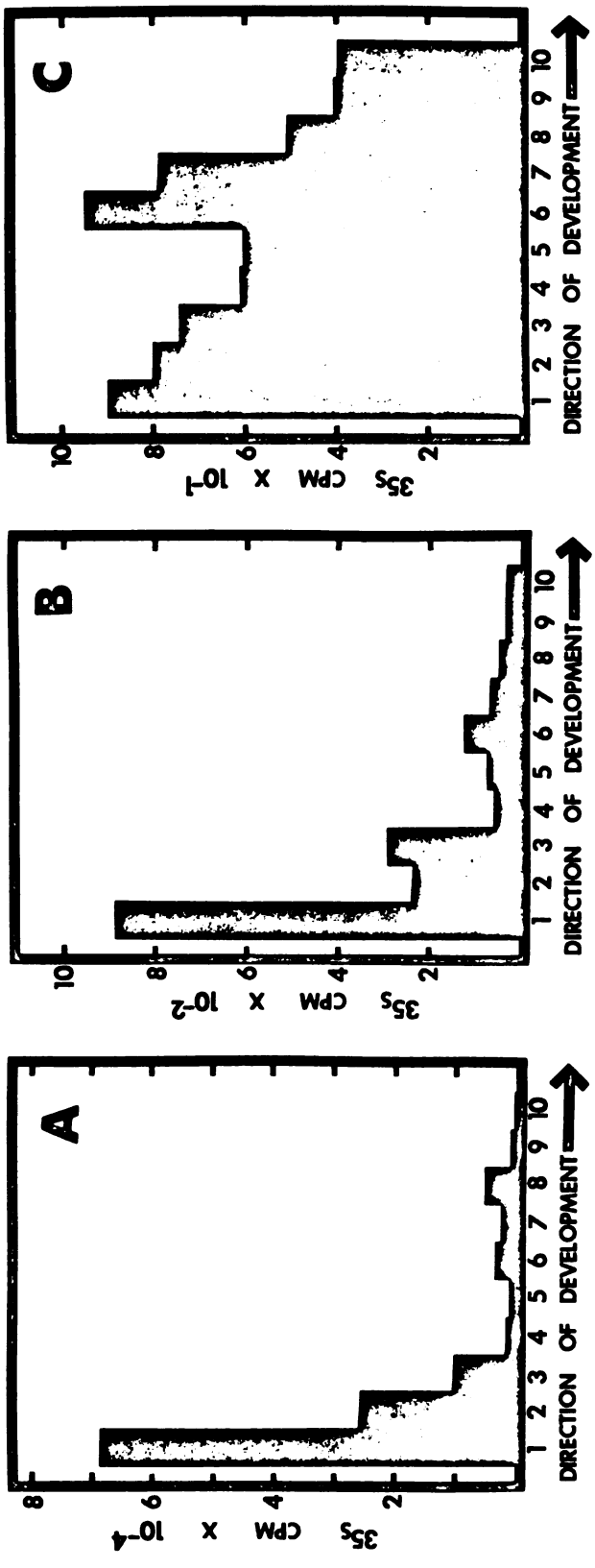

A
B
C
DIRECTION OF DEVELOPMENT
1 2 3 4 5 6 7 8 9 10
35S CPM X 10-4
35S CPM X 10-2
35S CPM X 10-1
2 4 6 8 10

Figure 14.

Figure 15.--Thin layer chromatography of inoculated pmx wheat leaves labeled with H-methionine from 20 to 25 hours after inoculation.

(A) leaf tissue,

(B) epidermal strips.

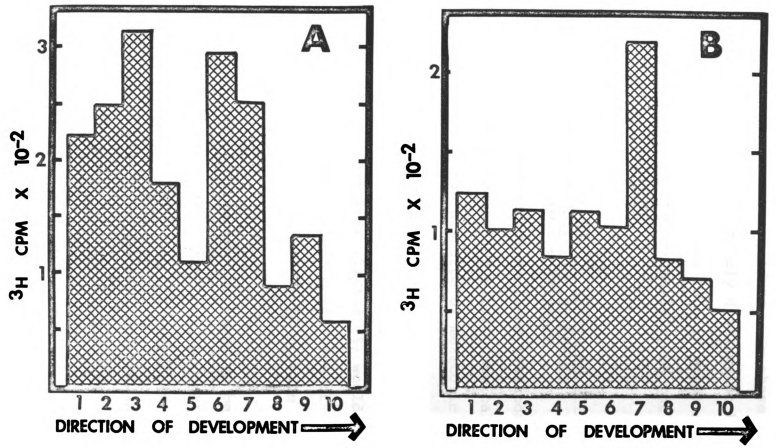


Figure 15.

A yellow spot also developed to a R_F of .33 corresponding to cysteine or one of the cystine residues. In these experiments it was impossible to accurately distinguish between inoculated and noninoculated leaves in both amino acid content and the distributions of radioactivity on the thin layer plates.

Effect of Temperature on ^{35}S Uptake in the Leaf, Translocation to the Epidermis and Transfer to the Parasite

Three criteria for active mediated transport are: (1) transport against a chemical or electrochemical gradient, (2) transport dependent upon a source of metabolic energy, and (3) transport that is unidirectional or vectorial in its normal energy requiring function (50). In the powdery mildew disease complex, criteria (1) and (3) would be difficult to establish under normal conditions of primary infection. Criterion (2), however, could provide suggestive evidence for either active or passive transfer.

