Abstract:
Triticum vulgare L. "Rego" plants were grown under controlled environmental conditions and were inoculated 10 days after planting with lyophilized uredospores of Puccinia striiformis West. The starch content in diseased leaves decreased from 5 to 9 days, increased from 9 to 12 days to twice that of healthy leaves, and decreased from 12 to 15 days after inoculation. Electron microscopy revealed the presence of starch granules in chloroplasts adjacent to fungal hyphae at 12 days after inoculation. Sugar phosphates, ATP, and inorganic phosphate (Pi) were determined during the infection process. Sugar phosphates in diseased plants fluctuated in concentration during the infection process; ATP increased in diseased leaves at 11 days after inoculation; and Pi, an inhibitor of ADP-glucose pyrophosphorylase, increased from 8 to 10 days, decreased from 10 to 11 days, increased from 11 to 14 days after inoculation. ADP-glucose pyrophosphorylase was extracted and partially purified from healthy and diseased leaves and fungal uredospores. The enzyme from healthy and diseased leaves was activated 14.5- and 42-fold, respectively, by 3-phosphoglycerate (3-PGA). Pi inhibited enzyme activity. ADP-glucose pyrophosphorylase concentration in diseased leaves was 1.5 times greater than in healthy leaves at 10 and 12 days after inoculation, and decreased to 0.3 that of healthy leaves at 14 days. When proportionate concentrations of sugar phosphates and Pi found in healthy and diseased leaves during the infection process were placed in the assay mixture, ADP-glucose pyrophosphorylase activity closely resembled the pattern of starch accumulation in diseased leaves during the infection process. Pyrophosphorylase activity was detected in P. striiformis uredospores; it appeared to be more specific for UDP-glucose, and was not activated by 3-PGA. The accumulation of starch from 10 to 12 days after inoculation is explained on the basis of regulation of ADP-glucose pyrophosphorylase by changes in metabolite levels at critical times during the infection process. The decrease in the concentration of Pi from 10 to 11 days after inoculation appeared to release inhibition of ADP-glucose pyrophosphorylase and to allow starch synthesis to occur. A contributing factor to starch accumulation might be activation of ADP-glucose pyrophosphorylase by 3-PGA in combination with the decrease in Pi.

This decrease in Pi may be the result of conversion to ATP in oxidative phosphorylation, from the increased respiration rates observed in diseased plants. The decrease in starch content in diseased leaves from 12 to 14 days may be the result of Pi concentrations increasing to levels resulting in inhibition of starch biosynthesis, and to the activity of starch degradive enzymes. ADP-glucose pyrophosphorylase from diseased plants appeared more sensitive to activators than the enzyme from healthy plants.

RELATIONSHIP OF ADP-GLUCOSE PYROPHOSPHORYLASE TO THE REGULATION OF STARCH

ACCUMULATION IN WHEAT LEAVES INFECTED WITH PUCCINIA STRIIFORMIS WEST.

by

PAUL WILLIAM MAC DONALD

A thesis submitted to the Graduate Faculty in partial
fulfillment of the requirements for the degree
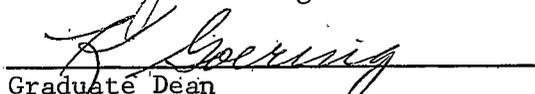

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ABSTRACT

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enzyme from healthy plants.

INTRODUCTION

Obligate plant parasite colonies behave like metabolic "sinks" or foreign meristematic regions, exerting a "field of dominance" on adjacent host tissue. As a result, starch, products of CO_2 fixation, metal ions and other metabolites accumulate at the infection site at the expense of host tissue (11, 23). The magnitude of this parasitic sink parallels the pathogen's growth, first becoming evident several days after inoculation, building to a climax at sporulation and decreasing thereafter (11).

Starch accumulation is a commonly observed phenomenon in diseased plants. It has been found to accumulate in plants infected with fungi (1, 2, 4, 5, 18, 25, 26, 44), bacteria (23), and viruses (7, 20, 40). Generally, starch accumulates in granules in the chloroplasts of photosynthetic tissue, as Akai et al. (1) found in chloroplasts associated with hyphal vesicle formation of Scleropthora macrospora (Sacc.) Thir., Shaw, and Naras. on rice, and in host cytoplasm in non-photosynthetic tissue, as Williams et al. (47) found in cabbage hypocotyls infected with Plasmodiophora brassicae Wor.

