PHOTOSYNTHETIC COMPETENCE OF BEAN LEAVES WITH RUST AND ANTHRACNOSE

By

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To Ronaldo and Maria José

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TABLE OF CONTENTS

page

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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The effects of two important diseases of common bean (*Phaseolus vulgaris*) on leaf photosynthesis were studied under controlled conditions. *Uromyces appendiculatus* and *Colletotrichum lindemuthianum*, the causal agents of rust and anthracnose, respectively, were inoculated separately or together in the same leaf, and several levels of disease intensity were considered in the study.

The photosynthetic rate of leaves with rust was reduced mainly within the area of the lesion, and thus there was little effect of the disease in the remaining green area of the leaf. Increased rates of respiration and loss of chlorophyll from the leaf tissue apparently were the major factors responsible for the reduction of photosynthetic rates on diseased leaves. The optimal quantum yield and the electron transport rate, which are parameters of chlorophyll fluorescence related to the efficiency of the photosynthetic apparatus, were reduced in leaves with high rust severity after the appearance of the fleck symptoms. Both

chlorophyll content and color of the leaves were well correlated to relative photosynthetic rates on healthy and diseased leaves with different nutritional status. Leaf color and chlorophyll content could, thus, be used as potential predictors of leaf photosynthetic rate.

The reduction in the photosynthetic rates of bean leaves with anthracnose was greater than that caused by rust. The photosynthesis in the green area beyond the necrotic symptoms of anthracnose was severely impaired shortly after the appearance of the symptoms. Factors associated with the reduction in the rate of photosynthesis were decreased stomatal conductance and increased rates of respiration.

No obvious interaction was observed between the rust and anthracnose pathogens, when both were simultaneously inoculated in the same leaf. The photosynthetic rate and the electron transport rate of leaves with both diseases were determined by the proportion of leaf tissue with anthracnose.

The impact of rust and 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.

CHAPTER 1
INTRODUCTION

The fact that plant diseases cause crop losses was the very reason for the birth of plant pathology as a science, in the last century. Nevertheless, after 150 years, it is still difficult to make accurate predictions about the magnitude of loss that a specific disease (or diseases) is capable of causing in a particular season. Reliable estimates of the impact of a disease on yield are a prerequisite to the establishment of any crop protection strategy (Zadoks and Schein, 1979).

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, leaf area) are not considered (Rouse, 1988; Waggoner and Berger, 1987).

Alternatively, a mechanistic approach to loss models would be to describe the various physiological processes of plant growth and to incorporate the effects of diseases on these processes, usually as a crop simulation model. The use of mechanistic models is of paramount importance for a fuller understanding of yield response to disease (Gaunt, 1987; Loomis et al., 1979; Loomis and Adams, 1983; Pinnschmidt et al., 1994). 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 (Boote et al., 1983). The first four categories would represent major effects of pests and pathogens on radiation interception, and the last three represent major effects on radiation use efficiency (Johnson, 1987). 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.

In several publications on crop growth physiology, crop production has been shown to be closely related to the amount of leaf area available (Watson, 1947), the duration of this leaf area (Watson, 1952), and the amount of insolation the plant is able to use during the season (Charles-Edwards, 1982; Gallagher and Biscoe, 1978; Monteith, 1972; Monteith, 1981). Monteith (1977) defined crop productivity as the product of radiation interception and radiation use efficiency. As early as 1955, plant pathologists began to notice the importance of leaf area to disease assessments (Last, 1955). Diseases reduced the leaf area duration and the green leaf area (Lim and Gaunt, 1981; Lim and Gaunt, 1986a; Lim and Gaunt, 1986b). Waggoner and Berger (1987) subtracted the diseased leaf area from the leaf area integrated over time and came up with the concept of healthy leaf area duration (HAD). Since yield is determined by the energy absorbed during the season, the concept of healthy leaf area absorption (HAA) was also introduced (Waggoner and Berger, 1987).

Waggoner and Berger (1987) proposed that HAD and HAA were much better predictors of yield compared to disease intensity as a predictor of yield loss, since HAD

and HAA add biological realism and flexibility to the empirical approaches. These concepts proved valid for many different pathosystems, such as *Phytophthora infestans* on potato (Haverkort and Bicamumpaka, 1986; Rotem et al., 1983a; Rotem et al., 1983b; van Oijen 1990), *Alternaria solani* on potato (Johnson and Teng, 1990), *Aschochyta fabae* on *Vicia faba* (Madeira et al., 1988), *Pyricularia oryzae* on rice (Pinnschmidt and Teng, 1993), *Erysiphe graminis* on wheat (Daamen and Jorritsma, 1990), and *Phaeoisariopsis griseola* on common bean (Bergamin et al., 1997).

The concepts of HAD and HAA should also apply to crops with multiple pathogens and even with insect pests (Berger, 1988). Under field conditions, the attack of more than one plant pathogen at the same time is very common, especially in tropical areas (Savary and Zadoks, 1991). Nevertheless, the list of published studies on multiple pathogens, based on the synecological approach (Kranz and Jörg, 1989), in relation to crop loss, is not very extensive (Johnson, 1992; Johnson et al., 1986; Savary et al., 1988; Savary and Zadoks, 1991; Savary and Zadoks, 1992a; Savary and Zadoks, 1992b; Savary and Zadoks, 1991; Simkin and Wheeler, 1974; van de Wal and Cowan 1974; van der Wal et al., 1975}. Disease assessment becomes more complicated with more than one disease in the same plant. Several authors agree that to assess the effects of a complex of diseases, the healthy leaf area of the plant should be the focus of attention (Berger, 1988; Johnson and Teng, 1990; Kranz and Jörg, 1989). The duration and absorption of the healthy leaf area can provide a valuable measure to integrate all components in the complex (Berger, 1988).

Johnson (1987) raised several interesting questions concerning the relation of HAA and yield. First, is it enough to know the amount of intercepted radiation by the

green portion of a canopy to predict yield? For many pests and diseases this was demonstrated to be so (Waggoner and Berger, 1987), but for some other pathosystems it may be different (Johnson, 1987). Second, how do different pathogens affect radiation interception and radiation use efficiency, the factors that determine productivity? In fact, there are examples of pathogens that cause a greater reduction in photosynthesis than that expected by the severity alone (Bastiaans, 1991; Bastiaans et al., 1994; Boote et al., 1980; Bourgeois and Boote, 1992).

The determination of radiation use efficiency has been done under field conditions, by estimating the slope of the line that relates yield to HAA , in plots with different disease intensities. If the slopes are constant for the different situations, the radiation use efficiency is considered not to be affected by the disease (Aquino et al., 1992; Waggoner and Berger, 1987). However, some researchers believe that only direct measurements of photosynthetic rates in healthy and diseased plants can show whether the photosynthetic activity of the green leaf area is being affected by disease. Examples of crop loss studies that were based on the effects of the harmful agent on physiological processes are given by Rabbinge and Rijsdijk (1981), Boote et al. (1983), Rabbinge et al. (1985), van Roermund and Spitters (1990), and Bastiaans (1991).

The concept of a virtual lesion, introduced by Bastiaans (1991), can help in the classification of pathogens according to their effect on the radiation use efficiency of their hosts. According to Bastiaans (1991), 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 severely reduced. The ratio between virtual and visual lesion size is defined as the parameter β, which characterizes the effect of the pathogen on leaf

photosynthesis for an entire range of disease severities. A β value of 1.0 would be interpreted as no detrimental effect on photosynthesis beyond the lesioned area.

Thus, to extend the use of HAD and HAA to different pathosystems and to study the effect of multiple pathogens on the same plant, there is a need to determine, for specific and multiple plant-pathogen interactions, how the green tissue of the diseased plant is being affected. To address this question, the pathosystems *Phaseolus vulgaris-Uromyces appendiculatus* and *P. vulgaris-Colletotrichum lindemuthianum* were chosen as model systems for the work presented here. The effects of each disease on the photosynthetic competence of bean leaf and also the effects of the interaction of these two fungi on the same leaf were quantified. Photosynthetic competence of a diseased leaf is defined here as the ability of that leaf to perform photosynthesis when compared to a similar healthy leaf.

CHAPTER 2
LITERATURE REVIEW

Photosynthesis and Stress Physiology

Dry matter production, and consequently crop yield, is largely determined by the amount of solar radiation intercepted by the green leaf area and by radiation use efficiency (Monteith, 1977; Monteith, 1981). Thus, photosynthesis is the key physiological process to understand crop yield potential and how this potential can be affected by various stresses. Most stress factors impact photosynthesis, even if they do not affect the composition of the photosynthetic apparatus directly (Lichtenthaler, 1996).

Plant stress is a very broad concept and may be defined as "any unfavorable condition or substance that affects or blocks a plant's metabolism, growth or development" (Lichtenthaler, 1996, p.4). It includes natural stress factors, such as low or high temperatures, shortage or excess of water, attacks by pests and diseases; and anthropogenic factors, such as herbicides, excess of nutrients and air pollutants. To understand this concept, it is important to differentiate between the effects of short-term and long-term stress, as well as between low stress events, which could be overcome, and chronic stress events, which could lead to irreparable damage.

Photosynthetic rate is generally reduced in plants subjected to stress, but the mechanisms underlying this reduction are dependent on the stress factor involved. For example, water stress may cause an increase in the carbohydrate status of the plant, which

indirectly may induce the photosynthetic rate to fall. Also, stomatal closure is believed to be influential in this case (Chaves, 1991). High temperatures will directly damage the photosynthetic apparatus, which causes a loss of thylakoid membrane function and the inactivation of photosystem II (PSII) (Paulsen, 1994). Similarly, low temperatures will alter the function of membranes and enzymes in chilling-sensitive plants, which can cause damage to PS II reaction centers (Guy, 1994).

Disease as a Stress Factor

Very few generalizations can be made regarding the ways pathogens affect photosynthesis. In most host/pathogen interactions, both net and gross photosynthetic rate decline, respiration rate increases and chlorophyll is lost from the tissue as infection progresses (Scholes, 1992).

Due to the scope of the work, only the reported effects of plant pathogenic fungi will be reviewed here. Plant pathogenic fungi can be divided in two groups, based largely on their nutritional behavior: biotrophic and necrotrophic fungi. Obligate fungal pathogens, such as rusts and powdery mildews, do not kill their host plants immediately; these biotrophic fungi are dependent upon viable host tissue to complete their development. Necrotrophic fungi, such as the blight and rotting fungi, usually invade only structural tissues killed before the spread of the pathogen, and they can survive just as well on decaying or dead host tissue (Agrios, 1997). As the pattern of response and the actual reduction in photosynthesis are related to the type of trophic relationships (Shtienberg, 1992), biotrophic and necrotrophic fungi are treated separately here.

Necrotrophic Fungi

The major effect of disease in depressing crop yields is through the reduction on light interception (Haverkort and Bicamumpaka, 1986; Madeira et al., 1988; Waggoner and Berger, 1987), which clearly happens when leaves turn completely brown and necrotic as a result of infection by a necrotrophic fungus. Necrotic lesions are photosynthetically useless. However, there may be an additional effect on the photosynthesis of non-infected areas of a diseased leaf, on symptomless leaves of diseased plants, and on infected areas before cell death and necrosis occur.

For *Rhynchosporium secalis* on barley leaves, Martin (1986) observed larger reductions in maximum net photosynthesis than would be expected simply from the reduction in green leaf area. He suggested that reduced photosynthetic rates occurred in the green parts of the leaf, as well as in lesioned tissue. Also, for higher severities, there was a decrease in photorespiration; however, there was an increase in the carbon dioxide compensation point, the resistance of the mesophyll, and the resistance of the stomata to the diffusion of CO_2. Bourgeois and Boote (1992) also found a reduction in the photosynthesis of peanut leaflets infected with *Cercosporidium personatum* that was greater than expected, when the area with visible symptoms was considered. They observed that a disease severity of 15% caused a 65% reduction in photosynthetic rate. Leaf blast, caused by *Pyricularia oryzae*, also reduced net photosynthesis and transpiration of infected rice leaves beyond what would be explained by severity alone (Bastiaans, 1991; Bastiaans, 1993). Conversely, van Oijen (1990) reported that photosynthesis was not impaired in healthy tissue of potato plants infected with *Phytophthora infestans*. In this latter study, differently from the previously mentioned

studies, the CO_2 assimilation was measured on the symptomless leaves of diseased plants and not on the green area of partially diseased leaves.

Some necrotrophic fungi reduce photosynthesis by inducing defoliation (leaf senescence accelerators) and thereby reducing light interception. Boote et al. (1980) demonstrated that this happens with Cercospora leafspot on peanut. Photosynthesis of diseased canopies was reduced by loss of leaves, which abscised as a result of the infection; also, diseased leaves that remained on the plants were less efficient in fixing CO_2.

Vascular wilt fungi are necrotrophic fungi that systemically infect plants, occupying the xylem vessels and causing wilt symptoms. There is some evidence that, although stomatal conductance was reduced in plants infected with these fungi, the reduction in photosynthesis is not always due to fungal-induced water stress. From analysis of CO_2 assimilation (A) versus intercellular CO_2 concentration (Ci) response curves, it has been shown that some wilts directly affect carboxylation efficiency (Hampton et al., 1990; Pennypacker et al., 1990). Hampton et al. (1990) suggested that *Verticillium dahliae* reduced photosynthesis in cotton initially (before visible symptoms) through nonstomatal processes, which were not directly mediated by water-deficit stress. In contrast, leaves with symptoms exhibited decreased photosynthesis due to combined chlorosis, water stress, and stomatal closure. They concluded that the resultant decrease in photosynthetic capacity was the product of two distinct mechanisms of pathogenicity. Pennypacker et al. (1990), working with *Verticillium albo-atrum* on alfalfa, indicated that the reduction in the amount and activity of carboxylase enzyme (Rubisco) was the factor

responsible for reduced net photosynthesis of infected plants, rather than the reduced internal CO_2 concentrations caused by reduced stomatal conductance.

Biotrophic Fungi

It can not be assumed that all biotrophic agents act in a similar manner concerning the mechanisms of infection. Most powdery mildews form haustoria only in epidermal cells. Thus, carbohydrates and other nutrients must pass through these cells prior to uptake by the fungus. In contrast, rust and downy mildews parasitize epidermal, mesophyll and bundle sheath cells; therefore, they have a more direct access to pools of carbohydrates. Such differences in growth within the leaf may have profound consequences on the mechanism of inhibition of photosynthesis (Scholes, 1992).

Many different mechanisms have been proposed to explain reductions in the rate of photosynthesis in infections by obligate fungi. Reduced net photosynthesis associated with large increases in mesophyll resistance were reported in barley with brown rust (Owera et al., 1981) and in sugar beet with powdery mildew (Gordon and Duniway, 1982). This increase in mesophyll resistance to CO_2 diffusion was suggested to be caused by metabolic alterations within chloroplasts or changed amounts of photosynthetic machinery.

Rusts and powdery mildews were reported to affect photosynthetic electron transfer in two ways: inhibiting non-cyclic photophosphorylation and inducing loss of components of the photosynthetic electron transfer chain. Powdery mildew on sugar beet and rust on broad bean induced an inhibition of non-cyclic photophosphorylation in isolated chloroplasts (Magyarosy et al., 1976; Montalbini and Buchanan, 1974). In both of these pathosystems, inhibition resulted from a diminution of electron flow from water

to NADP. Magyarosy and Malkin (1978) observed that the cytochrome content of the electron transport chain was decreased by about one-third in chloroplasts from powdery mildew-infected sugar beet plants. However, from measurements of chlorophyll fluorescence in leaves of barley with powdery mildew, caused by *Blumeria graminis* f.sp. *hordei*, there was no direct effect of the pathogen on the capacity for electron transfer in this pathosystem (Scholes et al., 1990).

Loss of chlorophyll from the leaf tissue is commonly reported for plants infected with biotrophic pathogens; it is usually closely correlated with decreases in photosynthetic rate (McGrath and Pennypacker, 1990; Tang et al., 1996). For chloroplast measurements made within individual pustules of *Uromyces muscari* on bluebell leaves, the chloroplast volume, the chlorophyll concentration, and the ratio of chlorophyll a:b declined, but there was little reduction in chloroplast number per unit area. This was interpreted as a loss of chlorophyll from individual chloroplasts (Scholes and Farrar, 1985). In contrast, Ahmad et al. (1983) reported that the reduction in the rate of net photosynthesis in barley leaves infected with brown rust was not due to reduced CO_2 fixation per chloroplast, but was ascribed to a decrease in the number of functional chloroplasts. In isolated chloroplasts from diseased and healthy plants, Ahmad et al. (1983) found that surviving chloroplasts in diseased leaves functioned at least as well as those from healthy leaves.

RuBP carboxylase/oxygenase (Rubisco) and other enzymes of the Calvin cycle also can be affected. The infection of barley with *B. graminis* f.sp. *hordei* decreased RuBP carboxylase activity per unit area, which was attributed to a decrease in the amount of enzyme and in enzyme activity (Scholes et al., 1994; Walters and Ayres, 1984). The

activity of other enzymes of the pentose phosphate pathway (3-phosphoglycerate-kinase and glyceraldehyde-3-phosphate dehydrogenase) was also reduced after inoculation with mildew. The net result of this reduction in enzyme activity would be a decrease in the regeneration of RuBP and possibly a reduction in the storage of various carbohydrates (Walters and Ayres, 1984). Roberts and Walters (1988) found that the activity of RuBP carboxylase was reduced in rust pustule areas of leek leaves infected with *Puccinia allii* . Losses in total soluble protein and chlorophyll were observed within the pustule area but not in the region between pustules.