Plant and fungal metabolism is known to be extremely low at four C. A clearer idea of the role of active transport should be evident by comparing the amount of ^{35}S in the epidermis of labeled plants incubated at four C and 22 C and in the fungus at both four C and 22 C. Rates of ^{35}S transfer from host to parasite have been shown to be dependent upon the stage of morphological development of the parasite. During the five hour host labeling period the fungus develops considerably. Comparing rates of transfer when inoculated

plants were given $^{35}SO_4^{2-}$ at four C and at 22 C presented a variable in fungal growth since growth of E. graminis is very limited at four C. To allow for this variable, inoculated pmx wheat leaves were given 100 uc/ml $^{35}SO_4^{2-}$ either in a growth chamber in the dark at 22 C from 15 to 20 hours after inoculation or in a well ventilated regrigerator in the dark, four C, from 18 to 23 hours after inoculation. The average stage of development of the parasite should be about equivalent for these two periods. Determination of ^{35}S activity in leaf sections, epidermal strips and in the parasite was as reported in materials and methods, chapter 2.

Less radioactivity was found in inoculated pmx leaves labeled at four C, as compared to the radioactivity in plants labeled at 22 C (Table 14). Inoculated leaves labeled at four C had 12,000 fewer ^{35}S CPM than those leaves labeled at 22 C. Radioactivity in epidermal strips of inoculated leaves labeled at four C had less than half the activity of those labeled at 22 C. ^{35}S activity in the parasite was reduced to 20 percent by labeling plants at four C compared to plants labeled at 22 C.

To determine if the parasite accumulates, retains or loses ^{35}S after the five hour labeling period of 19 to 24 hours, inoculated plants were transferred to vials with distilled water. Radioactivity in the leaf tissue, epidermal strips and in the parasite were determined at zero, two, four and nine hour intervals thereafter. The radioactivity of leaf tissue and epidermal strips remained the same when inoculated plants labeled with $^{35}SO_4^{2-}$ from 19 to 24 hours

TABLE 14.--The effect of temperature on ^{35}S activity in pmx inoculated leaves and transfer to E. graminis tritici.

Source	Reps	^{35}S Radioactivity (CPM)	
		Dark, 4 C	Dark, 22 C
Leaf tissue	7	34,000	46,000
Epidermis	2	5,000	10,500
Transfer to Fungus	4	90	435

at 22 C were transferred to vials containing distilled water for an additional zero, two, four, or nine hours (Figure]6). However, the ^{35}S activity in the parasite more than doubled with an additional nine hour association with the host. The radioactivity in the parasite as a result of prolonged feeding on the labeled host failed to approach the activity in the epidermis.

Parlodion strips taken from inoculated wheat seedlings labeled with $^{35}SO_4^{2-}$, and which contained the ectoparasitic portion of the fungus, were placed in distilled water or a 5.0 mM $CaSO_4$ solution to determine how much ^{35}S radioactivity could be washed out or exchanged. The parlodion strips were placed in the exchange solutions for 30 minutes and rinsed twice. The ^{35}S that does enter the parasite appears to be bound and not readily free for exchange. Solutions of 5.0 mM $CaSO_4$ and distilled water were able to leach out 6.3 and three percent of the fungal radioactivity, respectively.

Figure 16.--Accumulation of ^{35}S in E. graminis tritici at various times after removing wheat seedlings from radioactive solution. Inoculated plants were labeled from 19 to 24 hours after inoculation, then removed from radioactive solution (time 0), and placed in distilled H_2O for two, four, or nine hours.

(A) radioactivity in fungus (10 reps),

(B) radioactivity in epidermis (5 reps), and

(C) radioactivity in leaf tissue (2 reps).