There is little detailed information available on the pattern of starch accumulation in diseased plants. Quantitative data is scanty. Usually, there is a decrease in starch content around parasite colonies soon after infection followed by a subsequent increase before and during sporulation and then a decrease thereafter.

Mains (25) reported that starch gradually disappeared from parenchyma sheaths of corn leaves infected with Puccinia sorghi Schw. Ruth Allen (5) reported a decrease in starch content of wheat leaf cells around colonies of Puccinia recondita Rob. ex Desm. soon after infection followed by a subsequent increase, and P. J. Allen (4) reported an increase around Erysiphe graminis tritici Em. Marchal colonies on wheat. Bushnell and Allen (6) observed similar changes in barley infected with mildew. Shaw and Samborski (40) observed the accumulation of various labelled metabolites, including starch, around lesions caused by tobacco mosaic virus and around pustules of sunflower rust and Carroll and Kosuge (7) observed that starch granules accumulated in chloroplasts of hypersensitive plants of Nicotiana tabacum L. undergoing shock necrosis as result of tobacco mosaic virus infection. Inman (17) found that starch accumulated at infection sites of Uromyces phaseoli var. typica Arth. in bean leaves through the flecking stage and then decreased. Mirocha and Zaki (26), on the other hand, reported that starch decreased initially, increased sharply just before and during sporulation and then dropped sharply after sporulation in rusted bean leaves. Akai et al. (2) showed that starch in rice leaves infected with Cochliobolus miyabeanus (Ito and Kuribay) Dickson was depleted in the immediate area of infection, but accumulated at the periphery of the lesion. Keen and

Williams (18) reported that starch accumulated in the host cytoplasm of cells infected with Plasmodiophora brassicae Wor. during vegetative growth and decreased during sporulation of the fungus.

Several physiological mechanisms have been proposed to explain changes in starch content in diseased plants. Schipper and Mirocha (36) found a β-amylase activator present in ungerminated uredospores and cell-free extracts of uredospores of Uromyces phaseoli var. typica capable of causing starch hydrolysis in the leaves of Phaseolus vulgaris L. prior to penetration of the fungus. They suggest that this is possibly a mechanism by which the fungus obtains metabolites after endogenous reserves in the spore have been depleted early in the infection process. This mechanism helps to explain starch degradation but not its accumulation. Tanaka and Akai (43) proposed that increased starch in rice leaves infected with Cochliobolus miyabeanus was due to a decrease in β-amylase activity, and Lovrekovich et al. (23) found a causal relationship between starch decrease and inhibition of starch phosphorylase in tobacco leaves infected with Pseudomonas tabaci (Wolf and Foster) F. L. Stevens.

Keen and Williams (18) found increased specific activities of the starch synthetic enzymes, UDPG pyrophosphorylase and starch synthetase, during starch accumulation in cabbage hypocotyls infected with Plasmodiophora brassicae; and increased starch phosphorylase

activity, and decreased pyrophosphorylase and starch synthetase activity during starch decrease at sporulation. These enzymes were believed to be of host origin since they were undetected in the fungus. They did not offer an explanation for these changes in enzyme activities.

Several mechanisms of starch regulation in non-diseased plants have been proposed. Gibberellic acid in barley aleurone has been found to initiate α-amylase synthesis (29). This helps to explain starch degradation on the basis of hormonal control in germinating seeds; however, gibberellic acid applied to bean leaves produced no increase in the activities of α-amylase, β-amylase or starch phosphorylase (10).

