Effects of leaf blast on photosynthesis of rice.
1. Leaf photosynthesis

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Abstract

The effect of *Pyricularia oryzae*, the causal organism of leaf blast in rice, on photosynthesis characteristics of rice leaves was measured in two greenhouse experiments. Leaf blast reduced photosynthesis not only through a reduction in green leaf area, but also through an effect on photosynthesis of green leaf tissue surrounding the lesions. The assimilation rate at light saturation (P_{max}) was more affected than the initial light use efficiency (ϵ). Dark respiration (R_d) increased as a result of infection. The experimental data were used to derive relations between leaf blast severity and P_{max}, ϵ and R_d.

Introduction

Infection of rice leaves by *Pyricularia oryzae* Cavara, the causal organism of leaf blast in rice (*Oryza sativa* L.), leads to formation of typical spindle-shaped lesions. After infection, the physiology of rice leaves is affected. Toyoda and Suzuki (1957) have observed an increased respiration in the green tissue surrounding blast lesions. This increase was connected with a shift from the glycolytic to the pentose phosphate pathway, a phenomenon generally observed in plants infected by biotrofic pathogens (Shaw and Samborski, 1957; Smedegaard-Petersen, 1984). In contrast, Burrell and Rees (1974) did not detect an increased respiration of rice leaves after infection by *P. oryzae*. Bastiaans (1991) measured photosynthetic rate of healthy and infected leaves at light saturation. The measurements showed that leaf blast reduced leaf photosynthetic rate not only through a reduction in green leaf area, but also through an effect on photosynthesis of the remaining green leaf tissue.

Knowledge of plant physiological processes and the way they are affected by a disease can be used to analyse and predict the effect of the disease on crop growth and production. Rabbinge and Rijsdijk (1981) introduced an eco-physiological approach in which effects of diseases on plant physiological processes are introduced in a mechanistic crop growth model to estimate their effect on yield. The present study deals with the first step of this approach; the quantification of the effect of the disease on relevant plant physiological processes. After inoculation with *P. oryzae*, the effect of leaf blast on photosynthesis-light response characteristics of rice leaves was determined. The experimental data were used to derive relations between leaf blast severity and the assimilation rate at light saturation (P_{max}), the initial light use efficiency (ϵ) and the rate of dark respiration (R_d). In a further study, these relations will be introduced in a model for canopy photosynthesis, to compu-

te and explain effects of leaf blast on photosynthesis of rice canopies (Bastiaans and Kropff, 1993).

Materials and methods

Plant material and inoculation. During the summers of 1988 and 1989 plants of *O. sativa* were cultivated in a greenhouse, at the DLO Centre for Agrobiological Research (CABO-DLO), Wageningen, the Netherlands. Rice plants of cultivar IR50 were grown in 21 cm diameter closed pots. Before sowing, seeds were kept in moist petri dishes for 5 days. Five germinated seeds were sown per pot. In 1988, pots were filled with sand. Nitrogen fertilizer was added at a rate of 500 mg NH_4NO_3 per pot at 12 and 22 days after sowing (DAS). In 1989, pots were filled with clay. Fertilizer was added at a rate of 350 mg N (urea), 321 mg K (K_2SO_4), and 78 mg P (KH_2PO_4) prior to sowing. From 24 DAS till 30 DAS, additional fertilizer was applied as Hoagland solution at a rate of 100 ml per pot per day (in total: 105 mg N, 118 mg K, and 16 mg P). All pots were inundated. Plants were raised without supplementary radiation. Average global radiation inside the greenhouse was 10.7 MJd^{-1} in 1988 and 7.2 MJd^{-1} in 1989. Average daylength in both years was 16 h. Temperature ranged between 30 °C and 18 °C (day/night) and relative humidity varied between 60% and 95% (day/night).

Plants were inoculated with *P. oryzae* (isolate PO6-6) at 27 (1988), 30 and 32 DAS (1989). The fungus was grown on prune agar at a temperature of 28 °C. Inoculum was prepared as described by Mackill and Bonman (1986) and the spore density was adjusted to 50 000 (1988) and 250 000 (1989) conidia ml^{-1}. Gelatin was added to the inoculum in a concentration of 2.5 gL^{-1}. Plants were sprayed until runoff, using a portable air compressor, and incubated in a moist chamber for 36 h at 23 °C. In each experiment the inoculations were restricted to one inoculum-density, since inoculations with *P. oryzae* generally lead to a large variation in lesion density (E. Roumen; pers. comm., 1988). Control plants were sprayed with a gelatin solution.

