The Effects of Rust and Anthracnose on the Photosynthetic Competence of Diseased Bean Leaves

Daniela B. Lopes and Richard D. Berger

Department of Plant Pathology, University of Florida, 1453 Fifield Hall, Gainesville 32611.

Accepted for publication 9 November 2000.

ABSTRACT

Lopes, D. B., and Berger, R. D. 2001. The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. *Phytopathology* 91:212-220.

The effects of rust (caused by *Uromyces appendiculatus*) and anthracnose (caused by *Colletotrichum lindemuthianum*) and their interaction on the photosynthetic rates of healthy and diseased bean (*Phaseolus vulgaris*) leaves were determined by gas-exchange analysis, in plants with each disease, grown under controlled conditions. The equation $P_x/P_0 = (1-x)^\beta$ was used to relate relative photosynthetic rate (P_x/P_0) to proportional disease severity (x), where β represents the ratio between virtual and visual lesion. The β values obtained for rust were near one, indicating that the effect of the pathogen on the remaining green leaf area was minimal. The high values of β obtained for anthracnose (8.46 and 12.18) indicated that the photosynthesis in the green area beyond the necrotic symptoms of anthracnose was severely impaired. The impact of anthracnose on bean leaf photosynthesis should be considered in assessments of

the proportion of healthy tissue in diseased leaves. The accurate assessment of the healthy portion of the leaf could improve the use of concepts such as healthy leaf area duration and healthy leaf area absorption, which are valuable predictors of crop yield. The equation used to analyze the interaction between rust and anthracnose on the same leaf was $P_z = P_0 (1-x)^{\beta_x} \times (1-y)^{\beta_y}$, where P_z is the relative photosynthetic rate of any given leaf, P_0 is the maximum relative photosynthetic rate, x is anthracnose severity, y is rust severity, β_x is the β value for anthracnose in the presence of rust, and β_y is the β value for rust in the presence of anthracnose. From the resulting response surface, no interaction of the two diseases was observed. Dark respiration rate increased on diseased leaves compared with control leaves. The remaining green leaf area of leaves with both diseases was not a good source to estimate net photosynthetic rate because the effect of anthracnose extended far beyond the visual lesions, whereas the effect of rust on photosynthesis was essentially limited to the pustule plus halo.

Empirical loss models are used commonly to search for a relationship between disease intensity and yield loss, at one specific time or at several times, during a growing season. However, this relationship is often inconsistent, primarily because the highest possible yield is different for each field, locale, and season due to differences in environmental and edaphic factors. Also, the relationship between crop yield and intensity of disease can be disappointing, particularly if factors related to the host (developmental stage, defoliation, and leaf area) are not considered (39,52).

Alternatively, mechanistic approaches describe the various physiological processes of plant growth and incorporate the effects of diseases on these processes, often in a crop simulation model. The use of mechanistic models is of paramount importance for a fuller understanding of yield response to disease (17,27,28,34,44). Effect of pests and pathogens on crop carbon flow processes can be classified into seven groups: tissue consumers, leaf senescence accelerators, stand reducers, light stealers, photosynthetic rate reducers, assimilate sappers, and turgor reducers (11). The first four categories represent major effects of pests and pathogens on radiation interception, and the last three represent major effects on radiation use efficiency (22). The quantification of the damaging effects of pathogens on crop growth would make it possible to couple these effects to crop growth simulators to predict reductions in yield.

Waggoner and Berger (52) proposed that healthy leaf area duration (HAD) and healthy leaf area absorption (HAA) were much

better predictors of yield compared with disease intensity as a predictor of yield loss, because HAD and HAA add biological realism and flexibility to the empirical approaches. The concepts of HAD and HAA proved valid for the yield loss assessment of many different pathosystems, such as *Phytophthora infestans* on potato (20,38), *Alternaria solani* on potato (23), *Aschochyta fabae* on *Vicia faba* (29), *Pyricularia oryzae* on rice (33), *Blumeria graminis* on wheat (13), and *Phaeoisariopsis griseola* on common bean (8). Moreover, the use of the healthy leaf area of the host as a variable that integrates the components of the multiple disease complex has been regarded as a promising approach to multiple, crop-loss relationships (9,23,24).

In addition to its affect on the amount of intercepted radiation, some foliar pathogens can affect the efficiency of radiation use by the plant (6,7,11,12,22). In such reported cases, the assessment of HAD and HAA can be less than accurate if the effects of the disease on the healthy area are not considered (22).

The direct assessment of the photosynthetic competence of a diseased leaf, defined here as the ability of that leaf to perform photosynthesis when compared with a similar healthy leaf, allows for quantification of the impact of one or more diseases on the remaining green area of diseased leaves. Some researchers believe that this type of assessment is the most appropriate way to integrate physiological information into crop-loss studies (6,10,35,36,50,51).

Thus, our study focuses on the quantification of the effects of rust (*Uromyces appendiculatus* (Pers.:Pers) Unger) and anthracnose (*Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi. and Cav.), occurring singly or simultaneously on the same leaf, on the photosynthesis of leaves of common bean (*Phaseolus vulgaris* L.) to verify whether assessments of HAA and HAD for bean plants with these diseases should take into account any effects of each disease on radiation use efficiency.

Corresponding author: D. B. Lopes; E-mail address: dblo@uol.com.br

Florida Agricultural Experiment Station Journal Series Paper R-07635

Publication no. P-2000-1215-01R

© 2001 The American Phytopathological Society

MATERIALS AND METHODS

Plant material and inoculum. Bean plants (*Phaseolus vulgaris*) of susceptible cv. Rosinha were used in all experiments. The plants were grown, one plant per pot, in 4-liter pots filled with Metromix (Scott-Sierra Horticultural Products Co., Marysville, OH), and watered daily to field capacity. The plants were fertilized every other day with Peter's fertilizer (Peters Professional Soluble Plant Food, All Purpose 20-20-20, Grace-Sierra Horticultural Products Co., Milpitas, CA; 1 g/liter of water), unless stated otherwise. The growing point of each plant was removed above the fourth or fifth leaf, to restrict the indeterminate growth of the cultivar and to facilitate the handling of the plants.

Urediniospores of race 86 of *U. appendiculatus* were collected periodically from pustules formed on 'Rosinha' plants, allowed to dry for 24 h in a closed silica gel container, and stored at -4°C in glass vials sealed under vacuum. The viability of the spores was tested before each inoculation. A diluted suspension of urediniospores was plated on water agar, and the percentage of germinated spores after a 12-h period was determined. To prepare the inoculum, urediniospores were suspended in sterile distilled water with 0.01% Tween 20, and the suspension was agitated for 30 min. The concentration of spores was determined with a hemacytometer, and the suspension was adjusted to the desired concentration by dilution.