Preiss' group has found that regulation of α-1,4-glucan biosynthesis (reactions 1 and 2)

$$ATP + \alpha\text{-D-glucose-1-phosphate} \rightleftharpoons ADP\text{-glucose} + PP_i \qquad (1)$$

$$ADP\text{-glucose} + \alpha\text{-1,4-glucan} \rightleftharpoons \alpha\text{-1,4-glucosyl-glucan} + ADP \quad (2)$$
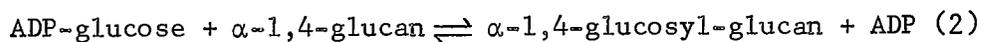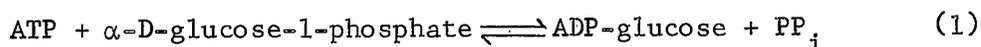
in green algae (31, 34) and higher plants (14, 15, 16) occurs at the level of ADP-glucose pyrophosphorylase (reaction 1). The regulation of ADP-glucose pyrophosphorylase involves activation by glycolytic intermediates and inhibition by inorganic phosphate. 3-Phosphoglycerate is the most potent activator. No activation of plant

α-1,4-glucan synthetases by glycolytic intermediates has been observed (13, 15). In contrast, mammalian (21) and yeast (3) glycogen trans-glucosylases utilize UDP-glucose as the glucosyl donor and regulation occurs at the transglucosylase level (reaction 2). Glucose-6-phosphate activates mammalian and yeast transglucosylases (9, 33).

Preiss et al. (30) postulated the following mechanism to explain regulation of starch synthesis in chloroplasts. In the light, the levels of P_i and ADP are decreased because of photophosphorylation. Because of carbon assimilation, the levels of 3-phosphoglycerate and other glycolytic intermediates would increase. Simultaneously, there would be an increase in reducing power in the cell. This sequence of events would lead to an increase in the formation of ATP as well as glycolytic intermediates, and would also activate ADP-glucose pyrophosphorylase. An increase in the rate of ADP-glucose synthesis would direct the flow of carbon to the formation of atarch. In the dark, the P_i and ADP concentrations would increase, and glycolytic intermediates would decrease because of cessation of photosynthetic carbon assimilation. These events would result in the inhibition of ADP-glucose synthesis. Conceivably, the increased phosphate concentrations might also cause starch breakdown by the enzyme phosphorylase. No information is available on the concentrations of effector molecules at the actual site of ADP-glucose

pyrophosphorylase to support this hypothesis. They added that this hypothesis does not preclude other mechanisms participating in the regulation of starch synthesis. There may be regulation at the site of synthesis of the two enzymes, ADP-glucose pyrophosphorylase and ADP-glucose: α-1,4-glucan transferase.

The goal of this research was to establish the pattern of starch accumulation in wheat leaves infected with Puccinia striiformis West., and to investigate the validity of the hypothesis that changes in effector molecule concentrations as a result of the host-parasite interaction are at least partially responsible for regulation of starch accumulation at the level of ADP-glucose pyrophosphorylase.

MATERIALS AND METHODS

Seed, Environmental Conditions, Spore Collection, Inoculation of
Plants

Triticum vulgare L. "Rego" was chosen for this investigation. The host plants were grown in a walk-in environment chamber rigidly controlled and programmed for temperature, relative humidity, and light. The diurnal temperature profile was $15^{\circ}C/24^{\circ}C$ (dark/light). The relative humidity was about 95% during the dark period and 65% during the light. The photoperiod was 12 hr. Light intensities were increased stepwise from 300 to 1,800 to a maximum of 3,500 ft-c at a middle of the photoperiod and then decreased through a similar range. Under these conditions Rego produces a 3-infection type (moderately susceptible: uredia abundant, chlorosis). The host plants were grown in four-inch clay pots in sandy loam-peat moss-sand (1:1:1).

The primary leaves of host plants were inoculated in a settling tower 10 days after planting with lyophilized uredospores of Puccinia striiformis (ATCC PR No. 35) according to the method of Sharp (38); a CO_2 gun was used where plants were horizontally oriented to a spore shower. Another method of inoculation was also used in which the spores were applied to the leaves with a camel hair brush.

After inoculation, the plants were placed in a dark dew chamber for 48 hr for spore germination to occur, and then returned to the

controlled-environment chamber. Control (non-inoculated) plants were also placed in the dew chamber to keep treatments similar. The term "infection process" is used in this investigation to include all phases of development of host-parasite interaction from inoculation through pathogen sporulation.

Materials and General Methods

Sugars, sugar phosphates, nucleotide phosphates, pyridine nucleotides, and enzymes for sugar phosphate analyses were purchased from Sigma Chemical Co., St. Louis, Missouri. Sodium carbonate $-^{14}C$ and sucrose-U-^{14}C were purchased from Nuclear Chicago Corporation, and sodium pyrophosphate $-^{32}P$ was purchased from Amersham/Searle, Des Plaines, Illinois.

