

## Alteration of Gas Exchange in Rice Leaves Infected with *Magnaporthe grisea*

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**Infection with rice blast fungus (*Magnaporthe grisea*) significantly reduced foliar net photosynthesis (A) of rice cultivars: Ilpoom, Hwasung, and Choochung in greenhouse experiments. By measuring the amount of diseased leaf area with a computer image analysis system, the relation between disease severity (DS) and net photosynthetic rate was curvilinearly correlated ( $r=0.679$ ). Diseased leaves with 35% blast symptom can be predicted to have a 50% reduction of photosynthesis. The disease severity was linearly correlated ( $r=0.478$ ) with total chlorophyll (chlorophyll a and chlorophyll b) per unit leaf area (TC). Light use efficiency was reduced by the fungal infection according to the light response curves. However, dark respiration (Rd) did not change after the fungal infection ( $p=0.526$ ). Since the percent of reduction in photosynthesis greatly exceeded the percent of leaf area covered by blast lesions, loss of photosynthetic tissue on an area basis could not by itself account for the reduced photosynthesis. Quantitative photosynthetic reduction can be partially explained by decreasing TC, but cannot be explained by increasing Rd. By photosynthesis (A) - internal CO<sub>2</sub> concentration (C<sub>i</sub>) curve analysis, it was suggested that the fungal infection reduced ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity, ribulose-1,5-bisphosphate (RuBP) regeneration, and inorganic phosphate regeneration. Thus, the reduction of photosynthesis by blast infection was associated with decreased TC and biochemical capacity, which comprises all carbon metabolism after CO<sub>2</sub> enters through the stomata.**

**Keywords :** photosynthesis, rice blast, disease severity, total chlorophyll.

Rice blast, caused by *Magnaporthe grisea* (Hebert) Barr (anamorph: *Pyricularia grisea*) is one of the most destructive diseases of rice. The blast infected rice leaf has been reported to have a much greater loss in photosynthetic capacity than the amount of necrotic leaf area would suggest (Goto, 1965; Bastiaans, 1991, 1993). Virtual diseased

area, which includes both the surrounding, healthy-looking tissue, as well as necrotic parts, was suggested to explain quantitative changes in infected rice leaf physiology and function. The photosynthetic reduction came from increased dark respiration (Rd) (Toyoda and Suzuki, 1957; Bastiaans, 1993) or decreased chlorophyll content partially (Magyarosy et al., 1976; Mitchell, 1979) or completely (Wynn, 1963; Berghaus and Reisener, 1985).

The amount of plant material occupied by disease symptoms needs to be measured to clarify the relation between physiological deterioration and visible symptoms. As computer technology improves, color image analyzing system for disease assessment is easily available for storing and retrieving the digitized image. Indeed, image analysis by computer is straightforward for discriminating diseased tissue and measuring surface area. While several studies (Lindow and Webb, 1983; Pennypacker et al., 1990) have examined disease discrimination with black and white image systems using light filters. While, Gaunt (1990) recommended color images were superior for discriminating diseased from healthy areas.

Non-destructive gas exchange measurement of plant tissue can assess quantitative plant functions involved in pathogenesis on an organ or tissue level. By controlling light and CO<sub>2</sub> concentration levels in a chamber, it is possible to investigate the detailed physiological processes of damaged leaves. Net photosynthesis (A)- internal leaf CO<sub>2</sub> concentration (C<sub>i</sub>) curve fitting can be used to explain the stomatal and biochemical processes of CO<sub>2</sub> assimilation (Ball et al., 1987; Flanagan and Jefferies, 1989). Reduction in the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and in the regeneration of its sugar substrate, ribulose-1,5-bisphosphate (RuBP), is responsible for reduced net photosynthesis in drought-stress plants (Farquhar and Sharkey, 1980; Vu et al., 1987) and *Verticillium* infected alfalfa (Pennypacker et al., 1990).

