Inhibition of Photosynthesis and Transpiration in Soybean Infected by Microsphaera diffusa

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ABSTRACT

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Net photosynthesis, transpiration, and dark respiration were determined by gas exchange of attached leaves of soybean (*Glycine max* 'Harosoy 63') infected by *Microsphaera diffusa*, which causes powdery mildew. In this variety, the presence of mycelia was not accompanied by visible host symptoms. By the time 82% of the leaf area was covered by *M. diffusa*, rates of photosynthesis and transpiration were reduced to less than half those of healthy controls. The diffusive conductivity of the leaf for CO_2 was reduced significantly, but neither internal CO_2 nor leaf porosity was altered by infection. Photosynthesis reduction was not therefore attributed to availability of internal CO_2 nor to reduced stomatal aperture. A decrease in the O_2 concentration to 1% increased net photosynthesis by 1.4 times relative to the rate in normal air (21% O_2), regardless of disease levels. This indicated that increases in rates of light-induced respiration did not account for decreased net photosynthesis. Change in rates of dark respiration were insufficient to affect net photosynthesis. Also, removal of surface mycelia did not increase photosynthesis or transpiration. There was no loss of photosynthetic tissue due to necrosis or early senescence (chlorosis). The lack of respiratory effects, stomatal effects, and changes in internal CO_2 concentration suggest that *M. diffusa* inhibited photosynthesis at the chloroplast level. Because the fungus was restricted to the epidermis, a translocatable factor produced by the fungus or host may have caused the chloroplast response.

Additional key words: dark respiration, photorespiration, powdery mildew, stomatal behavior.

The pathogen, *Microsphaera diffusa* Cke. & Pk., causes powdery mildew of soybean and a number of other higher plant species (26). In soybean, epidermal cells are invaded in leaves, stems, and pods. In some cultivars, symptoms include leaf chlorosis, green islands, rusty patches under the mycelial mat, and occasional defoliation; in other cultivars (eg, Harosoy 63), luxuriant growth of the fungus occurs without visible host symptoms. Powdery mildew has become increasingly prevalent in the USA during the last 5 yr, and losses in yield have been attributed to the disease (3,11,14,15,27,28).

The effects of *M. diffusa* on the metabolic activities of soybean have not been studied. Other obligate foliar parasites either increase (23,32,33) or decrease (1,16,23,24,33) rates of photosynthesis in the host. The decreased rates of photosynthesis were associated with increased rates of dark respiration (29). Photosynthesis increased during early infection (23,33) and at high CO_2 concentrations (16). In later stages of infection, photosynthesis decreases (1,16,23,33). Similarly, both increases (10,19,21,30,31,33) and decreases (21,30,31,33) in transpiration have been reported. Where increases occurred, water loss was attributed to rupture of the cuticle (10,12).

Because losses in yield caused by *M. diffusa* must have a metabolic basis, we measured photosynthesis, transpiration, and respiration in attached soybean leaves under environmental conditions resembling those in the field.

MATERIALS AND METHODS

Growth conditions. Soybean seeds were dusted with Thiram and inoculated with *Rhizobium japonicum*. Seeds then were planted in 15 cm pots containing steamed soil and later thinned to one plant per pot. Nitrate-free nutrient solution (20) was applied every 10 days, but one application of nitrate-containing medium (12 meq/L) was given 7 days after sowing. Control and infected plants were kept in two separate controlled environment chambers, to avoid cross contamination. Growing conditions were constant at 25 C, 65% RH, and 14 hr photoperiod (fluorescent bulbs, daylight-type) with an irradiance of 450 μEin/m^2/sec (400–700nm).

Method of inoculation. Primary leaves were inoculated with *M. diffusa* by dusting conidia over each leaf. In addition, the heavily infected Harosoy 63 plants that served as the source of inoculum were kept in the growth chamber for the duration of the experiments. Therefore, developing leaves were inoculated by movement of spores in air currents in the growth chamber. Three infected plants were selected for uniformity of infection and leaf size, and three healthy plants were selected for uniformity of leaf size. Measurements of photosynthesis, transpiration, and dark respiration were repeated on the same trifoliolate leaf of each plant at various times. Disease severity was estimated visually as the percentage of leaf area covered by mycelia.