Conidia of *C. lindemuthianum* race kappa were produced in Mathur's C modified medium (4). Conidia were harvested from 10- to 14-day-old cultures by rinsing with sterile distilled water to prepare the concentrated spore suspension, which was diluted to obtain the desired concentrations. Viability of harvested conidia was determined when a diluted suspension of spores was spread onto the surface of water-agar medium, and the percentage of germinated conidia was observed after 12 h of incubation.

Measurements of gas exchange on bean leaves with various levels of rust severity. Bean plants were grown outdoors in two experiments. The plants were fertilized every other day and watered daily to field capacity. In a third experiment, under greenhouse conditions, plants in the greenhouse were fertilized weekly with Peter's fertilizer (2 g/liter of water). Temperatures ranged from 14 to 30°C for the outdoor experiments and from 18 to 30°C for the experiment in the greenhouse. For the experiments conducted outdoors, both sides of the expanding third trifoliate leaf were sprayed with a suspension of urediniospores, amended with 0.01% of Tween 20 and 0.05% of the humectant Metamucil (Proctor and Gamble, Cincinnati, OH). For the greenhouse experiment, the second trifoliate leaf was inoculated and each plant was enclosed for 15 h in a clear plastic bag that served as a dew chamber. Suspensions of urediniospores of different concentrations (0 , 2×10^2 , 2×10^3 , or 2×10^4 viable spores per ml) were used to obtain leaves with a wide range of rust severity.

The net photosynthetic rate at light saturation of healthy and diseased leaves was determined by gas-exchange measurements with a portable photosynthesis system (LI-6200; Li-Cor, Lincoln, NE). For the outdoor experiments, measurements were taken at a range of light intensities from 1,000 to 1,500 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (400 to 700 nm). In the greenhouse, light intensity ranged from 800 to 1,000 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. At 10 to 12 days after inoculation, when symptoms were well developed, a portion of a bean leaf still attached to the plant was placed in a 1-liter leaf chamber for 20 s, while the infrared gas analyzer measured the rate of assimilation of CO_2 . Sensors in the chamber measured CO_2 concentration, air temperature, photosynthetically active radiation, relative humidity, and vapor pressure, which were used by the instrument to calculate the intercellular CO_2 concentration, the net photosynthetic rate, expressed in micromole of CO_2 per square meter per second, and the stomatal conductance, expressed in mole of H_2O per square meter per second. The leaf was detached from the plant, and later, the rust

severity on the portion of the leaf enclosed in the chamber was determined by the number of lesions (pustule plus halo) and an estimate of the average lesion size obtained with an ocular micrometer. Chlorophyll pigments of the leaves were extracted in 80% acetone and quantified with a spectrophotometer, according to the methodology described by Arnon (1).

Measurement of gas exchange on bean leaves with various levels of anthracnose severity. The experiment was conducted twice with bean plants grown under greenhouse conditions. The temperature ranges in the greenhouse, 18 to 21°C during the night and 24 to 35°C during the day, were similar for both experiments. The light intensity inside the greenhouse ranged from 700 to 1,200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ during the experiments.

In the first experiment, the conidial suspensions used in the inoculation had 10^4 , 5×10^4 , 10^5 , or 5×10^5 viable spores per ml. These suspensions were sprayed onto both surfaces of first trifoliate leaves. Control plants were sprayed with sterile distilled water. The plants were then enclosed in plastic bags and transferred to a growth room at 21°C , where they were maintained for 30 h and returned to the greenhouse. For the second experiment, the third trifoliate leaf was inoculated and the plants were maintained in the greenhouse, enclosed in plastic bags for 16 h during the night and early morning.

Gas-exchange measurements were taken 6 and 7 days after inoculation, when symptoms were well developed. The net photosynthetic rate at light saturation and the stomatal conductance of healthy and infected leaves were determined by a portable photosynthesis system (LI-6200; Li-Cor). Measurements were taken at a range of light intensity of 700 to 1,000 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$, with the same method described for the rust experiments. In the first experiment, the severity of the measured leaves was estimated by a diagrammatic scale of anthracnose symptoms (8). In the second experiment, the necrotic lesions of each leaf were traced onto transparent plastic; the plastic, as well as the actual leaf from where the lesions were traced were run through a portable area meter (LI-3000; Li-Cor). The severity was calculated as the proportion of leaf area with lesions.

After the determination of severity on the detached leaves, the chlorophyll content of the leaves was assessed with a chlorophyll meter (SPAD-502; Minolta Co. Ltd., Ramsey, NJ). Fifteen readings of 6 mm^2 each were taken and averaged for each leaf. The readings were taken on nonnecrotic areas of the diseased leaves.

Measurement of gas exchange on bean leaves with simultaneous infection of rust and anthracnose. The experiment was conducted twice with bean plants grown under greenhouse conditions. The temperature ranges in the greenhouse, 19 to 21°C during the night and 23 to 31°C during the day, were similar for both experiments. The light intensity inside the greenhouse ranged from 700 to 1,200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ during the experiments.

Concentrated spore suspensions of *U. appendiculatus* and *C. lindemuthianum* were prepared with sterile distilled water and 0.01% Tween 20. Nine spore suspensions, resulting from the combination of three concentrations of *U. appendiculatus* spore suspension (0 , 10^3 , and 10^4 viable spores per ml) and three concentrations of *C. lindemuthianum* spore suspension (0 , 3×10^3 , and 3×10^4 viable spores per ml), were prepared by dilution. The suspensions were sprayed onto the underside of first and second trifoliate leaves in the first experiment, and onto third and fourth trifoliate leaves in the second experiment. The plants were then individually enclosed in transparent plastic bags for 16 h. Because leaves with high severity of anthracnose usually wilt rapidly after the appearance of symptoms, the three suspensions that had the higher concentration of *C. lindemuthianum* conidia were prepared and used for inoculations 3 days after the first inoculations. This sequence was used to ensure that leaves with high severity of anthracnose would be available for the measurements at the same time as leaves with the low concentrations of inoculum and the noninoculated control.

Gas-exchange measurements were taken 8 and 9 days after the first inoculation, when symptoms of rust and anthracnose were well developed. The net photosynthetic rate at light saturation of healthy and diseased leaves was determined with a portable photosynthesis system (LI-6200; Li-Cor). Measurements were taken on healthy leaves and on both inoculated leaves of each plant at a light intensity range of 700 to 1,000 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$, as described previously.

Dark respiration rate (micromole of CO_2 per square meter per second), which is the efflux of CO_2 that occurs when the leaf is in the dark, was also measured in the second experiment by the same methodology described for the determination of photosynthetic rate. To measure the dark respiration rate, healthy and inoculated leaves grown under the same conditions were adapted to the dark (0 to 2 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$) for 20 min before the readings.

After the gas-exchange measurements, the leaves were detached from the plants for the assessment of leaf area and disease severity. The leaf area was obtained by passing the leaves through a portable area meter (LI-3000; Li-Cor). Rust severity was determined from the number of lesions (pustule plus halo) per leaf and an estimate of the average lesion size. For the assessment of anthracnose severity, the necrotic lesions of each leaf were traced onto transparent plastic; the sheet was then passed through the area meter and severity was calculated as the proportion of leaf area with lesions.