The following solvent systems were used in decending paper chromatography on Whatman No. 1 filter paper: Solvent A, n-butanol-acetic acid-H_2O (4:1:5); Solvent B, n-butanol-pyridine-water (6:4:3); and Solvent C, 95% ethanol-pyridine-water (8:2:1).

Radioactivity was quantitatively measured with a Nuclear Chicago liquid scintillation counter. The scintillation fluid contained 3.0 ml absolute methanol and 12.5 ml toluene containing 40 ml Spectrofluor and 50 mg p-bis-2(5-phenyloxazolyl)-benzene per liter. Radioactivity on planchets was counted on a Nuclear Chicago Gas Flow Counter at 1480 volts.

Cpm were converted to dpm for ^{14}C by the channel ratio method using a standard curve; for ^{32}P, cpm were converted to dpm by dividing by the percent efficiency which was determined by counting samples of known radioactivity. The loss in radioactivity of ^{32}P each day was corrected for on the basis that ^{32}P has a half-life of 14.3 days.

Starch Determinations

One gram fresh weight of primary leaf blades from both healthy and diseased plants was harvested at the same time each day at 10 am, cut into 15 mm sections, and boiled three times in 80% ethanol to remove chlorophyll and soluble sugars.

The extracted leaf sections were placed in large test tubes. The starch remaining behind in the leaves was then hydrolyzed with 15 ml 52% perchloric acid for 24 hr in a New Brunswick Psychrotherm Shaker at 10 rpm. Two ml samples were taken and neutralized with an equal volume of 9N NaOH.

Glucose concentrations were determined by the arsenomolybdate method of Nelson (28). A standard curve was established using α-D-glucose. The glucose concentrations obtained were multiplied by 0.9 to calculate starch concentrations. Rates of hydrolysis of known amounts of amylose, amylopectin and cellulose were determined. It was found that 80% of amylose, 70% of amylopectin and 20% of cellulose were hydrolyzed after 24 hr. Hydrolysis of cellulose was not

corrected for in starch determinations.

To determine products of hydrolysis, sample hydrolysates were passed through 1.0 x 5.0 cm Dowex 50W-X8 (H^+ form), 200-400 mesh, and Dowex 1-X8 (formate form), 200-400 mesh, columns to remove salt ions, and the neutral fraction taken to dryness, dissolved in a small amount of water, and spotted on Whatman No. 1 filter paper with reference sugars for paper chromatography. Chromatograms were developed in Solvents A, B, and C. Reducing sugars on chromatograms were detected by the $AgNO_3$ method of Trevelyan et al. (45).

Labelling Experiments

The purpose of labelling experiments was to demonstrate carbohydrate accumulation at infection sites. Primary leaves were inoculated only on the top two cm of the apical portion for this experiment.

$^{14}CO_2$ labelling was done in a manner similar to Shaw and Maclachlan (39). Potted plants were placed in a 6.1 liter plexiglas cabinet having two outlets. The cabinet contents were placed under a vacuum of 2 mm of Hg and closed off. $^{14}CO_2$ was generated in a closed system outside one outlet by placing 0.25 ml sodium carbonate $-^{14}C$ (specific activity 56 mc/m mole) in a well, and adding 2 ml 3 N H_2SO_4 which generated 25 μc of $^{14}CO_2$. This gas was leaked into the chamber by releasing the vacuum. The cabinet was then closed and placed in

the growth chamber for $2\frac{1}{2}$ hr to allow fixation of $^{14}CO_2$. At the end of the fixation period the cabinet was flushed out by bubbling the evacuated air through NaOH, and the plants were returned to the growth chamber. For dark fixation of $^{14}CO_2$, the plants were kept in the dark for $1\frac{1}{2}$ hr before administering label.

Leaves were sampled daily following labelling. They were placed between filter paper, pressed between glass plates, frozen at $-15^{o}C$ for 48 hr, and then autoradiographed on Kodak No-Screen X-ray film at $-15^{o}C$ for 24 hr (14 days in the case of dark fixation).

