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Effects of Powdery Mildew Infection on the Efficiency of CO_2 Fixation and Light Utilization by Sugar Beet Leaves

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ABSTRACT

Sugar beet leaves (*Beta vulgaris* L.) infected with powdery mildew (*Erysiphe polygoni* D.C.) show declining rates of net photosynthesis as the disease develops; relative to healthy controls, reductions of 35, 70, and 75% were observed at 9, 16, and 22 days after inoculation, respectively. A leaf gas exchange procedure in which an air stream flowed through the leaf showed that mesophyll conductance declined in parallel with photosynthesis in mildew-infected leaves. Viscous flow conductance of diseased leaves also declined over the same period suggesting that stomatal aperture was reduced. From the magnitude and time course of disease effects on stomatal aperture and mesophyll conductance, it appears that the effects at the mesophyll level were primarily responsible for mediating the decline in net photosynthesis. Changes in mesophyll conductance were closely correlated with reduced activity of ribulose-1,5-bisphosphate carboxylase on a leaf area basis. This decrease could be attributed to a reduction in the concentration of the enzyme, a reduction which was greater than the reduction in total soluble protein. The quantum efficiency of light use was also decreased by the disease. Mildew-infected leaves had quantum yields that were reduced, relative to healthy leaves, by 17 and 22% at 14 and 18 days after inoculation, respectively.

The powdery mildews are obligate fungal parasites which infect a number of economically important crops. The fungus grows superficially on infected leaves with invasive growth by subtending haustoria being restricted to the epidermal cells. Leaves infected with powdery mildews commonly show decreased rates of net photosynthesis (1, 3, 6, 10, 18). To evaluate the factors potentially limiting photosynthesis in diseased leaves requires measurements of stomatal and mesophyll conductances to CO_2 uptake. These are ordinarily obtained by calculations based on rates of diffusive CO_2 and water vapor exchange. Mildew on the leaf surface renders this approach somewhat problematical because the mycelium contributes an unknown amount of water vapor to the transpiration stream. However, we used an inoculation procedure which restricted mildew development to the adaxial surface of sugar beet leaves (6). This allowed us to use gas exchange data from the uninfected abaxial surface to show that, in spite of the mildew on the adaxial surface, rates of water vapor loss from the adaxial surface could still be used to give reasonably accurate values of adaxial stomatal condutance (6). We were therefore able to deduce the mesophyll component of conductance to CO_2 in mildewed leaves. The results revealed mesophyll conductance to be of primary importance in limiting light-saturated rates of net photosynthesis in mildew-infected sugar beet leaves (6).

In the present study we have used a flow through the leaf procedure to investigate the effects of powdery mildew on photosynthesis at the mesophyll level in sugar beet leaves. This technique provides a measure of mesophyll conductance which is not influenced by stomatal aperture and hence is free of any uncertainties engendered by the presence of powdery mildew. At the same time, viscous flow conductance of the leaf was used to evaluate mildew effects on stomatal aperture. Towards an understanding of the basis for disease effects on mesophyll conductance we have examined the activity and concentration of RuBPCase[1] in crude leaf extracts. Quantum yields of photosynthesis by mildew-infected and healthy sugar beet leaves are also reported.

MATERIALS AND METHODS

Sugar beets (*Beta vulgaris* L. var USH 10) were grown from seed in sterilized soil in a controlled environment chamber. At 7 weeks of age the youngest fully expanded leaf was inoculated on its adaxial surface with conidia of the powdery mildew fungus *Erysiphe polygoni* D.C. The abaxial surface remained free of visible infection for the duration of the experimental period. Additional information on growth conditions and inoculation is given elsewhere (6).

Parameters of leaf gas exchange were determined on individual attached leaves at various times after inoculation using the gas exchange system described by Gordon and Duniway (6). The leaf under study was enclosed within a double-walled Plexiglas chamber similar to the one described by Jarvis and Slatyer (12). Measurements were made on the 31 cm^2 of leaf area within the inner compartment of the leaf chamber. The leaf blade divided the inner compartment into separate, upper and lower halves, each supplied with a separate air stream.