More recently, feedback inhibition of photosynthesis following carbohydrate accumulation has been investigated as a possible mechanism of reduction in photosynthesis (Scholes, 1992; Scholes et al., 1994). The pathosystems studied in these cases were *B. graminis* on barley and on wheat. Based on measurements of the rate of photosynthesis, chlorophyll-a fluorescence, and freeze clamping of leaves throughout the infection cycle, an increase in the activity of acid invertase and, consequently, an accumulation of glucose, fructose, and sucrose, was observed. These events resulted in the inhibition of the rate of photosynthesis in infected leaves due to loss of activity and amount of photosynthetic enzymes of the Calvin cycle. Scholes et al. (1994) suggested that the down-regulation of the Calvin cycle occurred either as a result of end-product inhibition or, more probably, as a direct effect of carbohydrates on the expression of genes encoding photosynthetic enzymes. In the sequence, electron transfer would be down-regulated in response to a decreased demand for ATP and NADPH, and photoinhibition and accelerated loss of chlorophyll from the leaf would occur. In conclusion, this pathogen was thought to be altering the source/sink relations in the leaf

(Scholes, 1992). Increased activity of acid invertase and accumulation of carbohydrate were also detected in leaves of *Arabidopsis thaliana* infected with *Albugo candida*, and these were closely correlated with the decrease in photosynthesis, chlorophyll content, and Rubisco activity in those leaves (Chou et al., 1995; Tang et al., 1996)

Measuring Photosynthesis *in vivo*

Measurements of photosynthesis *in vivo*, under the environmental conditions in which the plant is growing, is an important step to determine the status of radiation-use efficiency. The most commonly used methods to measure photosynthesis in vivo in the last decade were carbon dioxide exchange measurements and chlorophyll fluorescence emission. The principles and applications of these techniques are discussed here.

CO_2 Exchange Measurements

Measurement of CO_2 exchange with infrared gas analyzers is an instantaneous and non-destructive technique that provides an unambiguous and direct measure of the net rate of photosynthetic carbon assimilation (Long and Hallgren, 1993). Leaves, individual plants, or a stand of plants are usually enclosed in a transparent chamber and the rate of CO_2 assimilation by the plant material is determined by measuring the change in the CO_2 concentration of the air flowing across the chamber. Portable infrared gas analyzer systems have made this technique very popular for measurements of individual leaves in field conditions.

The principle of infrared gas analysis is based on the absorption of infrared radiation, at specific wavelengths, by hetero-atomic gas molecules, such as CO_2, H_2O, and NH_3 (Hall and Rao, 1994; Long and Hallgren, 1993). Gas molecules consisting of

two identical atoms, such as O_2 or N_2, do not absorb infrared radiation. The main absorption peak for CO_2 is at 4.26 μm, with secondary peaks at 2.66, 2.77, and 14.99 μm. The only other hetero-atomic gas present in air with an absorption spectrum overlapping that of CO_2 is water vapor, which could interfere significantly with the determination of CO_2 concentration. This interference can be overcome simply by drying the air or by the use of interference filters that preferentially isolate the 4.3-μm peak for detection. An infrared gas analyzer has three basic parts: an infrared source; two parallel cells, the analysis and the reference cell; and an infrared radiation detector. Equal amounts of radiation pass into the two cells. There is a continuous flow of the sample gas through the analysis cell and CO_2-free air is used in the reference cell. The detector is divided into two chambers, linked to the cells, but separated by a thin metal diaphragm. Radiation passing through the reference cell enters one chamber and radiation passing through the analysis cell enters the other. Both chambers, also filled with CO_2, will absorb radiation, the amount available for absorption being proportional to the amounts absorbed within the cells. Pressure changes within the chambers will vibrate the diaphragm and produce a voltage reading. The CO_2 assimilation rate is expressed as the amount of CO_2 assimilated per unit leaf area and time (μmol CO_2 m^{-2} s^{-1}).

The environment of the enclosed leaf or plant is influenced by the design of the chamber; thus, it is important to monitor and, if possible control, the environmental variables that are associated with the determination of CO_2 assimilation rates (Cheeseman and Lexa, 1996; Long and Hallgren, 1993). Commonly, there is a system of air-circulation within the chamber, and sensors are included to determine leaf and air

temperature, vapor pressure and relative humidity, and light intensity (photosynthetically active radiation).

The parameters that commonly can be obtained by gas exchange systems are photosynthetic rate, transpiration rate, intercellular CO_2 concentration, and stomatal conductance or resistance. The calculations of these parameters in commercial equipment are done by pre-programmed models that link the disappearance of CO_2 from the chamber to the activity of the photosynthetic apparatus (Cheeseman and Lexa, 1996).

Other models are required to interpret the measurements and the interdependencies among the variables measured. The models developed by Farquhar and his group (Farquhar et al. 1980; Farquhar and von Caemmerer, 1982) summarized more than 10 years of research in this area and are still the basis for interpretation of gas-exchange measurements. The relationships of CO_2 assimilation (A) to external limiting factors, such as quantity of incident light, ambient temperature, and CO_2 concentration, allow quantitative assessments of the effects of environmental variables on different steps in the diffusion pathway and are widely used in ecophysiological studies. For example, the initial slope of the assimilation versus intercellular CO_2 concentration (A:Ci) curve reflects the activity status of Rubisco; CO_2 assimilation at saturating light and saturating CO_2 is assumed to be limited by the potential rate of regeneration of the substrate of carboxylation (ribulose-1,5-bisphosphate, RuBP). Further, the initial slope of the relationship A vs. incident light is an estimate of the maximum light-use efficiency (or quantum efficiency) of the light reaction of photosynthesis. The light-saturated assimilation rate is considered a measurement of the photosynthetic capacity of the leaf,

as it varies with almost all environmental variables that influence photosynthesis (Beyschlag and Ryel, 1998; Long and Hallgren, 1993).

Chlorophyll Fluorescence

Chlorophyll fluorescence, obtained *in vivo* with a fluorometer, has been called, in the last decade, the "plant physiologist's stethoscope" because it measures the efficiency of the photosynthetic activity and thus can provide approximate estimates of the vitality and vigor of a plant in its environment (Bolhàr-Nordenkampf and Öquist, 1993; Foyer, 1993; Hall and Rao, 1994). Measurements of chlorophyll fluorescence have been used widely, not only in routine studies on photosynthesis, but also in other related areas, such as studies on environmental stresses, screening for stress tolerance in plant breeding, and studies on herbicide and air pollution (Foyer, 1993). The non-invasiveness, speed of data acquisition, and high sensitivity are often cited as outstanding advantages of this technique (Foyer, 1993; Schreiber et al., 1998).

When a molecule of chlorophyll absorbs light, it becomes excited to a higher energy state. An excited molecule is not stable and the electrons return rapidly to their ground energy level, releasing the absorbed photon energy basically in three ways: (1) the electronic excitation energy is transferred by resonance to another acceptor molecule, which results in photosynthetic electron transport; (2) it can release part of the excitation as heat; and (3) it can emit the rest of the energy as a photon of lower energy content and higher wavelength, a phenomenon called fluorescence (Hall and Rao, 1994). Since these decay processes are competitive, changes in the photosynthetic activity and dissipative heat emission will cause complementary changes in the intensity of the emitted fluorescence (Bolhàr-Nordenkampf and Öquist, 1993). In limiting low light and

maximum quantum yield, about 80 to 90% of the absorbed light energy will be dissipated via photosynthetic quantum conversion, 5 to 15% by heat emission and only about 0.5 to 2% by fluorescence (Lichtenthaler, 1996). Most of the fluorescence emitted at room temperature emanates from photosystem II (PSII), and thus fluorescence yield is related to the efficiency of electron transport through PSII.

Chlorophyll fluorescence displays characteristic changes in intensity, termed the Kautsky effect (Kautsky and Franck, 1943), that follow the induction of photosynthesis in previously dark-adapted plants. Upon illumination, the fluorescence yield rises quickly in the first second and then it may take several minutes to decline to a terminal level. The fast rise in fluorescence yield is related to primary processes of PSII, whereas the decline in yield is related to interactions between processes in the thylakoid membranes and metabolic processes in the stroma, primarily carbon metabolism (Bolhàr-Nordenkampf and Öquist, 1993).

The parameters used in the quantification of fluorescence emission are derived from the Kautsky curve of induction (Bolhàr-Nordenkampf and Öquist, 1993; Schreiber et al., 1998; van Kooten and Snel, 1990). Minimal fluorescence, or F_0, is an immediate rise in fluorescence following weak illumination of dark-adapted photosynthetic tissues; minimal fluorescence is measured when all reaction centers are open for primary photochemistry, and it provides a reference for all other fluorescence parameters of the induction curve. When a sufficiently strong light is applied, fluorescence increases from F_0 to a peak level, which is the maximum fluorescence, F_m. This rise reflects a gradual increase in the yield of chlorophyll fluorescence, as the reaction centers become increasingly reduced and the rate of photochemistry concurrently declines. Maximum

fluorescence is the maximum level of fluorescence, when all the reaction centers are closed and no photochemistry is taking place. Variable fluorescence, F_v, equals the fluorescence increase from F_0 to F_m. The ratio F_v/F_m is a measure of the efficiency of excitation energy capture by open PSII reaction centers, and it is usually called maximal quantum yield of PSII. This ratio has a typical range of 0.75-0.85 in non-stressed healthy plants. Quantum yield also can be assessed during illumination, and in this case it is called effective quantum yield (Genty et al., 1989). There is a ubiquitous relationship between the effective quantum yield and the quantum efficiency of photosynthetic carbon assimilation, which is the photosynthetic efficiency at any light intensity, mol CO_2/mol of photon (Genty et al., 1989; Seaton and Walker, 1990). The introduction of the effective quantum yield parameter also made it possible to estimate electron transport rate through PSII. Electron transport rate can be obtained by multiplying the effective quantum yield by the fraction of incident irradiance absorbed by PSII, which is considered to be about 84% of the incident irradiance.

The fluorescence yield is lowered, or quenched, by two fundamentally different mechanisms. The first type of reduction in fluorescence yield is essentially linked to photochemistry, as it is controlled by the rate of re-oxidation of the first stable electron acceptor to PSII. This is called photochemical quenching (qP). The second type of quenching mechanism is called non-photochemical quenching (qN), and this is mainly determined by de-excitation through heat generation. The largest contributor to non-photochemical quenching is the energy-dependent quenching created by the hydrogen ion concentration gradient across the thylakoids. This is induced in response to light saturation and it is indicative of photo-protective regulations (Bolhàr-Nordenkampf and

Öquist, 1993; Scholes and Horton, 1993). The quenching parameters are also derived from the photosynthetic induction curve (Scholes and Horton, 1993).

It is well documented that PSII antenna and reaction centers are particular sensitive to a number of stress factors, such as high temperatures, chilling, freezing, drought, and excessive radiation (Bolhàr-Nordenkampf and Lechner, 1988; Cornic and Briantais, 1991; Cornic, 1994; Öquist and Ogren, 1985; Scheuermann et al., 1991). Few reports, in this last decade, have dealt with the effects of biotic stress factors, such as plant pathogens, on the activity of PSII (Balachandran et al., 1994b; Moll et al., 1995; Raggi, 1995). Since all these stresses affect the function of PSII, directly or indirectly, fluorescence can be used as a tool to quantify the stress response and to understand stress response mechanisms (Bolhàr-Nordenkampf and Öquist, 1993).

The recent progress in chlorophyll fluorescence research is closely linked to the development of modulated fluorometers, which allow detection of fluorescence yield under normal daylight, whereas the previous techniques had to be applied only in dark environments. Numerous reviews of the principles and details of the modulated light fluorescence and the equipment developed to measure it are available in the literature (Bolhàr-Nordenkampf and Öquist, 1993; Foyer, 1993; Schreiber et al., 1986; Schreiber et al., 1993; Schreiber and Bilger, 1993).

Although commercial devices for measurement of chlorophyll fluorescence provide valuable information on the state of the photosynthetic apparatus in healthy and stressed plants, they have an intrinsic disadvantage. Fluorescence data can be collected only from one leaf point per measurement, and the researcher needs to determine the fluorescence signals of various leaf points to obtain an approximate realistic figure of the

photosynthetic metabolism (Kramer and Crofts, 1996; Lichtenthaler, 1996). Fluorescence video imaging has been used recently in situations where spatially resolved fluorescence information is desirable. The ability to take video images during saturation pulses superimposed onto continuous illumination has allowed the mapping of spatial variations in the efficiency of PSII or in qN over the leaf surface (Daley et al., 1989; Genty and Meyer, 1994; Lichtenthaler, 1996). This approach seems to be particularly suitable to study changes in the photosynthetic metabolism of diseased leaves (Balachandran et al., 1992; Balachandran et al., 1994a; Daley et al., 1989; Peterson and Aylor, 1995; Rolfe and Scholes, 1995; Scholes and Rolfe, 1995; Scholes and Rolfe, 1996). Infected leaves are often heterogeneous, consisting both of cells directly invaded by the pathogen and cells that are not invaded but that can be modified by the pathogen's presence. The changes in the photosynthetic metabolism in the different regions of an infected leaf can be quantified with fluorescence imaging systems.

CHAPTER 3
THE PHOTOSYNTHETIC COMPETENCE OF BEAN LEAVES WITH RUST

Introduction

Bean rust, caused by *Uromyces appendiculatus* (Pers.: Pers.) Unger, inflicts major production problems worldwide (Stavely and Pastor-Corrales, 1989). It is especially damaging in humid areas, where periodic severe epidemics are common. Reported yield losses range from 18-100% and these losses are directly related to the time and severity of disease (Stavely, 1984; Stavely, 1991; Townsend, 1939; Venette and Jones, 1982). Under greenhouse conditions, severe yield reduction occurred when plants were inoculated in the pre-flowering, flowering, and pod-filling stages (Almeida et al., 1977). Reliable estimates of future losses depend on the understanding of the epidemiological variables that intensify the progress of the disease (Amorim et al., 1995; Berger et al., 1995) and on accurate assessment of the impact of rust on the performance of the bean crop (Bergamin and Amorim, 1996; Iamauti, 1995).

Waggoner and Berger (1987) proposed that healthy leaf area duration (HAD) and healthy leaf area absorption (HAA) were much better predictors of yield compared to disease intensity as a predictor of yield loss, since 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 (Haverkort and Bicamumpaka, 1986; Rotem et al., 1983a; Rotem et

al., 1983b; van Oijen 1990), *Alternaria solani* on potato (Johnson and Teng, 1990), *Aschochyta fabae* on *Vicia faba* (Madeira et al., 1988), *Pyricularia oryzae* on rice (Pinnschmidt and Teng, 1993), *Blumeria graminis* on wheat (Daamen and Jorritsma, 1990), and *Phaeoisariopsis griseola* on common bean (Bergamin et al., 1997). The yield of bean plants affected by rust was related to HAD and HAA, but yield had no significant relationship with the area under the disease progress curve (AUDPC) (Iamauti, 1995). The AUDPC has been considered as the best measure of the intensity of an epidemic (Campbell and Madden, 1990).

In addition to affecting the amount of intercepted radiation, some foliar pathogens can affect the efficiency of radiation use by the plant (Bastiaans, 1991; Bastiaans et al., 1994; Boote et al., 1980; Bourgeois and Boote, 1992; Johnson, 1987). 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 (Johnson, 1987). The determination of radiation use efficiency of diseased plants, from the slope of the line that relates yield to HAA, has been done under field conditions in plots with different disease intensities. If the slopes are constant for the different situations, the radiation use efficiency is considered to be unaffected by the disease (Aquino et al., 1992; Waggoner and Berger, 1987). However, some researchers believe that only direct measurements of photosynthetic rates in healthy and diseased plants can show whether the photosynthetic activity of the green leaf area is being affected by disease (Bastiaans, 1991; Boote et al., 1983; Rabbinge and Rijsdijk, 1981; Rabbinge et al., 1985; van Oijen 1990; van Roermund and Spitters 1990). Thus, the present study focused on the quantification of the effects of rust on the photosynthesis of leaves of common bean (*Phaseolus vulgaris*)

to verify whether assessments of HAA and HAD for bean plants with rust should take into account any effects of the disease on radiation use efficiency.

Material and Methods

Plant Material and Inoculum

Bean plants (*Phaseolus vulgaris*) of the susceptible cultivar Rosinha were used in all experiments. The plants were grown, one plant per pot, in 4-liter pots filled with Metromix, and watered daily to field capacity. The plants were fertilized every other day with Peter's fertilizer (20-20-20, 1 g/l 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. This procedure did not affect the relative photosynthetic rate of diseased plants (Appendix A).

Urediniospores of race 86 of *Uromyces appendiculatus* were collected periodically from pustules formed on 'Rosinha' plants, allowed to dry for 24 hours in a closed silica gel container, and stored under -4^{o}C in glass vials submitted to vacuum. The viability of the sample was tested before each inoculation. A diluted suspension of urediniospores was plated on water-agar, and the percentage of germinated spores after a 12-hour 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 minutes. The concentration of spores was determined with a haemocytometer, and the suspension was then adjusted to the desired concentration by dilution.

Determination of Virtual Lesion Size

Bean plants were grown outdoors for Experiments 3-1 (November) and 3-2 (October), or under greenhouse conditions, for Experiment 3-3 (March). The plants were fertilized weekly with Peter's fertilizer (20-20-20, 2 g/l of water), and watered daily to field capacity. Temperatures ranged from 14 to 30^{o}C for the outdoor experiments, and from 18 to 30^{o}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. For the experiment in the greenhouse, the second leaf was inoculated and each plant was enclosed for 15 hours in a clear plastic bag, which served as a dew chamber. Suspensions of urediniospores of different concentrations (0, $2x10^2$, $2x10^3$, or $2x10^4$ viable spores/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 using the LI-6200 Portable Photosynthesis System (LI-COR, Inc.). Measurements were taken at a range of light intensities from 1000 to 1500 μmol photon $m^{-2}s^{-1}$ of photosynthetically active radiation (400-700 nm) for the experiments outdoors, and 800 to 1000 μmol photon $m^{-2}s^{-1}$ for the experiment in the greenhouse. The measurements were taken 10-12 days after the 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 seconds, while the infra-red gas analyzer measured the rate of assimilation of CO_2. Simultaneously, sensors in the chamber measured CO_2 concentration, air temperature, photosynthetically active radiation, relative humidity, and vapor pressure, which were used to calculate the net photosynthetic rate, expressed in

μmol CO_2 $m^{-2}s^{-1}$, and the stomatal conductance, expressed in mol H_2O m^{-2} s^{-1}. The leaf was then detached from the plant and, later, the rust severity was determined by the number of lesions (pustule + halo) and an estimate of the average lesion size obtained with an ocular micrometer. Chlorophyll pigments of the leaves were then extracted in 80% acetone and quantified with a spectrophotometer, according to the methodology described by Arnon (Arnon, 1949).