Following autoradiography, chlorophyll and soluble sugars were extracted from the leaves in boiling 80% ethanol. The extracted leaves were again autoradiographed, cut into two cm sections from tip to base, and each section counted in the liquid scintillation counter.

Healthy and diseased plant cuttings were exposed to sucrose-U-^{14}C (specific activity 10.6 mc/m mole) to demonstrate accumulation of carbohydrate at infection sites. Leaf cuttings including the leaf sheath were harvested daily from six days after inoculation with a razor blade during the infection process, placed in test tubes containing 0.1 ml sucrose-U-^{14}C (0.11 μc), and allowed to take up the label. Distilled water was added to the tubes after the labelled material was taken up. After 12 hr the plants were harvested, frozen, and autoradiographed on x-ray film.

Electron Microscopy

Electron microscopy was used to determine the location of the starch in diseased leaf tissue. The following procedure was used: Leaf sections, 2 mm^2, were cut with a razor blade from healthy and diseased leaves and fixed for 1 hr in 2.5% glutaraldehyde in potassium phosphate buffer pH 7.3, followed by three-15 min washing in phosphate buffer. The sections were then fixed 4 to 12 hr in 2% osmium tetroxide followed by three-15 min washings in phosphate buffer.

The sections were dehydrated at room temperature in 20, 50, 70, and 100% acetone for 5, 10, 10 and 15 min, respectively, and in propylene oxide liquid for 15 min.

The sections were then infiltrated with embedding plastic and propylene oxide (1:1 v/v) for one hr at 40°C. The embedding plastic consisted of CIBA Araldite epoxy resin (#6005) and DDSA hardener (1.43 vol. of resin/1.0 vol. of hardener), and BDMA accelerator (0.034 vol. accelerator/2.43 vol. resin-hardener mixture). The specimens were embedded at 60°C for 24 hr to allow polymerization of plastic.

Specimen blocks were trimmed with a razor blade, thin-sectioned with glass knives on a Reichert Om Us ultramicrotome, and mounted on copper grids. Sections were then stained with 2% aqueous uranyl acetate for ½ hr and with Reynold's lead citrate (32) for 3-5 min.

Sections of several hundred cells from four specimen blocks at

each sampling date were examined using a Zeiss EM-9A electron micro-

scope operating at 60 kv. Photographs were taken on Agfa-Gevaert

(Scientia) or on Kodak LR Estar Safety Base film.

Photosynthesis and Respiration

A Gilson Differential Respirometer equipped with a light bar was

to measure photosynthesis and respiration rates. Measurements were

made at 25°C. Readings were taken at 10 min intervals for 50 min

following a 30 min equilibrium period. The change in O_2 occurring

between 20 and 40 min was used to calculate rates. This change was

converted to μl dry gas at standard conditions. Gross photosynthesis

was calculated by adding the respiration rate to the photosynthetic

rate.

For photosynthetic measurements the CO_2 concentration was main-

tained at 0.03% in the flasks using a 0.2M $NaCO_3$-$NaHCO_3$ buffer pH 9.9

(46). Four or five freshly harvested primary leaf blades were placed

in 3.0 ml of the buffer; buffer only was used as a blank. Respiration

rates were measured in the dark. Center wells contained 0.2 ml 20%

KOH to absorb CO_2 in the system, and strips of filter paper were

placed in the center wells to increase the surface area of the KOH.

Four or five leaf blades were placed in each flask with 3.0 ml dis-

tilled water. Following measurements leaves were oven dried and

weighed. All experiments were replicated five times.

Determination of Sugar Phosphates, ATP, and Inorganic Phosphate

Leaves from healthy and diseased plants were harvested, weighed,
and frozen at -15°C until all samples were obtained. Then they were
lyophilized overnight, weighed and boiled twice in 80% ethanol. The
ethanol extracts were combined, extracted twice with petroleum ether
(ligroin 30-60°C) to remove chlorophyll, vacuum evaporated to approx-
imately 5 ml, taken to dryness with an air blower at room temperature,
and stored desiccated at -15°C. The dried material was dissolved in
1 ml distilled water and passed through 1.0 cm x 2.0 cm columns of
Dowex 50W-X8 (H^+ form), 200-400 mesh, then Dowex 1-X8 (formate form),
200-400 mesh. The anion fraction which contained the sugar phosphates,
ATP, and inorganic phosphate was eluted off Dowex 1 with 6 N formic
acid, taken to dryness with the air blower, and stored at -15°C.