Leaf photosynthesis measurements. Equipment comparable to the type described by Louwerse and Van Oorschot (1969) was used to analyse the effect of *P. oryzae* on photosynthesis-light response characteristics of leaves. Light response curves of youngest, completely unfolded leaves were measured 7–11 days after inoculation (DC 27-29; Decimal Code, Zadoks et al., 1974). Five to six leaves were fixed in a leaf chamber. In 1988, irradiance was decreased in 5 steps from 300 to 0 $Jm^{-2}s^{-1}$ of photosynthetically active radiation (PAR; 400–700 nm). Radiation intensity was modified after stabilisation of the reading of CO_2-uptake, after approximately 30 minutes. In 1989, irradiance was increased in 5 steps from 0 to 180 $Jm^{-2}s^{-1}$ PAR. Average conditions within the leaf chamber were as follows: 340 ppm CO_2, temperature of 23 °C, and RH between 45–60%. After photosynthesis was measured, disease severity, specific leaf weight and N-content were determined. Disease severity was defined as the fraction of leaf area covered with lesions and determined according to the procedure described by Bastiaans (1991). Disease severity ranged from 0.00 to 0.08 in 1988, and from 0.00 to 0.15 in 1989.

Data-analysis. The CO_2 assimilation-light response curve for individual leaves can be described by a negative exponential function, which is characterized by the photosynthesis parameters P_{max} (net CO_2 assimilation rate at light saturation) and ϵ (initial light use efficiency), and the respiration parameter R_d (dark respiration) (Goudriaan, 1982). Dark respiration was defined as the CO_2-production measured after a dark period of 25 minutes. P_{max} and ϵ were determined by fitting the negative exponential function to the

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measured data of net photosynthetic rate, using the non linear regression option of the Genstat statistical package (Genstat 5 Reference Manual, 1987).

To analyse the effect of leaf blast, all three parameters were related to disease severity (x). For the photosynthesis parameters (P_{max}, ϵ) the concept of the virtual lesion was used (Bastiaans, 1991) :

$$P_x = P_0(1-x)^\beta \tag{1}$$

in which P_0 and P_x are the photosynthesis parameters for healthy and diseased leaf area, respectively. Parameter β is a proportionality constant, which expresses the ratio between the measured reduction in photosynthetic rate due to a single lesion and the expected reduction assuming that only the lesion is photosynthetically inactive. Dark respiration was related to disease severity by:

$$R_x = R_0(1-x)+\rho R_0 x \tag{2}$$

in which R_0 and R_x are the rate of dark respiration of healthy and diseased leaf area, respectively. This function is based on the observation that in general a respiratory increase is restricted to the area of the lesion and its near surrounding area (Samborski and Shaw, 1956; Bushnell and Allen, 1962). Here it is assumed that an increase is restricted to the visible lesion area. The parameter ρ expresses the ratio between the respiration of a lesion and that of an identical area of healthy leaf tissue. The Genstat statistical package was used to determine βp_{max}, β_ϵ and ρ.

Results

The negative exponential function gave an accurate description of the net CO_2 assimilation-light response for both healthy and diseased leaves. Estimated values for P_{max}, ϵ and R_d of healthy leaves are given in Table 1. Significant differences between both years were found for all three parameters.

Leaf blast was found to have a clear effect on the parameters which characterize the net CO_2 assimilation light-response curve (Fig. 1). The disease decreased both P_{max} and ϵ. The values of βp_{max} and β_ϵ differed significantly from one, indicating that the reduction was larger than could be explained through the reduction in green leaf area (Table 2). In both years the effect on P_{max} was larger than the effect on ϵ, but only in 1989 this difference was significant.

Dark respiration increased with increasing disease levels. Although there was a large

Table 1. Estimated parameter values ($\pm$ standard error) of net CO2 assimilation rate at light saturation (P_{max}), initial light use efficiency (ϵ), and dark repiration (R_d) of healthy leaves.

Parameter	1988 (No. = 4)			1989 (No. = 6)		
P_{max} ($\mu g\ CO_2\ m^{-2}s^{-1}$)	1406.	$\pm$ 27	(a)[1]	820.	$\pm$ 38	(b)
ϵ ($\mu g\ CO_2\ J^{-1}$)	13.7	$\pm$ 0.5	(a)	15.8	$\pm$ 0.4	(b)
R_d ($\mu g\ CO_2\ m^{-2}s^{-1}$)	28.2	$\pm$ 1.1	(a)	20.2	$\pm$ 1.9	(b)
Leaf N-content (g N m^{-2})	1.77	$\pm$ 0.03	(a)	1.11	$\pm$ 0.03	(b)
Specific leaf weight (g m^{-2})	29.1	$\pm$ 0.7	(a)	28.8	$\pm$ 0.8	(a)