Mesophyll conductance was taken as the slope of the line relating the rate of photosynthesis to the CO_2 concentration in the internal air spaces of a leaf under conditions of light saturation and CO_2 limitation (11). The measurements were made in both 1 and 21% O_2; the intercept on the CO_2 concentration axis was used to estimate the CO_2 compensation point in 21% O_2. The necessary data were obtained by passing the air stream used for gas exchange measurements directly through the leaf blade in the manner described by Lake and Slatyer (14). CO_2 concentration within the leaf was estimated by interpolating between the CO_2 concentrations above and below the leaf. To minimize the uncertainty in this estimate, the CO_2 differential across the leaf was maintained at ≤ 20 μl $CO_2 \cdot l^{-1}$. The direction of air flow through the leaf was from the lower to the upper surface. The required flow rates were obtained with trans leaf pressure differentials of less than 1.5 kPa. Air entering the lower chamber was humidified to a dew point of 15°C. Leaf temperature during flow through measurements was 25.4 ± 0.5°C.

Stomatal opening was estimated by measuring the viscous flow conductance of the leaf using the flow through procedure described above, except that ambient CO_2 levels were maintained at

[1] Abbreviations: RuBPCase, ribulose-1,5-bisphosphate carboxylase; RuBP, ribulose-1,5-bisphosphate.

320 ± 6 μl $CO_2 \cdot l^{-1}$ and the pressure gradient across the leaf was ≤1.0 kPa. Viscous flow conductance ($cm^4 \cdot s \cdot g^{-1}$) was calculated by dividing the rate of air flow through the leaf ($cm^3 \cdot s^{-1}$) by the pressure gradient across the leaf (dynes · cm^{-2}). To render these values more comparable to diffusive conductance they were raised to the power 0.4 (11).

Light-saturated rates of diffusive CO_2 uptake were measured separately for the two leaf surfaces and summed to give a whole leaf rate of net photosynthesis. During these measurements, conditions in the upper chamber were as follows: air temperature 29.8 ± 1.4°C; leaf to air vapor pressure gradient 1.01 ± 0.13 kPa; and CO_2 concentration 300 ± 5 μl $CO_2 \cdot l^{-1}$. For the lower chamber conditions were: air temperature 27.8 ± 0.6°C; leaf to air vapor pressure gradient 0.93 ± 0.16 kPa; and CO_2 concentration 300 ± 5 μl $CO_2 \cdot l^{-1}$. Leaf temperature was maintained at 28.1 ± 0.4°C. Incident photon flux density was 130 to 150 nE · cm^{-2} · s^{-1} of PAR.

Quantum yield was determined from the light dependence of photosynthesis at ambient CO_2 concentration. The leaf was illuminated with light passed through a red, RG-645 filter (Schott Optical Glass, Duryea, PA) at a photon flux density of 8.0 nE · cm^{-2} · s^{-1} until a steady-state rate of CO_2 uptake was achieved. The photon flux was then reduced stepwise until net CO_2 exchange was equal to zero. These measurements were then repeated without the use of a red filter, *i.e.* in white light. During the measurements, leaf temperature was 28.5 ± 0.9°C and CO_2 concentration was 365 ± 4 μl $CO_2 \cdot l^{-1}$ at both leaf surfaces.

At the conclusion of light dependence measurements, two discs of interveinal leaf tissue were removed for absorptance determinations using an Ulbricht sphere (6, 21). Light entering the sphere originated from a 2.5 kw xenon arc lamp and passed through a red (RG-645) filter. The red filter rendered light used for absorptance determinations nearly identical to the light used for light dependent photosynthetic measurements with respect to PAR.

Absorptance values (average of two leaf discs) were used to convert the incident photon flux, recorded during light dependence measurements, to the flux of quanta actually absorbed by the leaf. Quantum yield was taken as the slope of the line relating rate of CO_2 uptake to flux density of absorbed quanta.