Our objectives were to study the alteration of physiological performance in rice leaves infected by *M. grisea* and to quantitatively relate gas exchange measurements with leaf blast severity using an image analysis system. Using the relation between physiological change and digitized severity of diseased leaves, it is possible to determine rice yield

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loss by *M. grisea* infection in epidemiological studies. After leaf blast infection, changes in photosynthetic rate, light response, and  $A/C_i$  curve were measured to understand  $CO_2$  assimilation processes in terms of biochemical capacity. The investigation of quantitative relations between disease severity (DS: %) and photosynthesis, total chlorophyll (TC, chlorophyll a and b:  $mg\ cm^{-2}$ ),  $Rd$  can be used to explain disease severity with or without visible injury.

## Materials and Method

**Rice cultivars and inoculation of the blast fungus.** The three rice cultivars: Ilpoom, Hwasung, and Choochung, commonly susceptible to *M. grisea* race KI-197 were used in this study. They were surface-sterilized and sown in  $5 \times 10$  cm plastic pots in the greenhouse at 20-35°C. Rice plants were inoculated at the 4-5 leaf stage with a spore suspension ( $5 \times 10^5$  conidia  $ml^{-1}$ ) of *M. grisea*. Inoculated plants were placed in a dew chamber with 100% RH at 25°C for 1 day, then moved into the greenhouse. Only the cultivar, Choochung, was selected for quantitative disease assessment because the physiological changes after infection of all three cultivars was quite similar.

**Net photosynthesis measurement.** One-week after inoculation, net photosynthesis was measured under greenhouse conditions on two consecutive days with a Li-Cor 6400 photosynthesis measurement system (Li-Cor, Lincoln, NE). The measured leaves were fully expanded, 10-14 days old leaves. The leaf, still attached to the plant, was inserted into the leaf chamber and allowed to acclimate for about 2 minutes. To precisely control gas exchange measurements, the leaf cuvette was maintained at  $1000\ \mu mol\ m^{-2}\ \cdot\ s^{-1}$  with the light emitting diode (LED) at 25°C. The Li-Cor system measured  $CO_2$  change, water vapor change, light intensity, leaf temperature, and temperature inside the chamber. Based on these measurements and gas exchange theory (Farquhar and Sharkey, 1982), net photosynthesis ( $A$ :  $\mu mol\ CO_2\ m^{-2}\ \cdot\ s^{-1}$ ) was automatically calculated by the microprocessor. Since the size of the leaf chamber of the system was 2 cm wide and 3 cm long, the measured leaf length was 3 cm. After gas exchange measurement, the 3 cm long tissue areas in the chamber were measured using a Li-Cor 3100 leaf area meter. Rice leaf stomatal ratio between adaxial and abaxial sides was checked with a porometer (model 1600, Li-Cor). Since the transpiration on both sides was similar, the stomatal ratio was about 1. To quantify the relation between DS, photosynthesis and TC content, 38 diseased and 10

healthy leaves on the first day and 12 diseased and 6 healthy leaves on the second day were measured, respectively (Table 1).

**Light control in the leaf chamber for light curve.** We obtained light response curves by controlling the LED light intensity levels inside the chamber. Irradiance was decreased in 7 steps from 1000, 700, 400, 300, 200, 100, 0  $\mu mol\ PAR\ m^{-2}\ \cdot\ s^{-1}$  illumination on the adaxial side inside the chamber. Once a leaf was inserted into the chamber, gas exchange measurements were automatically conducted via the programmed setting of the 7 steps irradiations. Each measurement required a minimum of 30 minutes including time to match the two infra red gas analyzers (IRGA). Since three leaves were sequentially measured per treatment in each cultivar, a total of eighteen light response curves were obtained. On the second day, light response curves of twelve diseased and six healthy leaves were drawn to measure  $Rd$ . Dark respiration was measured in terms of the amount of  $CO_2$  production at 0  $mmol\ CO_2\ m^{-2}\ \cdot\ s^{-1}$ . The chamber environment was controlled at: 380 ppm  $CO_2$ , temperature of 25°C, and RH between 30-60%.