The equation $P_x/P_0=(1-x)^{\beta}$ was used to relate relative photosynthetic rate (P_x/P_0) to disease severity (x), where β represents the ratio between virtual and visual lesion sizes (Bastiaans, 1991). 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 parameter β was obtained by non-linear regression, using the procedure PROC NLIN (method DUD) of SAS (SAS Institute, Cary, NC; release 6.12 for personal computers). Analysis of covariance, using time as a covariate, was used with a linearized form of the Bastiaans' model (see Appendix B) to verify if the results of the three experiments could be pooled.

Effects of Fertilization on the Photosynthetic Competence of Bean Leaves with Rust

Bean plants growing under greenhouse conditions received the following fertilization treatments, applied every other day: (a) no fertilizer, (b) half of the recommended dosage of Peter's fertilizer for daily fertilization (0.5 g/l of water), (c) full recommended dosage of the fertilizer (1 g/l of water). The experiment was conducted twice. Temperatures inside the greenhouse ranged from 25 to 35^{o}C during the day, and from 22 to 25 ^{o}C during the night, for Experiment 3-4 (June). For Experiment 3-5

(March), the range of temperature was from 18 to 30^{o}C during the day, and around 18^{o}C during the night.

The expanded first trifoliate leaf of all plants was inoculated with suspensions of urediniospores. Spore concentrations of 0, $2x10^{3}$, or $2x10^{4}$ viable spores/ml were used to obtain several levels of rust severity. Control leaves of each treatment received only water with 0.01% Tween-20. Gas-exchange and fluorescence parameters were measured 10-12 days after inoculation, when symptoms were fully developed. The range of light intensity during gas-exchange and fluorescence measurements was 800-1000 μmol photon $m^{-2}s^{-1}$.

The chlorophyll content and average color of control and diseased leaves were determined. The objective of the quantification of chlorophyll and color was to verify if these variables were related to photosynthetic rate or fluorescence parameters. If there was such a relationship, it would be possible to use chlorophyll or color, which are easier to assess, to make inferences about the photosynthetic status of a specific leaf. The estimated chlorophyll content of the leaves was determined using a SPAD-502 chlorophyll meter (Minolta Co., Ltd.). The SPAD-502 measures peak chlorophyll absorbance at 650 nm and non-chlorophyll absorbance at 940 nm. A microprocessor calculates a SPAD value, which is proportional to the relative optical density, based on the ratio of absorbancies of the two wavelengths. The SPAD values correspond to actual amounts of chlorophyll present in the tissue (Appendix C); lower values of SPAD represent less chlorophyll in the tissue and more yellowing. The area measured by the equipment is very small (6 mm^{2}), thus a minimum of ten readings were taken for each leaf and then averaged.

The average color of the same leaves was determined using a Color Reader CR-10 (Minolta). The color reader has a built-in light source, which ensures uniform illumination of the object. The CR-10 expresses colors numerically in the L*a*b color space, also referred to as CIELAB. In this color space, L indicates lightness, and a and b are chromaticity coordinates, representing the red-green direction and the yellow-blue direction, respectively (Minolta, 1989). Six readings were taken and averaged for each leaf. The average reading of each leaf was then compared to the average color of the control leaves, which in this case were the healthy leaves of plants fertilized with 100% of the recommended rate. These latter leaves had the darkest green color and the most chlorophyll. The color difference (ΔEab) between any specific leaf and the average color of the controls was calculated by using the equation $\Delta Eab = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$, where ΔL, Δa, and Δb are, respectively, the differences in L, a, and b values between the leaf color and the average color of the control leaves.

The equation $P_x=P_0(1-x)^{\beta}$ was applied to relate relative photosynthetic rate (P_x/P_0) to disease severity (x), where P_x is the photosynthetic rate of a diseased leaf, and P_0 is, in this case, the average photosynthetic rate of the healthy control leaves for each fertilizer treatment. The β parameter for each fertilizer treatment was obtained by non-linear regression, using SAS. The equation $R_x=R_0(1-x)+\sigma R_0 x$, introduced by Bastiaans and Kropff in 1993, was used to relate dark respiration R_x to disease severity (x) at each fertilizer level, where R_0 is the rate of dark respiration of healthy leaves and σ expresses the ratio between the respiration of a lesioned area and that of an identical area of healthy

tissue. This function assumes that an increase in respiration is restricted to the visible lesion area (Bastiaans and Kropff, 1993).

Analysis of covariance was used with a linearized form of the Bastiaans' model to verify the significant differences among the levels of fertilizer, and also to determine if the results of the experiments could be pooled. The non-linear estimation module of the software package STATISTICA (release 5.1 for Windows, StatSoft, Inc.) was used to obtain the best fitting model to describe the relationships between color or chlorophyll to the photosynthesis variables. The evaluation of model fitness was based on the values of the coefficients of determination and the homogeneity of distribution of the residuals.

Measurement of Chlorophyll Fluorescence during Disease Development

Bean plants were grown under 300-400 μmol photon $m^{-2}s^{-1}$ and a photoperiod of 12 hours in a growth room. The temperature in the room ranged from 13 to 18^{o}C during the night, and from 24 to 32^{o}C during the day. To obtain plants with different levels of severity, suspensions of urediniospores of either of two concentrations (10^3 or 10^4 viable spores/ml) were sprayed onto both surfaces of the first trifoliate leaf. One of the three leaflets was protected from inoculation and was considered as a non-inoculated area of inoculated leaves. Control plants were sprayed with water with 0.01% Tween-20. Inoculated leaves were enclosed in clear plastic bags for 18 hours in the dark.

This experiment was conducted three times, under similar environmental conditions. In Experiments 3-6 and 3-7, three circular areas of 2 cm in diameter, on inoculated and on non-inoculated areas, were randomly marked on each leaf after inoculation. These areas contained lesioned tissue, as well as green asymptomatic tissue. Severity assessments and fluorescence measurements were performed on these marked

areas. Rust severity was determined 12-14 days after inoculation by the number of pustules and the average lesion area. The lesion area was considered to be the area of the sporulating pustule and the adjacent chlorotic halo. Leaves with 18-30% symptomatic area were considered as low severity, and leaves with 65-85% symptomatic area were considered as high severity. In Experiment 3-8, chlorophyll fluorescence was measured in smaller areas delimited by an aluminum foil frame that exposed a circular area of 1 mm in diameter. The use of this frame made it possible to measure fluorescence specifically on the lesioned area, as well as in areas between lesions, in leaves with low severities. In leaves with high severity, only measurements on lesioned areas were taken, since there were few isolated green areas in the affected tissues. In Experiment 3-8, leaves with 30-38% symptomatic area were considered as low severity, and leaves with 65-80% were high severity.

Minimal fluorescence (F_0), maximal fluorescence (F_m), optimal quantum yield (F_v/F_m), effective quantum yield, and electron transport rate were the parameters of chlorophyll fluorescence measured in attached leaves, using the PAM-2000 modulated light fluorometer (Walz, Germany). The PAM-2000 is a portable instrument that can be used to measure in vivo fluorescence of photosynthesizing plant tissue. Modulated chlorophyll fluorescence techniques use the repetitive application of brief saturated light pulses in addition to the continuous actinic illumination used to drive photosynthesis (Foyer, 1993). Minimal fluorescence, maximal fluorescence, and optimal quantum yield were determined on leaves that were dark-adapted for 20 minutes. A modulated light beam of very low intensity was applied to the dark-adapted leaf, which induced a weak initial rise in fluorescence to a low level (F_0). This response is considered to be the

emission of fluorescence which occurs when all PSII reaction centers are open. A brief strong light pulse was then added to the modulated beam to cause light saturation and to close all reaction centers at once (F_m). Optimal quantum yield was calculated from F_0 and F_m , and it is considered as an indication of the potential photosynthetic efficiency of a leaf. Measurements similar to the ones taken on dark-adapted leaves were then obtained when the leaves were re-adapted to 300-400 μmol photon $m^{-2}s^{-1}$, to calculate the effective quantum yield and the electron transport rate.

The fluorescence parameters were determined on all plants before inoculation and then at frequent intervals during a period of 14 days. For Experiment 3-7, the chlorophyll of leaves of similar age and severity as those of the measured leaves was extracted in 80% acetone and quantified on days 7, 10, and 14 after inoculation, according to the procedure described by Arnon (1949). The response of each fluorescence parameter over time, for healthy plants and plants with low or high severity, was analyzed with the SAS repeated measures procedure.

Results

Net Photosynthetic Rate, Chlorophyll Content, and Stomatal Conductance

In all three experiments, the net photosynthetic rates were reduced in leaves with more than 5% rust severity (Table 3.1). Reductions of photosynthetic rate were proportional to the area affected by the disease. In Experiment 3-3, leaves that had

Table 3.1. Net photosynthetic rate and total chlorophyll content for bean leaves with different levels of rust severity.

	Experiment 3-1		Experiment 3-2			Experiment 3-3		
Rust severity[a]	No. of leaves	Photosynthetic rate μmol CO_2 $m^{-2}s^{-1}$	No. of leaves	Photosynthetic rate μmol CO_2 $m^{-2}s^{-1}$	Total chlorophyll[c] mg/cm^2	No. of leaves	Photosynthetic rate μmol CO_2 $m^{-2}s^{-1}$	Total chlorophyll mg/cm^2
0.00	5	18.49±0.48[b]	10	17.55±0.40	0.023±0.0015[d]	9	13.46±0.55	0.024±0.0012
<0.01	5	20.04±1.59	0	-	-	2	14.07±0.65	0.025±0.0027
0.01-0.05	6	18.45±0.86	7	16.99±1.13	0.021±0.0016	4	12.06±0.39	0.022±0.0005
0.05-0.10	0	-	7	14.78±0.97	0.019±0.0024	0	-	-
0.11-0.20	4	11.69±3.23	9	13.18±1.16	0.022±0.0011	8	11.91±0.45	0.024±0.0012
0.21-0.30	7	12.54±0.81	8	11.5±0.91	0.021±0.0007	3	10.46±0.50	0.021±0.0002
0.31-0.40	0	-	5	9.37±0.65	0.015±0.0017	0	-	-
0.41-0.50	2	8.93±0.96	3	7.26±0.73	0.015±0.0039	0	-	-
0.51-0.70	0	-	0	-	-	5	5.29±1.01	0.011±0.0024
0.71-0.90	0	-	0	-	-	5	2.37±0.44	0.007±0.0010

[a] Proportion of leaf area with rust.
[b] Mean net photosynthetic rate ± standard error of the mean.
[c] Chlorophyll was extracted from individual leaves and quantified using methodology described by Arnon (1949).
[d] Mean chlorophyll content ± standard error of the mean.

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 (Figure 3.1a). The ratio of intercellular CO_2 to ambient CO_2 (Ci/Ca), 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 (Figure 3.1b).

Virtual Lesion Size

The values of β, for the relationship of relative photosynthetic rate to rust severity, ranged from 0.88 to 1.54 (Figure 3.2). These values were either not significantly different from one or were very close to one, 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 were similar or higher than the values reported in the literature for this type of experiment (Bastiaans, 1991; Goodwin, 1992). The higher coefficient of determination for the experiment conducted in the greenhouse (Experiment 3-3) than in the experiments conducted outside is probably due to the better control of environmental conditions.

Effects of Fertilization on the Photosynthetic Competence of Bean Leaves with Rust

The nutritional condition of the leaf determined its level of photosynthetic activity. The absolute average values of net photosynthetic rate, chlorophyll content, and

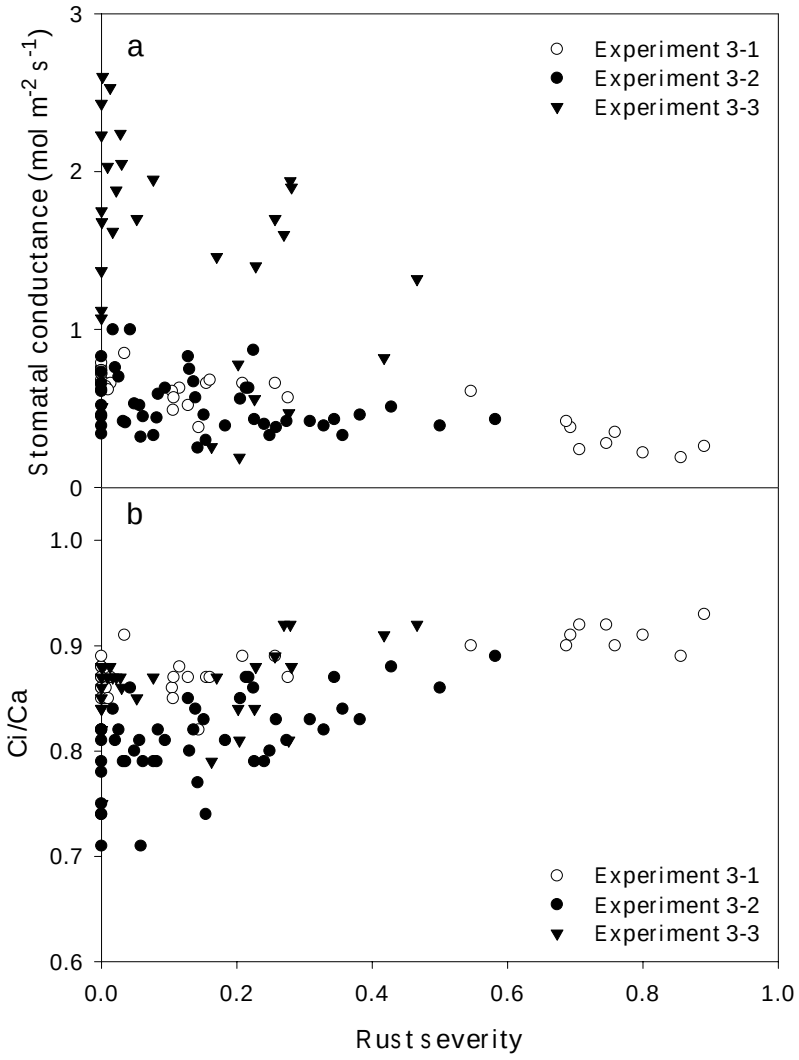

Figure 3.1. Effects of rust severity on bean on (a) stomatal conductance and on (b) ratio of intercellular to ambient CO_2 concentration (Ci/Ca). The regression lines for (a) are y=0.69-0.50x (r^2=0.77) for Exp. 3-1, y=0.59-0.4x (r^2=0.10) for Exp. 3-2, and 1.75-1.97x (r^2=0.16) for Exp. 3-3; for (b) the equations are y=0.86+0.06x(r^2=0.54) for Exp. 3-1, y=0.79+0.16x (r^2=0.33) for Exp. 3-2, and y=0.85+0.1x (r^2=0.14) for Exp. 3-3.

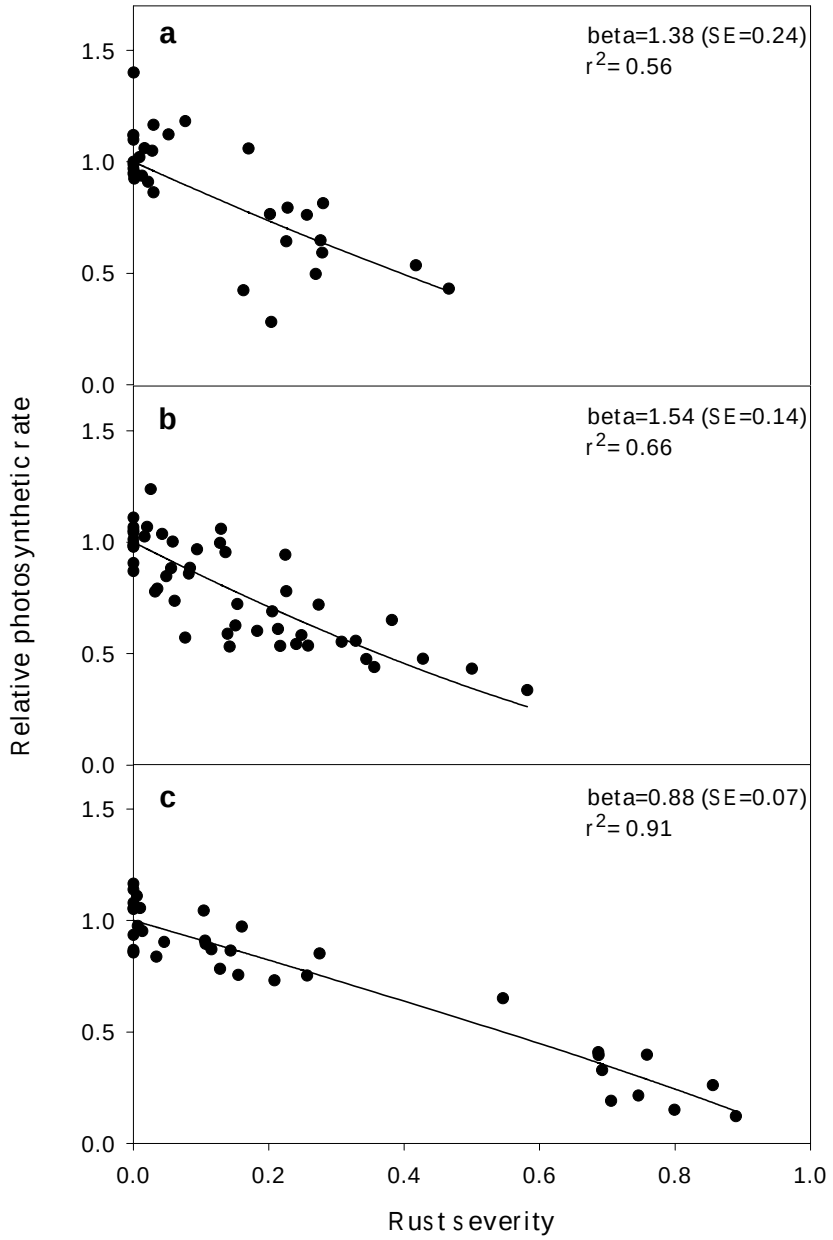

Figure 3.2. Effects of rust severity (x) on the relative photosynthetic rate (P_x/P_0) of bean leaves. The values of beta (β) were obtained with the model $P_x/P_0 = (1-x)^{\beta}$; (a) Experiment 3-1, (b) Experiment 3-2, (c) Experiment 3-3.

electron transport rate for the healthy control leaves increased with increasing rates of fertilizer (Table 3.2). Dark respiration was higher in diseased plants than in the healthy controls in all fertilizer treatments (Figure 3.3) in Experiment 3-5. According to the equation proposed by Bastiaans (1993), $R_x=R_0(1-x)+\sigma R_0 x$, respiration rates in the lesions were 4.8, 6.3, and 5.1 times higher than respiration of green leaf tissue, respectively, for plants with 0%, 50%, and 100% of the recommended rate of fertilizer (Figure 3.3a). Also, almost twice as much chlorophyll was lost in non-fertilized plants than in fertilized ones in both Experiments 2-4 and 2-5, as rust severity increased (Figure 3.3b).