The enzyme RuBPCase was extracted from 180 to 220 mg interveinal leaf tissue (eight leaf discs); mildew was removed from diseased leaves with a moist cotton swab before leaf discs were taken. Leaf tissue was homogenized with a mortar and pestle in 7.0 ml grinding buffer, in the presence of 20% (w/w) PVP. The composition of the grinding buffer was 200 mM Hepes, 5 mM DTT, and 8 mM $MgCl_2$; the pH was adjusted to 8.0 at 4°C with 10 N KOH. The homogenate was centrifuged at 30,000*g* for 20 min. The supernatant fluid was the source of the enzyme. The concentration of total soluble protein in the supernatant, determined with a Lowry assay (16) as described by Terry (22), was 1.2 ± 0.2 mg · ml^{-1} and 1.0 ± 0.1 mg · ml^{-1} for extracts from healthy and diseased leaves, respectively. All procedures after harvest were carried out at 0 to 4°C.

The assay for RuBPCase activity was conducted at substrate saturation at 30°C. The composition of the reaction medium after the addition of 100 μl of the enzyme preparation, was 200 mM Hepes, 4.0 mM DTT, 15 mM $MgCl_2$, 0.3 mM RuBP, and 45.0 mM $NaH^{14}CO_3$ (0.2 Ci/mol); the total reaction volume was 350 μl. The enzyme was activated in the presence of the assay medium for 10 min prior to the addition of RuBP (15). The reaction was stopped after 60 s by the addition of 100 μl 6 N acetic acid. Radioactivity in the acid-stable products was determined with a liquid scintillation counter in the usual manner (13).

RuBPCase protein was assayed serologically. The anti-RuBPCase serum was prepared against the enzyme purified from spinach leaves (19). The serum was used to quantify the amount of RuBPCase in leaf extracts by means of a quantitative precipitin test, as described by Kleinkopf *et al.* (13). The efficacy of this technique was established by showing that, at equal enzyme activities equal amounts of RuBPCase were precipitated by the antibody from a purified preparation from a healthy sugar beet leaf as from crude extracts from either a healthy or mildew-infected sugar beet leaf (13). Purified enzyme was obtained by using the procedure of Hall and Tolbert (9). Concentration and activity of the enzyme in the purified preparation were determined in the same manner as for the crude preparation.

RESULTS

Sporulating powdery mildew completely covered the adaxial surface of inoculated leaves by 6 days after inoculation, as described previously (6). The abaxial surface remained free of visible infection throughout the experimental period.

Whole leaf photosynthetic rates, representing a summation of the rates measured for the two leaf surfaces separately, declined substantially during disease development (Fig. 1). Healthy leaves changed comparatively little over the same time period. Mesophyll conductance (Fig. 1) was determined on the same leaves for which photosynthetic rates are given, one plant being used for each day of measurement. Mesophyll conductance declined in parallel with net photosynthesis in diseased leaves, while it remained substantially unaltered in healthy leaves. Mesophyll conductances (Fig. 1) are those obtained using 21% O_2. In 1% O_2, both diseased and healthy leaves had mesophyll conductances between 7 and 15% higher than the same leaves in 21% O_2. Subsequently, measurements of mesophyll conductance (in 21% O_2) and net photosynthesis were repeated on one healthy and four diseased plants. The results confirmed those shown in Figure 1.

To verify that the flow through measure of mesophyll conductance was comparable to that obtained from rates of diffusive CO_2 and water vapor exchange, we compared the two techniques using a healthy leaf in 21% O_2. First, mesophyll conductance was determined separately for both surfaces from their respective rates of diffusive gas exchange under CO_2 limiting and light-saturating conditions. These two values were then summed to give a whole leaf mesophyll conductance of 0.455 cm · s^{-1}. Finally, flow through procedures were used on the same leaf and a mesophyll conductance of 0.329 cm · s^{-1}, was obtained. A repeat of this experiment on a second healthy leaf gave values of 0.415 and 0.311 cm · s^{-1} for diffusive and flow through measures of mesophyll conductance, respectively. Thus, the flow through measure of mesophyll conductance underestimates the value obtained by measurements of diffusive leaf-gas exchange by about 25%.