**$A/C_i$  response curve.** The various levels of  $CO_2$  concentration inside the measurement chambers were controlled with the  $CO_2$  injector system (Li-Cor 6400-01, Li-Cor) and liquid  $CO_2$ . This system can deliver precisely controlled  $CO_2$  concentrations in the chamber ( $C_a$ ). Because  $CO_2$  changes are very rapidly detected and compensated by the injector system,  $C_a$  can be held at precise levels. Autoprograms within the Li-Cor 6400 system can automatically change setpoints to 1200, 800, 400, 200, 100, 0 ppm  $C_a$  and log data for each setpoint. Internal  $CO_2$  concentration ( $C_i$ ) was calculated based on the gas exchange theory (Farquhar and Sharkey, 1982). Eighteen leaves were sequentially measured on three varieties and two treatments. The measured chamber was controlled at:  $1000\ \mu mol\ PAR\ m^{-2}\ \cdot\ s^{-1}$ , temperature of 25°C, and RH between 30-60%.

**Disease severity measurement.** After gas exchange measurement on two consecutive days, a total of sixty-six photographs of leaf pieces were taken and the images were digitized using a color image analyzer (KS 400 version 3.0, Kontron Elektronik, Germany). The severity of injury of each leaf piece was assessed with a computer-controlled video image analyzing system, which consists of a personal computer, a color video camera, a monitor, and an image storage unit. In this experiment, disease area was defined as abnormal leaf color, including typical and mimic symptoms. Accordingly, disease severity was defined as the percent of leaf area covered by visible lesions.

**Total chlorophyll contents.** After the 66 diseased leaf images were taken, the gas exchange- measured, 3-cm long, leaf pieces

**Table 1.** Chlorophyll content ( $mg\ ml^{-1}$ ) of rice leaf with or without rice blast infection

Measurement	Treatment	No. of leaf	Chlorophyll a	Chlorophyll b	Total chlorophyll
Day 1	Diseased	41	16.24	3.95	20.19
	Healthy	10	23.84	7.83	30.67
		P-value*	0.254	0.212	0.216
Day 2	Diseased	12	15.33	5.35	20.67
	Healthy	6	21.85	7.56	29.41
		P-value*	0.003	0.007	0.003

were soaked in 80% acetone for 72 hours. The amount of total chlorophyll, chlorophyll a, and chlorophyll b extracted from these leaf pieces was determined through optical density readings at 645, 652, and 663 nm (MacKinney, 1941). Total chlorophyll content was determined by adding chlorophyll a and chlorophyll b per unit leaf area ( $\text{mg chlorophyll cm}^{-2}$ ).

**Statistical analysis.** Effects of the fungal infection were checked by a one way analysis of variance using Minitab statistical software (State College, PA). The regressions of DS and photosynthesis, chlorophyll content, dark respiration were analyzed GLM using Minitab.

## Results

Net photosynthesis ( $A$ ) of the healthy leaves was significantly ( $p < 0.001$ ) higher than that of the diseased (Fig. 1) in each of the three cultivars. The standard deviations of photosynthesis for diseased leaves were 2-10 times greater than those for healthy ones depending on the cultivars. This large variation came from 30-50% of various DS of diseased leaves. Regardless of cultivar, photosynthesis was significantly ( $p < 0.001$ ) decreased due to the fungal infection.

Figure 2 shows the unique light response curves of 9 diseased and 9 healthy leaves. Light saturation point of the healthy leaves was between 300-400  $\mu\text{mol PAR m}^{-2} \cdot \text{s}^{-1}$ , while that of the diseased leaves was approximately 100-200  $\mu\text{mol PAR m}^{-2} \cdot \text{s}^{-1}$ . The maximum photosynthetic rate of the healthy leaves was about 3-4 times higher than that of the diseased leaves, thus, infection by *M. grisea* tended to reduce the light use efficiency (LUE,  $\text{mol CO}_2$  fixed per mol PAR). Infection had no significant effect on  $R_d$  at 0  $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ . Since the rate of photosynthesis was different at 100  $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$  between the two treatments, the slope from compensation to saturation was different.