In both experiments, the values of β, the parameter that describes the relationship between relative photosynthetic rate and disease severity, were significantly lower for the group of plants that received no fertilizer (1.66 for Exp. 3-4 and 1.14 for Experiment 3-5) than the values obtained for the plants that received 100% of the recommended rate of fertilizer (2.42 for Experiment 3-4 and 1.82 for Experiment 3-5) (Figure 3.4). For Experiment 3-4, β values were 1.66, 2.15, and 2.42 for 0, 50%, and 100% fertilizer rates, respectively. These values were all significantly different from one. For Experiment 3-5, the β values of 1.14 for the treatment with no fertilizer and 1.13 with 50% of the fertilizer rate were not significantly greater than one, but the β value of 1.82 for the treatment with 100% of the fertilizer rate was greater than one. The values of the β parameter different from one were interpreted as a virtual lesion area slightly larger than the visual lesion in these experiments.

For the combined data of Experiments 3-4 and 3-5, the apparent quantum yield of CO_2 assimilation (mol CO_2 assimilated/mol of quanta absorbed) of healthy and diseased

Table 3.2. Absolute average values of net photosynthetic rate, chlorophyll content, and electron transport rate for healthy control leaves of bean.

Fertilizer treatment	Experiment 3-4			Experiment 3-5		
	Photosynthetic rate μmol CO_2 $m^{-2}s^{-1}$	Chlorophyll content SPAD values[b]	Electron transport rate μmol CO_2 $m^{-2}s^{-1}$	Photosynthetic rate μmol CO_2 $m^{-2}s^{-1}$	Chlorophyll content SPAD values	Electron transport rate μmol CO_2 $m^{-2}s^{-1}$
No fertilizer	9.61±0.7[a]	31.02±1.7	62.26±5.5	8.85±0.3	25.92±0.6	72.97±3.5
50% fertilizer rate	13.10±0.4	34.13±0.6	72.50±5.2	16.34±0.8	31.17±0.7	105.34±4.9
100% fertilizer rate	16.73±1.2	38.52±2.2	108.25±5.7	18.00±0.8	38.00±0.9	133.55±8.7

[a] Mean net photosynthetic rate ± standard error of the mean.
[b] Values obtained with the Minolta Chlorophyll Meter SPAD-502; 15 readings on non-necrotic areas were taken for each leaf.

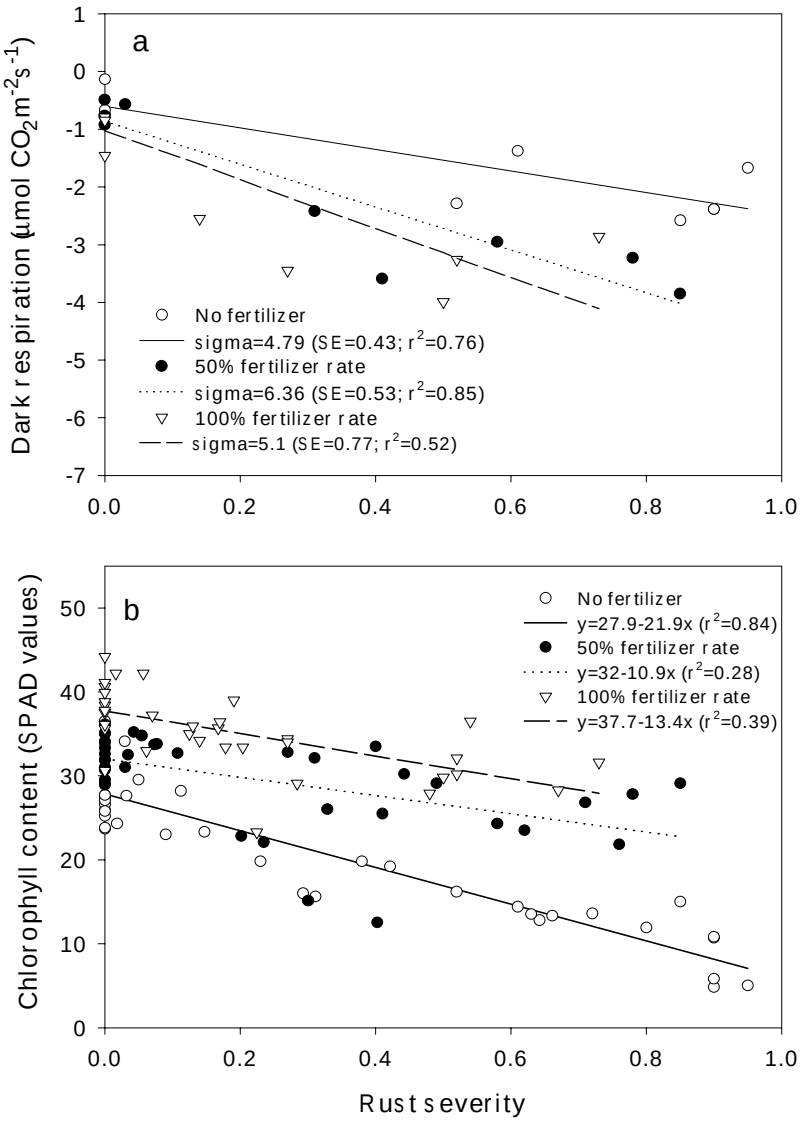

Figure 3.3. Effects of rust severity on (a) dark respiration (R_x) (Experiment 3-5) and (b) chlorophyll content of bean leaves (combined data from Experiments 3-4 and 3-5) with different nutritional status. Sigma (σ) values were obtained with the equation $R_x=R_0(1-x)+\sigma R_0 x$, where R_0 is the average dark respiration of the healthy leaves, and x is the rust severity, expressed as a proportion of the leaf area. SPAD values are the values given by the chlorophyll meter (SPAD-502).

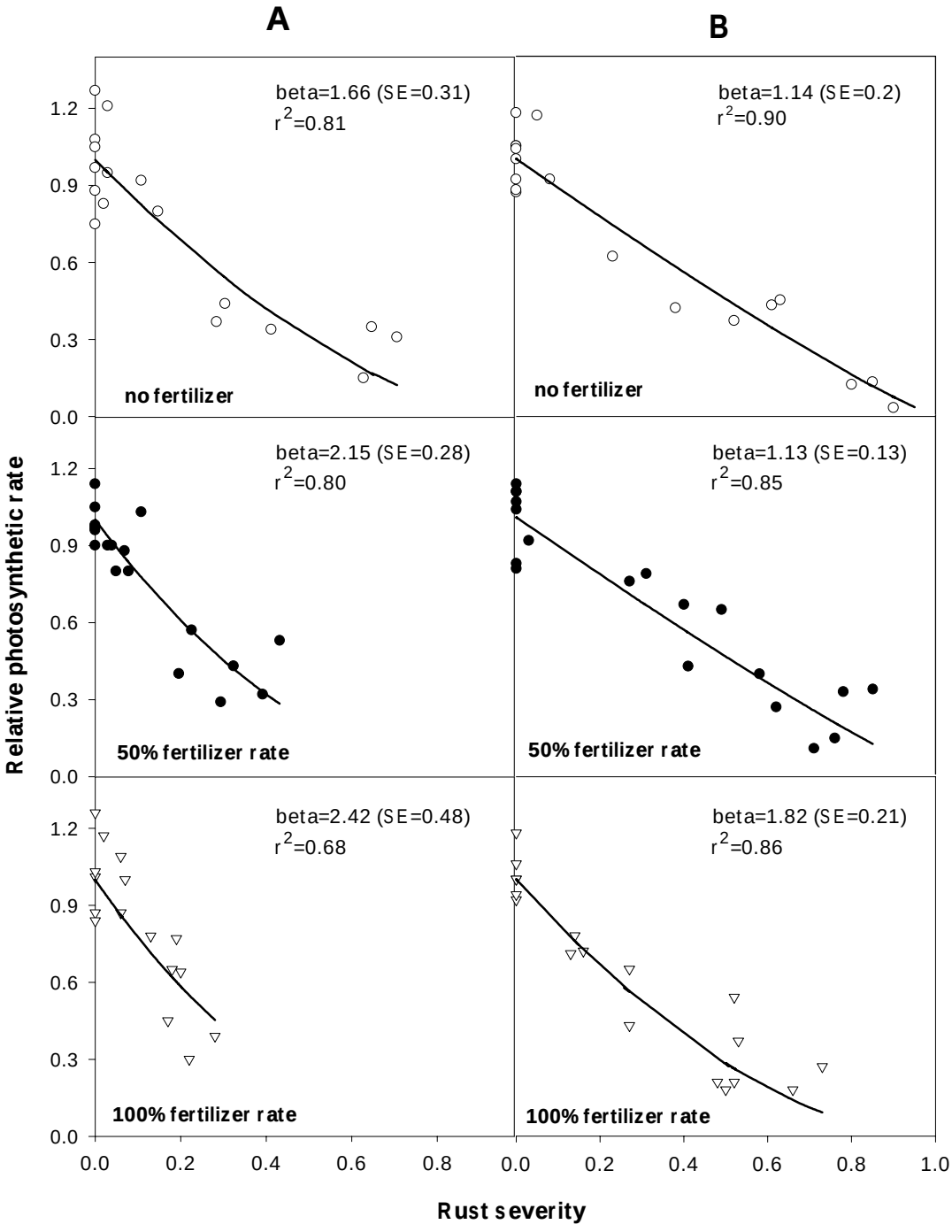

Figure 3.4. Effects of rust severity (x) on the relative photosynthetic rate (P_x/P_0) of bean leaves with different nutritional status; The values of beta (β) were obtained with the model $P_x/P_0 = (1-x)^{\beta}$; (A) Experiment 3-4; (B) Experiment 3-5.

leaves with the three levels of nutrition correlated well with the effective quantum yield obtained through fluorescence measurements (Figure 3.5). Also, color differences and estimated chlorophyll content had good relationships with both relative photosynthetic rate and relative electron transport rate, best described by a negative exponential model (Figures 3.6 and 3.7). The greater the difference between the color of a diseased leaf and the average color of healthy, well fertilized leaves, the lower the photosynthetic and electron transport rates. Higher contents of chlorophyll corresponded to higher photosynthetic and electron transport rates.

Measurement of Chlorophyll Fluorescence during Disease Development

Experiments 3-6 and 3-7 had similar results; thus, only the results of Experiment 3-7 are presented here. The data from Experiment 3-6 are presented in Appendix D. Relative changes in the parameters of chlorophyll fluorescence were first observed during the fleck stage, 5 to 7 days after inoculation, for the group of leaves that later had 65-85% of rust severity (Figures 3.8 and 3.9). All the fluorescence parameters were reduced in the presence of high rust severity. Fourteen days after inoculation, when disease symptoms were very evident, the values of minimal fluorescence (F_0), maximal fluorescence (F_m), optimal quantum yield (F_v/F_m), effective quantum yield, and electron transport rate were, respectively, 69%, 39%, 74%, 57%, and 56% of the average values of these parameters in healthy control leaves.

The average chlorophyll content of the leaves with high severity (65-85% severity) was 54% lower than the content of healthy control leaves at the fleck stage (Figure 3.10). At 14 days after inoculation, when chlorotic halos surrounding the

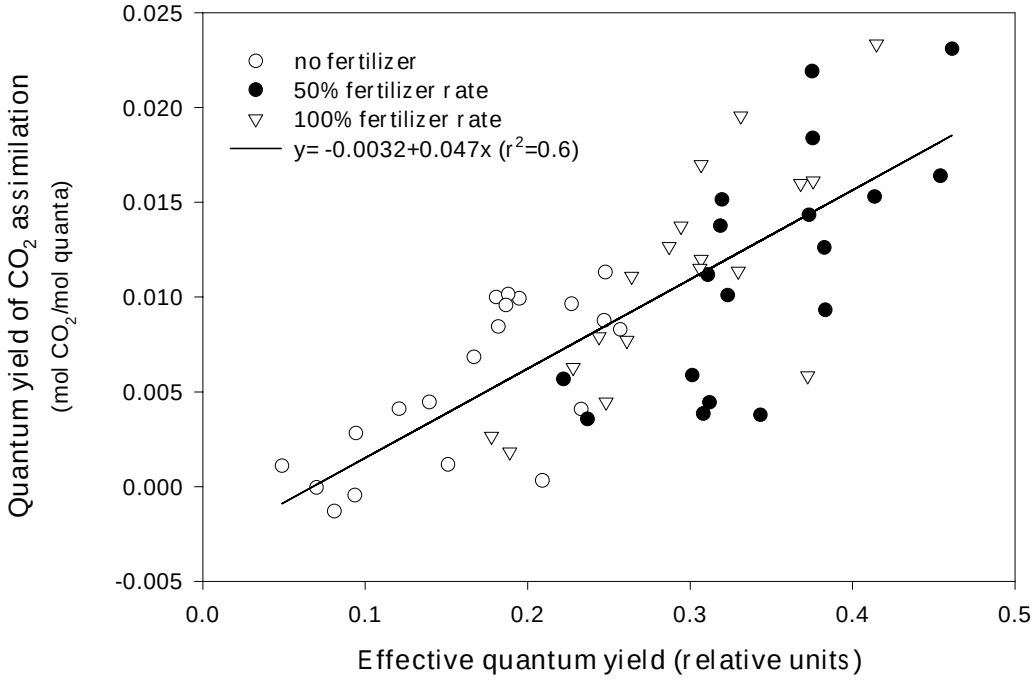

Figure 3.5. Relationship between effective quantum yield of photosystem II and quantum yield of CO_2 assimilation in bean plants with different levels of rust severity and nutritional status.

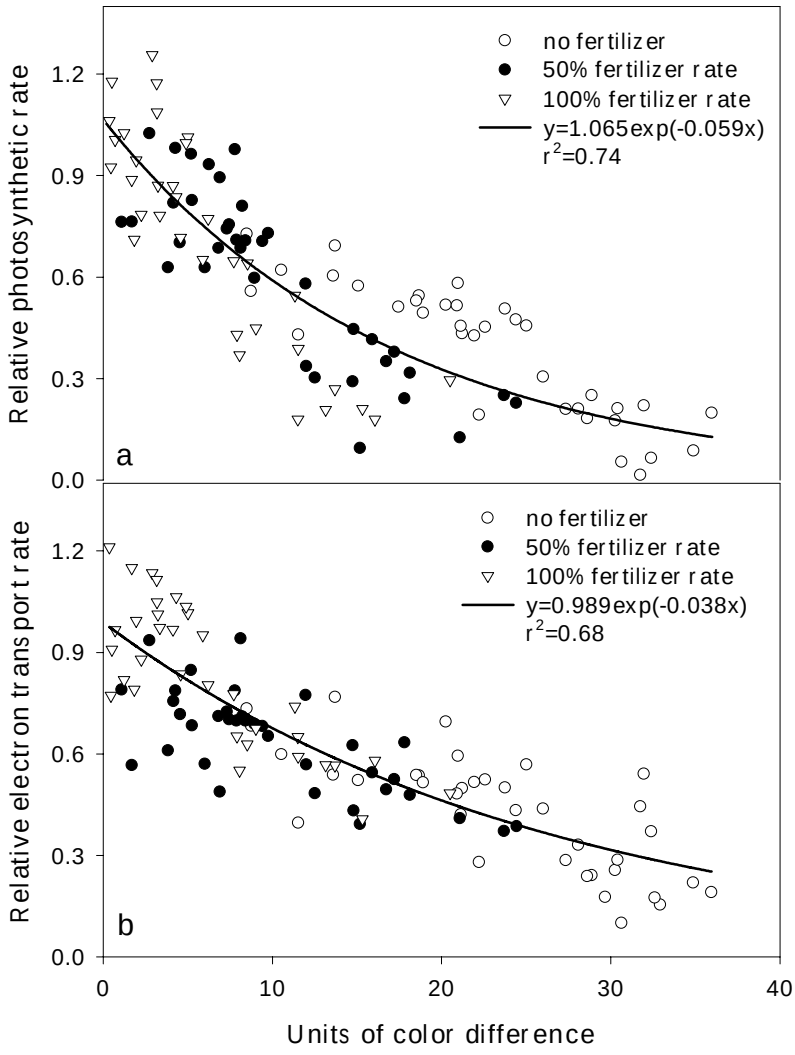

Figure 3.6. Responses of (a) relative photosynthetic rate and (b) relative electron transport rate to units of color difference, at the different levels of fertilizer. Both rates are expressed as fractions of the average value of all healthy bean leaves at the recommended fertilizer rate of 100%. Data of Experiments 3-4 and 3-5 were combined in these graphs.

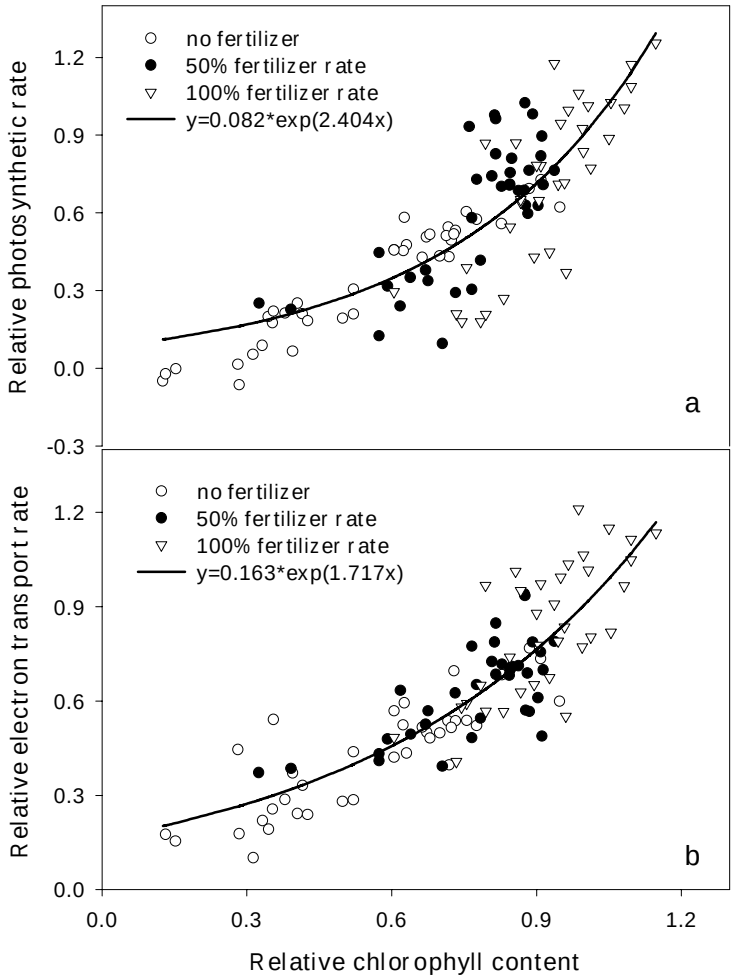

Figure 3.7. Responses of (a) relative photosynthetic rate and (b) relative electron transport rate to relative chlorophyll content (SPAD values), at the different levels of fertilizer. Both rates and the values of SPAD are expressed as fractions of the average value of all healthy bean leaves at the recommended fertilizer rate of 100%. Data of Experiments 3-4 and 3-5 were combined in these graphs.