The CO_2 compensation point was not greatly different in diseased and healthy leaves. Values for healthy leaves ranged from

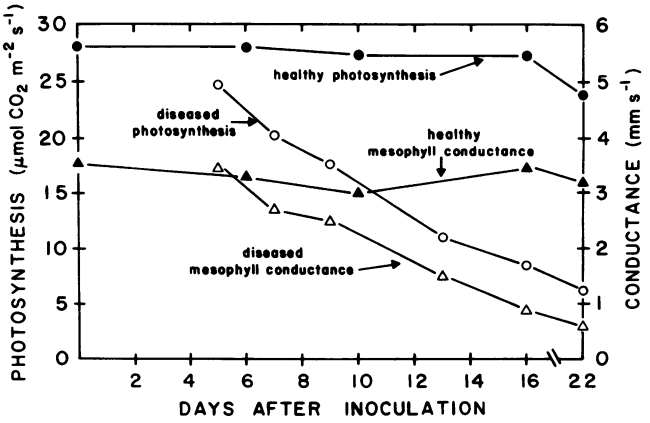

FIG. 1. Net photosynthesis and mesophyll conductance for healthy and powdery mildew-infected sugar beet leaves measured at various times after infected leaves were inoculated.

48 to 55 $\mu l \cdot CO_2 \cdot l^{-1}$. For the diseased leaves referred to in Figure 1, the CO_2 compensation point was 53, 58, 44, 73, 59, and 89 $\mu l \cdot CO_2 \cdot l^{-1}$ at 5, 7, 9, 13, 16, and 22 days after inoculation, respectively.

Viscous flow conductance, determined for the same diseased leaves on which photosynthetic parameters were measured (Fig. 1), was reduced relative to healthy controls (Fig. 2). Presumably this results from a decrease in stomatal aperture on one or both leaf surfaces.

The depressed mesophyll conductance observed for diseased leaves suggested a diminished capacity of the mesophyll to fix CO_2. For this reason, we assayed the activity of RuBPCase in extracts from diseased and healthy leaves. Measurements of net photosynthesis were made on one healthy and four diseased leaves. Within 3 h after the conclusion of the gas exchange measurements, the same leaf for which photosynthesis had been measured was sampled for the activity of RuBPCase. The results of these experiments showed that the activity of RuBPCase declined along with photosynthesis as the disease developed (Fig. 3). These experiments were repeated on both a healthy and a diseased leaf on each of three different days after inoculation. In addition to measuring the activity of the enzyme, its concentration was determined with a quantitative precipitin test. The results (Table I) indicate that decreased concentration of RuBPCase is closely correlated with the measured decrease in activity. Measurements of the activity and concentration of RuBPCase were repeated on yet a different set of healthy and diseased leaves over the same time period without the accompanying photosynthetic measurements. The results showed a similarly close correlation between the activity and concentration of the enzyme. Total soluble protein decreased by no more than 25% in diseased leaves relative to healthy controls (Table I).