The sugar phosphate and ATP concentrations were determined
according to the method of Latzko and Gibbs (19). The compounds were
assayed in the following sequence: glucose-6-phosphate, fructose-6-
phosphate, glucose-1-phosphate, ATP, dihydroxyacetone-phosphate,
glyceraldehyde-3-phosphate, fructose-1,6-diphosphate, and 3-
phosphoglyceric acid.

Concentrations of compounds were determined in a final volume
of 3.0 ml using a Beckman DU Spectrophotometer at 340 mμ; distilled

water was used as a blank.

Glucose-6-P, fructose-6-P, glucose-1-P, and ATP were assayed as follows. The following solutions were added per ml: initially 100 μ moles triethanolamine-HCl buffer pH 7.6; 0.2 μ moles TPN; 5 μ moles MgCl$_2$; 0.5 unit glucose-6-P dehydrogenase ($\triangle$ A = glucose-6-P), then the addition of 0.5 unit phosphohexoseisomerase ($\triangle$ A = fructose-6-P), then the addition of 0.5 unit phosphoglucomutase ($\triangle$ A = glucose-1-P), and finally the addition of 3 μ moles glucose and 0.5 unit hexokinase ($\triangle$ A = ATP).

Dihydroxyacetonephosphate, glyceraldehyde-3-P, fructose-1,6-di P were assayed as follows. Initially, 100 μ moles triethanolamine-HCl buffer pH 7.6; 50 μ moles EDTA pH 7.6; 0.1 μ moles DPNH; 0.5 unit α-glycerophosphate dehydrogenase ($\triangle$ A = dihydroxyacetonephosphate), then the addition of 0.27 unit triose-P isomerase ($\triangle$ A = glyceraldehyde-3-P), and finally the addition of 0.55 unit aldolase ($\triangle$ A = dihydroxyacetonephosphate + glyceraldehyde-3-P from fructose-1,6-di P).

Glycerate-3-P was assayed as follows. 100 μ moles triethanolamine HCl buffer pH 7.6; 5 μ moles MgCl$_2$; 0.1 μ mole DPNH; 2.8 units glyceraldehyde-3-P dehydrogenase ($\triangle$ A = 1,3-PGA); and then the addition of 0.2 unit glycerate-3-P kinase ($\triangle$ A = 3-PGA).

Inorganic phosphate was determined by the method of Fiske and SubbaRow (12). A standard curve was established using pure K$_2$HPO$_4$

in distilled water.

Preparation of ADP-Glucose Pyrophosphorylase

The enzyme was prepared by a method from Ghosh and Preiss (16). Protein concentration was measured by the method of Lowry et al. (24).

Step 1. Ten gram quantities of healthy primary leaves were cut into 1 cm sections, placed in an OmniMixer (Sorvall) with 70 ml chilled acetone and homogenized at full speed for 1 min. The homogenate was passed under vacuum through a Büchner funnel containing Whatman No. 42 filter paper and followed by several volumes of chilled acetone. The acetone powder was dried on the filter paper and stored desicated at -15°C. The filter papers were cut up into small pieces and taken up in 0.05 M Tris-HCl buffer pH 7.5 containing 1 mM EDTA and 2.5 mM reduced glutathione (GSH), passed through two layers of cheesecloth to remove cellulose fibers and filter paper, and centrifuged at 40,000x g for 15 min. The supernatant fluid was used as the source of pyrophosphorylase.

Step 2. ADP-glucose pyrophosphorylase was further purified by heat denaturation at 65°C for 5 min in a water bath then quickly cooled in cold water. Denatured protein was removed by centrifugation at 20,000x g for 15 min.

Step 3. The supernatant, which contained all enzymatic activity, was fractionated with solid ammonium sulfate into 0-40% and 40-60%

saturation fractions, centrifuged at 20,000x g for 15 min, taken up in

0.05 M Tris-HCl buffer containing 1 mM EDTA and 2.5 mM reduced glut-

athione, and dialyzed overnight against the same buffer at 4°C. The

40-60% ammonium sulfate fraction was used for enzyme assays. No

further purification of the enzyme was made. The ammonium sulfate

fraction was stored at 4°C.