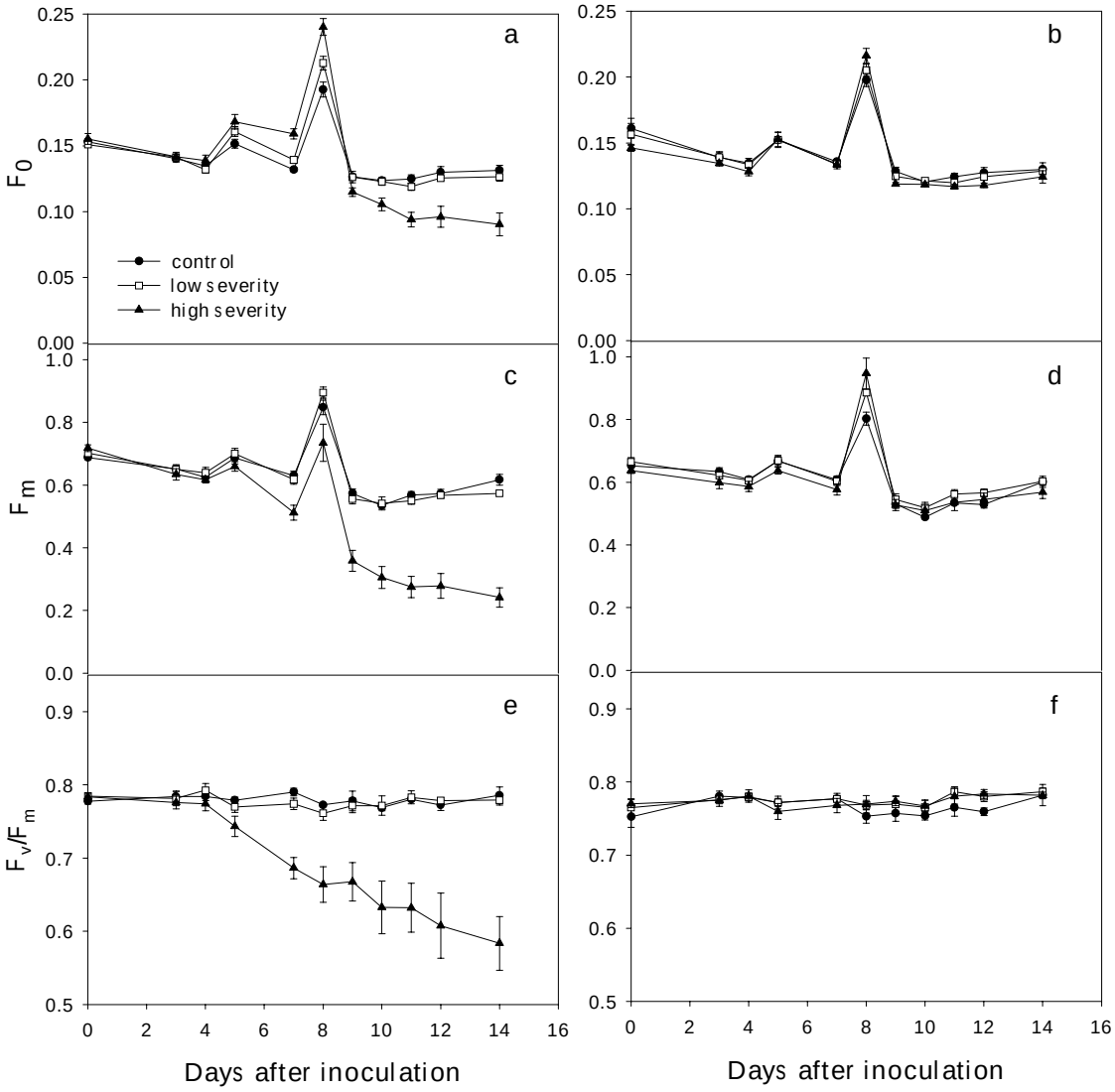

Figure 3.8. Minimal fluorescence (F_0), maximal fluorescence (F_m), and optimal quantum yield (F_v/F_m) during rust development, in inoculated (a, c, e) and non-inoculated (b, d, f) areas of bean leaves with different rust severities. The fluorescence parameters are expressed in relative units. The percentage of area affected by rust was 18-30% in leaves with low severity, and 65-85% in leaves with high severity.

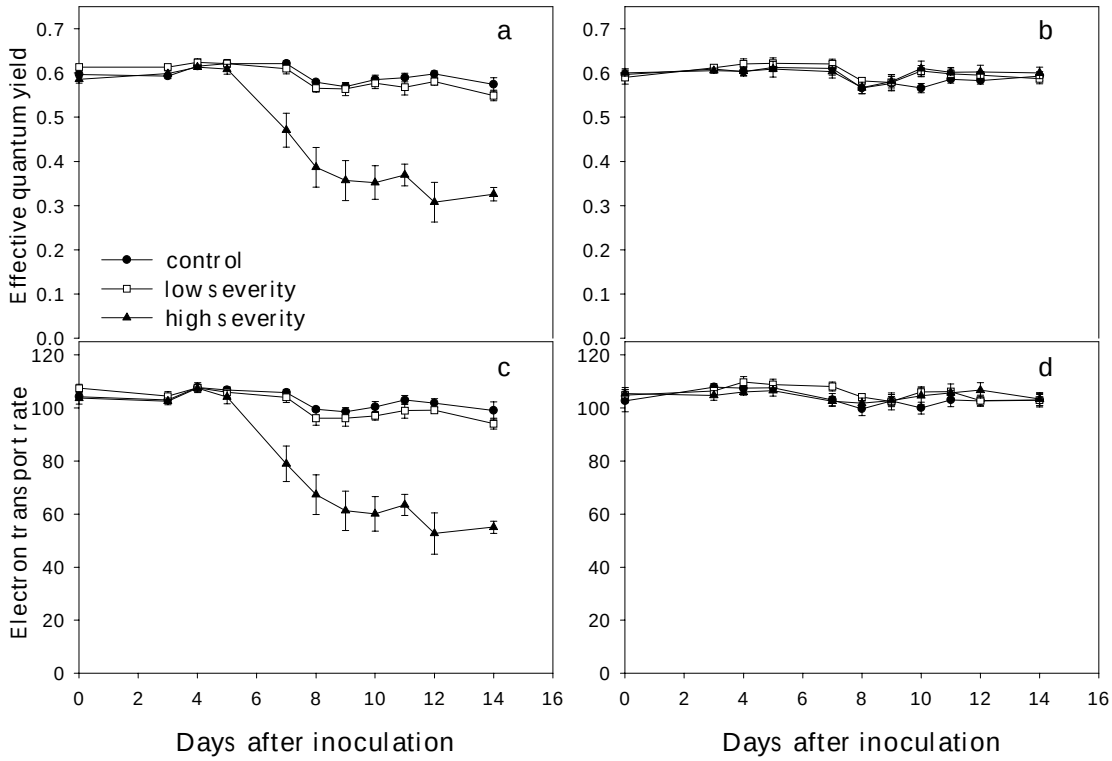

Figure 3.9. Effective quantum yield, in relative units, and electron transport rate (μmol $m^{-2}s^{-1}$) during rust development, in inoculated (a, c) and non-inoculated (b, d) areas of bean leaves with different rust severities. The percentage of area affected by rust was 18-30% in leaves with low severity, and 65-85% in leaves with high severity.

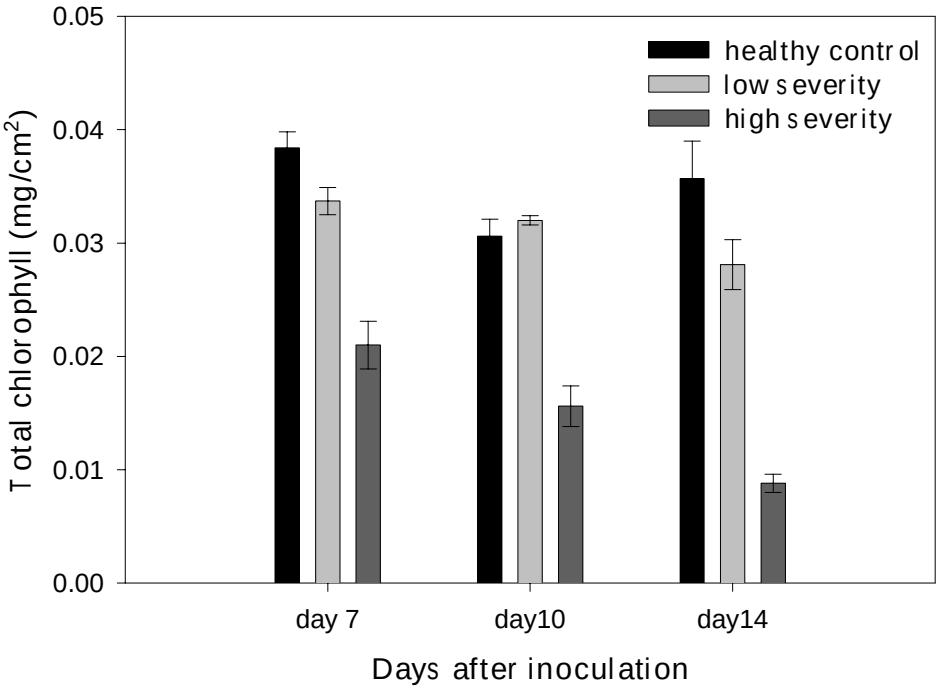

Figure 3.10. Chlorophyll content of bean leaves with different rust severities during disease development. The percentage of area affected by rust was 18-30% in leaves with low severity and 65-85% in leaves with high severity.

pustules were well developed, the chlorophyll content of those leaves was around 25% of the control.

Leaves with 18-30% severity did not differ significantly from the healthy control leaves in any of the measured parameters during disease development. Rust development in symptomatic areas of inoculated leaflets did not interfere with the fluorescence parameters of the non-inoculated leaflet of the same leaf (Figures 3.8 and 3.9). The noticeable peaks in the graphs for minimal and maximal fluorescence, on day 8 after inoculation, occurred one day after the apical portion of all plants was removed to avoid shading of the leaves of interest. Whatever the reason for this sudden rise in fluorescence, it probably did not interfere with the effects of rust on leaf photosynthesis, as it occurred for all three groups of plants, on both inoculated and non-inoculated areas. On the following day, fluorescence emission returned to the levels observed prior to the removal of the apical portion.

For Experiment 3-8, where fluorescence was measured on the lesioned area and areas between lesions in leaves with low severity, maximal fluorescence and optimal quantum yield were reduced inside the lesions, beginning at 10-11 days after inoculation (Figure 3.11). Effective quantum yield and electron transport in the chlorotic areas of leaves with low severity were reduced on day 11, but were not significantly different from the other treatments on day 12. Fluorescence in the green area between lesions on leaves with low severity remained unaffected when compared to the healthy control leaves. All fluorescence parameters were reduced on leaves with high severity when compared to the healthy control group. Minimal fluorescence on leaves with high

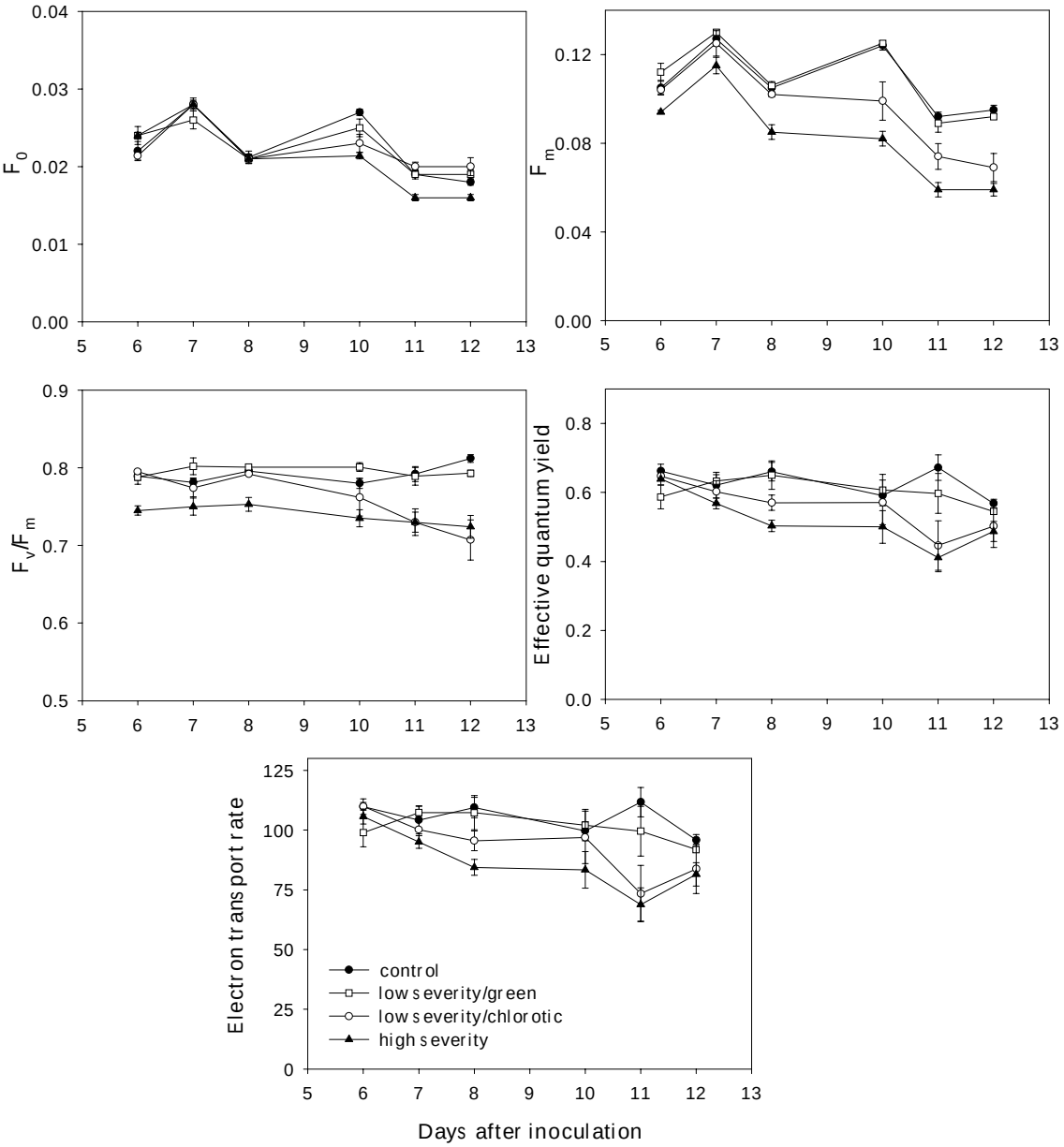

Figure 3.11. Minimal fluorescence (F_0), maximal fluorescence (F_m), optimal quantum yield (F_v/F_m), effective quantum yield, and electron transport rate during rust development in healthy control and inoculated bean leaves with different rust severities. In leaves with low severity (30-38%), fluorescence was measured in green areas (between lesions) and chlorotic areas (inside the lesions). The percentage of area affected by rust was 65-80% in leaves with high severity.

severity was significantly lower than the control leaves on days 10 to 12. Maximal fluorescence and optimal quantum yield were significantly reduced from the onset of the fleck stage, 6 days after inoculation. Effective quantum yield and electron transport rate on the same leaves were significantly reduced on days 8 to 12.

Discussion

The effects of rust on the photosynthetic competence of common bean have been described by various researchers. Decreased photosynthetic rates (Livne, 1964; Raggi, 1980), increased respiration (Daly et al., 1961; Livne, 1964; Raggi, 1980), changes in the patterns of translocation of photosynthates (Livne and Daly, 1966), and increase in invertase activity (Wagner and Boyle, 1995) were the main effects observed during infection by the rust pathogen. Also, Moll et al. (1995) reported loss of chlorophyll in the pustule area and diminished chlorophyll fluorescence during rust development, which indicated reduced photosynthetic activity. In most of these studies, the effects of rust on the physiology of the bean plant were investigated using only heavily infected leaves. Rates of photosynthesis, respiration, and loss of chlorophyll are dependent upon infection density (Scholes, 1992). Thus, the quantification of disease effects on plant physiology must take the density of infections into consideration.