Data analysis. The equation $P_x/P_0 = (1 - x)^\beta$ was used to relate relative photosynthetic rate (P_x/P_0) to disease severity (x), in the experiments where rust and anthracnose were analyzed separately. The parameter β represents the ratio between virtual and visual lesion sizes (6). Relative photosynthetic rate is the ratio of the photosynthetic rate of a diseased leaf to the average photosynthetic rate of the healthy control leaves. According to Bastiaans (6), the virtual lesion is the proportion of leaf tissue equal to or larger than the visual lesion (proportion of leaf tissue with visible symptoms), in which photosynthesis is impaired and assumed to be zero. The size of the virtual lesion is chosen such that the reduction in leaf photosynthetic rate is fully accounted for. The ratio between virtual and visual lesion size is defined as parameter β , which characterizes the effect of the pathogen on leaf photosynthesis over a range of disease severities. A β value of 1.0 would be interpreted as no detrimental effect on photosynthesis beyond the lesioned area. The parameter β was obtained by nonlinear regression, using the procedure PROC NLIN (method DUD) of SAS (SAS Institute, Cary, NC; release 6.12 for personal computers). *T* tests were applied to verify whether the β values were significantly different from one. Analysis of covariance was used to verify whether the results of the experiments could be pooled.

For the experiment with the interaction of the two diseases, the photosynthetic rates determined on healthy leaves, single-infected leaves, and double-infected leaves were plotted against the severity of rust and anthracnose in a tridimensional space. An equation derived by B. Hau (B. Hau, *personal communication*) was examined in which the multiple infection transformation was employed for the individual diseases and for the overlap of lesions of the two diseases on the same leaf. The equation to calculate the effect of both diseases on the same leaf on photosynthesis was $P_z = P_0 (1 - x)^{\beta x_2} \times (1 - y)^{\beta y_2}$, where P_z is the relative photosynthetic rate of any given leaf, P_0 is the maximum relative photosynthetic rate, x is anthracnose severity, y is rust severity, βx_2 is the β value for anthracnose in the presence of rust, and βy_2 is the β value for rust in the presence of anthracnose. The values of βx_2 and βy_2 were compared with the values of β (βx_1 and βy_1) obtained when the model $P_x/P_0 = (1 - x)^\beta$ was applied to the data for the separate diseases (single-infected leaves). This comparison was used to verify whether there was any effect of the interaction on the photosynthetic competence of the double-infected leaves. Additionally, the software Table Curve 3D (SPSS, Chicago; version 1.0) was used to compare the fit of B. Hau's equation to the fit of approximately 4,000 equations available in this software. The choice of the equation with the best fit was based on the values of the coefficient of determination, number of parameters, and the value of the *F* statistic.

RESULTS

Experiments with rust. Based on the analysis of covariance, the experiments with rust were too different to pool the results. In all three experiments, the net photosynthetic rates were reduced in leaves with more than 5% rust severity (Table 1). Reductions of photosynthetic rate were proportional to the area affected by the disease. In the third experiment, leaves that had between 70 and 90% severity had net photosynthetic rates close to zero. The chlorophyll content markedly decreased only in leaves with rust severity higher than 30%.

Stomatal conductance, which represents the degree of stomatal opening and is directly proportional to the transpiration rate, also decreased with increases in disease severity (Fig. 1A). The ratio of intercellular CO_2 to ambient CO_2 (C_i/C_a), which describes the diffusion of CO_2 from the atmosphere to intercellular spaces and is responsive to mesophyll photosynthetic capacity, slightly increased at higher severities in all three experiments (Fig. 1B).

The values of β , for the relationship of relative photosynthetic rate to rust severity, ranged from 0.88 to 1.54 (Fig. 2). These values were either not significantly different from one (0.88 and 1.38) or were statistically different (1.54) but very close to one,

TABLE 1. Net photosynthetic rate and total chlorophyll content for bean leaves with different severities of rust (*Uromyces appendiculatus*)^a

Rust severity ^b	Experiment 1		Experiment 2			Experiment 3		
	No. of leaves	Photosynthetic rate ^c $\mu\text{mol of CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	No. of leaves	Photosynthetic rate ^c $\mu\text{mol of CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	Total chlorophyll ^d mg cm^{-2}	No. of leaves	Photosynthetic rate ^c $\mu\text{mol of CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	Total chlorophyll ^d mg cm^{-2}
0.00	5	18.49 $\pm$ 0.48 ^c	10	17.55 $\pm$ 0.40	0.023 $\pm$ 0.0015	9	13.46 $\pm$ 0.55	0.024 $\pm$ 0.0012
<0.01	5	20.04 $\pm$ 1.59	0	...	...	2	14.07 $\pm$ 0.65	0.025 $\pm$ 0.0027
0.01 – 0.05	6	18.45 $\pm$ 0.86	7	16.99 $\pm$ 1.13	0.021 $\pm$ 0.0016	4	12.06 $\pm$ 0.39	0.022 $\pm$ 0.0005
0.05 – 0.10	0	...	7	14.78 $\pm$ 0.97	0.019 $\pm$ 0.0024	0	...	...
0.11 – 0.20	4	11.69 $\pm$ 3.23	9	13.18 $\pm$ 1.16	0.022 $\pm$ 0.0011	8	11.91 $\pm$ 0.45	0.024 $\pm$ 0.0012
0.21 – 0.30	7	12.54 $\pm$ 0.81	8	11.50 $\pm$ 0.91	0.021 $\pm$ 0.0007	3	10.46 $\pm$ 0.50	0.021 $\pm$ 0.0002
0.31 – 0.40	0	...	5	9.37 $\pm$ 0.65	0.015 $\pm$ 0.0017	0	...	...
0.41 – 0.50	2	8.93 $\pm$ 0.96	3	7.26 $\pm$ 0.73	0.015 $\pm$ 0.0039	0	...	...
0.51 – 0.70	0	...	0	...	...	5	5.29 $\pm$ 1.01	0.011 $\pm$ 0.0024
0.71 – 0.90	0	...	0	...	...	5	2.37 $\pm$ 0.44	0.007 $\pm$ 0.0010

^a The first and second experiments were conducted with plants growing outdoors and the third experiment was conducted in a greenhouse.

^b Proportion of leaf area with rust.

^c Mean net photosynthetic rate $\pm$ standard error of the mean, measured on second or third trifoliate leaves at a light intensity range of 800 to 1,500 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$, 10 to 12 days after inoculation.

^d Chlorophyll was extracted from individual leaves and quantified using methodology described by Arnon (1). Mean chlorophyll content $\pm$ standard error of the mean.

which is an indication that the virtual lesions and the visual lesions were of similar sizes for this combination of rust isolate and bean cultivar. The values of the coefficient of determination obtained with the model $P_x = P_0 (1 - x)^\beta$ for the three experiments (0.56, 0.66, and 0.91) were similar or higher than the values reported in the literature for this type of experiment (6,18).