Gas exchange measurements. All measurements were made on attached trifoliolate leaves at the fourth node above the cotyledons at various times during the development of infection. A semiclosed compensating system for measuring CO_2 and H_2O vapor exchange by the leaves (4) consisted of an assimilation chamber into which an attached leaf could be inserted and sealed, leaving the rest of the plant outside the chamber. The chamber conditions were: air temperature, 25 ± 0.25 C; relative humidity, 67 ± 2%; CO_2 concentration, 306 μl/L, and O_2 concentration, 21%. For some experiments, the oxygen concentration was decreased to 1% by partially flushing the assimilation chamber with N_2. The O_2 concentration was monitored with an oxygen analyzer. Diffusive resistance to CO_2 in the boundary layer around the leaves was kept low (0.1 sec/cm) by stirring the air at 1.3 m/sec, which also assured that leaf and air temperatures were similar. Leaf temperatures, monitored with a thermocouple junction (0.076 mm) pressed against the underside of the leaf, were within 2.5 C of the air temperature. These temperatures were within 0.1 C of those indicated by a thermocouple threaded directly into the leaf mesophyll. Incandescent lights (300 W) filtered through 10 cm of water provided an irradiance of 1.6 times that of full sun

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(2500–2900 μEin/m/sec, 400–700 nm) at leaf height, which was saturating for soybean. Rates of photosynthesis were determined from the time required for the CO_2 concentration to decrease 6 μl/L (usually 20–70 sec) after the CO_2 control system was deactivated. Rates of dark respiration were determined similarly from the time required for the CO_2 concentration to increase by the same amount. The rate of transpiration was measured simultaneously from the time required for the relative humidity to rise 2% after the humidity control system was deactivated. Transpiration rate was calculated by comparison with the rate of water loss (weight loss) from wet sponges placed under identical conditions in the assimilation chamber.

The diffusive conductivity of the leaf for CO_2, ie, the ability of CO_2 to diffuse from the leaf surface to the surface of the photosynthesizing cells within the leaf, was determined from the simultaneous outward diffusion of water vapor during transpiration (18). The diffusion of water vapor out of the leaf during transpiration (T, g/dm^2/sec) is generally expressed (18) according to the equation, $T = -(C_l - C_a)/(r_l + r_a)$, where C_l is the saturation vapor concentration (g/cm^3) in the leaf at the temperature of the leaf, C_a is the vapor concentration in the bulk air outside the boundary layer surrounding the leaf (g/cm^3), r_l is the diffusive resistance of the leaf to H_2O vapor (including cuticular and stomatal effects, sec/cm), and r_a is the diffusive resistance of the boundary layer (sec/cm). The negative sign accounts for the negative transpiration flux.

Because the diffusive resistance for CO_2 is 1.6 times that for water vapor (25), the diffusive conductivity of the leaf (L, cm/sec) was calculated from the equation: $L = r'_l = 1/[1.6\,(r_l + r_a) - r'_a] = -T/[1.6\,(C_l - C_a) + Tr'_a]$, where r'_l and r'_a are the diffusive resistances to CO_2 in the leaf and boundary layer, respectively. The diffusive resistance r_a was evaluated from the evaporation rate of wet filter paper substituted for the leaf. A similar approach was used to study a vascular wilt disease (13).

In addition to these measurements on leaves covered by mycelia, gas exchange and diffusive resistances also were measured after the mycelia were removed by wiping the leaf surface with tissue paper under a stream of water and permitting the leaf to dry.

Stomatal behavior. The relative stomatal aperture was estimated with a viscous flow porometer (2) immediately after measuring the rates of photosynthesis and transpiration. Three porometer measurements of the time (t, sec) for a standard pressure change were made for each leaf. The average was expressed as the reciprocal of the cube root of the time (8) according to the equation, Porosity $= (k \ln P/P_o)(t^{-0.33})$, where P is the initial pressure (3 cm of water below ambient pressure), P_o is the final pressure (2 cm of water below ambient pressure), and k is an arbitrary constant. Because $k \ln P/P_o$ was constant, $t^{-0.33}$ was proportional to the porosity of the leaf and was reported directly to permit results for viscous flow to be compared with diffusive conductivity (7,8).

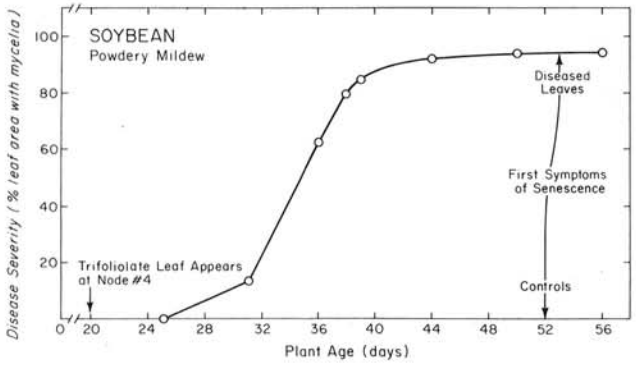

Fig. 1. Development of *Microsphaera diffusa* mycelia on the test leaf at node 4 of soybean plants (Harosoy 63), and the time of first senescence in diseased and control leaves.