A virtual lesion consists of a visual lesion and an adjacent area, in which the photosynthetic activity is zero. This concept was introduced by Bastiaans (1991), as a basis for a model that quantifies photosynthetic competence of diseased leaves. 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 remaining 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 (Bastiaans, 1991). However, diffusible toxins have not been associated with infections by biotrophic pathogens (Gay, 1984), 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 (Bastiaans, 1991, based on data from Spitters et al., 1990), and 8.74 ± 1.70 for *Blumeria graminis* on winter wheat (Bastiaans, 1991, based on data from Rabbinge et al., 1985). The reported β values for facultative parasites are 3.04 ± 0.18 and 3.74± 0.19 for *Pyricularia oryzae* on rice (Bastiaans, 1991); 2.68 ± 0.22 for *Xanthomonas campestris* pv. phaseoli on common bean (Goodwin, 1992); 7.24 ± 0.75 for *Colletotrichum lindemuthianum* on common bean (Bassanezi et al., 1997); 11.0 ± 3.5 for Cercospora leafspot of peanut (van der Werf et al., 1990, based on data from Boote et al., 1980); 2.1 ± 0.61 for *Rhynchosporium secalis* on barley (van der Werf, 1990, based on data from (Martin, 1986); and 1.66 ± 0.34 for *Septoria nodorum* on wheat (van der Werf, 1990, based on data from Rooney, 1989). In the present study, most of the β values obtained were not different from one; the remaining values were between one and two (Figures 3.2 and 3.4). These values of β are similar to the value of 1.26 ± 0.16 obtained for brown rust of wheat (Spitters et al., 1990), the only other reported value for a pathosystem involving a rust pathogen. The conclusion, in the present study, was that

there was little effect of rust infection on the remaining green area of leaves of 'Rosinha' bean, but some environmental factors may cause variation in this response. Light and temperature conditions during plant growth and symptom development, and the nutritional status of the plant are factors that may have contributed to the observed variation between experimental outcomes.

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: (a) photosynthetic rates should be determined at light saturation to ensure that the maximum rate (P_{max}) is obtained (Bastiaans, 1991); (b) if possible, leaves of similar physiological age or position in the canopy should be selected for the measurements (Goodwin, 1992; Shtienberg, 1992); and (c) the environmental conditions in which the measurements are taken should not be very different from the conditions under which the plants were grown (Daly, 1976). The aspects listed above are suggested to reduce the variation in the values of photosynthetic rate obtained, as 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 interpreting the β values.

Assessments of severity at early stages of symptom development may result in a different β value than the one obtained when symptoms are fully advanced. In a preliminary experiment (results not shown), the β value, determined for bean leaves inoculated with rust showing fleck symptoms, was 4.7 ± 0.77 (r^2=0.49). The maximum severity estimated at that stage was 5%. Four days later, when the symptoms were well developed, with chlorotic halos surrounding the sporulating pustules, the maximum severity was estimated at 63%, and the β value was 1.27 ± 0.07 (r^2=0.95).

Chlorophyll fluorescence, obtained in vivo with a fluorometer, has been called, in the last decade, the "plant physiologist's stethoscope" because it measures the efficiency of the photosynthetic activity, and thus can provide approximate estimates of the vitality and vigor of a plant in its environment (Bolhàr-Nordenkampf and Öquist, 1993; Foyer, 1993; Hall and Rao, 1994). Measurements of chlorophyll fluorescence have been used widely, not only in routine studies on photosynthesis, but also in other related areas, such as studies on environmental stresses, screening for stress tolerance in plant breeding, and studies on herbicide and air pollution (Foyer, 1993). The non-invasiveness, speed of data acquisition, and high sensitivity are often cited as outstanding advantages of this technique (Foyer, 1993; Schreiber et al., 1998). Plant diseases certainly can be considered stressful to the physiology of the plant, and, thus, chlorophyll fluorescence may provide valuable information on the photosynthetic competence of diseased leaves.

Chlorophyll fluorescence reflects the efficiency of light utilization on photosystem II (PSII). It was shown that there is an ubiquitous curvilinear or biphasic relation between the effective quantum yield of fluorescence and the apparent quantum yield of CO_2 assimilation (mol CO_2/mol quanta) (Cornic and Briantais, 1991; Genty et al., 1989;

Seaton and Walker, 1990). This relationship may allow good estimates of photosynthetic rate through measurements of fluorescence, without recourse to analysis of gaseous exchange between leaf and environment. Inhibition of primary photochemistry, electron transport, or carbon metabolism caused by environmental stresses will affect the function of PSII and will be expressed as changes in fluorescence yield. These changes in fluorescence yield can provide quantitative information about plant responses to the duration and intensity of the stress (Bolhàr-Nordenkampf and Öquist, 1993).

Minimal fluorescence (F_0) represents the emission from molecules of chlorophyll a on the light harvesting antenna associated with PSII, prior to the excitation energy being used for electron transport (Krause and Weis, 1984). It is therefore independent of photochemical events, but dependent upon the chlorophyll content of the tissue and on the ultrastructure of the thylakoid membrane. There was a slight increase in F_0 on the bean leaves infected by the rust pathogen during the fleck stage, but it later decreased during sporulation. Similar results were reported by (Scholes and Farrar, 1985), for measurements taken on bluebell leaves infected with rust, caused by *Uromyces muscari*. These authors proposed two hypotheses to explain the temporary increase in F_0 before sporulation. It could be due to an inactivation of some PSII reaction centers, caused by a reducing compound produced transiently by the pathogen; or it could be caused by a disorientation of a proportion of the chlorophyll a molecules within the thylakoid membrane during colonization of the tissue by the pathogen. The subsequent decrease in F_0 may be explained by the loss of chlorophyll from the tissue, which was very pronounced at the onset of sporulation (Figure 3.10).

Maximal fluorescence (F_m) is a measure of the oxidation-reduction status of the electron acceptors between PSII and PSI, and is a direct indicator of PSII activity (Scholes and Farrar, 1985). Environmental stresses that cause thylakoid damage, such as heat and freezing stresses, usually lower F_m. The F_m values of leaves with high rust severity were reduced beginning at fleck stage. After sporulation, these values were less than 40% of the control values (Figure 2.8c). In leaves with lower rust severity, F_m was reduced only in the chlorotic areas, but not in areas between lesions (Figure 3.11b). Maximal fluorescence was also reduced in plants infected by other pathogens, such as *Uromyces muscari* on bluebell (Scholes and Farrar, 1985), *Taphrina deformans* on peach (Raggi, 1995), and tobacco mosaic virus on tobacco (Balachandran and Osmond, 1994).

The optimal quantum yield (F_v/F_m) provides information on the potential photosynthetic efficiency of PSII, and it is often discussed as a vitality index (Moll et al., 1995). It has a range of 0.75 to 0.85 for healthy leaves of many plant species and ecotypes, and decreases under stress (Bolhàr-Nordenkampf and Öquist, 1993). In this study, there was a reduction in F_v/F_m on bean leaves with high rust severity (Figure 3.8e) and on the lesioned areas of leaves with low severity (Figure 3.11c), largely due to the decrease in F_m values. These results are in accordance to the findings of (Moll et al., 1995), with the same pathosystem.

Effective quantum yield is the quantum yield of the non-cyclic electron flow (Foyer, 1993; Genty et al., 1989). It is proportional to the concentration of the open PSII reaction centers and to the efficiency with which these centers capture and use excitation energy. It is the basis to calculate the electron transport rate. Both effective quantum yield and electron transport rate suffered similar reductions on highly diseased leaves, but

these variables were not affected outside the area of the pustules in leaves with lower severities (Figures 3.8 and 3.11d and 3.11e).

Moll et al. (1995) suggested that the diminished efficiency of use of the excitation energy may be due to ultrastructural changes within the chloroplasts. Another hypothesis to explain the depression in photochemical efficiency is the down-regulation of PSII photochemistry, as a consequence of a reduced demand for NADPH and ATP in the chloroplasts, resulting from inhibition of the Calvin cycle (Krupa et al., 1993).

On bean leaves with high rust severity, reductions in the fluorescence parameters were first noticeable at the fleck stage (5 to 7 days after inoculation). Similarly, alterations in photosynthetic rate and dark respiration were not noticed before the fleck stage for this same pathosystem (Daly et al., 1961; Livne, 1964; Raggi, 1980). However, (Peterson and Aylor, 1995), through chlorophyll fluorescence imaging, were able to observe enhanced fluorescence emission 3 days after inoculation of bean leaves with rust, when symptoms were not visible. This observation was interpreted as an early sign of alteration in light utilization for photosynthesis.

A reduction in the F_v/F_m ratio is considered symptomatic of a phenomenon called photoinhibition, which is a light-dependent inhibition of photosynthesis (Baker, 1993; Foyer, 1993; Krause, 1988). When a healthy, non-stressed plant is exposed to light levels considerably higher than the light conditions experienced during growth, this plant will face an excess of absorbed excitation energy. The thylakoids have a mechanism to increase the rate of dissipation of excitation energy as heat, when the rate of electron transport cannot meet the rate of excitation of the reaction centers. The end-result of this

process is an inhibition of photosynthesis, as a consequence of a decreased efficiency of light utilization with the increase in light intensity.

Stressed plants will face the same problems described above, but at lower light intensities (Baker, 1993; Bolhàr-Nordenkampf and Öquist, 1993; Osmond, 1994). Photoinhibition is thus considered in the study of stress physiology, as a secondary stress response, usually triggered by other stress factors that reduce the ability of the plant to assimilate CO_2. The accumulation of the end-products of the light-reactions of photosynthesis (ATP and reductants) will result in an inhibition of electron transport. Under such conditions, damage to the reaction-center proteins can occur if the excess of excitation energy in the pigment antenna is not successfully dissipated. Osmond (1994) called this condition chronic photoinhibition, which can have two possible outcomes: photon protection or photon damage. Photon protection will be the result of a decrease in the efficiency of photosynthesis by means of dissipating the excess of photons as heat, before these photons can reach the reaction centers. A decline in dark-adapted F_v/F_m values, accompanied by a decline in F_0 is considered diagnostic of mechanisms that engage photon protection, and result in little photon damage. Photon damage occurs when the photoprotective capacity is exceeded, and the excess of photons irreversibly degrades certain components of the PSII reaction centers. In this case, F_v/F_m declines, but F_0 increases.

Balachandran and Osmond (1994) reported that chlorotic tissue of expanding leaves of tobacco infected with TMV were chronically photoinhibited (low F_v/F_m, high F_0). These authors concluded that, in tissue infected with viral particles, photoinhibitory damage was occurring as a result of illumination of chloroplasts having impaired PSII.

The photon damage then led to photooxidation of chlorophyll and, thus, to patchy chlorosis. From the results with bean rust in the present study, it is concluded that the reduced carbon assimilation resulting from the infection induced chronic photoinhibition on bean leaves. The F_v/F_m ratio was clearly reduced in severely diseased leaves, and in chlorotic areas of leaves with low severity, in comparison to the control leaves (Figures 3.8e and 3.11c). The transient increase in F_0 during the fleck stage and its subsequent decrease during sporulation (Figure 3.8a) was interpreted as both photon damage and photon protection may occur in this system under moderate light conditions (400 μmol photon m^{-2} s^{-1}).

Low availability of essential mineral elements for growth impairs the photosynthetic capacity of a plant. The question in the present study was whether bean rust would have a greater impact on the photosynthesis of non-fertilized plants than on well-fertilized plants. The answer was that increases in disease severity did not cause greater reductions on the photosynthetic rates of plants that received no fertilizer, when compared to the reductions on plants with 100% of fertilizer. In fact, the opposite was true in the present experiments. The photosynthetic rates of healthy control plants that received no fertilizer were 43-51% lower than the rates of well-fertilized control plants. Although the healthy control plants of the three fertilizer treatments initially had different photosynthetic rates, all plants with 90-100% rust severity, independently of their nutritional status, would have rates of photosynthesis close to zero or negative values, according to the regressions performed. Consequently, leaves of well fertilized plants lost more efficiency per unit area than leaves of non-fertilized plants, when both had the same area affected by disease. The impairment of photosynthesis in areas with higher

relative efficiency was the cause for the larger proportional reductions of photosynthetic rates on plants with 100% fertilizer, and their slightly higher β values (Figure 3.4).

Physiological processes other than photosynthesis, but closely related to it, were also affected by rust infection. In the present study, decreased stomatal conductance, increased respiration, and losses of chlorophyll from leaf tissue were observed in response to increases in rust severity.

Stomatal functioning is believed to be integrated with photosynthesis in such a way that it optimizes the use of water while only marginally limiting the photosynthetic process (Farquhar and Sharkey, 1982). The stomatal conductance of bean leaves decreased with their reduced photosynthetic capacity, as rust severities increased (Figure 3.2a). This positive linear relationship between conductance and carbon assimilation was also observed for leaves with various nutritional and water status, age, and levels of viral infection (Hall and Loomis, 1972; Schulze and Hall, 1982; Wong et al., 1979). 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 (Farquhar and Sharkey, 1982; Rabbinge et al., 1985). Carboxylation resistance is the resistance of the mesophyll to CO_2 diffusion. Stomatal resistance, which is the inverse of stomatal conductance, represents the 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 is an indication that the flow of CO_2 from the stomatal cavities to the carboxylation sites was not affected (Figure 3.2b). Consequently, it is possible to conclude that mesophyll resistance to carboxylation increased in diseased leaves.

Increased rates of dark respiration in bean leaves with rust are in accordance with previous reports on respiratory changes for this pathosystem (Daly et al., 1961; Raggi, 1980). The higher rates of respiration reported were observed during sporulation, but were believed to be related mostly to host respiration (Daly et al., 1961). The contribution of fungal respiration to the increases in the rate of respiration was considered negligible.

The rate of respiration in the light is comparable to the rate of respiration in the dark (Azcón-Bieto and Osmond, 1983). Respiration rate contributes significantly to the total CO_2 exchange in illuminated leaves. Increased respiration was partially responsible for the reduced photosynthetic rates observed in bean leaves in the present study, because the rate obtained by gas-exchange measurements was a net photosynthetic rate, which included CO_2 effluxes and refixation, besides the CO_2 assimilation by the leaf tissue. Rates of dark respiration inside the lesions were determined to be five to six times higher than in non-infected green tissue (Figure 3.3a), according to Bastiaans' equation (Bastiaans and Kropff, 1993). Possible mechanisms of increased respiration in tissues infected by biotrophic pathogens include: (a) wound respiration due to regenerative activity of tissue physically damaged by the invading pathogen; (b) enhanced consumption of ATP and reductants through increased biosynthesis; and (c) increased levels of starch and soluble sugars that accumulate in the cells as a result of blocked translocation (Hutcheson and Buchanan, 1983; Smedegaard-Petersen, 1984).

The percentage of losses of chlorophyll from diseased leaves in relation to control leaves had similar values to the proportion of the tissue visually chlorotic, which was the basis for assessments of severity. This was true for all experiments (Table 3.1, Figure

3.3b, Figure 3.10), independent of the methodology used to assess chlorophyll content of the tissue. However, Moll et al. (1995) reported that chlorotic areas in bean leaves with rust still retained a certain amount of chlorophyll, and that the pigment was also reduced in green areas between lesions, in comparison to non-infected control leaves. Leaf chlorophyll content had a good relationship with net photosynthetic rates and electron transport rates in bean leaves with different nutritional status and rust severities (Figure 3.7). This is in accordance with reports in which the leaf chlorophyll content is often well correlated with leaf nitrogen status, Rubisco activity, and photosynthetic activity (Evans, 1983; Seeman et al., 1987; Thompson et al., 1996). Also, chlorophyll is believed to be a sensitive indicator of many types of plant stress. Leaf color, which is largely dependent upon chlorophyll content, was also a good indicator of the photosynthetic status of bean leaves. When expressed as the difference in color from a healthy control standard, relative leaf color was strongly related to photosynthetic rates and electron transport rates of healthy and diseased bean leaves (Figure 3.6).

The relationships obtained between leaf color, chlorophyll content, and the photosynthetic parameters were obtained for healthy and rust-infected leaves, with different levels of nutrition. Thus, if the relationships found here could be validated in field experiments, estimates of photosynthetic activity of plants with rust could be done on larger scales. A few healthy, well-fertilized plants, growing in the same conditions as the plants of interest, could be used as healthy control standards. The instruments used to determine leaf color and chlorophyll content of the leaves, respectively the Color Reader CR-10 and the SPAD-502 Chlorophyll Meter, are portable, easy to operate, and relatively

inexpensive when compared to the equipment necessary to measure gas-exchange or fluorescence.

The photosynthetic competence of bean leaves infected with rust was reduced, beginning at fleck stage, when assessed under controlled conditions. Loss of chlorophyll and higher respiration rates in the diseased tissue are believed to be the main factors determining this reduction. The reduced rates of photosynthesis observed were proportional to the proportion of leaf tissue with visual symptoms, for most of the experiments in the present study. However, in a few cases, the reduction was slightly higher than what could be explained by rust severity. 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 noticed, though, that these conclusions need to be verified for other combinations of bean cultivar and pathogen race.

The quantitative determination of the effects of a disease on the physiology of individual leaves is the first step towards a broader understanding of crop losses (Bastiaans et al., 1994). The future steps would be to determine the effects of disease on the physiology of whole plants and, then, to integrate this information on plant performance over an entire season.

CHAPTER 4
THE PHOTOSYNTHETIC COMPETENCE OF BEAN LEAVES WITH ANTHRACNOSE

Introduction

Severe epidemics of bean anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi. and Cav., usually occur when contaminated seeds are planted in locations with cool to moderate temperatures, frequent rains, and high humidity, as in temperate and subtropical zones and high altitudes in the tropics (Tu, 1992). Reported yield losses due to bean anthracnose were up to 100% for highly susceptible cultivars and between 27 and 88% for moderately susceptible cultivars (Guzman et al., 1979; Shao and Teri, 1985). Serious losses occurred when bean plants became infected in the first 5 weeks of development (Guzman et al., 1979).

In the past, the control of bean anthracnose relied heavily on race-specific resistance(Tu, 1992). However, *C. lindemuthianum* has high pathogenic variability and new races of the pathogen are reported frequently (Menezes and Dianese, 1988). Thus, integrated disease management is considered the most effective approach to minimize the yield losses to anthracnose. The control measures in an integrated disease management program include use of non-infected seeds, race non-specific resistance, and chemical treatment. Reliable estimates of yield losses are essential to assess the success of any disease management program. The reliability of estimates of yield loss due to a disease depends on the understanding of the epidemiological variables that intensify the progress

of disease and on accurate assessment of the impact of the disease on crop performance (Bergamin and Amorim, 1996; Berger et al., 1995).