Preparation of Pyrophosphorylase from Fungal Spores

Non-lyophilized P. striiformis uredospores from another col-.

lection, were homogenized in a Braun MSK cell homogenizer for 10 min

using 0.10-0.11 mm glass beads and 0.05 M Tris-HCl buffer, pH 7.5,

containing 1 mM EDTA and 2.5 mM reduced glutathione. The homogenate

was centrifuged at 40,000x g for 15 min, and the supernatant used as

the enzyme source. The enzyme preparation was further purified by

heat denaturation and ammonium sulfate fractionation as described for

the plant enzyme preparation.

Assay of Pyrophosphorylase Activity

Pyrophosphorylase activity was assayed according to the method

of Shen and Preiss (42) whereby pyrophosphorolysis of ADP-glucose

was followed by the formation of ATP-^{32}P in the presence of $P^{32}P_i$.

Sodium pyrophosphate-^{32}P (specific activity 189.5 mc/m mole) was taken

up in 53 ml of 0.01 M sodium pyrophosphate to yield 5 x 10^4 dpm/µl at

the commencement of experiments. The reaction mixture, which contained 30 μ moles of Tris-HCl buffer (pH 7.5), 3 μ moles of $MgCl_2$, 0.2 μ mole of ADP-glucose, 0.5 μ mole of $P^{32}P_i$ (specific activity 0.4-2.2 $\mu c/\mu$ mole), 5 μ moles of KF, and the enzyme preparation in a final volume of 0.5 ml, was incubated in conical centrifuge tubes at $37^{\circ}C$ for 10 min in a water bath. The reaction was stopped by the addition of 3 ml of 5% cold trichloroacetic acid (TCA), and 0.1 ml 0.1 M unlabelled sodium pyrophosphate was added to dilute the $P^{32}P_i$. Then, 0.1 ml Norit A suspension (150 mg/ml) was added to absorb the ATP-^{32}P formed. The Norit A suspension was centrifuged in a clinical centrifuge and the supernatant discarded, washed twice more with 3 ml of cold 5% TCA and once with 3 ml cold distilled water. After washing the Norit A was suspended in 2 ml of aqueous solution of 50% ethanol containing 0.1% NH_3. One ml of this suspension was dried in a planchet and counted in the gas-flow counter; or alternatively, the dried Norit A was suspended in 15 ml of Cab-O-Sil gel (Beckman Instruments) dissolved in liquid scintillation fluid and counted in the liquid scintillation counter.

RESULTS

Preliminary results using IKI staining indicated the presence of starch in diseased wheat leaves. The positive reaction for starch was most pronounced 12 days after inoculation indicating the highest concentration of starch at that time. Under the microscope the starch appeared to be located in chloroplasts of host cells adjacent to intercellular fungal hyphae, and in immature uredospores at the periphery of pustules. The chloroplasts appeared more "granular" with starch and smaller than those in healthy leaves.

Pattern of Starch Accumulation

The starch content of wheat leaves inoculated with <u>Puccinia</u> <u>striiformis</u> was followed from 5 through 15 days after inoculation (Fig. 1). Starch content decreased from 5 to 9 days during the flecking stage, increased from 9 to 12 days with the peak concen-tration occurring at 12 days, and then decreased sharply from 12 to 15 days. At 12 days after inoculation the starch content of diseased leaves was 1.8 times greater than in healthy leaves.

Labelling Experiments

Autoradiographs are shown in Figures 2 and 3 of ethanol-extracted healthy and diseased leaves following $^{14}CO_2$ labelling. Leaves were inoculated at the tips to demonstrate movement of label with time. Both healthy and diseased leaves appeared uniformly