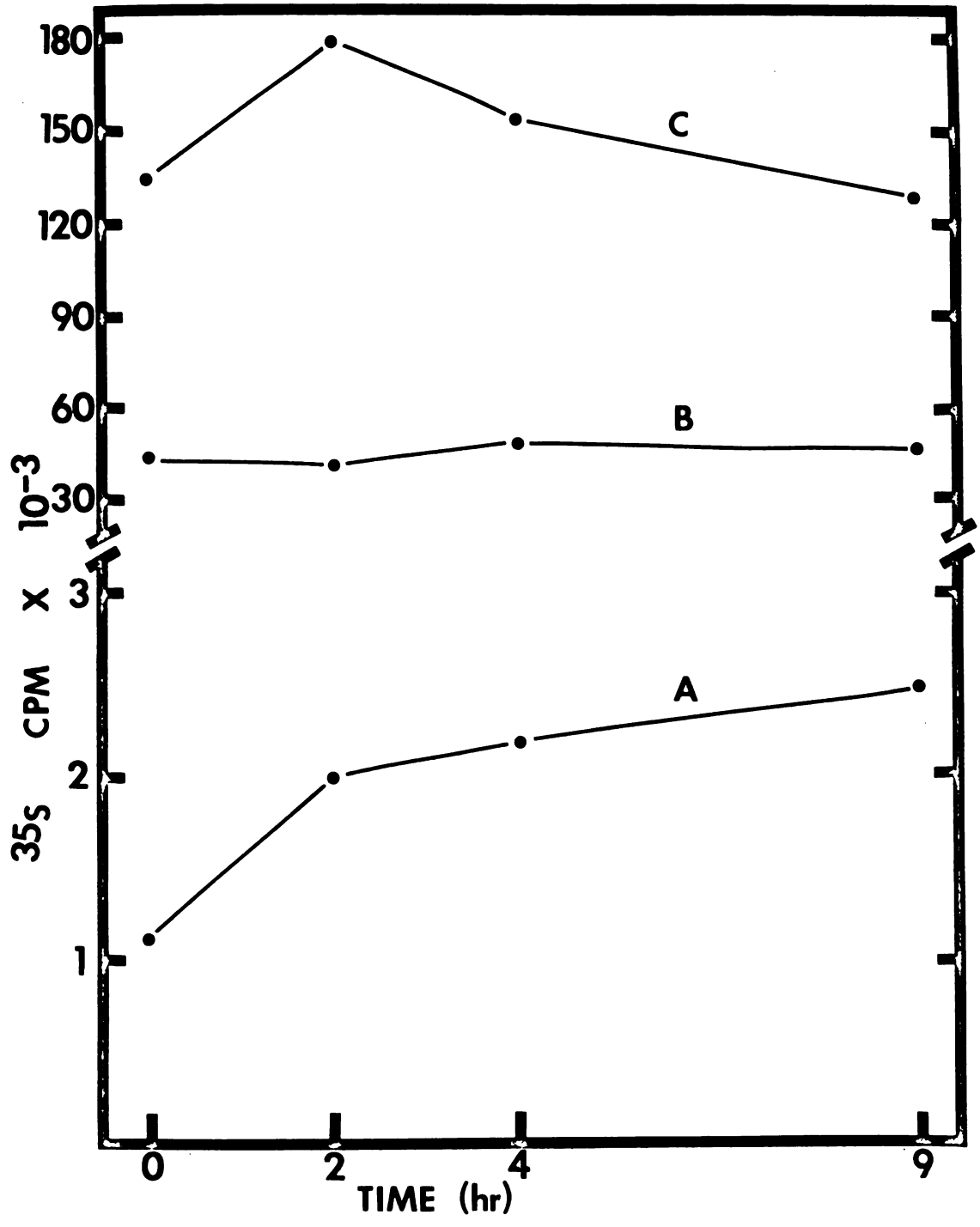


Figure 16.

DISCUSSION

The environmental conditions necessary for high infection efficiency and synchronous parasite development in the different stages of primary infection of wheat and barley by E. graminis have been studied (55, 58, 62). This defined system has permitted studies of the initial host-parasite interactions during primary infection and could be used in determining the effects of environment or other factors upon the establishment of a successful infection (78). The production of elongating hyphae >10 u by the parasite population was used as a criterion of a successful infection.

Initial studies were concerned with the kinetics of secondary hyphal elongation with time with compatible and incompatible parasite/host genotypes. Discussion of the results of this study are presented in chapter 1.

Another criterion for measuring successful infection has utilized rates of ^{35}S transfer from host to parasite (44, 58, 80, 87). Rates of ^{35}S transfer from host to parasite were shown to be reduced to varying degrees when comparing incompatible with compatible parasite/host genotypes (80, 87). More quantitative data on rates of transfer were desired. Rates of ^{35}S transfer were placed on a per spore basis, and a per unit of label in the epidermis which was considered

available to the parasite. To achieve this perspective of the data, spores inoculated onto the leaf were counted and results of uptake and translocation of ^{35}S in the host with the environmental conditions used for synchronous parasite development were examined. Rates of ^{35}S transfer were then correlated with the morphological stage of development of the parasite. Discussion of the results of this study are presented in chapter 2.

The effect of inoculation on host metabolism has received considerable attention. Unfortunately many of these studies have been directed at late stages of disease development, long after compatibility or incompatibility have been expressed. Little has been known about the effect of inoculation on the host before or at the time incompatibility is determined. Would inoculation on the host affect ^{35}S uptake by the host, and if so, how would this effect be expressed and would there be differences in ^{35}S uptake between compatible and incompatible parasite/host genotypes?

The data presented in this thesis show that one effect of inoculation of E. graminis f. sp. tritici on wheat is to reduce uptake of ^{35}S by leaves during primary infection. The reduction in uptake of ^{35}S by inoculated leaves appears to be independent of the compatibility or incompatibility of the parasite/host genotype and is expressed as early as six hours after inoculation (Figure 7). Furthermore, this

effect appears to be nonspecific as spores of other form species of E. graminis elicit the same response (Table 4, personal communication with T. J. Martin). The reduction in the uptake of ^{35}S by inoculated leaves was not dependent upon spore density at two spore concentrations tested. The effect of inoculation to reduce ^{35}S uptake by the leaf is not considered to be a physical effect, i.e. plugging of stomates, since T. J. Martin (personal communication) found that viable Erysiphe spores were necessary for reduction of uptake. Spores killed by UV-irradiation or chalk dust did not cause reduced uptake by inoculated leaves. These results suggest the need for further investigations of host-parasite interactions prior to penetration. Clearly there is transfer of information from the parasite to the host prior to penetration of the host by the parasite.

Rates of uptake of ^{35}S by cut seedlings are primarily thought to be a function of host transpiration rates. Good correlations were observed between loss of $^{35}SO_4^{2-}$ solution in vials and radioactivity taken up by leaves. A difference in radioactivity in leaves due to treatments such as light, inoculation, etc., was accompanied by a correlation with the amount of $^{35}SO_4^{2-}$ solution taken up from the vial. Duniway and Durbin (18) found that transpiration rates and stomatal aperatures of primary bean leaves infected with rust were reduced four to six days after inoculation when compared with healthy primary bean leaves. Majerink (52) also reported that low water output with a high water deficiency

occurs in the whole plant during the pathogenesis of mildew. During fungal development, total and stomatal transpiration were lowered but cuticular transpiration increased with time in fungus-infected tissues (52). The results here are consistent with reports of Majerink (52) and Duniway and Durbin (18) but suggest these effects are evident before fungal penetration. It appears quite likely that spores inoculated onto the leaf may result in the partial closing of the stomates, perhaps as a result of production of some diffusate, thus reducing transpiration and uptake by leaves. The results presented herein imply that an effect of inoculation is to put stress on the host regardless of the compatibility or incompatibility of the parasite/host genotype.