Waggoner and Berger (1987) proposed that healthy leaf area duration (HAD) and healthy leaf area absorption (HAA) were much better predictors of yield compared to disease intensity as a predictor of yield loss, since 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 (Haverkort and Bicamumpaka, 1986); Rotem et al., 1983a and 1983b; van Oijen, 1990), *Alternaria solani* on potato (Johnson and Teng, 1990), *Aschochyta fabae* on *Vicia faba* (Madeira et al., 1988), *Pyricularia oryzae* on rice (Pinnschmidt and Teng, 1993), *Blumeria graminis* on wheat (Daamen and Jorritsma, 1990), and *Phaeoisariopsis griseola* on common bean (Bergamin et al., 1997). The yield of bean plants affected by anthracnose, expressed in number of pods per plant or grams of seeds per plant, was positively correlated to HAD and HAA, but yield had no significant relationship with the area under the disease progress curve (AUDPC) (Nunes and Bergamin, 1996). The AUDPC has been considered as the best measure of the intensity of an epidemic (Campbell and Madden, 1990).

In addition to affecting the amount of intercepted radiation, some foliar pathogens can affect the efficiency of radiation use by the plant (Bastiaans, 1991; Bastiaans et al., 1994; Boote et al., 1980; Bourgeois and Boote, 1992; Johnson, 1987). In the 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 (Johnson, 1987). The determination of radiation use efficiency of diseased plants has been done under field conditions, from the

slope of the line that relates yield to HAA , in plots with different disease intensities. If the slopes are constant for the different situations, the radiation use efficiency is considered to be unaffected by the disease (Aquino et al., 1992; Waggoner and Berger, 1987). However, some researchers believe that only direct measurements of photosynthetic rates in healthy and diseased plants can show whether the photosynthetic activity of the green leaf area is being affected by disease (Bastiaans, 1991; Boote et al., 1983; Rabbinge and Rijsdijk, 1981; Rabbinge et al., 1985; van Oijen 1990; van Roermund and Spitters 1990). Thus, the present study focused on the quantification of the effects of anthracnose on the photosynthesis of leaves of common bean (*Phaseolus vulgaris*) to verify whether assessments of HAA and HAD for bean plants with anthracnose should take into account any effects of the disease on radiation use efficiency.

Materials and Methods

Plant Material and Inoculum

Bean plants (*Phaseolus vulgaris*) of the susceptible cultivar Rosinha were used in all experiments. The plants were grown, one plant per pot, in 4-liter pots filled with Metromix, and watered daily to field capacity. The plants were fertilized every other day with Peter's fertilizer (20-20-20, 1 g/l of water). 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.

Conidia of *C. lindemuthianum*, race kappa, were produced in Mathur's C modified medium (Balardin et al., 1997). A suspension of conidia was spread on the

surface of the medium to obtain maximum sporulation. Ten- to fourteen-day-old cultures were rinsed with sterile distilled water to prepare the concentrated spore suspension, which was then diluted to obtain the desired concentrations. The viability of the conidia was determined prior to the preparation of the suspensions. A diluted suspension of conidia was spread on the surface of water-agar medium, and the percentage of germinated conidia was determined after 12 hours of incubation.

Measurement of Gas-Exchange on Bean Leaves with various Levels of Anthracnose Severity

The experiment was conducted twice (Experiments 4-1 and 4-2) with bean plants grown under greenhouse conditions. The temperature ranges in the greenhouse, 18-21^{o}C during the night and 24-35^{o}C during the day, were similar for both experiments. In Experiment 4-1, the conidial suspensions used in the inoculation had 10^4, 5 x 10^4, 10^5, or 5 x 10^5 viable spores/ml. These supensions 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^{o}C, in which they were maintained for 30 hours, and then returned the greenhouse. For Experiment 4-2, the third trifoliate leaf was inoculated and the plants were maintained in the greenhouse, enclosed in plastic bags for 16 hours 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 using the LI-6200 Portable Photosynthesis System (LI-COR Inc.). Measurements were taken at a range of light intensity of 700 to 1000 μmol photon m^{-2} s^{-1} , with the method described in Chapter

3. For Experiment 4-1, the severity of the measured leaves was estimated using a diagrammatic scale of anthracnose symptoms (Godoy et al., 1997). For Experiment 4-2, 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 then run through an area meter (LI-3000 Portable Area Meter, LI-COR). The severity was calculated as the proportion of leaf area with lesions.

In Experiment 4-2, other variables were also determined in healthy and diseased leaves. The electron transport rate and effective quantum yield were determined under the same conditions as the photosynthetic rate with the PAM-2000 fluorometer (Walz, Germany). After the plants were adapted for 30 minutes in the dark, the rate of dark respiration was determined with the LI-6200.

After the determination of severity on the detached leaves was concluded, the chlorophyll content of the leaves was assessed with the SPAD-502 Chlorophyll Meter (Minolta Co., Ltd.). Fifteen readings were taken and then averaged for each leaf. The readings were taken on non-necrotic areas of the diseased leaves. The average leaf color was also determined on non-necrotic areas, with the CR-10 Color Reader (Minolta), and then expressed as a value of color difference in relation to the average color of the healthy leaves. The objective of the quantification of chlorophyll and color was to verify if these variables were related to photosynthetic rate or fluorescence parameters. If there is such a relationship, it may be possible to use chlorophyll or color to make inferences about the photosynthetic status of healthy and diseased leaves.

The equation $P_x/P_0=(1-x)^{\beta}$ was used to relate relative photosynthetic rate (P_x/P_0) to disease severity (x), where β represents the ratio between virtual and visual lesion sizes

(Bastiaans, 1991). Relative photosynthetic rate was the ratio of the photosynthetic rate of a leaf to the average rate of the healthy control leaves. The parameter β was obtained by non-linear regression, using the procedure PROC NLIN (method DUD) of SAS (SAS Institute, Cary, NC; release 6.12 for personal computers). The equation $R_x=R_0(1-x) + \sigma R_0 x$, introduced by Bastiaans and Kropff in 1993, was used to relate dark respiration R_x to disease severity (x), where R_0 is the rate of dark respiration of healthy leaves and σ expresses the ratio between the respiration of a lesion and that of an identical area of healthy tissue. This function assumes that an increase in respiration is restricted to the visible lesion area (Bastiaans and Kropff, 1993).

Effects of Fertilization on the Photosynthetic Competence of Bean Leaves with Anthracnose

Bean plants growing under greenhouse conditions received the following fertilization treatments, every other day: (a) no fertilizer, (b) half of the recommended dosage of Peter's fertilizer for daily fertilization (0.5 g/l of water), or (c) full recommended dosage of the fertilizer (1 g/l). The experiment was conducted twice (Experiments 4-3 and 4-4). Temperatures inside the greenhouse ranged from 25 to 30^{o}C during the day and from 18 to 20 ^{o}C, during the night, for both experiments.

The expanded third trifoliate leaf in all plants was inoculated with suspensions of conidia, sprayed onto both leaf surfaces. Two suspensions with different concentrations (5×10^3 or 5×10^4 viable conidia/ml) were used to obtain levels of anthracnose severity. Control leaves of each treatment were sprayed with sterile distilled water. All plants were enclosed in plastic bags for 16 hours during the night and early morning. Measurements were taken 6 and 7 days after inoculation, when symptoms were fully developed. The

range of light intensity during the measurements was 700-900 μmol photon m^{-2} s^{-1}. Net photosynthetic rate and stomatal conductance were determined by gas-exchange measurements using the LI-6200 Portable Photosynthesis System. Electron transport rate and effective quantum yield were measured with the PAM-2000 fluorometer, under the same environmental conditions. Estimates of chlorophyll content and average color of control and diseased leaves were obtained as described above.

The equation $P_x=P_0(1-x)^{\beta}$ was applied to relate relative photosynthetic rate (P_x/P_0) to disease severity (x), where P_x is the photosynthetic rate of a diseased leaf, and P_0 is, in this case, the average photosynthetic rate of the healthy control leaves for each fertilizer treatment. The β parameter for each fertilizer treatment was obtained by non-linear regression, using SAS. Analysis of covariance was used with a linearized form of the Bastiaans' model to verify the significant differences among the levels of fertilizer and also to determine if the experiments could be pooled. The non-linear estimation module of the software package STATISTICA (release 5.1 for Windows, StatSoft, Inc.) was used to obtain the best fitted model to describe the relationships between color or chlorophyll to the photosynthesis variables. Evaluation of model fitness was based on the values of the coefficients of determination and the homogeneity of the distribution of the residuals.

Measurement of Chlorophyll Fluorescence during Disease Development

Two independent experiments (Experiments 4-5 and 4-6) were conducted to determine the changes in fluorescence parameters during the development of anthracnose symptoms. Bean plants were grown under 300-400 μmol photon m^{-2} s^{-1} and a photoperiod of 12 hours in a growth room. The temperature in the room ranged from 13 to 18^{o}C during the night, and from 24 to 32^{o}C during the day. To obtain plants with

different levels of severity, suspensions of conidia of different concentrations (5×10^4 or 5×10^3 viable spores/ml, for Experiment 4-5; and 7.5×10^5, 7.5×10^4, or 7.5×10^3 viable spores/ml, for Experiment 4-6) were sprayed onto both surfaces of the first trifoliate leaf, in different groups of plants. One of the three leaflets was protected from inoculation by a plastic bag, and was then considered as a non-inoculated area of inoculated leaves. Control plants were sprayed with sterile distilled water. Inoculated plants were enclosed in clear plastic bags for 18 hours in the dark.

In Experiment 4-5, leaves with low severity had a range of 0.1 to 0.4% of disease and leaves with high severity had from 1 to 7% of disease. For Experiment 4-6, leaves with low, medium, and high severity had, respectively, 0.8-2%, 3.6-10%, and 16-25% of their area with symptoms of anthracnose. Disease severity was estimated at the end of the experiments using a diagrammatic scale of anthracnose symptoms (Godoy et al. 1997).

Minimal fluorescence (F_0), maximal fluorescence (F_m), optimal quantum yield (F_v/F_m), effective quantum yield, and electron transport rate were the parameters of chlorophyll fluorescence measured in attached leaves, using the PAM-2000 modulated light fluorometer. The PAM-2000 is a portable instrument that can be used to measure *in vivo* fluorescence of photosynthesizing plant tissue. Modulated chlorophyll fluorescence techniques use the repetitive application of brief saturated light pulses in addition to the continuous actinic illumination used to drive photosynthesis (Foyer, 1993). Minimal fluorescence, maximal fluorescence, and optimal quantum yield were determined on leaves that were dark-adapted for 20 minutes. A modulated light beam of very low intensity was applied to the dark-adapted leaf, which induced a weak initial rise in fluorescence to a low level (F_0). This response is considered to be the emission of

fluorescence which occurs when all PSII reaction centers are open. A brief strong light pulse was then added to the modulated beam to cause light saturation and to close all reaction centers at once (F_m). Optimal quantum yield was calculated from F_0 and F_m , and this is considered an indication of the potential photosynthetic efficiency of a leaf. Measurements similar to the ones taken on dark-adapted leaves were then obtained when the leaves were re-adapted to 300-400 μmol photon m^{-2} s^{-1} to calculate the effective quantum yield and the electron transport rate.

The first readings were taken before inoculation (day 0), and then at frequent intervals during 12 days for Experiment 4-5. In Experiment 4-6, the same fluorescence parameters were measured from day 0 to day 6 after inoculation. After day 6 the leaves with high severities of anthracnose collapsed due to disease development. In both experiments the parameters of chlorophyll fluorescence were measured at random locations on the leaves before the appearance of symptoms. The necrotic areas of the leaf were avoided when symptoms were visible. The responses of each fluorescence parameter over time for healthy leaves and diseased leaves were analyzed with the SAS repeated measures procedure.

Results

Net Photosynthetic Rate and Related Variables

In both experiments, the reduction in the net photosynthetic rate of leaves with anthracnose was greater than the increase of severity (Table 4.1). The average net photosynthetic rate of leaves with 20% severity was reduced in both experiments to about 30% of the rate for the healthy control leaves. For the same leaves, the electron transport

rate in Experiment 4-2 was reduced to 74% of the rate for the healthy control leaves,. The estimated chlorophyll content of non-necrotic areas in Experiment 3-2 did not decrease significantly in leaves with up to 20% severity. The dark respiration rate in the diseased areas increased 26 fold in negative values when compared to healthy tissue (Figure 4.1).

Stomatal conductance, which represents the degree of stomatal opening and is directly proportional to the transpiration rate, decreased with increases in disease severity (Figure 4.2). This reduction in stomatal conductance was described by a negative exponential model (Figure 4.2a). The ratio of intercellular CO_2 to ambient CO_2 (Ci/Ca), 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 (Figure 4.2b).

The apparent quantum yield of CO_2 assimilation, expressed as mol CO_2/ mol of quanta, was obtained by dividing the absolute values of photosynthetic rate by the light intensity at the specific moment of the measurement. The apparent quantum yield of CO_2 assimilation was positively correlated to the effective quantum yield, which is a fluorescence parameter related to the efficiency of the photosystem II (Figure 4.3).

Virtual Lesion Size

The value of β, for the relationship of relative photosynthetic rate to anthracnose severity, was 8.46 for Experiment 4-1, and 12.18 for Experiment 4-2 (Figure 4.4). 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

Table 4.1. Net photosynthetic rate, electron transport rate, and estimated chlorophyll content for bean leaves with different levels of anthracnose severity.

Experiment 4-1			Experiment 4-2				
Anthracnose severity[a]	No. of leaves	Photosynthetic rate μmol CO_2 $m^{-2}s^{-1}$	Anthracnose severity	No. of leaves	Photosynthetic rate μmol CO_2 $m^{-2}s^{-1}$	Electron transport rate μmol CO_2 $m^{-2}s^{-1}$	Chlorophyll content SPAD values[c]
0.00	13	19.27±0.52[b]	0.00[a]	9	16.43±0.55	121.4±2.45	36.53±0.63[d]
<0.01	6	14.78±1.14	<0.01	5	15.06±0.87	124.4±5.07	36.52±0.63
0.01-0.05	6	10.29±0.89	0.01-0.02	9	14.25±0.62	118.8±3.56	36.41±0.41
0.05-0.10	7	12.37±0.88	0.02-0.03	6	12.09±0.34	109.8±4.60	35.33±0.96
0.11-0.20	8	5.99±1.42	0.03-0.05	7	10.39±0.77	107.0±7.26	35.24±0.58
0.21-0.35	3	2.75±1.30	0.05-0.2	4	5.065±1.68	90.4±7.89	35.70±1.48

[a] Proportion of leaf area with symptoms.
[b] Mean photosynthetic rate ± standard error.
[c] Chlorophyll was estimated with the Minolta Chlorophyll Meter SPAD-502; 15 readings on non-necrotic areas were taken for each leaf.
[d] Mean amount of chlorophyll ± standard error.

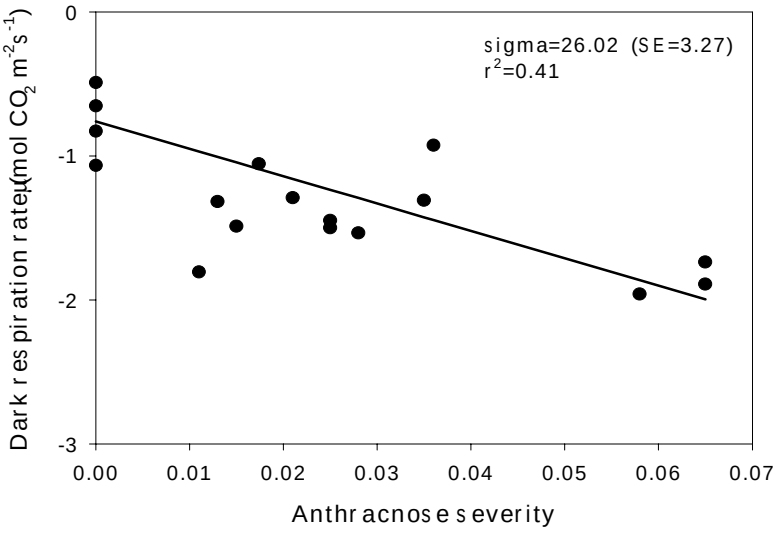

Figure 4.1. Effects of anthracnose severity on dark respiration rate (R_x) of bean leaves. Sigma (σ) values were obtained with the equation $R_x=R_0(1-x) + \sigma R_0 x$, where R_0 is the average dark respiration rate of the healthy leaves, and x is anthracnose severity, expressed as a proportion of the leaf area.

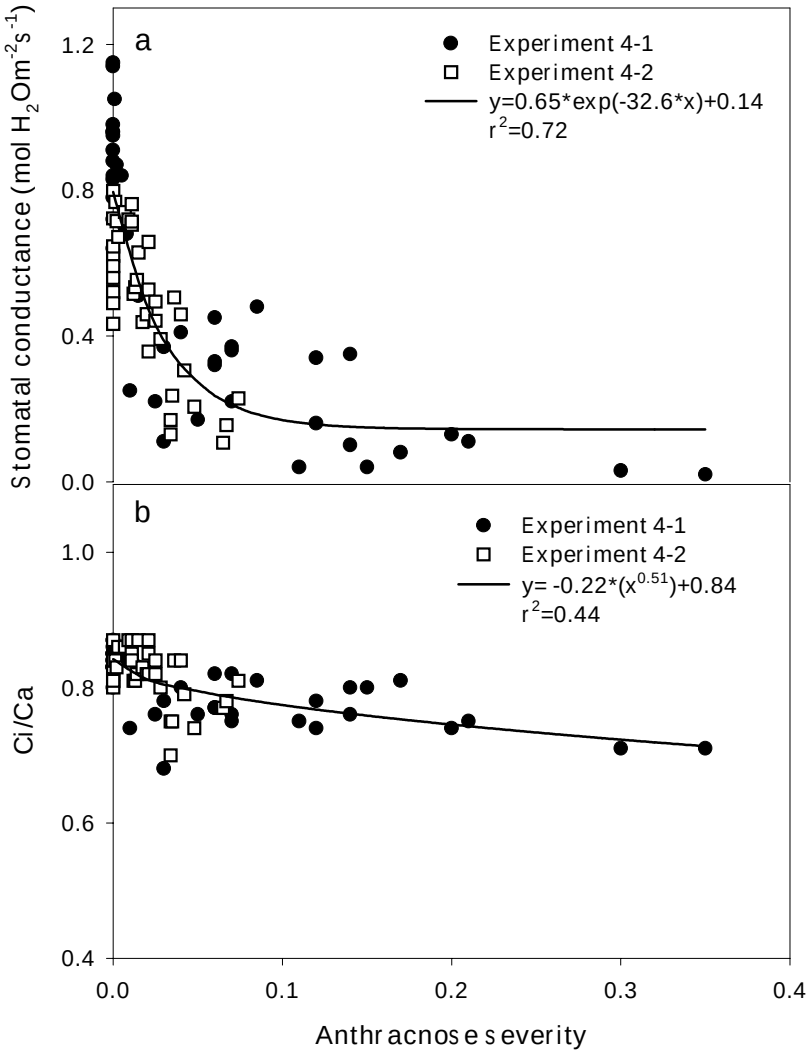

Figure 4.2. Effects of anthracnose severity in bean leaves on (a) stomatal conductance and on (b) ratio of intercellular to ambient CO_2 concentration (Ci/Ca).