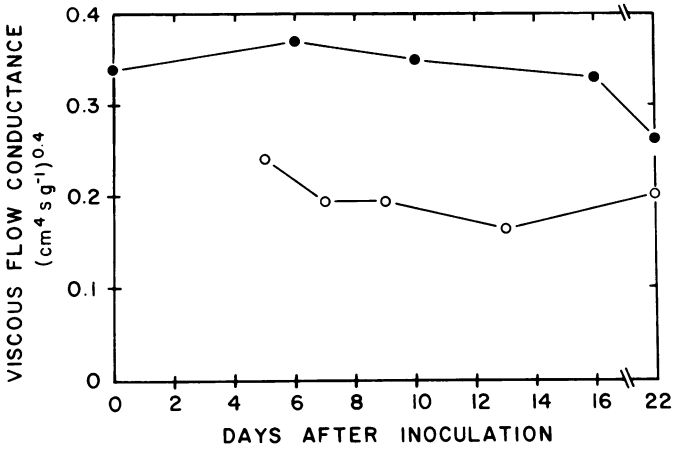

FIG. 2. Conductance to viscous flow of air through a leaf blade, raised to the power 0.4, for healthy (●) and powdery mildew-infected (○) sugar beet leaves at various times after infected leaves were inoculated.

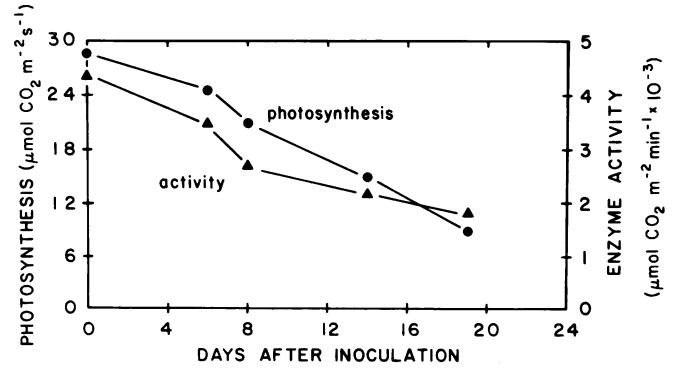

FIG. 3. Net photosynthesis and RuBPCase activity of sugar beet leaves measured at various times after inoculation with powdery mildew. Enzyme activity per unit leaf area was assayed in extracts from leaves for which photosynthetic measurements had been made. Day zero represents an uninoculated sugar beet leaf.

Table I. *Effect of Powdery Mildew on Net Photosynthesis, Mesophyll Conductance, RuBPCase Activity, RuBPCase Concentration and Total Soluble Protein of Sugar Beet Leaves*

Relative values are given, diseased/healthy, for each day of measurement.

Time After Inoculation	Net Photosynthesis	Mesophyll Conductance	RuBPCase Activity	RuBPCase Concentration[a]	Total Protein[b]
days					
6	0.76	0.86	0.76	0.82	0.89
10	0.54	0.62	0.56	0.61	0.81
13	0.38	0.33	0.49	0.52	0.75

[a] Healthy leaves had 0.27 ± 0.02 mg RuBPCase/cm^2 leaf area.
[b] Healthy leaves had 0.85 ± 0.08 mg total soluble protein/cm^2 leaf area.

Quantum yields of diseased leaves measured in red light (≥ 645 nm) at 7, 14, and 18 days after inoculation were 0.065, 0.064, and 0.060 nmol CO_2 fixed per nE of absorbed light, respectively. Healthy leaves at ages corresponding to 0 and 14 days after inoculation had quantum yields of 0.076 and 0.078 nmol CO_2 fixed per nE of absorbed light. Quantum yields based on incident white light were determined for these same diseased and healthy leaves. The values were all somewhat lower than those measured on the basis of absorbed red light but the same pattern of decline with time after inoculation was apparent.

DISCUSSION

Attempts to identify the factors limiting net photosynthesis in mildew-infected leaves have been hampered by the presence of the fungus on the leaf surface. This fungal tissue contributes a relatively small but still unknown amount of moisture to the rate of transpiration measured in the light (6), making it difficult to use such data to precisely assess stomatal conductance. The physical influence of a powdery mildew on boundary layers may further complicate gas exchange analyses (1). Uncertainties inherent in calculated values of stomatal conductance will carry over to mesophyll conductance when the latter is determined as a residual term (11). We have circumvented these difficulties by, first, using viscous flow conductance as a measure of stomatal aperture, since it does not rely on water vapor exchange data, and then, measuring mesophyll conductance by the flow through procedure, which required no presumptions about stomatal aperture.