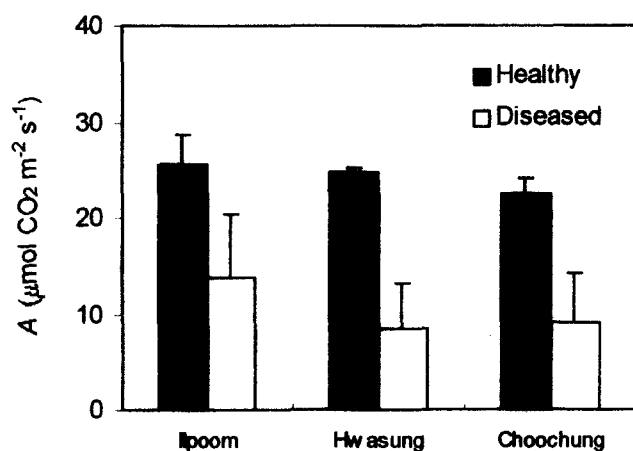


Fig. 1. Net photosynthesis ( $A$ ;  $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ ) change on the healthy and rice blast infected leaves of the three varieties.

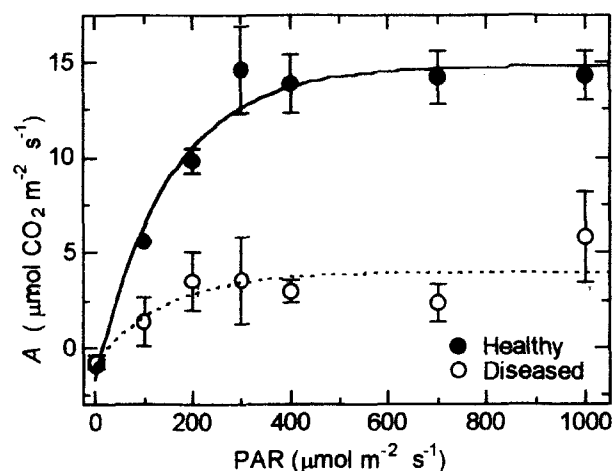


Fig. 2. Effect of leaf blast infection on the response of photosynthesis to variation in photosynthetically active radiation (PAR) in rice leaf. Chamber temperature was 25°C.

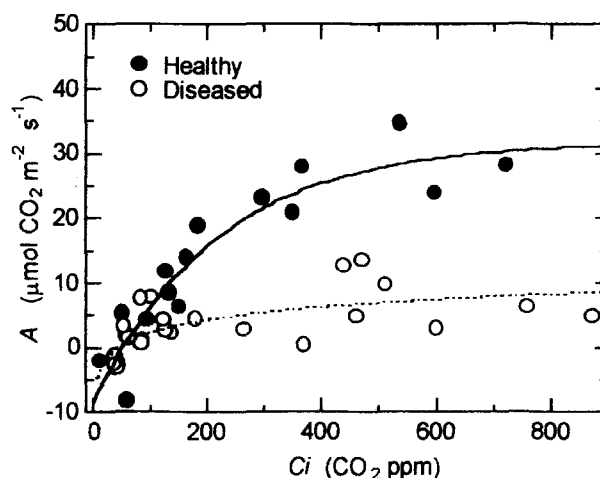


Fig. 3. Effect of leaf blast infection on the response of photosynthesis to variation in intercellular  $\text{CO}_2$  concentration in rice leaf. The curves represent a biochemical model fitted to the data. Measurement condition were: chamber temperature 25°C, photon flux density 1000  $\mu\text{mol PAR m}^{-2} \cdot \text{s}^{-1}$ .

As supplied  $\text{CO}_2$  concentrations decreased in the measuring chamber, leaf photosynthesis decreased. The healthy and the diseased  $A/C_i$  curve fittings were significantly different (Fig. 3). The slope of the diseased leaves'  $A/C_i$  curve was only one-third that of the healthy one. Also, the maximum photosynthetic level of the diseased was 10  $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ , whereas that of the healthy was around 30  $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ . The results show that photosynthetic rates in the healthy leaves with increase in  $\text{CO}_2$  concentration, while photosynthetic activity in the diseased leaves would not.

