PHOTOSYNTHESIS OF BARLEY LEAVES INFECTED WITH BARLEY STRIPE MOSAIC VIRUS

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ABSTRACT

The primary leaves of ten day old Hordeum vulgaris L. var. Blackhulless were inoculated with barley stripe mosaic virus. Approximately ten days later, third leaves of healthy and diseased plants were used for photosynthetic studies. The latter were based on 0_2 evolution or $C^{14}0_2$ uptake and indicated that such factors as light intensity, temperature, $C0_2$ partial pressure, and the units chosen for comparison of photosynthesis affected the rates by healthy and infected third leaves. Expression of 0_2 evolution on either an area or a fresh weight basis indicated that no significant differences existed between healthy and infected leaves. However on a chlorophyll basis, measurement of 0_2 evolution showed that infected leaves photosynthesized at a greater rate than healthy leaves. In contrast, expression of $C^{14}0_2$ uptake on a fresh weight basis revealed that healthy leaves incorporated more radioactivity than infected leaves. A study of three photochemical activities by chloroplasts from infected leaves showed that they reacted differently to the virus. Both the Hill reaction and $C^{14}0_2$ fixation rates by infected chloroplasts were lower than those of healthy chloroplasts. In contrast, photophosphorylation was greater in infected than healthy chloroplasts. The possibility is considered that virus-induced photosynthetic alterations could account for the reduced growth and vigor of BSMV-diseased plants.

LIST OF ABBREVIATIONS

ADP	adenosine diphosphate
ATP	adenosine triphosphate
BSMV	barley stripe mosaic virus
EDTA	ethylenediaminetetraacetic acid
glucose-6-P	glucose-6-phosphate
H	healthy
Hepes	N-2-hydroxyethylpeperazine-N'-ethane-sulfonic acid
I	infected
MES	2-(N-morpholine)ethanesulfonic acid
NADP	oxidized nicotinamide adenine dinucleotide phosphate
$NADPH_2$	reduced NADP
P_i	inorganic phosphate
6-PGA	6-phosphogluconic acid
PMS	phenazine methosulfate
PPO	2,5-diphenyloxazole
POPOP	1,4-bis-2-(5-phenyloxazolyl)benzene
RNA	ribonucleic acid
TCA	trichloroacetic acid
Tris	tris(hydroxymethyl)aminomethane

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The green plant is capable of photosynthetically assimilating inorganic minerals, carbon dioxide, and water into substances which are required for its growth and development. Consumption of these assimilates by the respiratory process provides the necessary energy to carry out such metabolic activities as the building up of pectic and cellulosic substances for cell walls, fat and starch reserves, and proteins and nucleoproteins for the protoplasm and the nucleus. Under normal conditions, numerous genetically programmed enzyme systems rigidly control these activities. This results in the maintenance of a well-balanced rhythm of growth, multiplication, and reproduction in the plants. However, many agents are present in the biosphere which are potentially capable of disrupting these regulatory systems (82, 89). In each case the end result is similar, namely the undue expenditure of host reserves with the concomitant expression of physiological disorders and/or structural abnormalities. With inanimate agents such as soil and meteorological conditions, the disruption of host mechanisms is generally temporary, and these readily revert to normal with the onset of conditions more favorable for growth. On the other hand, certain animate and viral agents, normally regarded as obligate parasites, tend to induce marked changes in the metabolic activities of the host. Striking changes in the respiratory

metabolism and, to a lesser degree, photosynthesis have been reported. Whereas the respiration of diseased plants has been studied extensively (21,23,39,43), the interactions of pathogen development and photosynthesis has received little attention (15,23,39,85).

The peculiar nature of virus-host interactions offer distinct advantages over such studies in bacterial or fungal diseases. In direct contrast to the latter, viral pathogens lack an independent metabolic activity. Despite this deficiency, infecting virus particles are still capable of disrupting host metabolic activities (15,23,39,85).

A literature survey revealed very few metabolic studies on the BSMV-barley host combination (39,68). Preiss (68) showed that maximum differences in respiration between infected and healthy plants occurred approximately 9 to 12 days after inoculation. At this time the third leaves of infected plants showed extensive chlorotic mottling as well as the appearance of a characteristic necrogenous 'V' shaped mark. These foliar symptoms suggested that the photosynthetic activities of infected plants must undoubtedly be altered and this investigation was undertaken to reveal the extent to which BSMV had affected the photosynthetic activities of susceptible barley plants.

LITERATURE REVIEW

Photosynthetic studies have generally stressed the effect of the pathogen on the chlorophyll content and on the overall rate of photosynthesis by the plant. However, biochemical studies of the photosynthetic apparatus in the chloroplast have received less attention.