[1] Means in the same row, followed by the same letter, are not significantly different according to t-test ($P < 0.05$).
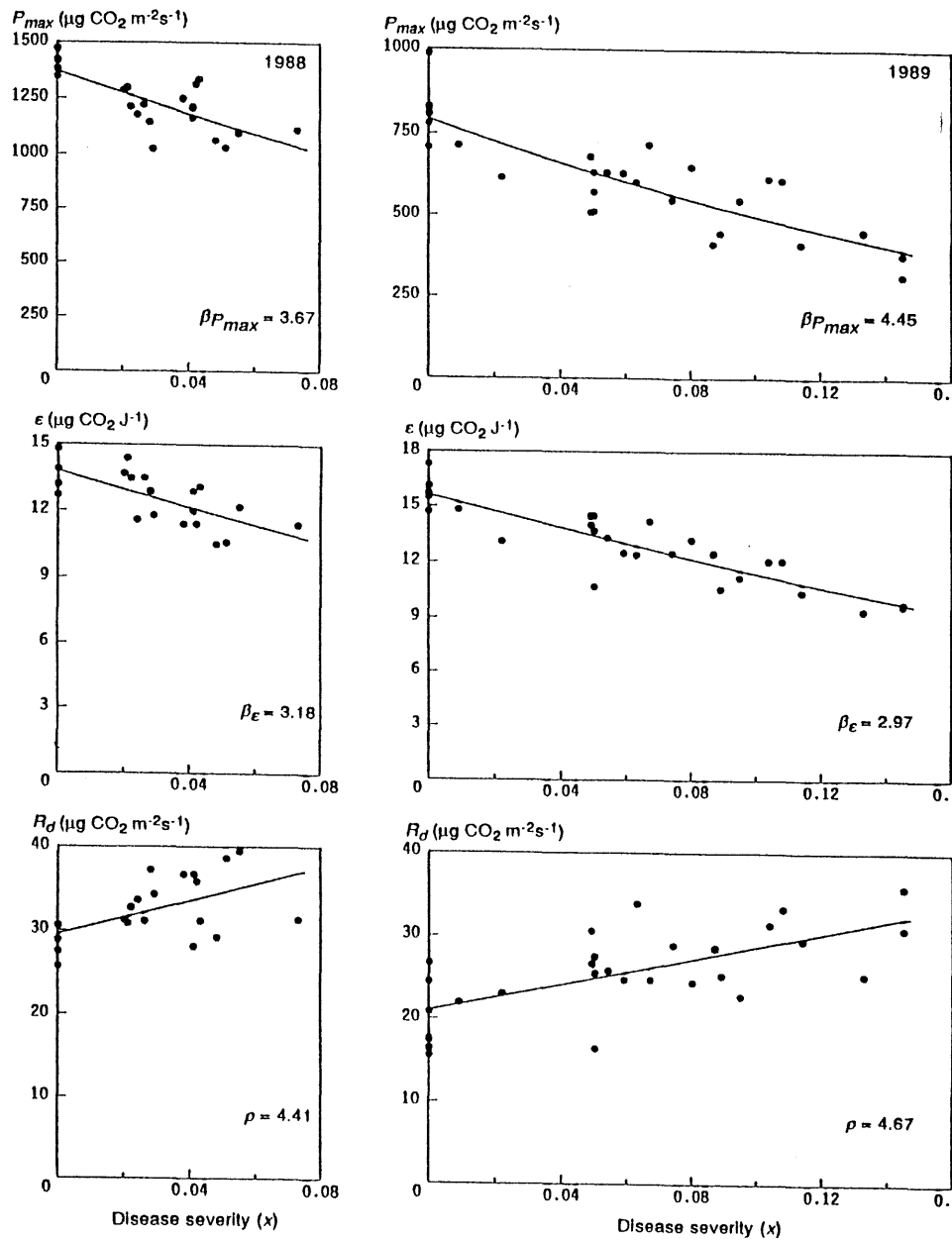
Fig. 1. Photosynthesis and respiration parameters of rice leaves infected by *Pyricularia oryzae* in relation to disease severity, as measured in two greenhouse experiments (●, observed; P_{max} is assimilation rate at light saturation; ϵ is initial light use efficiency; R_d is dark respiration). Equations 1 and 2 were used to describe the relation between disease severity and photosynthesis and respiration parameters, respectively (————).

Table 2. Estimated values ($\pm$ standard error) of parameters which characterize the effect of leaf blast on net CO_2 assimilation rate at light saturation (βp_{max}), initial light use efficiency (β_ϵ) and dark respiration (ρ) of rice leaves. For an explanation of the parameters see text.