Experiments with anthracnose. Based on the analysis of covariance, the results of the two experiments with anthracnose were too different to pool the results. In both experiments, the reduction in the net photosynthetic rate of leaves with anthracnose was greater than the increase of severity (Table 2). The average net photosynthetic rate of leaves with 20% severity was reduced in both experiments to approximately 30% of the rate for the healthy control leaves. The estimated chlorophyll content of nonnecrotic areas in the second experiment did not decrease significantly in leaves with up to 20% severity.

Stomatal conductance decreased with increases in disease severity (Fig. 3). This reduction in stomatal conductance was described by a negative exponential model (Fig. 3A). The ratio of intercellular CO_2 to ambient CO_2 (C_i/C_a), which describes the diffusion of CO_2 from the atmosphere to intercellular spaces, also decreased at higher severities in both experiments, according to a power function (Fig. 3B).

Fig. 1. A, Effects of severity of rust (*Uromyces appendiculatus*) on bean in stomatal conductance and **B,** on ratio of intercellular (C_i) to ambient (C_a) CO_2 concentration. The regression lines for **A** are $y = 0.69 - 0.50x$ ($R^2 = 0.77$) for experiment one, $y = 0.59 - 0.4x$ ($R^2 = 0.10$) for experiment two, and $y = 1.75 - 1.97x$ ($R^2 = 0.16$) for experiment three; The regression lines for **B** are $y = 0.86 + 0.06x$ ($R^2 = 0.54$) for experiment one, $y = 0.79 + 0.16x$ ($R^2 = 0.33$) for experiment two, and $y = 0.85 + 0.1x$ ($R^2 = 0.14$) for experiment three.

The value of β , for the relationship of relative photosynthetic rate to anthracnose severity, was 8.46 in the first experiment and 12.18 in the second experiment (Fig. 4). The β values were greater than one, which was an indication that the virtual lesion was much larger than the visual lesion for this combination of *C. lindemuthianum* isolate and bean cultivar. The values of the coefficient of determination for the two experiments (0.70 and 0.82) were very similar or higher than the values reported in the literature for this type of experiment (5,6,18). The higher coefficient of determination for the model in the second experiment compared

Fig. 2. Impact of severity (x) of rust (*Uromyces appendiculatus*) on the relative photosynthetic rate (P_x/P_0) of bean leaves. Relative photosynthetic rate is the ratio of the photosynthetic rate of a diseased leaf to the average photosynthetic rate of the healthy control leaves. The values of β were obtained with the model $P_x/P_0 = (1 - x)^\beta$; **A**, experiment one; **B**, experiment two; and **C**, experiment three.

with that for the first experiment was probably due to greater precision in the assessment of anthracnose severity in the second experiment, which may have reduced the variability of the data.

Experiments with the simultaneous infection of rust and anthracnose. The data of the two experiments were combined because there was no significant variation in the average rates of the measured variables for the healthy control plants in the two experiments. Net photosynthetic rates of single-infected or double-infected bean leaves were reduced when compared with healthy leaves (Figs. 5 and 6). Similar increases in disease severity caused greater decreases in photosynthetic rate on leaves with only anthracnose than on leaves with only rust, as described previously. The β_{x1} value for leaves with anthracnose alone was 9.039 ± 1.21 ($R^2 = 0.58$), which means that the virtual lesion was ninefold greater than the visual lesion. The calculated value of β_{y1} for the leaves with rust alone was 0.647 ± 0.078 ($R^2 = 0.60$). Values of β_{y1} less than 1.0 were interpreted as some photosynthesis occurred in the haloed areas.

When the proportion of diseased area of each leaf, including leaves with only one or both diseases, was subtracted from one, the proportion of asymptomatic tissue was obtained. This variable was not well correlated to net photosynthetic rate (Fig. 5C). The weak correlation between green leaf tissue and the physiological variables was due to the different impact of each disease on the remaining green leaf area.

The model of B. Hau, $P_z = P_0 (1 - x)^{\beta_{x2}} \times (1 - y)^{\beta_{y2}}$, had the best fit ($R^2 = 0.703$, $F = 264.02$) of the thousands of equations tested and provided accurate depiction of the zero values of net photosynthesis at high severities of anthracnose. This equation was used to build a response surface for the whole data set (Fig. 6). The values of three parameters that gave the best fit to the response surface were $P_0 = 0.99$ (SE = 0.016), $\beta_{x2} = 11.56$ (SE = 0.89), and $\beta_{y2} = 0.67$ (SE = 0.08). The β_{x2} (11.56) for anthracnose and β_{y2} (0.67) for rust that occurred simultaneously on the same leaf were not significantly different from β_{x1} (9.039) and β_{y1} (0.647) when only one of the diseases was present in the leaf.

Low severities of rust, anthracnose, and the combination of the two diseases induced increases in the dark respiration rate of diseased leaves (Fig. 7). Negative values of CO_2 exchange rate indicated efflux of CO_2 from the leaf into the equipment chamber; thus, lower values in the graph indicated higher rates of dark respiration.

DISCUSSION

Diseased leaves are not homogeneous because they have both cells directly invaded or killed by the pathogen and cells, which are not invaded but are affected by the presence of the pathogen in the tissue (42). Therefore, measurements of photosynthetic rate and related variables on the whole leaf are dependent upon the density of infection and must be compared with measurements

taken on healthy control leaves to allow the quantification of the impact of disease on leaf physiology. The virtual lesion concept, introduced by Bastiaans (6), is the basis for a model that quantifies photosynthetic competence of diseased leaves.

A virtual lesion consists of a visual lesion and an adjacent area, in which the photosynthetic activity is zero. The relation between disease severity and photosynthesis is described with a single parameter, β , the ratio of virtual and visual lesions. Values of β greater than one are interpreted as an indication that the disease not only reduced the leaf area capable of photosynthetic activity but also affected the photosynthesis in the surrounding green leaf tissue. For some pathosystems involving necrotrophic organisms, the virtual lesion could result from the production and diffusion of toxins in the surrounding area of the symptomatic tissue (6). However, diffusible toxins have not been associated with infections by biotrophic pathogens (30), and thus, biochemical studies complementary to photosynthetic measurements are needed to clarify the phenomenon.

The values of β reported for diseases caused by obligate parasites are 1.26 ± 0.17 for *Puccinia recondita* on winter wheat (6) and 8.74 ± 1.70 for *B. graminis* on winter wheat (6). The reported β values for facultative parasites are 3.04 ± 0.18 and 3.74 ± 0.19 for *Pyricularia oryzae* on rice (6), 2.68 ± 0.22 for *Xanthomonas campestris* pv. *phaseoli* on common bean (18), 7.24 ± 0.75 for *C. lindemuthianum* on common bean (5), 11.0 ± 3.5 for *Cercospora leafspot* of peanut (49), 2.1 ± 0.61 for *Rhynchosporium secalis* on barley (49), and 1.66 ± 0.34 for *Septoria nodorum* on wheat (49).