RESULTS

The test leaf at the fourth node appeared 20 days after planting, and the leaf displayed the first visible mycelia of *M. diffusa* 5 days later. Eighteen to 20 days after the appearance of the fungus, 95% of the upper leaf surface was covered with mycelia (Fig. 1). All measurements were made during this period of disease development (plant age, 25–45 days). Infection did not affect the time at which the leaves became senescent as judged from the appearance of leaf chlorosis (Fig. 1). Rapid growth of the fungus resulted in spread of colonies and appearance of new colonies, but caused no visible host symptoms (Fig. 2). The leaves appeared normal after the mycelia were removed by washing. Furthermore, shading effects of the mycelia were negligible; washing heavily infected leaves improved light transmissibility by only 20% (measured between 400 and 700 nm with a radiation sensor under the leaf). As infection increased, net photosynthesis (NP) was inhibited significantly (Fig. 3). By the time 82% of the leaf area was infected by the fungus, more than half of the photosynthetic activity of the leaf had

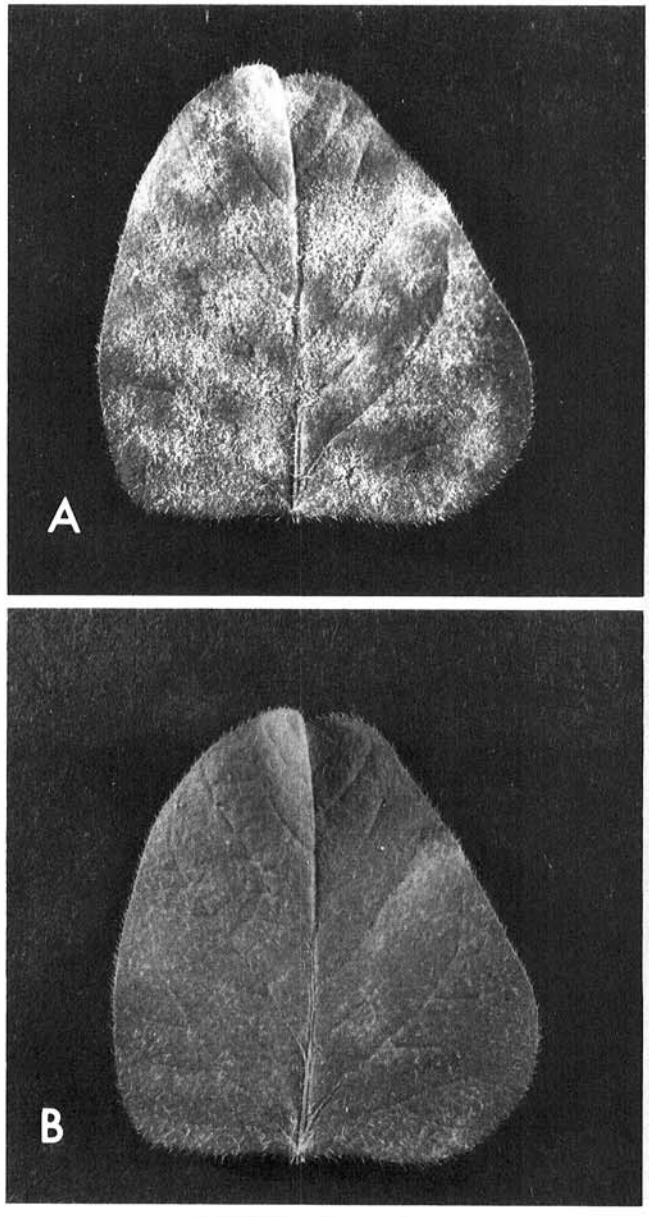

Fig. 2. Soybean (Harosoy 63) leaflet from node 2 on a plant 30 days old infected with *Microsphaera diffusa*) **A**) before (85% of leaf area covered by mycelia) and **B**) after mycelia were washed from the leaf surface. Note the lack of obvious symptoms associated with infection in (B).

been lost. The NP in the controls remained constant at 36.3 ± 1.1 mg $CO_2/dm^2/hr$ throughout the experiment. The diffusive conductivity for CO_2 in diseased leaves also was reduced significantly (Fig. 3), but the internal CO_2 concentration of the leaf (about 250 μl/L calculated by Gaastra's (18) methods was not altered (Fig. 3). Likewise, the leaf porosity was not affected by infection (Fig. 3).