The color changes typical of most diseases indicate either that chlorophyll is not synthesized at the same rate as in healthy plants or that some chlorophyll is destroyed as a consequence of infection. Peterson and McKinney (67) reported changes in the chlorophyll content and chlorophyllase activity in leaves infected with four viruses which produced mosaic symptoms of varying severity in tobacco. In uninfected leaves, chlorophyllase activity and chlorophyll content were directly proportional, whereas in infected leaves chlorophyllase was greatest in the leaves that contained the least chlorophyll. As all the pigments were not decreased by the same proportion, these authors suggested that chlorosis was the result of chlorophyll failing to mask the yellow pigments. Akai and Fukutomi (1,2) also reported differences between the protochlorophyll, chlorophyll a and b, carotene and xanthophyll contents of healthy and downy mildewed leaves of rice plants. In the healthy leaves, the pigments decreased with the descending order of the leaf arrangement, whereas in diseased plants they increased. The

difference in chlorophyll content between the healthy and diseased leaves was most marked in the newly developed leaves - the latter showing a yellowish tint. They concluded that the yellowish tint of diseased upper leaves was probably due to a decreased chlorophyll content and not to an increased carotenoid content. However, in 1925 Elmer, cited in Bawden's text Plant Viruses and Virus Diseases (12), reported that infection with tobacco mosaic virus doubled the concentration of carotene while decreasing the amounts of both chlorophyll and xanthophyll.

The chlorotic symptoms characteristic of many disease syndromes may actually result from an inhibition of chlorophyll production due to the secretion of metabolites by the invading pathogen. Ryan et al (73) found that culture extracts from certain clones of Alternaria tenuis Auct. induced albinism in citrus seedlings when the seeds were germinated in the extracts. Fuller et al (31) demonstrated that a metabolite secreted by this fungus only induced chlorosis in cotton seedlings when it was placed in contact with germinating cottonseed before the tissue was exposed to light. It was suggested that the inhibitor blocked biosynthesis of chlorophyll and did not actually destroy the pigment. Studies by Braun (17) on the action of the wildfire toxin of Psuedomonas tabaci (Wolf and Foster) F.L. Stevens

suggested that this toxin either destroyed chlorophyll or inhibited its synthesis. Spectroscopic and chemical analyses of the chlorophyll of toxin treated and untreated leaves, revealed that chlorophyll concentrations in treated tissues were much lower than in comparable untreated tissue. As the chlorophyll from both leaf types were spectroscopically similar, he concluded that the biological effect exerted by the toxin was not upon the chlorophyll molecule per se.

While invasion by a pathogen generally results in chlorosis and in a reduction in chlorophyll in restricted areas of the plant, infection may result in enhanced chlorophyll contents of infected areas or of whole leaves. Holmes (45) cited several examples of over-all color abnormalities in leaves affected by viral diseases. Wang (90) showed that "green islands" surrounding rust pustules on leaves of Pinto bean were the result of pigment retention in the host tissue within the area of influence of the parasite. Similarly, Fucikovsky (30) reported that the green rings surrounding lesions on soybean leaves caused by Cercospora sojina Hara were due to chlorophyll retention.

Generally, these changes in chloroplast pigments are associated with an alteration in the morphology of the chloroplast. Cytological studies of wheat leaves by Allen (5) showed that chloroplasts were much smaller in rust-affected

than in healthy leaves. However, electron microscopic studies of chloroplast alterations of this type have generally been restricted to virus infected plants.

Misawa and Ehara (60) reported that with the lapse of time after infection, chloroplasts from cucumber mosaic virus infected cucumber cells showed a marked distortion and degeneration. Six to eight days after inoculation, osmiophilic bodies in the chloroplast expanded and twelve days after inoculation, the chloroplasts appeared highly disorganized with the stromal material breaking down and numerous vacuolated areas forming. By this time the expanded osmiophilic bodies had faded away. Eighteen days after inoculation, the chloroplast membrane and stroma lamellae had collapsed; in addition the grana lamellae was greatly reduced. However, while this pattern of disintegrations was occurring, apparently normal chloroplasts could still be observed within the same cells.

Gerola et al (34,35,36) carried out a series of electron microscopic observations into ultrastructural alterations induced in chloroplasts of virus infected plants. They found that the lamellar system of parenchyma cell chloroplasts of wheat plants infected with maize rough dwarf virus were disarranged. The grana and intergrana lamellae were randomly oriented rather than regularly aligned

as in normal chloroplasts. They also found chloroplasts from leaves of Petunia hybrida Hort. infected with arabis mosaic virus were similarly disorganized and that chloroplasts isolated from Chinese cabbage leaves infected with turnip yellow mosaic virus were more deeply lobed, shorter and thicker than normal. These lobed portions often detached themselves from the chloroplasts to lie free in the cytoplasm. Similar degenerative changes in chloroplasts have been reported for other virus-host combinations such as barley stripe mosaic virus-barley (32), wheat striate mosaic virus-wheat (53), tobacco mosaic virus-tomato (79), turnip yellow mosaic virus-Chinese cabbage (19), sunflower mosaic virus-sunflower (9), California tobacco rattle virus-tobacco (22), and tobacco mosaic virus-Datura stramonium L. (18).