The form in which labeled materials are transferred from wheat to E. graminis f. sp. tritici has been unknown. Experiments designed to answer this question were to: examine the kinetics of ^{3}H-methionine uptake by the host and ^{3}H transfer to the parasite, label the host simultaneously with $^{35}SO_4^{2-}$ and ^{3}H-methionine, and determine TCA solubility and separation by thin layer chromatography of labeled compounds in both the host and parasite. The data presented herein suggest that both sulfate and methionine may be transported across membranes.

Rates of transfer of ^{3}H, when given to the plants as ^{3}H-methionine, were lower than transfer of ^{35}S given to the plants as $^{35}SO_4^{2-}$. One reason for these results is that the

counting efficiency for ^{3}H is low and that the specific activity is lower for ^{3}H than ^{35}S(1.6 C/mM versus 43 C/mM). Computation of the actual number of molecules that are transferred shows at least five times more methionine than sulfate molecules being transferred assuming that all of the ^{3}H is transferred as methionine and all the ^{35}S is transferred as sulfate. The actual number of methionine molecules transferred may be less than the calculated number because the ^{3}H label was on the methyl group (which is easily removed). However, the data from thin layer chromatography suggest that a large percentage of the ^{3}H given to the plants as ^{3}H-methionine is still in methionine in the epidermis.

Fungal mycelia have been reported (3, 5) to have the ability to take up sulfate and methionine. Depression of sulfate uptake in the presence of methionine has been reported in both plants and fungi (5, 28, 39, 75). When plants were given solution containing both $^{35}SO_4^{2-}$ and ^{3}H-methionine, transfer of both ^{35}S and ^{3}H to the parasite was observed with a compatible parasite/host genotype (Table 8). With a compatible parasite/host genotype there is an apparent inhibition ($\simeq$ 60 percent) of ^{35}S transfer from wheat to the parasite in the presence of ^{3}H-methionine. Considering specific activity, nearly 20 methionine molecules were transferred for every sulfate molecule transferred, again assuming all the ^{3}H is in methionine and all the ^{35}S in sulfate.

Whether the inhibition of sulfate uptake by methionine is occurring at the plant cell membrane, fungal membrane, or both in the results presented herein is unknown.

The TCA-insoluble fraction is assumed to be largely protein, but the fraction was not characterized and therefore is referred to as label in the TCA-insoluble fraction. E. graminis f. sp. tritici can take up and incorporate $^{35}SO_4^{2-}$ into a TCA-insoluble form (Table 10). The difference in percentage of TCA-insoluble ^{35}S between the parasite on the surface of the host and the parasite on an agar surface may be due to the mode of incubation or developmental stage of the parasite. A slightly higher percentage (1.6 versus 0.7) of the total ^{35}S taken up was converted to a TCA-insoluble form in the parasite on the surface of the host than in the epidermis, respectively. The low percentage of ^{35}S that is TCA-insoluble suggests that very little of the ^{35}S is in protein in either the host epidermis or in the parasite on the surface of the host. The difference of 1.6 versus 0.7 suggests that there may be more protein synthesis in the parasite on the surface of the host than in the host epidermis. However, the haustorial bodies of the fungus in the inoculated epidermis of the host may account for some of the radioactivity in the TCA-insoluble fraction. The low percentage of ^{35}S in a TCA-insoluble form precludes any easy tests on whether any ^{35}S is transferred from host to parasite in the form of protein. It seems more likely that ^{35}S given

as $^{35}SO_4^{2-}$ is either transferred as $^{35}SO_4^{2-}$ or a sulfur-containing amino acid.

Since methionine is more readily incorporated into a TCA-insoluble form than is sulfate (90), a greater percentage of radioactivity in the TCA-insoluble fraction may be expected in plants given ^{3}H-methionine than in plants given $^{35}SO_4^{2-}$. Several interpretations of the results obtained are possible. At a time of presumably rapid protein synthesis (one to six hours after inoculation of plants six days old) little if any difference occurs in the percentage of label in the TCA-insoluble fraction in leaf tissue between plants given ^{3}H-methionine or $^{35}SO_4^{2-}$. One interpretation is that there are different pool sizes from which ^{3}H and ^{35}S may be drawn for protein synthesis. For example, rapid efflux of ^{3}H-methionine from a large methionine pool would show similar amounts of label in the TCA-insoluble fraction as would slow efflux of $^{35}SO_4^{2-}$ from a small sulfate pool.

Plants labeled with either ^{3}H-methionine or $^{35}SO_4^{2-}$ had less radioactivity incorporated into the TCA-insoluble fraction at 21 to 26 hours than at one to six hours after inoculation. However, inoculated plants given ^{3}H-methionine from 21 to 26 hours after inoculation have twice the percentage of radioactivity in the TCA-insoluble fraction as plants given $^{35}SO_4^{2-}$. This may suggest a change in relative methionine and sulfate pool sizes or that inoculation of plants given $^{35}SO_4^{2-}$ caused a greater decrease in protein synthesis in plants from 21 to 26 than from one to six hours after

inoculation than in inoculated plants given ^{3}H-methionine for the same time period. The pool sizes of methionine and sulfate in the host were not examined. Measurement of pool sizes may have given an explanation for these results. A lower rate of protein synthesis from 21 to 26 hours as compared to one to six hours after inoculation due to inoculation of plants given either ^{3}H-methionine or $^{35}SO_4^{2-}$ may also be a result of increased translocation of nutrients to the infection site. If this were true, then epidermal strips of inoculated leaves should possess greater activity than epidermal strips of noninoculated leaves. However, data of ^{35}S activity in epidermal strips of inoculated leaves given $^{35}SO_4^{2-}$ show less ^{35}S activity than epidermal strips of noninoculated leaves given $^{35}SO_4^{2-}$. This increase of translocation of nutrients to the infection site is not a satisfactory interpretation of these results.