In the current study, the β values obtained for rust were not different from or were very close to one (Fig. 2), which led to the conclusion that there is little effect of rust infection on the remaining green area of leaves of ‘Rosinha’ bean. These values of β were similar to the value of 1.26 obtained for brown rust of wheat (6), based on data from Spitters et al. (47), the only other reported value for a pathosystem involving a rust pathogen. The values of β obtained for anthracnose (8.46 and 12.18) (Fig. 4) indicated that the virtual lesion induced by this pathogen was much larger than the visual lesion. Thus, there was a great impact of anthracnose on the photosynthesis of the remaining green tissue of bean leaves of ‘Rosinha’. These results are similar to the high value of β (7.24 ± 0.75) reported by Bassanezi et al. (5) for a different cultivar of common bean, ‘Carioca Comum’.

The determination of the β value, using the equation $P_x/P_0 = (1 - \text{severity})^\beta$, depends on (i) the reliability of the estimates of the average photosynthetic rate of healthy control plants (P_0), (ii) the photosynthetic rates of diseased plants (P_x) with a wide range of severities, and (iii) the proportion of leaf area affected by the disease. Some aspects related to the physiology of the plant that should be taken into consideration when determining β values include (i) photosynthetic rates should be determined at light saturation to ensure that the maximum rate (P_{max}) is obtained (6), because at high light intensities even large changes in light inten-

TABLE 2. Net photosynthetic rate and estimated chlorophyll content for bean leaves with different severities of anthracnose (*Colletotrichum lindemuthianum*)

Experiment 1			Experiment 2			
Anthracnose severity ^a	No. of leaves	Photosynthetic rate ^b μmol of CO ₂ m ⁻² s ⁻¹	Anthracnose severity ^a	No. of leaves	Photosynthetic rate ^b μmol of CO ₂ m ⁻² s ⁻¹	Chlorophyll content SPAD values ^c
0.00	13	19.27 ± 0.52	0.00	9	16.43 ± 0.55	36.53 ± 0.63
<0.01	6	14.78 ± 1.14	<0.01	5	15.06 ± 0.87	36.52 ± 0.63
0.01 – 0.05	6	10.29 ± 0.89	0.01 – 0.02	9	14.25 ± 0.62	36.41 ± 0.41
0.05 – 0.10	7	12.37 ± 0.88	0.02 – 0.03	6	12.09 ± 0.34	35.33 ± 0.96
0.11 – 0.20	8	5.99 ± 1.42	0.03 – 0.05	7	10.39 ± 0.77	35.24 ± 0.58
0.21 – 0.35	3	2.75 ± 1.30	0.05 – 0.20	4	5.07 ± 1.68	35.70 ± 1.48

^a Proportion of leaf area with symptoms.

^b Mean photosynthetic rate ± standard error, measured on first or third trifoliate leaves at a light intensity range of 700 to 1,000 μmol photon m⁻² s⁻¹, 6 to 7 days after inoculation.

^c Chlorophyll was estimated with a chlorophyll meter (SPAD-502; Minolta Co. Ltd., Ramsey, NJ); 15 readings on nonnecrotic areas were taken for each leaf. Mean amount of chlorophyll ± standard error.

sity have only small effects on photosynthetic rate; (ii) if possible, leaves of similar physiological age or position in the canopy should be selected for the measurements (18,45); and (iii) the environmental conditions in which the measurements are taken should not be very different from the conditions under which the plants were grown (14). The aspects listed above are suggested to reduce the variation in the values of photosynthetic rate obtained because an intrinsic variation among leaves is expected. The accurate assessment of the proportion of leaf area affected and the time of this assessment in relation to symptom development can also influence the β values obtained. For diseases that do not have clearly confined symptoms, it is certainly more difficult to assess severity accurately. The accuracy of disease assessment should be taken into consideration when the β values are interpreted. Also, studies on the response of the β value to low light intensities (early and late in the day or at lower depths in the canopy) and to different temperatures could help to refine the applicability of the virtual lesion concept to predictions of yield.

Several researchers have described the effects of rust on the photosynthetic competence of common bean. Decreased photosynthetic rates (25,26), increased respiration (15,25,26), changes in the patterns of translocation of photosynthates (26), and increase in invertase activity (53) were the main effects observed during infection by the rust pathogen. Also, Moll et al. (31) reported loss of chlorophyll in the pustule area and diminished chlorophyll fluorescence during rust development, which indicated

reduced photosynthetic activity. In our study, the percentage of losses of chlorophyll from leaves with rust in relation to control leaves had similar values to the proportion of the tissue visually chlorotic, which was the basis for assessments of severity (Table 1).

The stomatal conductance of bean leaves decreased with their reduced photosynthetic capacity, whereas the severities of rust increased (Fig. 1A). Stomatal functioning is integrated with photosynthesis in such a way that it optimizes the use of water while only marginally limiting the photosynthetic process (16). A positive linear relationship between conductance and carbon assimilation was observed for leaves with various nutritional and water status, age, and levels of viral infection (19,43,56). The simultaneous reduction of carbon assimilation and transpiration rate is generally caused by two different mechanisms; one based on an increase in carboxylation resistance, and a second based on an increase in stomatal resistance (16,35). Carboxylation resistance is the resistance of the mesophyll to CO_2 diffusion. Stomatal resistance, which is the inverse of stomatal conductance, represents the

Fig. 3. A, Effects of severity (x) of anthracnose (*Colletotrichum lindemuthianum*) on bean on stomatal conductance and B, on ratio of intercellular to ambient CO_2 concentration (Ci/Ca). The equations presented refer to the pooled data of experiments one and two.

Fig. 4. Impact of severity (x) of anthracnose (*Colletotrichum lindemuthianum*) on the relative photosynthetic rate (P_x/P_0) of bean leaves. Relative photosynthetic rate is the ratio of the photosynthetic rate of a diseased leaf to the average photosynthetic rate of the healthy control leaves. The values of β were obtained with the model $P_x/P_0 = (1 - x)^\beta$; A, experiment one; and B, experiment two.

degree of stomatal closure. If stomatal closure were the cause of the reduced assimilation, a reduction in the intercellular CO_2 would be observed. The slight increase in the Ci/Ca ratio in the case of leaves infected with rust is an indication that the flow of CO_2 from the stomatal cavities to the carboxylation sites was not affected (Fig. 1B). Consequently, it is possible to conclude that mesophyll resistance to carboxylation increased in leaves with rust.