Parameter	1988 (No.= 20)	1989 (No.= 28)
βp_{max}	3.67 ± 0.83 (a)[1]	4.45 ± 0.59 (a)
β_ϵ	3.18 ± 0.81 (a)	2.97 ± 0.31 (a)
ρ	4.41 ± 1.45 (a)	4.67 ± 0.99 (a)

[1] Means in the same row, followed by the same letter, are not significantly different according to t-test ($P < 0.05$).

variation between individual observations, the value of ρ was almost identical in both years and differed significantly from one (Table 2).

Discussion

The CO_2 assimilation-light response curve of healthy leaves of plants grown in 1988 differed significantly from the response curve of plants grown in 1989. Apart from the differences in P_{max}, ϵ and R_d, light saturation in 1989 was reached at a relatively low radiation intensity. During measurement, radiation was increased to a maximum of 180 $Jm^{-2}s^{-1}$ PAR, since at higher light intensities photoinhibition occured. This phenomenon may be explained by the lower level of radiation during the nursing of the plants grown in 1989. Plants grown under a low radiation regime tend to react as shade plants, with light saturation reached at low light intensities (Björkman, 1981). Apart from the lower radiation during the nursing of the plants, leaf N-content in 1989 was lower. Cook and Evans (1983) found a consistent and linear relation between photosynthetic rate at light saturation and leaf N-content of several *Oryza* species. Comparison with their data demonstrates that the difference in P_{max} can be attributed to the difference in leaf N-content.

The sequence of light intensities during measurement of photosynthetic rate may have contributed to the higher R_d in 1988. In 1988 light intensity was gradually decreased and dark respiration was determined at the end of the measurement period. As a result, dark respiration may have been enhanced by the production of carbohydrates during the preceding measurement of photosynthetic rate at high light intensities. A direct relation between photosynthesis in the preceding light period and the rate of dark respiration was for instance demonstrated in wheat (Azcón-Bieto and Osmond, 1983). In 1989 the measurements started with the determination of dark respiration, after which the light intensity was gradually increased.

The observed difference in ϵ between both years is surprising, since ϵ is a conservative value (Björkman, 1981). An analysis with the model of Marshall and Biscoe (1980) revealed that the leaf photosynthesis-light response in 1989 tended towards a Blackman-curve. Fitting a Blackman-type of response with a negative exponential function leads to an overestimation of ϵ, as was most likely the case in 1989. Nevertheless, the negative exponential was used, since the model of Marshall and Biscoe did not give a significantly better fit, even though it consists of one extra parameter.

Although the photosynthesis-light response curve of plants grown in 1988 differed from the response curve of plants grown in 1989, a similar effect of the pathogen was measured in both years. The reduction in P_{max} and ϵ surpassed the reduction in green leaf area, indicating that the effect of the disease is not limited to the visibly affected part of

the leaf, but exceeds the limits of the lesion. Previous measurements showed that the effect of a single blast lesion on leaf photosynthetic rate at light saturation corresponds to a reduction in leaf area of 3–4 times the area occupied by the visible lesion (Bastiaans, 1991). The present observations confirm this finding.

The different β values for the effects on P_{max} and ϵ allow some speculations to be made on the physiological basis of the effect of leaf blast on photsynthesis of rice leaves. Effects of pathogens on CO_2 assimilation can be divided into effects on photosynthetic events *per se* and effects on photosynthesis related processes (Buchanan et al., 1981). The first category of effects will lead to an increased carboxylation resistance, whereas an increased stomatal resistance is an example of the second category (Rabbinge et al., 1985). A reduced ϵ as a result of a direct effect on stomatal behaviour is unlikely. Even if diffusion of CO_2 into the leaf is impaired, it is doubtful whether CO_2 will become a limiting factor at low light intensities. Therefore, the reduction in ϵ is more likely to result from a hindered photosynthetic event *per se*. The same mechanism will be partly responsible for the reduction in P_{max}. However, the larger β value for the effect of leaf blast on P_{max} indicates that another mechanism is contributing to the overall effect at light saturation. This extra reduction may be due to either a reduced photosynthetic event *per se*, or a reduced diffusion of CO_2. Detailed physiological research is required if a better insight in the mechanisms responsible for the reduction in leaf photosynthetic rate is desired.

Toyoda and Suzuki (1957) observed an increase in the respiration of rice leaves after infection with *P. oryzae*. The present observations confirm this finding. If the increased respiration was solely attributed to an increased respiration in the visible lesion area, respiration of this part of the leaf would be 4.5 times as high as respiration of green leaf tissue. In a further study the measured effect of leaf blast on both photosynthesis and respiration will be introduced in a mechanistic model for canopy photosynthesis (Bastiaans and Kropff, 1993). This will enable a comparison of the relative importance of both damage mechanisms to the reduction in crop growth rate.

Acknowledgements

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