Incompatibility of several parasite/host genotypes (P1/Pm1, P3a/Pm3a, P4/Pm4) is expressed prior to 26 hours after inoculation. While the reduction of ^{35}S uptake by inoculation does not appear to depend on the compatibility or incompatibility of the parasite/host genotype it would be interesting to know in further experiments whether incompatible genotypes given $^{35}SO_4^{2-}$ or ^{3}H-methionine would differ from compatible genotypes in percentage of label in the TCA-insoluble fraction at 21 to 26 hours after inoculation. Based on the data and interpretations

presented herein, one may expect a difference at 21 to 26 hours after inoculation.

Large differences in percentages of ^{35}S and ^{3}H-methionine in the TCA-insoluble fraction were shown to occur with time after inoculation (Figure 12, Table 11). These differences are attributed to both (1) the different age of leaves (Figure 12, Table 12), i.e. those plants labeled from 21 to 26 hours after inoculation have begun initiation of a second leaf and are nearly one day older than plants labeled from one to six hours after inoculation, and (2) to the environmental conditions preceding the incubation period (Figure 12, Table 13), i.e. plants labeled from one to six hours had received at least eight hours of light preceding label uptake while plants labeled from 21 to 26 hours received 14 hours (from 6 to 20 hours) darkness preceding label uptake. Patterson and Smillie (63) have reported that the total RNA was less in six-day-old than in five-day-old wheat seedlings. The amount of radioactive leucine incorporated into fraction I protein was less in seven-day-old than in six-day-old wheat seedlings. Rates of uptake by rice, carrot and potato tissues, and release of CO_2 in the light, were also affected by the prior conditions used during growth (day length and night temperature), by the age of the leaves, and by the developmental stages of the plants (86). Crafts (14) states that there was a preferential delivery of nutrients to young leaves. Older leaves were bypassed. The results presented herein are consistent with these reports that age of the

plants and the environmental conditions prior to labeling has a major effect on the results obtained. These variables should be considered in all future work.

About four percent of the ^{35}S extracted from wheat leaves, which had been given $^{35}SO_4^{2-}$, moved to a position on TLC plates where methionine was expected. Therefore, it appears that about four percent of the ^{35}S is in methionine. Similar results were obtained in extracts of epidermal strips. A slightly higher percentage of the ^{35}S was in methionine in the parasite on the host leaf.

About 25 percent of ^{3}H activity extracted from wheat leaves which had been given ^{3}H-methionine moved to a position on TLC plates expected for methionine. About 38 percent of the ^{3}H activity in the epidermal cell was probably methionine. Since the kinetics of transfer of ^{35}S and ^{3}H (given to the plant as $^{35}SO_4^{2-}$ and ^{3}H-methionine, respectively) are similar, the results are consistent with the hypothesis that both SO_4^{2-} and methionine are transferred from host to parasite.

The presence of radioactivity in both leaf sections and epidermal strips in plants given $^{35}SO_4^{2-}$ at four C suggest some diffusive flow from cut stems into leaf sections and epidermal strips. The data (Table 14) indicate that diffusive flow of ^{35}S is a much greater factor in leaf sections than in epidermal strips. Diffusion and uptake in the leaves are probably by xylem vessels. The fact that radioactivity in plants labeled at four C does occur in the epidermis but at a reduced rate suggests that translocation

of ^{35}S may occur both by cell free space and through the symplasm (15). However, Giaquinta and Geiger (36) have reported that inhibition of translocation in beans by cold temperature is a result of physical blockage of sieve plates (through displacement of cytoplasmic material lining the sieve tube wall) rather than from direct inhibition of a metabolic process. I can not distinguish between these two possible explanations of the results presented herein.

On 20 percent as much transfer of radioactivity to the parasite was observed from plants labeled at four C compared to plants labeled at 22 C. If uptake by the fungus were simply free diffusion, 50 percent of the activity would have been expected since epidermal strips of plants labeled at four C had ca. 50 percent of the radioactivity of epidermal strips of plants labeled at 22 C (Table 14). The fact that a small amount of radioactivity does occur in the fungus when plants are labeled at four C suggests either some passive transfer or that a limited amount of metabolism occurs at four C in E. graminis.

Gerson and Poole (35) propose that in certain instances anion absorption by plant cells may occur by transport on a single carrier accompanied by parallel free diffusion. The interpretation given based on the above data, literature, and the fact that very little ^{35}S was removed from parasites during exchange experiments is that transfer from wheat to E. graminis is largely by an active, energy requiring transport mechanism.

The results presented in this thesis suggest that inoculation of E. graminis tritici on wheat causes an alteration of host metabolism which is dependent upon many variables: age of leaf, time after inoculation, environmental conditions employed, and in some instances the elements assayed. Incompatible parasite/host genotypes have been shown to uniquely affect the growth of the fungus on the leaf surface and the rate at which ^{35}S is transferred from the host to the fungus. The expression of incompatible parasite/host genotypes on host and parasite metabolism awaits future investigation. The more variables that can be identified from procedures used in studying host-parasite relationships, the better our understanding and interpretation of the results obtained will be.

SUMMARY

The data on the kinetics of secondary hyphae elongation with time after inoculation are interpreted to give further evidence of separate distinct interactions, rather than alteration of single interaction rates, of the four different incompatible parasite/host genotypes tested. The four different incompatible parasite/host genotypes differ from each other in the kinetics of secondary hyphal elongation, time of host-parasite interaction, transfer of ^{35}S from host to parasite, host reaction and final infection type.

The environmental conditions used for synchronous parasite development of E. graminis f. sp. tritici on wheat greatly affect the amount of label taken up by the host, translocated to the epidermis, and transferred to the parasite. Greatest ^{35}S transfer activity is correlated with an earlier stage of the morphological development of the parasite than was previously thought.