To check whether the decreases in NP could have resulted from an undetected decrease in internal CO_2, we measured NP when the internal CO_2 concentration of the leaves was increased to 326 μl/L by raising the external CO_2 concentration to 408 μl/L. The NP on severely infected leaves increased but still remained less than 50% of control NP.

To determine whether NP was altered because of a change in the oxygenase activity of ribulose biphosphate carboxylase, which supplies the substrates for light-induced respiration (22), the O_2 concentration around the leaf was decreased from 21%, which is characteristic of normal air, to 1%, which inhibits the oxygenase activity of the carboxylase (17). The NP was increased 1.3–1.4 times relative to the rate of 21% O_2 in both healthy and diseased leaves (Fig. 4). The increase was proportional to NP; the ratio of NP at 1%/21% O_2 remained constant even though NP decreased as a function of disease level (Fig. 3 and 4). Dark respiration always increased slightly as infection progressed in individual leaves, but the differences between the means for each infection level were not significant (Fig. 4).

Transpiration was inhibited significantly early in infection and continued to decrease as infection progressed (Fig. 3). In leaves having 82% of the surface covered by mycelia, transpiration was 36% of the control rate. Control transpiration remained constant at 5.5 ± 0.3 $g/dm^2/hr$ for the duration of the experiment.

There was no evidence of increased transpiration after the mycelia were removed from the leaf surface by washing. Instead, transpiration decreased to about 70% of the rate observed before washing. Likewise, NP decreased to about 90% of the prewashing rate. Similar decreases also occurred in the controls after washing the leaves.

DISCUSSION

Infection of soybean with *M. diffusa* caused substantial decreases in rates of net photosynthesis and transpiration. Such decreases became more severe as mycelia became more extensive, but neither visible symptoms nor early senescence were associated with the development of infection. Furthermore, rates of net photosynthesis and transpiration were virtually constant in the control leaves throughout the measurement period. Therefore, the losses in rates of net photosynthesis and transpiration were not caused by secondary effects of cell mortality or senescence but resulted instead from direct changes in metabolic activity induced by the pathogen.

Infrared gas analysis, manometric, and radioactive-labeling techniques previously were used to measure photosynthesis, transpiration, and respiration in excised host tissue of plants infected by foliar pathogens (1,5,9,16,23,24,29,31–33). Measurements often were hampered by inadequate control of environmental conditions such as tissue temperature, resistances to CO_2 diffusion, and concentrations of CO_2. The diversity of findings in this earlier work probably can be attributed in part to these problems (16). Close control of the environment in the gas exchange measurements in our study assured that leaf temperatures were close to air temperature, external resistances to CO_2 diffusion were small, and CO_2 concentration had minimum variability. The technique had the further advantage that single, attached leaves could be monitored repeatedly as disease progressed.

In soybean, *M. diffusa* penetrates the cuticle and forms haustoria in the epidermal cells but does not infect mesophyll cells or enter the stomatal chamber (26). Unlike other pathogens (19,21,30–33), *M. diffusa* did not increase transpiration and in fact caused substantial inhibition. Because the leaf porosity did not change significantly, we attribute this inhibition to factors other than stomatal closure. Previous studies (6) in this laboratory showed that Harosoy soybean exhibits substantial decreases in leaf porosity when water deficiencies cause losses in transpiration. There was no evidence of water deficiency in the diseased leaves, and large decreases in porosity should have been obvious if stomatal closure caused inhibition of transpiration. Changes in the cuticle also do not explain the inhibition of transpiration because changes in the cuticular conductivity would exert major control of water loss only in the dark, where stomata are relatively closed. In the dark, transpiration was so low that it was almost negligible. Because our measurements were made in the light, cuticular changes would have been masked by factors, other than the cuticle, that control rates of water loss in the light. Therefore, since neither stomatal closure nor cuticular alteration could account for the decrease in transpiration, we assume that the effect was caused by some other change in the transpiration path within the leaf.