C. lindemuthianum is a hemibiotrophic fungus. It exhibits a two-phase infection process involving an initial biotrophic phase, during which the pathogen establishes itself in living host cells,

Fig. 5. Net photosynthetic rate in healthy and diseased bean leaves. **A**, Leaves showing only rust symptoms; **B**, leaves showing only anthracnose symptoms; and **C**, leaves with rust, anthracnose, or both diseases. The proportion of asymptomatic tissue was obtained by subtracting rust or anthracnose severities from 1.0. **A**, The β value for rust (β_{y1}) was 0.65, and **B**, the β value for anthracnose (β_{x1}) was 9.04. The values of β were obtained with the model $P_x/P_0 = (1 - x)^\beta$.

followed by a visibly destructive phase (3,32). The impact of *C. lindemuthianum* on photosynthesis is associated with the necrotrophic phase of the infection. In plants of *Vigna sesquipedalis*, the photosynthetic rate and the chlorophyll content of the leaves were reduced and the respiration rate increased when necrotic symptoms appeared (58). Reduced photosynthetic and transpiration rates were reported for plants of *Phaseolus vulgaris* with different levels of anthracnose severity (5). *C. lindemuthianum* had no significant effect on the translocation of current photosynthate from diseased leaves of *Vigna sesquipedalis* (57). The pathogen induced only a slight accumulation of photosynthates during the first days of pathogenesis. In the present study, the stomatal conductance of leaves with anthracnose decreased sharply with the increases of severity (Fig. 3A). The drop in the Ci/Ca ratio for leaves with anthracnose (Fig. 3B) was an indication that the flow

Fig. 6. Effects of severity of anthracnose (x) and rust (y) on relative photosynthetic rate (P_z , micromole of CO_2 per square meter per second) of bean leaves. The equation for the response surface is $P_z = P_0 (1 - x)^{\beta_{x2}} \times (1 - y)^{\beta_{y2}}$, where P_0 is the maximum relative photosynthetic rate, β_{x2} is the β value for anthracnose in the presence of rust and β_{y2} is the β value for rust in the presence of anthracnose. The parameter values that gave the best fit were $P_0 = 0.99$ (SE = 0.016), $\beta_{x2} = 11.56$ (SE = 0.89), and $\beta_{y2} = 0.67$ (SE = 0.08), $R^2 = 0.703$.

Fig. 7. Dark respiration rate on healthy control leaves of bean (H), leaves with anthracnose (A) (1 to 7% severity), leaves with rust (R) (2 to 9% severity), and leaves with both diseases (A & R) (3 to 14% rust severity, 0.6 to 2% anthracnose severity). Negative values on the y axis are rates of CO_2 release. The values of the columns are averages of five to seven leaves and the vertical bars represent the standard errors of the means.

of CO₂ from the stomatal cavities to the carboxylation sites was affected and that stomatal closure was a major cause of reduced assimilation. The amounts of chlorophyll in the green area of infected leaves were not significantly affected by the disease at the levels of severity obtained (Table 2).

From the measurement of various physiological variables in healthy and diseased bean leaves, it was concluded in our study that rust and anthracnose have different patterns of impact on photosynthetic competence of individual leaves that were probably related to pathogenic events associated with the mode of nutrition of each pathogen. Anthracnose severely impairs the photosynthetic competence of bean leaves. A reduced capacity in performing photosynthesis is likely to reduce the efficiency with which the intercepted radiation is utilized by the plant. Thus, accurate assessments of HAD and HAA should account for the effects of the disease on the green leaf tissue adjacent to necrotic lesions of anthracnose. The ratio between the virtual and the visual lesion sizes for this pathosystem, which is the β parameter proposed by Bastiaans (6), could be incorporated in the calculation of HAA and HAD or in similar models used to predict yield. On the other hand, the reduction in the rates of photosynthesis of leaves infected with rust was proportional to the amount of leaf tissue with visual symptoms. It was concluded that there is probably very little interference of bean rust in the efficiency with which the intercepted radiation is utilized by the plant. Thus, it would not be necessary to correct severity assessments based on visual symptoms to have accurate assessments of HAD or HAA. It should be noted that these conclusions need to be verified for other combinations of bean cultivar and pathogen race, under field conditions.

We did not observe any interaction between anthracnose and rust on the impact of these diseases on the photosynthesis of bean leaves. The presence of rust did not change the impact caused by anthracnose on leaf photosynthesis and vice versa. This lack of an interaction was reflected on the similar β_x and β_y values obtained for leaves infected with only one of the pathogens (β_{x1} and β_{y1}) and for leaves infected with both pathogens (β_{x2} and β_{y2}).

Both rust and anthracnose, singly or combined, induced changes in the dark respiration rates of bean leaves (Fig. 7). Increased rates of dark respiration in bean leaves with rust are in accordance with previous reports on respiratory changes for this pathosystem (15,37). Wong and Thrower (57) also reported increased respiration rates at the onset of necrotic symptoms of anthracnose on *Vigna sesquipedalis*. The rate of respiration in the light is comparable with the rate of respiration in the dark (2) and contributes significantly to the total CO₂ exchange in illuminated leaves. Increased respiration was partially responsible for the reduced photosynthetic rates observed in bean leaves infected with rust and anthracnose in the present study, because the rate obtained by gas-exchange measurements was a net photosynthetic rate that included CO₂ effluxes and refixation, besides the CO₂ assimilation by the leaf tissue. Possible mechanisms of increased respiration in tissues infected by biotrophic pathogens include (i) wound respiration due to regenerative activity of tissue physically damaged by the invading pathogen; (ii) enhanced consumption of ATP and reductants through increased biosynthesis; and (iii) increased levels of starch and soluble sugars that accumulate in the cells as a result of blocked translocation (21,46). Besides the above-mentioned mechanisms, necrotrophic pathogens can also induce increases in respiration rates by causing loss of compartmentalized enzymes and key metabolites because of increased membrane permeability and uncoupling of mitochondrial electron transport from ATP synthesis (21). The last two mechanisms mentioned could be caused by pathogen toxins. There are no reports of toxins isolated from cultures of *C. lindemuthianum* or infected plant tissue; however, host nonspecific toxins that produce effects similar to the symptoms produced by the pathogens were isolated from other species of *Colletotrichum*, such as *C. nicotianae* and *C. capsici* (3).

The conditions of the experiments conducted in this study are only an indication of what can happen with natural infections in the field. Simultaneous infection of the same leaf by both pathogens is not likely to be the rule under natural conditions. Positive or negative effects of pathogen interaction on damage usually operate through changes in host physiology (54,55). Most of the synergistic interactions reported are related to sequential etiology, where the infection of a primary pathogen influences the susceptibility of the host to secondary pathogens. This phenomenon is termed biopredisposition. The negative interactions may be due to stimulation of active defense mechanisms in the plant or simply competition for plant resources.

The use of the healthy area of the host as a variable that integrates the components of the multiple disease complex has been regarded as a promising approach to multiple, crop-loss relationships (9,23,24,40). However, some diseases have unproportionally high physiological effects on crop yield, which may confuse the assessment of the healthy area (24). The proportion of healthy or green areas of a plant with two or more diseases can be assessed directly if the effects of the diseases on plant physiology are proportional to the visibly diseased tissue. However, if one or both diseases have an impact on the remaining green tissue such as bean anthracnose, the severities of each disease must be assessed separately. The size of the virtual lesion (6) could then be used to correct the assessments of disease severity and account for the impact on green tissue.