Figure 1.   Variation of starch content of leaves infected with

Puccinia striiformis from 5 to 15 days after inoc-

ulation expressed as the difference in starch content

between diseased and healthy leaves. The vertical

lines indicate the divisions of stages of host-
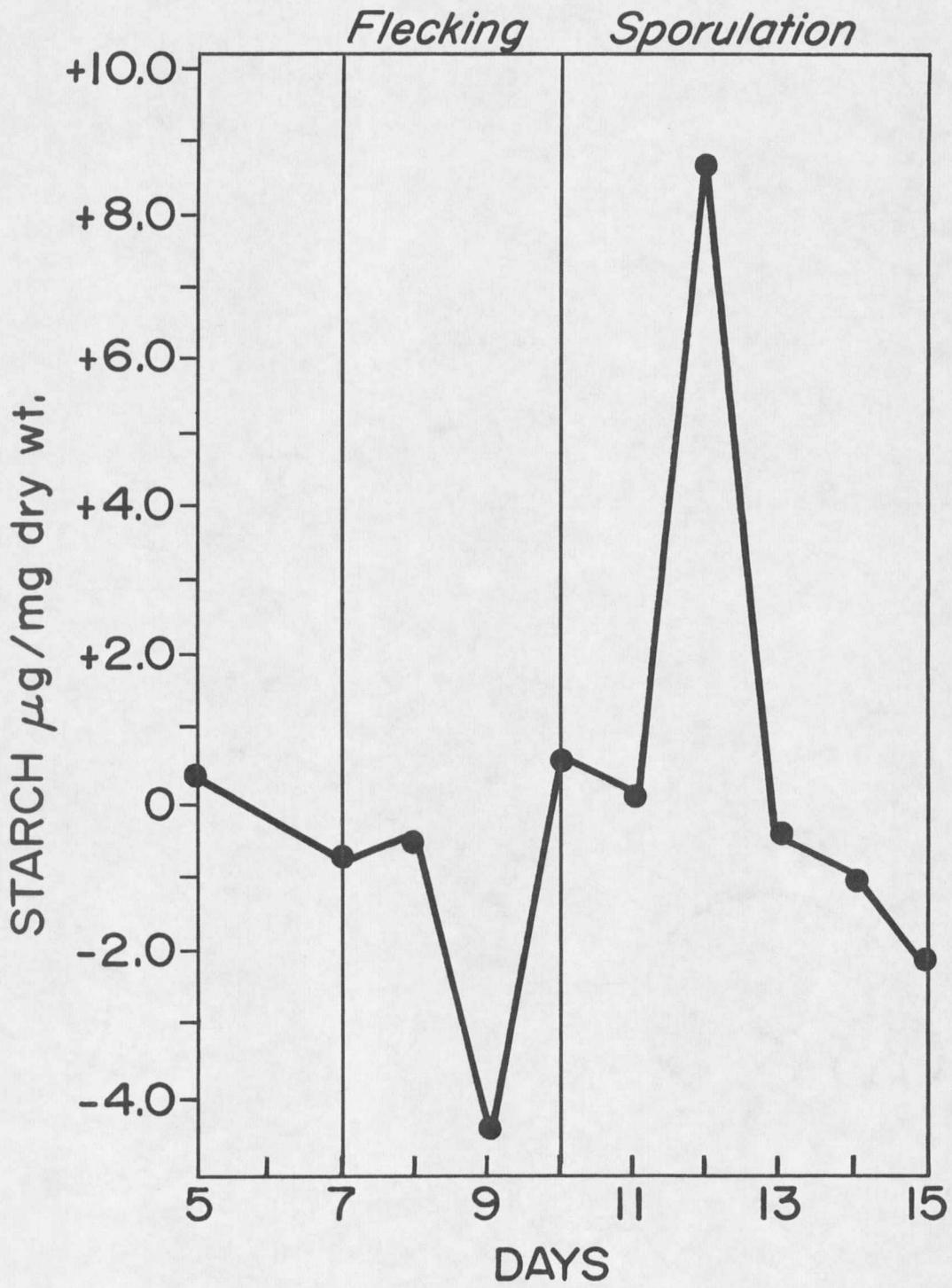
parasite interaction.

Figure 2.   Photograph of an autoradiograph of healthy and

Puccinia striiformis-infected wheat leaves fed $^{14}CO_2$

8 days after inoculation and sampled 8 to 12 days

after inoculation. Note accumulation of radio-

activity (white areas) at diseased leaf tips at 9,

10, 11, and 12 days, and in the new leaves in the

leaf sheath of all leaves. H = healthy leaves, and

D = diseased leaves. Numbers indicate days after

inoculation.