The measured leaf photosynthetic rate was plotted against disease severity (Fig. 4). The fitted line was a nega-

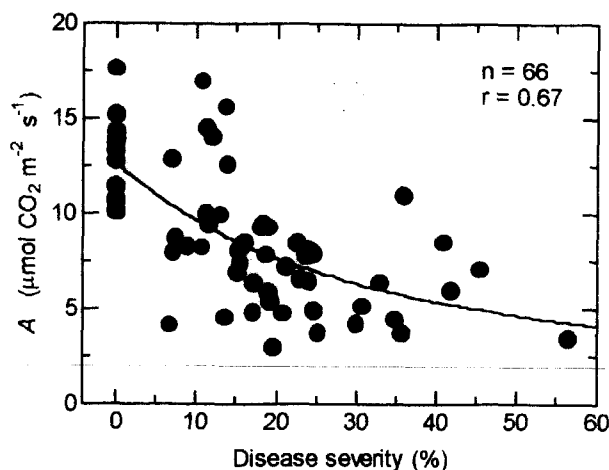


Fig. 4. Net photosynthetic rate of leaves infected by *Magnaporthe grisea* in relation to disease severity as measured in a greenhouse experiment.

tive exponential regression.

$$Y = 1.878 * \exp^{(104.98/(X + 54.82))}$$

where Y = net photosynthesis ( $\text{mmol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ )

X = disease severity (%)

The correlation coefficient for the entire 66 leaves was 0.674, including 19 healthy leaves. Residuals were homogeneously distributed around the fitted curves. The regression line showed that about 35% of leaf damage was enough to decrease net photosynthesis by 50%. Photosynthetic loss in damaged areas did never exceed 60%. Some samples with less than 10% injury had much greater loss in photosynthetic activity than predicted by the regression line.

Chlorophyll a, chlorophyll b, and total chlorophyll contents were measured separately after measuring photosynthesis. Total chlorophyll in rice leaves consisted of 80% chlorophyll a and 20% chlorophyll b (Table 1). The infection-damage effect was found only on Day 2. Since variation in chlorophyll content of 38 diseased leaves on Day 1 was 2 to 3 times that of the control treatment, the difference was not significant, although fungal infection was associated with a decrease in the average amount of total chlorophyll. The relation between disease severity and total chlorophyll was linear (Fig. 5) though the correlation coefficient was only 0.478. Chlorophyll a and chlorophyll b had a similar pattern (data are not shown). The linear fit is shown on Fig. 5.

$$Y = 24.51 - 0.268 X$$

where Y = total chlorophyll content ( $\text{mg cm}^{-2}$ )

X = disease severity (%)

Although the equation is simpler, it fits worse than the

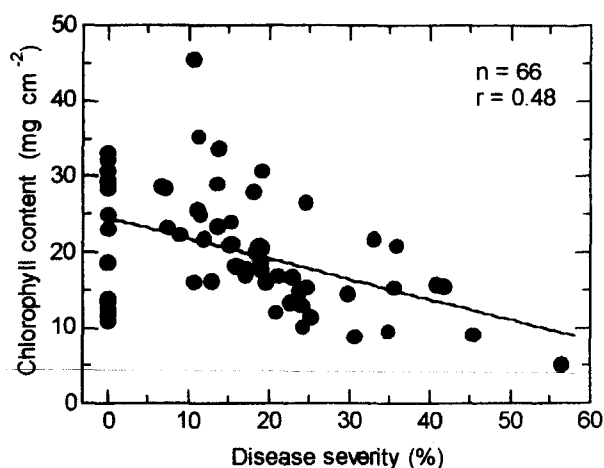


Fig. 5. Total chlorophyll (a+b) of leaves infected by *Magnaporthe grisea* in relation to disease severity as measured in a greenhouse experiment.