The results revealed that disease-induced reductions in light-saturated rates of net photosynthesis are accompanied by major decreases in mesophyll conductance (Fig. 1). Measurements of viscous flow conductance suggest that stomatal aperture is reduced by as much as 50% in mildew-infected sugar beet leaves (Fig. 2). Ayres (1) has reported reductions in stomatal aperture in mildew-infected pea leaves, while Mignucci and Boyer (18) found no change in the porosity of mildew-infected soybean leaves. The relative contribution of stomatal and mesophyll conductances, as reported here, to the total conductance to CO_2 uptake in diseased leaves cannot be characterized because the two parameters are expressed in different units. However, a 50% reduction in stomatal aperture, as suggested by our viscous flow conductance data, is consistent with findings based on stomatal diffusive conductance measured on the uninfected abaxial surface of sugar beet leaves infected with powdery mildew on their adaxial surface (6). In that

instance, stomatal conductance could be directly compared to mesophyll conductance for the same side of the leaf. Even with a 50% reduction in stomatal conductance, total conductance to CO_2 uptake was determined almost entirely by mesophyll conductance (6). Our present results support our previous conclusion that the reductions in stomatal aperture do not have an important effect on photosynthesis in mildew-infected sugar beet leaves. This implies that mesophyll conductance is limiting photosynthesis in diseased leaves and indeed, the magnitude and time course of the decline in mesophyll conductance, shown in Figure 1, are consistent with the conclusion that changes in mesophyll conductance are of primary importance in mediating the decline in net phtosynthesis by mildew-infected sugar beet leaves.

Reductions in mesophyll conductance resulting both from senescence (5) and from a virus infection (8) have been correlated with reductions in the activity of RuBPCase (7). Such a correlation is apparent in our results as well (Table I). The activity of other reductive pentose phosphate cycle enzymes may also be reduced by the disease, so RuBPCase activity is not necessarily responsible for limiting the flux of carbon through the reductive pentose phosphate cycle. Loss of RuBPCase activity can be traced to a reduction in the concentration of the enzyme. There is no apparent change in the specific activity of RuBPCase as a result of the disease.

The reduction in total soluble protein is somewhat less than the reduction in RuBPCase (Table I). The actual reduction in total leaf protein is probably greater than these numbers suggest, since fungal proteins from the haustoria are measured in the diseased leaf extracts along with host proteins. But it seems unlikely that this could account for more than a small part of the discrepancy. Thus, infection with powdery mildew seems to engender a somewhat preferential degradation of RuBPCase. This is similar to the situation in senescing leaves where loss of RuBPCase accounts for most of the decrease in total soluble protein (5, 20, 25). In fact, it has been suggested that powdery mildews cause a type of accelerated senescence (2, 4). Ironically, such speculation has usually been based on the chlorosis of colonized leaf tissue (2), and loss of Chl is the attribute of senescence most conspicuously lacking in mildew-infected sugar beets (6, 17). However, there are other examples where senescence, as evidenced by decreases in net photosynthesis, mesophyll conductance, and the concentration of RuBPCase, is not accompanied by a substantial loss of Chl (5, 23, 24).

The damaging effects of the disease extend to the efficiency of light utilization as revealed by lower quantum yields in diseased leaves. These reductions in quantum yield cannot be attributed to loss of Chl (6, 22). Magyarosy *et al.* (17) found reduced rates of noncyclic photophosphorylation in chloroplasts isolated from mildew-infected sugar beet leaves. They examined leaves only at an advanced stage of infection (30 days after inoculation) but it is possible that such an effect is operative earlier and contributes to the reduced quantum yields reported here.

The impact of the disease on quantum yield is less dramatic than the effect on light-saturated photosynthetic rates. However, in the field mildew is found predominantly on shaded leaves so reduced quantum efficiency may be an important component of the yield loss associated with powdery mildew infection of sugar beets.

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