A nonspecific interaction occurs before the time of penetration by E. graminis f. sp. tritici on wheat which results in decreased transpiration (as measured by less ^{35}S uptake) by inoculated tissues.

Both ^{3}H and ^{35}S are transferred to the parasite from inoculated plants given both ^{3}H-methionione and $^{35}SO_4^{2-}$ simultaneously. Methionine partially inhibits ^{35}S transfer.

E. graminis f. sp. tritici is able to incorporate SO_4^{2-} into a TCA-insoluble form. Leaves given ^{3}H-methionine or $^{35}SO_4^{2-}$ from one to six hours after inoculation have a higher percentage of label in the TCA-insoluble fraction than corresponding leaves given ^{3}H-methionine or $^{35}SO_4^{2-}$ from 21 to 26 hours after inoculation. This is apparently due in part to both the difference in environmental conditions preceding labeling and to the difference in age of the leaf. Leaves given ^{3}H-methionine from 21 to 26 hours after inoculation have a higher percentage of label in the TCA-insoluble fraction than leaves given $^{35}SO_4^{2-}$ for the same time period.

More radioactivity on thin layer chromatograms corresponds to the R_F value of methionine in the leaf and epidermis when plants are given ^{3}H-methionine than when plants are given $^{35}SO_4^{2-}$.

Less ^{35}S transfer from host to parasite occurs when plants are given $^{35}SO_4^{2-}$ at four C than at 22 C. Less ^{35}S is transferred from host to parasite at four C than at 22 C than would be expected if transfer occurred solely by simple diffusion. A largely active transport system from host to parasite is suggested.

LITERATURE CITED

LITERATURE CITED

1. Akai, S., H. Kunoh, and M. Fukutomi. 1968. Histological changes of the epidermal cell wall of barley leaves infected by Erysiphe graminis hordei. Mycopathol. Mycol. Appl. 35:175-180.

2. Allen, P. J. 1942. Changes in the metabolism of wheat leaves induced by infection with powdery mildew. Amer. J. Bot. 29:425-435.

3. Benko, P. V., T. C. Wood, and I. H. Segel. 1967. Specificity and regulation of methionine transport in filamentous fungi. Arch. Biochem. Biophys. 122:783-804.

4. Birnboim, H. C. 1970. Optimal conditions for counting of precipitated ^{3}H-RNA on glass-fiber filters. Anal. Biochem. 37:178-182.

5. Bradfield, G., P. Somerfield, T. Meyn, M. Holby, D. Babcock, D. Bradley, and I. H. Segel. 1970. Regulation of sulfate transport in filamentous fungi. Plant Physiol. 46:720-727.

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

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

8. Bushnell, W. R. 1971. The haustorium of Erysiphe graminis: An experimental study by light microscopy. In Morphological and Biochemical Events In Plant-Parasite Interaction. ed. S. Akai, S. Ouchi, pp. 229-254. Tokyo: Phytopathol. Soc. Japan. 415 pp.

9. Bushnell, W. R. 1972. Physiology of fungal haustoria. Ann. Rev. Phytopath. 10:151-176.

10. Bushnell, W. R., and P. J. Allen. 1962. Respiratory changes in barley leaves produced by single colonies of powdery mildew. Plant Physiol. 37:751-758.

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

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

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

14. Crafts, A. S. 1961. Translocation in plants. Holt, Rinehart, and Winston. New York. 181 pp.

15. Crowdy, S. H., and T. W. Tanton. 1970. Water pathways in higher plants. I. Free space in wheat leaves. J. Expt. Bot. 21:102-111.

16. Daly, J. M. 1972. The use of near-isogenic lines in biochemical studies of resistance of wheat to stem rust. Phytopathology. 62:392-400.

17. Daly, J. M., P. Ludden, and P. Seevers. 1971. Biochemical comparisons of resistance to wheat stem rust disease controlled by the Sr6 or Sr11 alleles. Physiological Plant Pathology. 1:397-407.

18. Duniway, J. M. and R. D. Durbin. 1971. Some effects of Uromyces phaseoli on the transpirational rate and stomatal response on bean leaves. Phytopathology. 61:114-119.

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

20. Edwards, H. H. 1971. Translocation of carbon in powdery mildewed barley. Plant Physiol. 47:324-328.

21. Edwards, H. H., and P. J. Allen. 1966. Distribution of products of photosynthesis between powdery mildew and barley. Plant Physiol. 41:683-688.

22. Edwards, H. H., and P. J. Allen. 1970. A fine structure of the primary infection process during infection of barley by E. graminis f. sp. hordei. Phytopathology. 60:1504-1509.

23. Ehrlich, M. A., and H. G. Ehrlich. 1970. Electron microscope radioautography of ^{14}C transfer from rust uredospores to wheat host cells. Phytopathology. 60:1850-1851.

24. Ehrlich, M.A., and H. G. Ehrlich. 1971. Fine structure of the host-parasite interfaces in mycoparasitism. Ann. Rev. Phytopath. 9:155-184.

25. Ellingboe, A. H. 1968. Inoculum production and infection by foliage pathogens. Ann. Rev. Phytopath. 6:317-330.

26. Ellingboe, A. H. 1972. Genetics and physiology of primary infection by Erysiphe graminis. Phytopathology. 62:401-406.

27. Farkas, G. L., L. Deszi, M. Howath, K. Kisban, and I. Udvardy. 1964. Common pattern of enzymatic changes in detached leaves and tissues attacked by parasites. Phytopath. Z. 49:343-354.

28. Ferrari, G., and F. Renosto. 1972. Regulation of sulfate uptake by excised barley roots in the presence of selenate. Plant Physiol. 49:114-116.