The effects of multiple constraints, such as pests, diseases, and weeds, on crop yield can be examined through response surface analysis (41,48). Savary and Zadoks (41) described the responses of peanut yield to various rust and leaf spot intensities at different attainable yields with response surfaces. The response surface analysis allows the visualization and modeling of the response of interest, when this response is influenced by several variables. In this study, the response surface was used to examine the response of physiological parameters related to leaf photosynthesis to different intensities and combinations of rust and anthracnose. The comparison of response surfaces of this kind obtained under various situations, such as different temperatures, light intensities, or at different layers of the canopy, may provide valuable information about the dynamics of pathogen interactions on the same plant.

The quantitative determination of the effects of a disease on the physiology of individual leaves is the first step toward a broader understanding of crop losses (7). The future steps are to determine disease effects on the physiology of whole plants, relating these effects to measures of plant yield, and to integrate this information on plant performance over an entire season.

ACKNOWLEDGMENTS

We thank T. Davoli for the invaluable help in the data collection and the Brazilian agency CNPq for sponsoring the Ph.D. program of D. B. Lopes.

LITERATURE CITED

1. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 14:1-15.
2. Azcón-Bieto, J., and Osmond, C. B. 1983. Relationship between photosynthesis and respiration. Plant Physiol. 71:574-581.
3. Bailey, J. A., O'Connell, R. J., Pring, R. J., and Nash, C. 1992. Infection strategies of *Colletotrichum* species. Pages 89-120 in: *Colletotrichum: Biology, Pathology, and Control*. J. A. Bailey and M. J. Jeger, eds. CAB International, Wallingford, U.K.
4. Balardin, R. S., Jarosz, A. M., and Kelly, J. D. 1997. Virulence and molecular diversity in *Colletotrichum lindemuthianum* from South, Central, and North America. Phytopathology 87:1184-1191.
5. Bassanezi, R. B., Martins, M. C., Godoy, C. V., Amorim, L., and Bergamin Filho, A. 1997. Efeito da antracnose na eficiência fotossintética do feijoeiro. Fitopatol. Bras. 22:520-524.
6. Bastiaans, L. 1991. Ratio between virtual and visual lesion size as a