It is not surprising that plants with this loss of chlorophyll and/or breakdown of the chloroplast should suffer impaired photosynthesis. However, few measurements have been made on photosynthesis by leaves infected with obligate parasites (15,23,39). Sempio (78) remarked that as early as 1904 Montemartini had discovered a gradual attenuation of photosynthesis in leaves infected with certain groups of the Uredinales. Parris (66) compared the rates of apparent photosynthesis by diseased and healthy bean leaflets and found

that bean leaflets infected with Colletotrichium lindemuthianium (Sacc. and Magn.) Bri. and Cav. assimilated 24% less carbon dioxide than did the companion healthy leaflets on the same plant. However, bean leaflets infected with Erysiphe polygoni D.C. did not reduce normal assimilation until yellowing was present. Excessive yellowing was then accompanied by a pronounced reduction in assimilation as compared with the assimilation of healthy leaflets. In mildewed wheat leaves, Allen (4) observed an initial high rate of photosynthesis followed by a drop in both chlorophyll content and photosynthesis and, by the ninth or tenth day, photosynthesis reached a very low level. Similar trends of a stimulated photosynthesis followed by a subsequent decline were reported for the same host-pathogen combination by Sempio (77) and Scott and Smillie (76), and for the Helminthosporium blight of rice plants by Akai and Tanaka (3).

Edwards and Allen (25) have refined the earlier techniques (4) by photosynthetically feeding $C^{14}O_2$ to the powdery mildew-barley complex. Photosynthesis by the complex was then found to decrease after inoculation as compared with healthy leaves. In addition they found that the ethanol soluble metabolites of the infected host tissues differed only slightly from those of the healthy, the major differences being a decreased amount of sucrose and an increased amount

of malic acid and serine. Similar decrease in photosynthetic assimilation of carbon dioxide have been reported for Yellow Rust infection of wheat (24) and for potato plants infected by Phytophthora infestans (Mont.) d By. (33).

In contrast to this general finding of a decreased photosynthesis, several workers have presented evidence of a stimulated photosynthesis by host plants following pathogenic attack. Wang (90) showed that, in rusted wheat, fixation was depressed at sporulation sites, but was stimulated in the surrounding zone. Likewise, Livne (54) observed a marked stimulation in photosynthesis of young bean trifoliate leaves during periods when rust-infected unifoliate leaves were inhibited. A similar effect was also found in diseased wheat and safflower. Livne suggested that inhibition of photosynthesis in infected tissue could be compensated in part by stimulation of tissues at a distance from the infected leaf.

Experimental evidence of an altered photosynthetic pattern in plants invaded by phytobacterial pathogens is lacking, except for the work of Beckman et al (13). They found that the reduced photosynthesis in leaves of banana plants attacked by Pseudomonas solanacearum E.F. Sm. was attributed to water stress, and was readily overcome when this stress was eliminated.

Effects on photosynthesis have been studied with only

a few viruses. Owen (63,65) measured apparent photosynthesis of virus-infected plants and found in most instances that there was no effect until symptoms appeared. However, when he inoculated tobacco leaves with tobacco mosaic virus a 10-15% reduction in photosynthetic activity occurred within one-half hour after inoculation (64). Why this immediate effect on photosynthesis should occur has not yet been resolved particularly in view of the fact the activities of chloroplasts isolated from such plants failed to show effects until seven days after inoculation (96). A subsequent attempt to verify Owen's work failed (95). Roberts and Corbett (72) studied photosynthesis of tobacco plants infected with tobacco ringspot virus and when based on leaf area, photosynthesis was significantly reduced in necrotic tissue but not in recovered tissue. Photosynthesis was, however, significantly reduced in recovered leaves when the results were expressed on a chlorophyll basis. These authors therefore concluded that decreased photosynthesis in virus-infected plants was due to early changes in the enzymatic components of the chloroplast.