29. Fincham, J. R. S., and P. R. Day. 1963. Genetics of pathogenicity. In Fungal Genetics. pp. 257-273. Davis Co., Philadelphia.

30. Flor, H. H. 1946. Genetics of pathogenicity in Melampsora lini. J. Agr. Research. 73:335-337.

31. Flor, H. H. 1955. Host-parasite interactions in flax rust--its genetics and other implications. Phytopathology. 45:680-685.

32. Flor, H. H. 1956. The complementary genetic systems in flax and flax rust. Advan. Genet. 8:29-54.

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

34. Futrell, M. C., and J. G. Dickson. 1954. The influence of temperature on the development of powdery mildew on spring wheats. Phytopathology. 44:247-251.

35. Gerson, D. F., and R. J. Poole. 1971. Anion absorption by plants. A unary interpretation of dual mechanisms. Plant Physiol. 48:509-511.

36. Giaquinta, R. T., and D. R. Geiger. 1973. Mechanism of inhibition of translocation by localized chilling. Plant Physiol. 51:372-377.

37. Graf-Marin, A. 1934. Studies on the powdery mildew of cereals. Cornell Univ. Agr. Exp. Sta. Mem. 157. 48 pp.

38. Hadwiger, L. A., S. L. Hess, and S. von Broembsen. 1970. Stimulation of phenylalanine ammonia-lyase activity and phytoalexin production. Phytopathology. 60:332-336.

39. Hart, J. W., and P. Filner. 1969. Regulation of sulfate uptake by amino acids in cultured tobacco cells. Plant Physiol. 44:1253-1259.

40. Hatchett, J. H., and R. L. Gallum. 1970. Genetics of the ability of the Hessian Fly, Mayetiola destructor, to survive on wheats having different genes for resistance. Annals. Ent. Soc. America. 63:1400-1407.

41. Heitefuss, R. 1966. Nucleic acid metabolism in obligate parasitism. Ann. Rev. Phytopath. 4:221-244.

42. Hirata, K., and M. Kojima. 1962. On the structure and the sac of the haustorium of some powdery mildews, with some considerations on the significance of the sac. Trans. Mycol. Soc. Japan. 3:43-46.

43. Howes, N. K., and K. J. Scott. 1972. Sulfur nutrition of Puccinia graminis f. sp. tritici in axenic culture. Can. J. Bot. 50:1165-1170.

44. Hsu, S. C., and A. H. Ellingboe. 1972. Elongation of secondary hyphae and transfer of ^{35}S from barley to Erysiphe graminis f. sp. hordei during primary infection. Phytopathology. 62:876-882.

45. Hymble, G. D., and K. Raschke. 1971. Stomatal opening quantitatively related to potassium transport. Plant Physiol. 48:447-453.

46. Jones, J. P. 1966. Absorption and translocation of ^{35}S in oat plants inoculated with labeled crown rust uredospores. Phytopathology. 56:272-275.

47. Large, E. C., and D. A. Doling. 1963. Effect of mildew on yield of winter wheat. Plant Pathology. 12:128-130.

48. Lauchli, A. 1972. Translocation of inorganic solutes. Ann. Rev. Plant Physiol. 23:197-218.

49. Lauger, P., and G. Stark. 1970. Kinetics of carrier-mediated ion transport across lipid bilayer membranes. Biochim. Biophys. Acta. 211:458-466.

50. Lehninger, A. L. 1970. Biochemistry. Worth Publishers, Inc. New York. 833 pp.

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

52. Majerink, O. 1965. Water balance changes of barley infected by Erysiphe graminis D. C. f. sp. hordei Marchal. Phytopath. Z. 53:145-153.

53. Masri, S. S. 1965. The development of appressoria, haustoria and secondary hyphae during the primary infection of wheat and barley by Erysiphe graminis. Ph.D. Thesis, Michigan State University. 89 pp.

54. Masri, S. S., and A. H. Ellingboe. 1966. Germination of conidia and formation of appressoria and secondary hyphae in Erysiphe graminis f. sp. tritici. Phytopathology. 56:304-308.

55. Masri, S. S., and A. H. Ellingboe. 1966. Primary infection of wheat and barley by Erysiphe graminis. Phytopathology. 56: 389-395.

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

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

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

59. Mount, M. S., and A. H. Ellingboe. 1969. ^{32}P and ^{35}S transfer from susceptible wheat to Erysiphe graminis f. sp. tritici during primary infection. Phytopathology. 59:235.

60. Mott, R. C., and F. C. Steward. 1972. Solute accumulation in plant cells. I. Reciprocal relations between electrolytes and non-electrolytes. Annals of Botany. 36:621-639.

61. Nair, K. R. S., and A. H. Ellingboe. 1962. A method of controlled inoculations with conidiospores of Erysiphe graminis var tritici. Phytopathology. 52:714.

62. Nair, K. R. S., and A. H. Ellingboe. 1965. Germination of conidia of Erysiphe graminis f. sp. tritici. Phytopathology. 55:365-368.

63. Patterson, B. D., and R. M. Smillie. 1971. Developmental changes in ribosomal ribonucleic acid and fraction I protein in wheat leaves. Plant Physiol. 47:196-198.

64. Pardee, A. B. 1968. Membrane transport proteins. Science. 162:632-637.

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

66. Person, C. and G. Sidhu. 1971. Genetics of host-parasite interrelationships. In Mutation Breeding for Disease Resistance. pp. 31-38. International Atomic Energy Agency. Vienna. 1971.

67. Person, C., D. J. Samborski, and R. Rohringer. 1962. The gene-for-gene concept. Nature. 194:561-562.

68. Pringle, R. B., and R. P. Scheffer. 1964. Host specific plant toxins. Ann. Rev. Phytopath. 2:133-156.

69. Rahe, J. E., J. Kuc, Chien-Mei Chuang, and E. B. Willaims. 1969. Correlation of phenolic metabolism with histological changes in Phaseolus vulgaris inoculated with fungi. Netherlands J. Plant Pathology. 75:58-71.