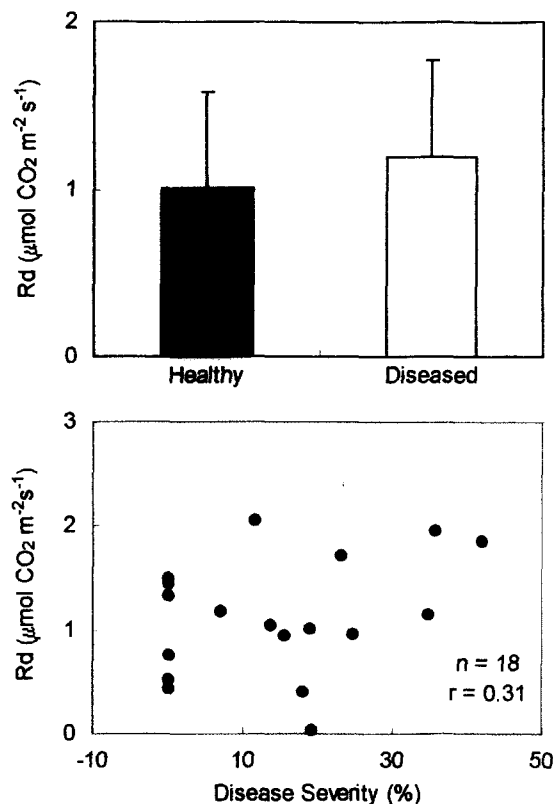


Fig. 6. Dark respiration ( $R_d$ ;  $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ ) of the healthy and the rice blast diseased leaves (top) and the relationship between respiration and disease severity among the diseased leaves (bottom).

photosynthesis-disease severity relation. The linear regression line can predict that a leaf with 46% visible injury will have a net decrease in total chlorophyll of 50%.

The dark respiration ( $R_d$ ) in 12 infected and 6 healthy

leaves was measured by controlling light intensity. The leaf respiration was slightly higher in the diseased leaves (Fig. 6), but was not statistically significant ( $p=0.526$ ). Because  $R_d$  levels were 1/10 or 1/20 of the maximum photosynthetic rate,  $R_d$  would not have a significant effect on the reduction of photosynthesis by the fungal infection. The regression analysis between respiration and disease severity among the diseased leaves was not statistically significant ( $p=0.214$ ). Therefore the regression line ( $r=0.308$ ) showed that respiration was not correlated with disease severity.

## Discussion

The quantitative relation between disease severity and net photosynthesis is proportionally greater than the corresponding reduction of healthy leaf area due to leaf blast. Carbon assimilation of infected leaves with 20% injury by *M. grisea* decreased by 50% compared to healthy leaves. It was similar to the previous results of Bastiaans and Roumen (1993). Bastiaans (1991) warned that the degree of rice plant injury by blast infection was underestimated if disease severity is considered merely by measuring visible damage alone. Maximum injury in this study was 60%, while 40% in the previous work. Many other pathosystems have been shown to have photosynthetic reductions caused by the fungal infection (Ellis et al., 1981; Rabbinge et al., 1985; McGrath and Pennypacker, 1990; Bowden and Rouse, 1991; Shtienberg, 1992).

Lower light use efficiency of diseased leaves (Fig. 2) may be explained by decreased  $\text{CO}_2$  diffusion due to increased damage to the cell walls of mesophyll cells. In addition, the surrounding, healthy-looking regions may have decreased chlorophyll content or damaged light reactions of photosynthesis by blocking electron flow in the light harvesting antennae (Novel, 1991). Our results confirmed the presence and effect of Bastiaans' virtual disease (1993) of rice leaf blast on the surrounding, healthy-looking region of infected leaves. It has been suggested that the fungus secretes enzymes or phytotoxic compounds diffusing to uncolonized parts of the leaf, or the fungus may absorb carbohydrates or nutrients from the healthy region of the infected leaves (Goto, 1965; Bastiaans, 1991).

The significantly different  $A/C_i$  curve responses of diseased and healthy leaves (Fig. 3) means the blast infection is associated with lower Rubisco enzyme activity, limitation of RuBP regeneration, and limitation of inorganic phosphate supply (Ball et al., 1987; Flanagan and Jefferies 1989). The saturation level of  $A/C_i$  means either the limitation is caused by the regeneration of RuBP or the limited supply of inorganic phosphate on ATP regeneration (Wullschleger, 1993). The rice blast infection in this study can explain the damage on Pi and RuBP supply in the dark

reaction of photosynthesis. The different slopes of  $A/C_i$  curves mean various Rubisco enzyme activities are under lower  $\text{CO}_2$  supply. Rubisco in the diseased leaves was damaged because of slower increase in carbon fixation as  $\text{CO}_2$  increased. Thus, the fungal infection affected all aspects of carbon metabolism after  $\text{CO}_2$  entered through stomata into intercellular air spaces (Ball et al., 1987). *Verticillium* sp. infection has been shown to decrease Rubisco activity on alfalfa using  $A/C_i$  curves (Pennypacker et al., 1990) and controlling ambient and 600 ppm  $\text{CO}_2$ . Due to an advanced technique, we could control various  $\text{CO}_2$  concentrations inside the chamber ( $C_a$ ): 1200, 800, 360, 200, 100 and 0 ppm with the precisely controlled  $\text{CO}_2$  injector system. We could draw more precise  $A/C_i$  curves of the diseased leaves than the before.