- measure to describe reduction in leaf photosynthesis of rice due to leaf blast. *Phytopathology* 81:611-615.
7. Bastiaans, L., Rabbinge, R., and Zadoks, J. C. 1994. Understanding and modeling leaf blast effects on crop physiology and yield. Pages 357-380 in: *Rice Blast Disease*. R. S. Zeigler, S. A. Leong, and P. S. Teng, eds. CAB International, Wallingford, U.K.
 8. Bergamin Filho, A., Carneiro, S. M. T. P. G., Godoy, C. V., Amorim, L., Berger, R. D., and Hau, B. 1997. Angular leaf spot of *Phaseolus* beans: Relationships between disease, healthy leaf area, and yield. *Phytopathology* 87:506-515.
 9. Berger, R. D. 1988. Measuring disease intensity. *Biol. Cultural Tests* 3:1-4.
 10. Boote, K. J., Jones, J. W., Mishoe, J. W., and Berger, R. D. 1983. Coupling pests to crop growth simulators to predict yield reductions. *Phytopathology* 73:1581-1587.
 11. Boote, K. J., Jones, J. W., Smerage, G. H., Barfield, C. S., and Berger, R. D. 1980. Photosynthesis of peanut canopies as affected by leafspot and artificial defoliation. *Agron. J.* 72:247-252.
 12. Bourgeois, G., and Boote, K. J. 1992. Leaflet and canopy photosynthesis of peanut affected by late leaf spot. *Agron. J.* 84:359-366.
 13. Daamen, R. A., and Jorritsma, I. T. M. 1990. Effects of powdery mildew and weather on winter wheat yield. II. Effects of mildew epidemics. *Neth. J. Plant Pathol.* 96:35-46.
 14. Daly, J. M. 1976. The carbon balance of diseased plants: Changes in respiration, photosynthesis and translocation. Pages 450-479 in: *Physiological Plant Pathology*. Encyclopedia of Plant Physiology. R. Heitefuss and P. H. Williams, eds. Springer-Verlag, Berlin.
 15. Daly, J. M., Bell, A. A., and Krupka, L. R. 1961. Respiratory changes during development of rust diseases. *Phytopathology* 51:461-471.
 16. Farquhar, G. D., and Sharkey, T. D. 1982. Stomatal conductance and photosynthesis. *Annu. Rev. Plant Physiol.* 33:317-345.
 17. Gaunt, R. E. 1987. A mechanistic approach to yield loss assessment based on crop physiology. Pages 150-159 in: *Crop Loss Assessment and Pest Management*. P. S. Teng, ed. The American Phytopathological Society, St. Paul, MN.
 18. Goodwin, P. H. 1992. Effect of common bacterial blight on leaf photosynthesis of bean. *Can. J. Plant Pathol.* 14:203-206.
 19. Hall, A. E., and Loomis, R. S. 1972. An explanation for the difference in photosynthetic capabilities of healthy and beet yellows virus-infected sugar beets (*Beta vulgaris* L.). *Plant Physiol.* 50:576-580.
 20. Haverkort, A. J., and Bicumupaka, M. 1986. Correlation between intercepted radiation and yield of potato crops infested by *Phytophthora infestans* in central Africa. *Neth. J. Plant Pathol.* 92:239-247.
 21. Hutcheson, S. W., and Buchanan, B. 1983. Bioenergetic and metabolic disturbances in diseased plants. Pages 327-345 in: *Biochemical Plant Pathology*. J. A. Callow, ed. John Wiley & Sons, New York.
 22. Johnson, K. B. 1987. Defoliation, disease, and growth: A reply. *Phytopathology* 77:1495-1497.
 23. Johnson, K. B., and Teng, P. S. 1990. Coupling a disease progress model for early blight to a model of potato growth. *Phytopathology* 80:416-425.
 24. Kranz, J., and Jörg, E. 1989. The synecological approach in plant disease epidemiology. *Rev. Trop. Plant Pathol.* 6:27-38.
 25. Livne, A. 1964. Photosynthesis in healthy and rust-affected plants. *Plant Physiol.* 39:614-621.
 26. Livne, A., and Daly, J. M. 1966. Translocation in healthy and rust-affected beans. *Phytopathology* 56:170-175.
 27. Loomis, R. S., and Adams, S. S. 1983. Integrative analysis of host-pathogen relations. *Annu. Rev. Phytopathol.* 21:341-362.
 28. Loomis, R. S., Rabbinge, R., and Ng, E. 1979. Explanatory models in crop physiology. *Annu. Rev. Plant Physiol.* 30:339-367.
 29. Madeira, A. C., Clark, J. A., and Rossall, S. 1988. Growth, light interception and disease in field bean (*Vicia faba*): The effect of late infection of *Ascochyta fabae*. *Ann. Appl. Biol.* 112:585-595.
 30. Manners, J. M., and Gay, J. L. 1982. Transport, translocation and metabolism of ^{14}C -photosynthates at the host-parasite interface of *Pisum sativum* and *Erysiphe pisi*. *New Phytol.* 91:221-244.
 31. Moll, S., Serrano, P., and Boyle, C. 1995. In vivo chlorophyll fluorescence in rust-infected bean plants. *Angew. Bot.* 69:163-168.
 32. O'Connell, R. J., and Bailey, J. A. 1991. Hemibiotrophy in *Colletotrichum lindemuthianum*. Pages 211-222 in: *Electron Microscopy of Plant Pathogens*. K. Mendgen and D. E. Lesemann, eds. Springer-Verlag, Berlin.
 33. Pinnschmidt, H. O., and Teng, P. S. 1993. Empirical versus mechanistic approaches to adjust yield expectations for multiple pest damage on rice. (Abstr.) *Intl. Congr. Plant Pathol.* 6:116.
 34. Pinnschmidt, H. O., Teng, P. S., and Yong, L. 1994. Methodology for quantifying rice yield effects of blast. Pages 381-408 in: *Rice Blast Disease*. R. S. Zeigler, S. A. Leong, and P. S. Teng, eds. CAB International, Wallingford, U.K.
 35. Rabbinge, R., Jorritsma, I. T. M., and Schans, J. 1985. Damage components of powdery mildew in winter wheat. *Neth. J. Plant Pathol.* 91:235-247.
 36. Rabbinge, R., and Rijsdijk, F. H. 1981. Disease and crop physiology: A modeler's point of view. Pages 201-221 in: *Effects of Disease on the Physiology of the Growing Plant*. P. G. Ayres, ed. Cambridge University Press, Cambridge.
 37. Raggi, V. 1980. Correlation of CO_2 compensation point with photosynthesis and respiration and CO_2 -sensitive compensation point in rust-affected bean leaves. *Physiol. Plant Pathol.* 16:19-24.
 38. Rotem, J., Kranz, J., and Bashi, E. 1983. Measurement of healthy and diseased haulm area for assessing late blight epidemics in potatoes. *Plant Pathol.* 32:109-115.
 39. Rouse, D. I. 1988. Use of crop growth-models to predict the effects of disease. *Annu. Rev. Phytopathol.* 26:183-201.
 40. Savary, S., and Zadoks, J. C. 1992. Analysis of crop loss in the multiple pathosystem groundnut-rust-late leaf spot. I. Six experiments. *Crop Prot.* 11:99-109.
 41. Savary, S., and Zadoks, J. C. 1992. Analysis of crop loss in the multiple pathosystem groundnut-rust-late leaf spot. II. Study of the interactions between disease and crop intensification in factorial experiments. *Crop Prot.* 11:110-120.
 42. Scholes, J. D. 1992. Photosynthesis: Cellular and tissue aspects in diseased leaves. Pages 85-106 in: *Pest and Pathogens: Plant Responses to Foliar Attack*. P. C. Ayers, ed. Bios Scientific Publishers, Oxford.
 43. Schulze, E. D., and Hall, A. E. 1982. Stomatal responses, water loss and CO_2 assimilation rates of plants in contrasting environments. Pages 181-230 in: *Physiological Plant Ecology*. Vol. 2. Encyclopedia of Plant Physiology. O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, eds. Springer-Verlag, New York.
 44. Shaw, M. W., and Royle, D. J. 1989. Estimation and validation of a function describing the rate at which *Mycosphaerella graminicola* causes yield loss in winter wheat. *Ann. Appl. Biol.* 115:425-442.
 45. Shtienberg, D. 1992. Effects of foliar diseases on gas exchange processes: A comparative study. *Phytopathology* 82:760-765.
 46. Smedegaard-Petersen, V. 1984. The role of respiration and energy generation in diseased and disease-resistant plants. Pages 73-85 in: *Plant Diseases: Infection, Damage and Loss*. R. K. S. Wood and G. J. Jellis, eds. Blackwell Scientific Publications, Oxford.
 47. Spitters, C. J. T., van Roermund, H. J. W., van Nassau, H. G. M. G., Schepers, J., and Mesdag, J. 1990. Genetic variation in partial resistance to leaf rust in winter wheat: Disease progress, foliage senescence and yield reduction. *Neth. J. Plant Pathol.* 96:3-15.
 48. Teng, P. S., and Gaunt, R. E. 1980. Modeling systems of disease and yield loss in cereals. *Agric. Syst.* 6:131-154.
 49. van der Werf, W., Bastiaans, L., Rossing, W. A. H., and Rabbinge, R. 1990. Modeling crop response to growth reducing factors. Pages 87-102 in: *Proc. Int. Symp. Biotic Stress Barley Arid Semi-Arid Environ.*, Montana State University, Bozeman.
 50. van Oijen, M. 1990. Photosynthesis is not impaired in healthy tissue of blighted potato plants. *Neth. J. Plant Pathol.* 96:55-63.
 51. van Roermund, H. J. W., and Spitters, C. J. T. 1990. Simulation of yield reduction by leaf rust in winter wheat, applied to the analysis of genetic variation in partial resistance. *Neth. J. Plant Pathol.* 96:17-28.
 52. Waggoner, P. E., and Berger, R. D. 1987. Defoliation, disease and growth. *Phytopathology* 77:393-398.
 53. Wagner, S., and Boyle, C. 1995. Changes in carbohydrate, protein and chlorophyll content, and enzyme activity during the switch from uredinio- to teliospore sporulation in the bean-rust fungus *Uromyces appendiculatus* (Pers.) Link. *J. Phytopathol.* 143:633-638.
 54. Waller, J. M., and Bridge, J. 1984. Effects of pathogen interactions on tropical crop production. Pages 311-320 in: *Plant Diseases: Infection, Damage and Loss*. R. K. S. Wood and G. J. Jellis, eds. Blackwell Scientific Publications, Oxford.
 55. Weber, G. E., Gulec, S., and Kranz, J. 1994. Interaction between *Erysiphe graminis* and *Septoria nodorum* on wheat. *Plant Pathol.* 43:158-163.
 56. Wong, S. C., Cowan, I. R., and Farquhar, G. D. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* 282:424-426.
 57. Wong, P. Y. O., and Thrower, L. B. 1978. Effect of *Colletotrichum lindemuthianum* on photosynthesis and respiration of *Vigna sesquipedalis*. *Phytopathol. Z.* 92:88-94.
 58. Wong, P. Y. O., and Thrower, L. B. 1978. Sugar metabolism and translocation in *Vigna sesquipedalis* infected by *Colletotrichum lindemuthianum*. *Phytopathol. Z.* 92:102-112.