70. Rains, D. W. 1968. Kinetics and energetics of light-enhanced potassium absorption by corn leaf tissue. Plant. Physiol. 43:394-400.

71. Reisener, H. J., and E. Ziegler. 1970. Uber den Stoffwechsel des parasitishen. Mycels und dessen Beziehungen zum Wirt bei Puccinia graminis auf Weizen. Angew. Bot. 44:343-346.

72. Sako, N., and M. A. Stahmann. 1972. Multiple molecular forms of enzymes in barley leaves infected with Erysiphe graminis f. sp. hordei. Physiological Plant Pathology. 2:217-226.

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

74. Scott, K. J. 1965. Respiratory enzymic activities in the host and pathogen of barley leaves infected with Erysiphe graminis. Phytopathology. 55:438-441.

75. Segel, I. H., and M. J. Johnson. 1961. Accumulation of intracellular inorganic sulfate by Penicillium chrysogenum. J. Bacteriol. 81:91-106.

76. Shaw. M. 1963. The physiology and host parasite relations of the rusts. Ann. Rev. Phytopath. 1:259-294.

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

78. Slesinski, R. S., and A. H. Ellingboe. 1969. The genetic control of primary infection of wheat by Erysiphe graminis f. sp. tritici. Phytopathology. 59:1833-1837.

79. Slesinski, R. S., and A. H. Ellingboe. 1970. Gene-for-gene interactions during primary infection of wheat by Erysiphe graminis. Phytopathology. 60:1068-1070.

80. Slesinski, R. S., and A. H. Ellingboe. 1971. Transfer of ^{35}S from wheat to the powdery mildew fungus with compatible and incompatible parasite/host genotypes. Can. J. Bot. 49:303-310.

81. Smith, R. C., and E. Epstein. 1964. Ion absorption by shoot tissue: kinetics of potassium and rubidium absorption by corn leaf tissue. Plant Physiol. 39:992-996.

82. Stahmann, M. A. 1967. Influence of host-parasite interactions on proteins, enzymes, and resistance. In the Dynamic Role of Molecular Constituents in Plant-Parasite Interaction. ed. by C. J. Mirocha and I. Uritani, pp. 357-372. Bruce Publishing Co., St. Paul, Minnesota.

83. Stakman, E. C. 1947. Plant diseases are shifty enemies. Am. Scientist. 35:321-350.

84. Stakman, E. C., and J. J. Christensen. 1953. Problems of variability in fungi. pp. 35-62. In Plant Diseases the Yearbook of Agriculture. U.S.D.A., Washington, D.C.

85. Stanbridge, B., J. L. Gay, and R. K. S. Wood. 1971. Gross and fine structural changes in Erysiphe graminis and barley before and during infection. In Ecology of Leaf Surface Microorganisms. ed. T. F. Preece, C. H. Dickenson, pp. 367-379. London & New York: Academic.

86. Steward, F. C., G. H. Craven, S. P. R. Weerasingbe, and R. G. S. Bidwell. 1971. Effects of prior environmental conditions on the subsequent uptake and release of carbon dioxide in the light. Can. J. Bot. 49:1999-2007.

87. Stuckey, R. E., and A. H. Ellingboe. 1972. ^{35}S uptake by Triticum aestivum and transfer to Erysiphe graminis f. sp. tritici during primary infection. Phytopathology. 62:791.

88. Stuckey, R. E., and A. H. Ellingboe. (1973) Elongation of secondary hyphae of Erysiphe graminis f. sp. tritici on wheat with compatible and incompatible parasite/host genotypes. In press.

89. Thatcher, F. S. 1943. Cellular changes in relation to rust resistance. Can. J. Res. Sect. C. 21:151-172.

90. Thompson, J. F. 1967. Sulfur metabolism in plants. Ann. Rev. Plant Physiol. 18:59-84.

91. Uritani, I. 1963. The biochemical basis of disease resistance induced by infection (with) Discussion led by Stahmann. In Perspectives of Biochemical Plant Pathology, ed. by S. Rich, pp. 4-19. Conn. Agr. Exp. Sta., New Haven, Conn.

92. von Broembsen, S. L., and L. A. Hadwiger. 1972. Characterization of disease resistance responses in certain gene-for-gene interactions between flax and Malampsora lini. Physiological Plant Pathology. 2:207-215.

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

94. Weber, D. J., and M. A. Stahmann. 1964. Ceratocystis infection in sweet potato: its effect on proteins, isozymes, and acquired immunity. Science. 146: 929-931.

95. White, N. H., and E. P. Baker. 1954. Host-pathogen relations in powdery mildew of barley. I. Histology of tissue reactions. Phytopathology. 44:657-662.

96. Wieneke, J., R. P. Covey, Jr., and N. Benson. 1971. Influence of powdery mildew infection of ^{35}S and ^{45}Ca accumulations in leaves of apple seedlings. Phytopathology. 61:1099-1103.

97. Yarwood, C. E. 1957. Powdery mildews. Bot. Rev. 23:235-301.

98. Ziegler, H. 1965. Isotopes and radiation in soil-plant studies. pp. 361-370. Wein: International Atomic Energy Agency.

99. Ziegler, H., and U. Luttage. 1967. Die salzdrusen von Limonium vulgare II. Mitteilung die lokalisierung des chlorids. Planta. 74:1-17.

100. Zucker, M. 1968. Sequential induction of phenylalanine ammonia-lyase and a lyase-inactivating system in potato tuber disks. Plant Physiol. 43:365-374.