The negative relation between TC and DS was linearly correlated ( $r=0.478$ ). Incidentally, the relation between net photosynthesis per unit of chlorophyll ( $A/TC$ ) and DS was not significantly correlated ( $r=0.218$ , data not shown). Thus, the quality of chlorophyll in rice leaves was not changed after the fungal infection. Only a decrease in the amount of total chlorophyll could be a convincing explanation of photosynthetic decline by the fungal infection. The reduction in light use efficiency of diseased leaves can be explained by a reduction in the amount of total chlorophyll and damaged in the photosynthetic processes. Infection by *Verticillium dahliae* decreased light use efficiency in potato leaves (Bowden and Rouse, 1991).

Apparent photosynthetic rate is equal to gross photosynthetic rate minus respiration rate. Therefore an increase in respiration of the diseased leaves may account for a small fraction of the observed reduction in apparent photosynthetic rate. The increase of leaf respiration after infection was only 5-10% of the maximum photosynthetic rate and it was not significant in this study. However, the previous works (Toyoda and Suzuki, 1957; Bastiaans, 1993) have documented significant increases in  $R_d$  of rice leaves after blast infection. Several studies involving other pathosystems have shown that there is only a small increase in  $R_d$  and photorespiration associated with infection (Hall and Loomis, 1972; Mignucci and Boyer, 1979; Mitchell, 1979).

The relation between water use efficiency (WUE, mol  $\text{CO}_2$  fixed per mol  $\text{H}_2\text{O}$  transpired) and DS were fit to a negative linear line ( $p<0.0001$ ,  $r=0.526$ , Fig. 7). Greater the percentage of leaf area covered by lesions, the more the leaf transpires water vapor per unit  $\text{CO}_2$  assimilation. Bowden and Rouse (1991) reported WUE increase on *Verticillium* wilt infected potato leaves, and they warned WUE results could be extrapolated to other crop systems because of the defoliating effect of the disease.

In summary, the blast infected leaves were investigated with precisely controlled gas-exchange instrument and

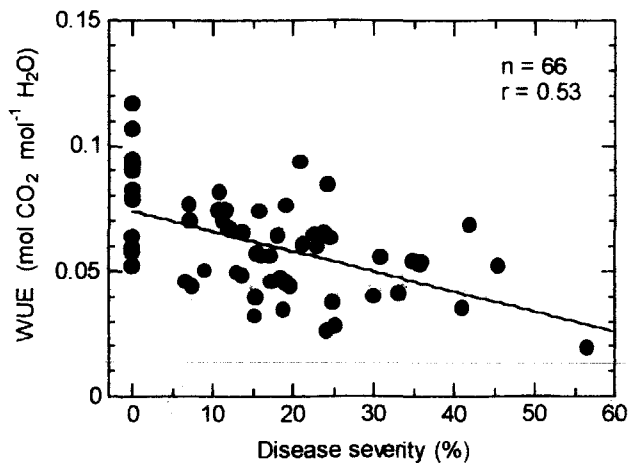


Fig. 7. Water use efficiency (WUE, mol CO<sub>2</sub> mol<sup>-1</sup> H<sub>2</sub>O) infected by *Magnaporthe grisea* in relation to disease severity as measured in a greenhouse experiment.

color image analysis system. Although there was fairly large variation on net photosynthesis in a same amount of disease, we could get a curvilinear relationship. It can be partially explained by chlorophyll contents. In addition, RuBP damage was found due to the fungal infection. However, dark respiration was not contributed to the photosynthetic decrease of the fungal infection. Our results show that the photosynthetic decrease due to blast infection on rice leaves was not a simple linear regression relationship with a large variation in nature.

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