The decreased exchange of water vapor undoubtedly represented a decreased capacity for CO_2 exchange by the leaf as well. However, the decline in photosynthesis could not be attributed to decreased CO_2 diffusion; the CO_2 concentrations within the leaf were unaltered by infection. Apparently, the rate of photosynthesis declined sufficiently that a lower flux of CO_2 occurred and the

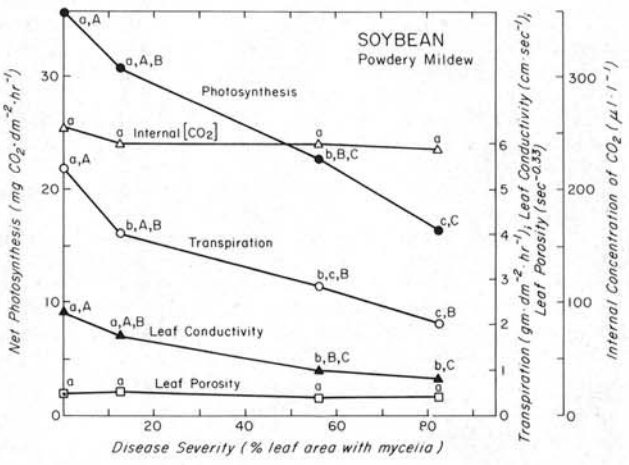

Fig. 3. Rates of transpiration and net photosynthesis of attached soybean leaves as infection with *Microsphaera diffusa* progressed on the epidermal cells. Leaf conductivity for CO_2 diffusion, internal CO_2 concentration, and stomatal behavior (porosity) are given for the same leaves. Each plotted number is a mean of several measurements taken on three different plants. Means on the same line having one common letter are not significantly different at $P = 0.05$ (lower case) and $P = 0.01$ (capital letter) according to the Fisher's least significant difference test.

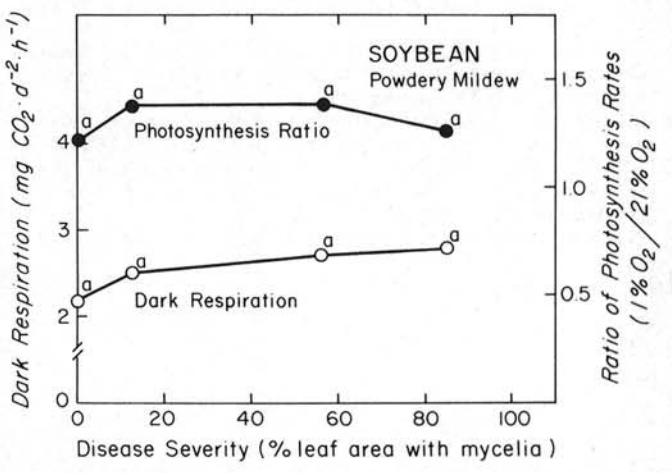

Fig. 4. Effects of *Microsphaera diffusa* on rates of photosynthesis at 21% and 1% O_2 and on rates of dark respiration of attached soybean leaves. Statistical comparisons as in Fig. 3.

internal CO_2 concentration remained unaltered. Therefore, the decline in photosynthesis must have been an independent event that occurred simultaneously with the decline in the conductivity of the leaf for CO_2. This idea was confirmed when the internal CO_2 concentration of the diseased leaves was increased. Despite an increase of CO_2 concentration by 1.3 times, rates of net photosynthesis hardly changed.

Changes in rates in dark respiration were too small to account for the changes in rates of net photosynthesis (Fig. 4). Similarly, the oxygen sensitivity of net photosynthesis was unaltered by the pathogen. Because the oxygen sensitivity indicates the rate at which substrates for light-induced respiration are formed (22), increased oxygen sensitivity would have indicated that an increase in light-induced respiration could lower the rates of net photosynthesis, as suggested for barley infected with *Erysiphe graminis* f. sp. *hordei* (16). However, the constancy of the oxygen sensitivity of photosynthesis as disease progressed (measured as the ratio of rates of net photosynthesis at 1%/21% O_2, Fig. 4) indicated that decreases in net photosynthesis are unlikely to be attributable to altered rates of light-induced respiration. For similar reasons, it is also unlikely that the competitive inhibition of the carboxylase by O_2 was altered by the disease.

Because *M. diffusa* does not penetrate leaf mesophyll cells and the disease-induced decrease in photosynthesis is not attributable to carbon dioxide supply, some translocatable factor probably inhibited chloroplast activity (24). Several metabolic alterations have been demonstrated in the mesophyll of mildew-infected leaves some distance from the site of infection (9,33). In theory, this factor could be released by the pathogen or produced by the host in response to the pathogen.

Because powdery mildew infections occur mostly on low, poorly illuminated leaves of field-grown soybeans, it is unlikely that the large decreases in photosynthesis observed in this work cause equally large reductions in yields. The losses in yield previously reported (15,27,28) are indeed less than the photosynthate losses found here. If favorable conditions for early infection by *M. diffusa* permit rapid invasion of upper leaves of the canopy, however, yield losses probably could become large.

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