The effects of plant pathogens on enzyme controlled reactions of photosynthesis such as the Hill reaction, photophosphorylation and $C0_2$ fixation, have received very little attention. Spikes and Stout (81) measured the Hill reaction of sugar beet chloroplasts by a potentiometric technique as

a function of light intensity in order to determine whether the beet yellows virus affected the rate-limiting photochemical reaction, the rate-limiting dark reaction, or both. They found the rates of both processes were decreased by approximately 50%. Zaitlin and Jagendorf (96) reported a decrease in the Hill reaction and in photophosphorylation by chloroplasts isolated from tobacco leaves inoculated with tobacco mosaic virus. However, it was demonstrated that this loss of enzymatic activity was due to a secondary effect of virus infection. Diseased plants supplemented with nitrogen fertilizer failed to show a decreased Hill reaction despite a significant increase in virus multiplication. The authors suggested that virus multiplication had affected chloroplast activities by inducing a state of nitrogen stress. Goffeau and Bové (37) demonstrated alterations in the Hill reaction and in cyclic and noncyclic photophosphorylation in chloroplasts isolated from turnip yellow mosaic virus-infected leaves. Only with chloroplasts from local lesion leaves were these workers able to observe an increase in photosynthetic activity in comparison with chloroplasts from control plants and this increased activity occurred at a time when the virus was in an active state of multiplication in these leaves. In contrast, decreased activity was recorded for chloroplasts from systemically infected leaves of the same

plants. This was in agreement with the results of Spikes and Stout (81) and Zaitlin and Jagendorf (96). These activities have also been partially characterized in rusted and mildewed host plants.

Wynn (94) studied the influence of infection by Puccinia coronata Cda. f. sp. avenae on photosynthetic incorporation of P_i into ATP. Chloroplasts isolated from infected oat seedlings six days after inoculation were found to photophosphorylate ADP at essentially the same rate as chloroplasts from comparable healthy leaves. This was observed regardless of whether the chloroplasts had been obtained from a susceptible or a resistant variety. Wynn suggested, therefore, that the primary or "light" reactions of photosynthesis which are centered around this process were not altered by rust infection. Scott and Smillie (76) found that the capacity of leaves infected with powdery mildew for partial reactions of photosynthesis, such as the Hill reaction and the photoreduction of NADP, decreased during the later stages of infection. Associated with these decreased activities was a decrease in the activities of other chloroplast enzymes such as aldolase and NADPH-diaphorase.

Varied results have been reported for the fixation of CO_2 by chloroplasts and/or the carboxylative enzymes isolated from infected leaves. Farkas et al (28), working with

turnip yellow mosaic virus, found no effect on the rate of fixation, even when there was a breakdown of chlorophyll and chloroplast degeneration. Malca et al (57) attempted to correlate the reported decrease in photosynthesis by mildewed barley (75) with decreased activities of the photosynthetic carboxylative enzymes. The authors compared the activities of phosphoriboisomerase, phosphoribulokinase and ribulose-1, 5-diphosphate carboxylase in cell-free extracts of healthy and infected barley leaves. The activity of these enzymes decreased considerably in infected leaves soon after the appearance of symptoms. Similar changes to these have also been reported for susceptible leaves of maize infected with Helminthosporium carbonum Ullstrup (56).

MATERIALS AND METHODS

A. GROWTH AND INOCULATION OF PLANT MATERIAL

The barley variety H. vulgaris var. Blackhulless was used exclusively as the host material. Seed of this variety was kindly provided by Dr. R. G. Timian, United States Department of Agriculture, Fargo, North Dakota and the supply was increased by planting some of the original seed in field plots.

All laboratory experiments employed plants grown from seeds which had been planted in a loam-sand mixture in six-inch plastic pots. The pots were placed in an environmental control room (Coldstream Growth Chamber) where the seeds germinated and the resulting plants allowed to grow for ten days at which time they were inoculated. In this controlled environment the temperature was held at 20±1°C and the light intensity, at the level of the seedlings, was 1200 to 1500 ft-c from combined fluorescent and incandescant bulbs. A 16-hour photoperiod was used, followed by an 8-hour dark period.

An unknown strain of BSMV was isolated from plants grown from infected barley seed (H. vulgaris var. Plush) kindly provided by Dr. W.A.F. Hagborg, Canada Department of Agriculture, Winnipeg, Manitoba. The virus was maintained in Blackhulless barley seedlings.

The inoculation procedure employed for this study was

similar to the one followed by Preiss (68). Leaves showing pronounced symptoms were harvested from the infected stock plants and pulped in a mortar with buffer, in the ratio of 1 g leaf tissue to 1.5 ml of phosphate buffer, pH 7.2. The liquid, from which most of the tissue was removed by passage through a double layer of cheesecloth, served as the virus suspension. To ensure a pronounced symptom appearance, 600-mesh Carborundum powder was dusted lightly over the surface of the primary leaves and the virus suspension was then rubbed over the entire surface of the primary leaf with a pair of tongs fitted with sponge-like plugs. Control plants were rubbed in a similar way with an extract from healthy plants.

B. STUDIES OF APPARENT PHOTOSYNTHESIS

(a) Photosynthesis by Leaf Discs as Measured by Oxygen Evolution

(1) Plant material

Leaf discs were cut, from the third leaves of healthy and infected plants, with a number three cork borer from three locations on each leaf, namely the base, the middle, and the tip.

(2) Measurement of apparent photosynthesis

The methods employed for this study were essentially those used by Forsyth and Hall (29). Standard