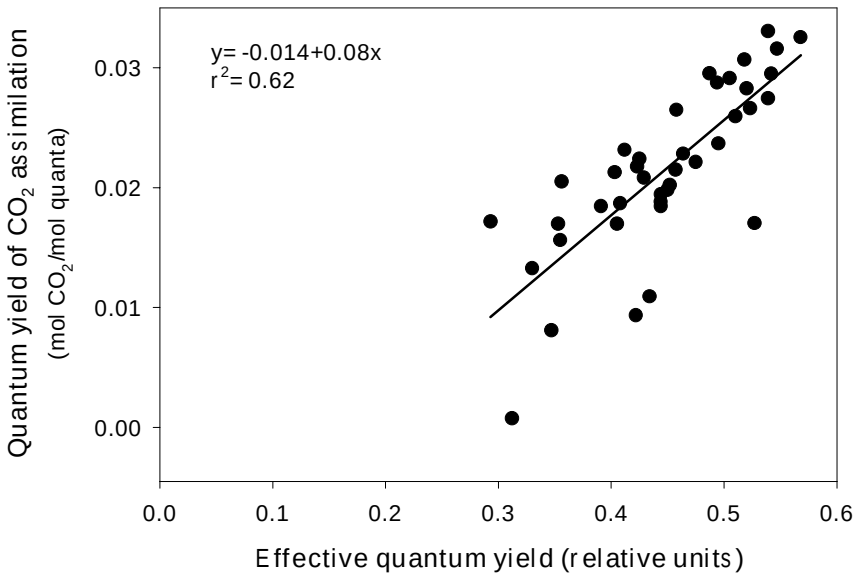

Figure 4.3. Relationship between effective quantum yield of the photosystem II and apparent quantum yield of CO_2 assimilation in bean plants with different levels of anthracnose severity.

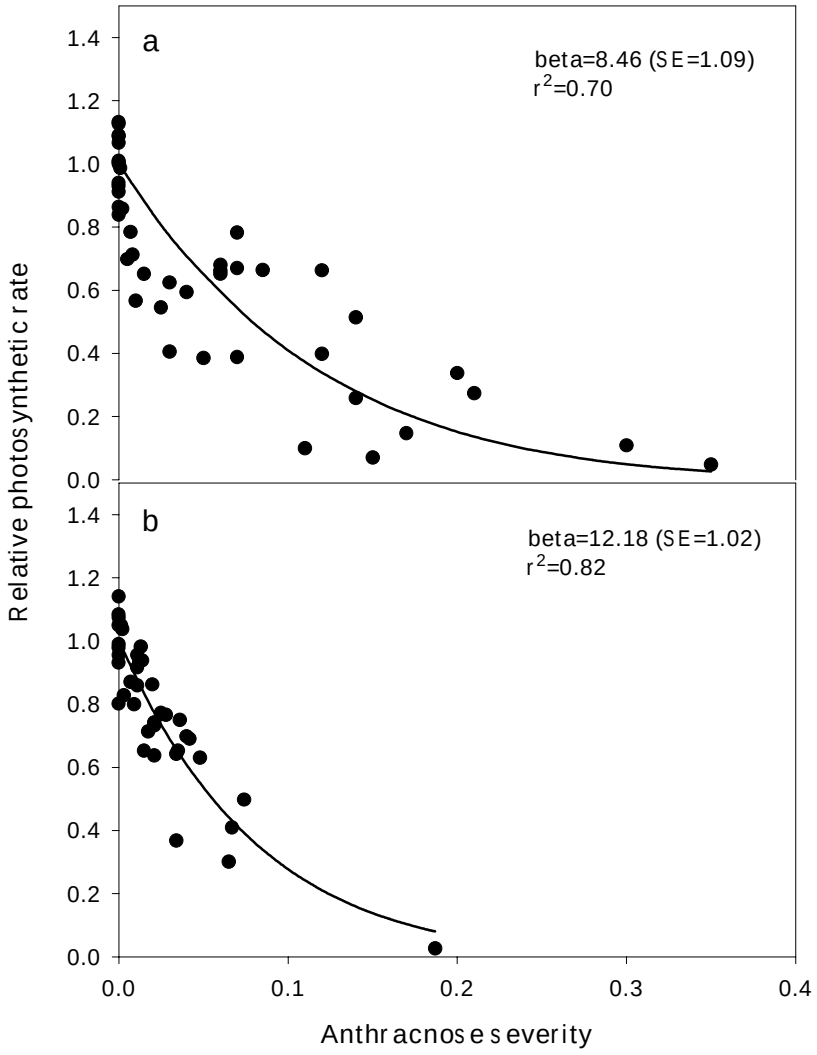

Figure 4.4. Effects of anthracnose severity (x) on the relative photosynthetic rate (P_x/P_0) of bean leaves. The values of beta (β) were obtained with the model $P_x/P_0 = (1-x)^{\beta}$; (a) Experiment 4-1, (b) Experiment 4-2.

cultivar. The values of the coefficient of determination for the three experiments were higher than the values reported in the literature for this type of experiment (Bastiaans, 1991; Goodwin, 1992). The higher coefficient of determination for the model in Experiment 4-2 compared to that for Experiment 4-1 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.

Effects of Fertilization on the Photosynthetic Competence of Bean Leaves with Anthracnose

The nutritional condition of the leaf determined its level of photosynthetic activity. The absolute average values of net photosynthetic rate, chlorophyll content, and electron transport rate for the healthy control leaves in Experiments 4-3 and 4-4 were significantly lower in plants that did not receive any fertilizer than in plants that received 50 or 100% of the recommended rate of fertilizer (Table 4.2). The results of the two experiments for the several variables analyzed were combined, based on lack of significant differences in the initial statistical analysis. At the levels of severity obtained in these experiments, there was no reduction on the chlorophyll content of diseased leaves, when compared to the healthy control leaves at all levels of fertilizer (Figure 4.5a). Leaf color also was not significantly affected by the levels of anthracnose severity observed (Figure 4.5b). Despite the absence of a significant relationship between anthracnose severity and leaf color or chlorophyll content, the two latter variables were correlated with relative photosynthetic rate and relative electron transport, when plants at all levels of fertilizer were considered (Figures 4.6 and 4.7).

Table 4.2. Absolute average values of net photosynthetic rate, chlorophyll content, and electron transport rate for healthy control bean leaves.

Fertilizer treatment	Experiment 4-3			Experiment 4-4		
	Photosynthetic rate μmol CO_2 $m^{-2}s^{-1}$	Chlorophyll content SPAD values[b]	Electron transport rate μmol $m^{-2}s^{-1}$	Photosynthetic rate μmol CO_2 $m^{-2}s^{-1}$	Chlorophyll content SPAD values	Electron transport rate μmol $m^{-2}s^{-1}$
No fertilizer	9.67±0.44[a]	22.20±0.85	69.40±4.08	11.22±0.81	27.24±0.94	88.42±3.91
50% fertilizer rate	15.44±0.44	31.81±0.70	106.57±4.0	15.2±0.78	32.48±1.17	112.56±4.3
100% fertilizer rate	14.10±0.65	31.54±1.33	95.46±3.23	17.66±0.58	35.25±0.69	126.52±2.9

[a] Means ± standard errors.
[b] Values obtained with the Minolta Chlorophyll Meter (SPAD-502); 15 readings on non-necrotic areas were taken for each leaf.

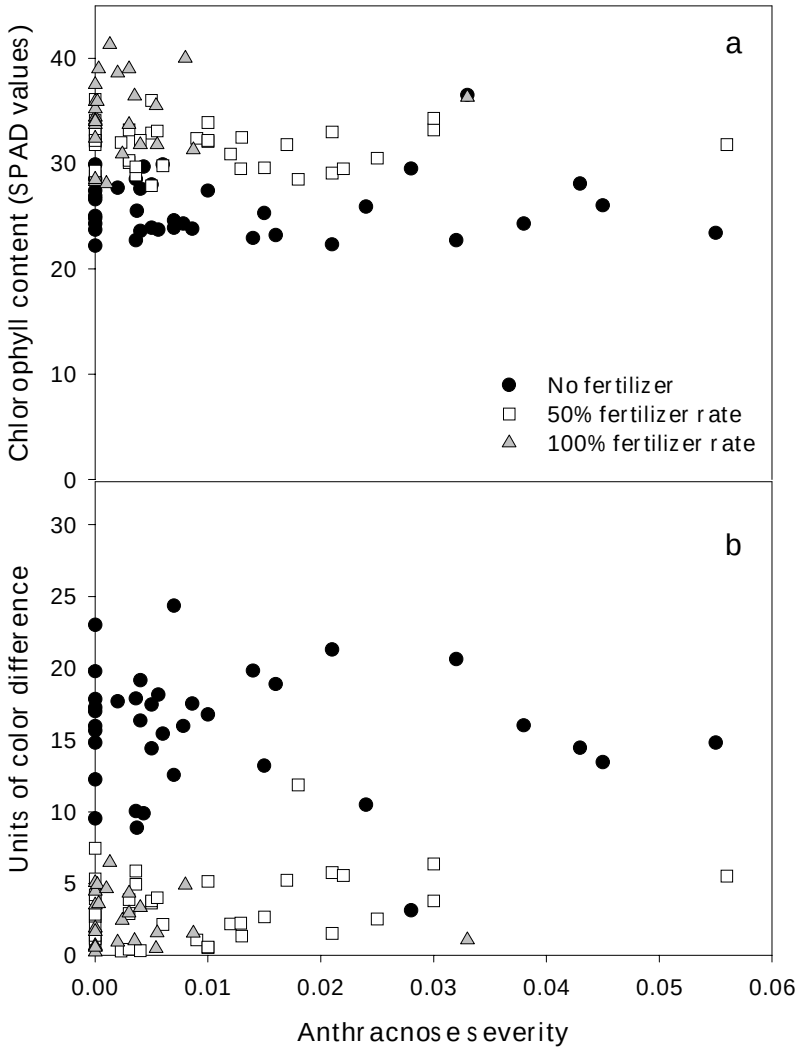

Figure 4.5. Effects of anthracnose severity on (a) chlorophyll content and (b) color of bean leaves with different nutritional status. SPAD values are the values given by the chlorophyll meter (SPAD-502, Minolta). Color is expressed as units of difference between the color of a given leaf and the average color of the well fertilized, healthy control leaves.

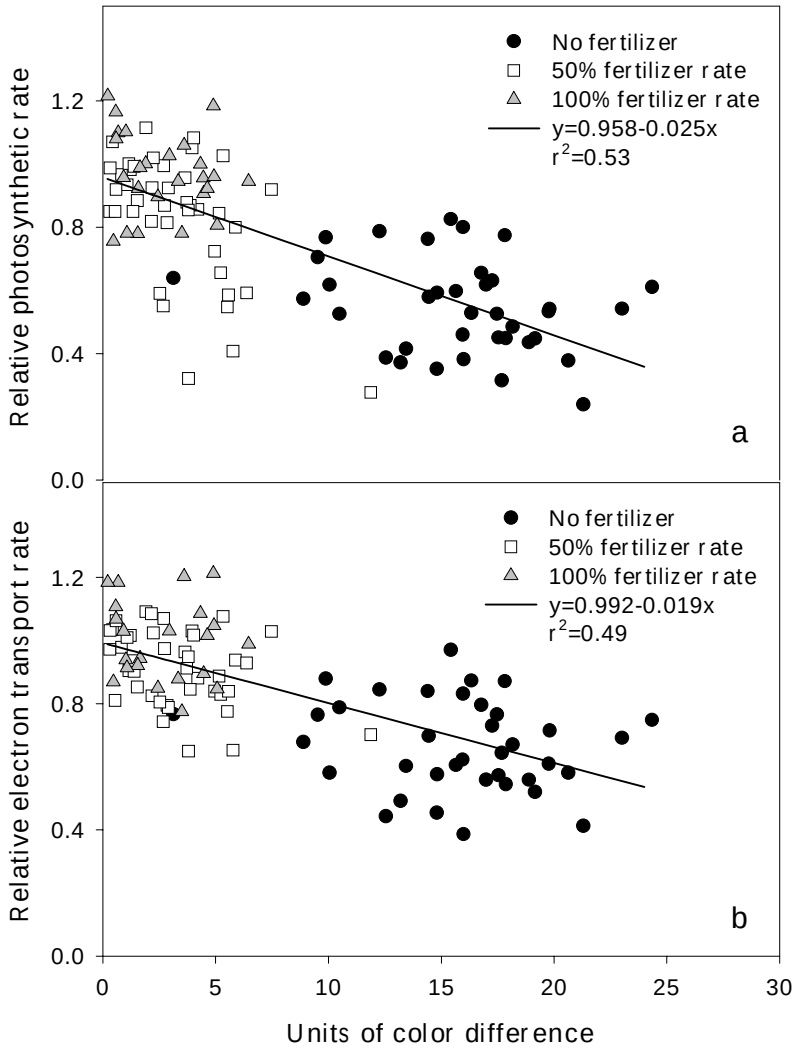

Figure 4.6. Responses of (a) relative photosynthetic rate and (b) relative electron transport rate to units of color difference at the different levels of fertilizer. Both rates are expressed as fractions of the average value of all healthy bean leaves at the recommended fertilizer rate of 100%. Data from Experiments 3-3 and 4-4 were combined in these graphs.

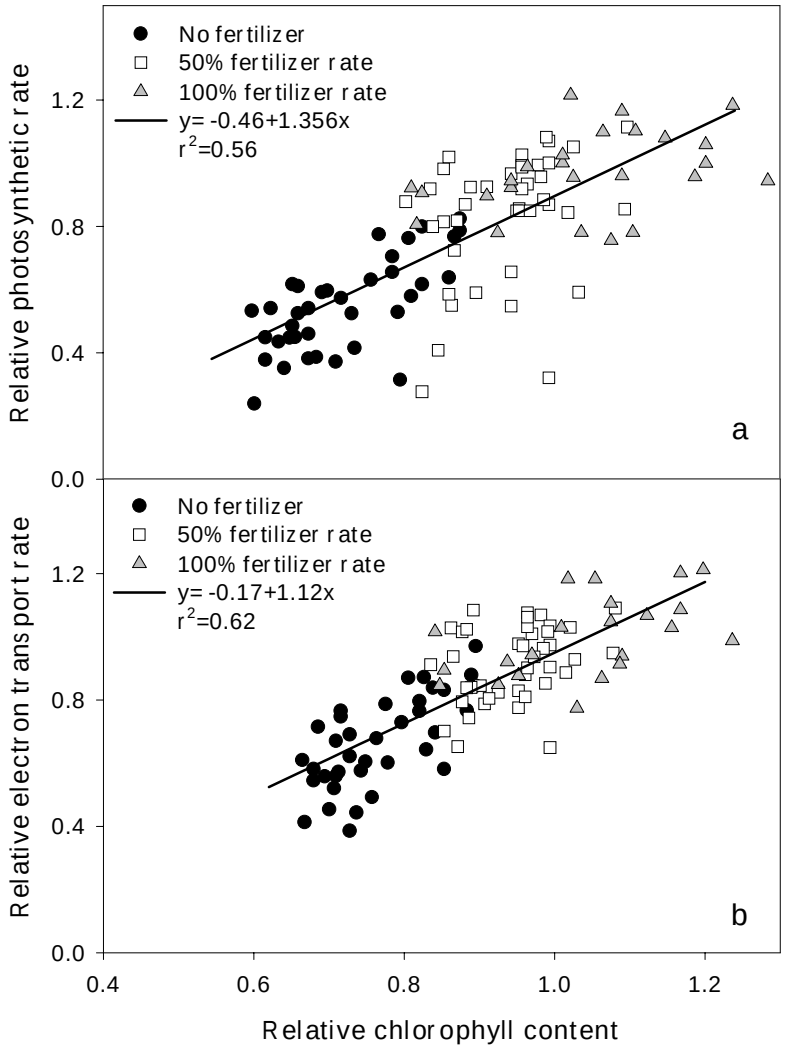

Figure 4.7. Responses of (a) relative photosynthetic rate and (b) relative electron transport rate to relative chlorophyll content (SPAD values), at the different levels of fertilizer. Both rates and the values of SPAD are expressed as fractions of the average value of all healthy bean leaves at the recommended fertilizer rate of 100%. Data from Experiments 4-3 and 4-4 were combined in these graphs.

The values of β, the ratio of virtual lesion to visual lesion size, for all three levels of fertilization were greater than one (Figure 4.8). The value of β for the group of plants that received no fertilizer (11.51) was significantly lower than the value obtained for the plants that received 50% of the recommended rate of fertilizer (18.56), but it did not differ significantly from the value for the treatment with 100% fertilizer (11.85). The treatments that received 50% and 100% of fertilizer rate did not have significantly different values of β. The variability observed in the data was high, and only a small part of this variability could be explained by the model that was used.

Measurement of Chlorophyll Fluorescence during Disease Development

For Experiment 4-5, there was a significant reduction over time in maximal fluorescence, electron transport rate, and effective quantum yield, in non-necrotic areas of inoculated leaves that had 1 to 7% severity (Figure 4.9b, 4.10a, 4.10b). These parameters were reduced in comparison to healthy tissue after the appearance of symptoms of anthracnose, 6 days after inoculation. Minimal fluorescence and optimal quantum yield were not reduced in leaves with 1 to 7% severity in comparison to healthy control leaves (Figure 4.9a, 4.9c). Leaves with 0.1-0.4% severity did not differ significantly from the healthy control leaves in any of the measured parameters during disease development. Anthracnose development in symptomatic areas of inoculated leaflets did not interfere with the fluorescence parameters in the non-inoculated leaflet of the same leaf (Figures 4.9d, e, f and 4.10c and d).

For Experiment 4-6, maximal fluorescence, optimal quantum yield, electron transport rate, and effective quantum yield were reduced in the non-necrotic areas of

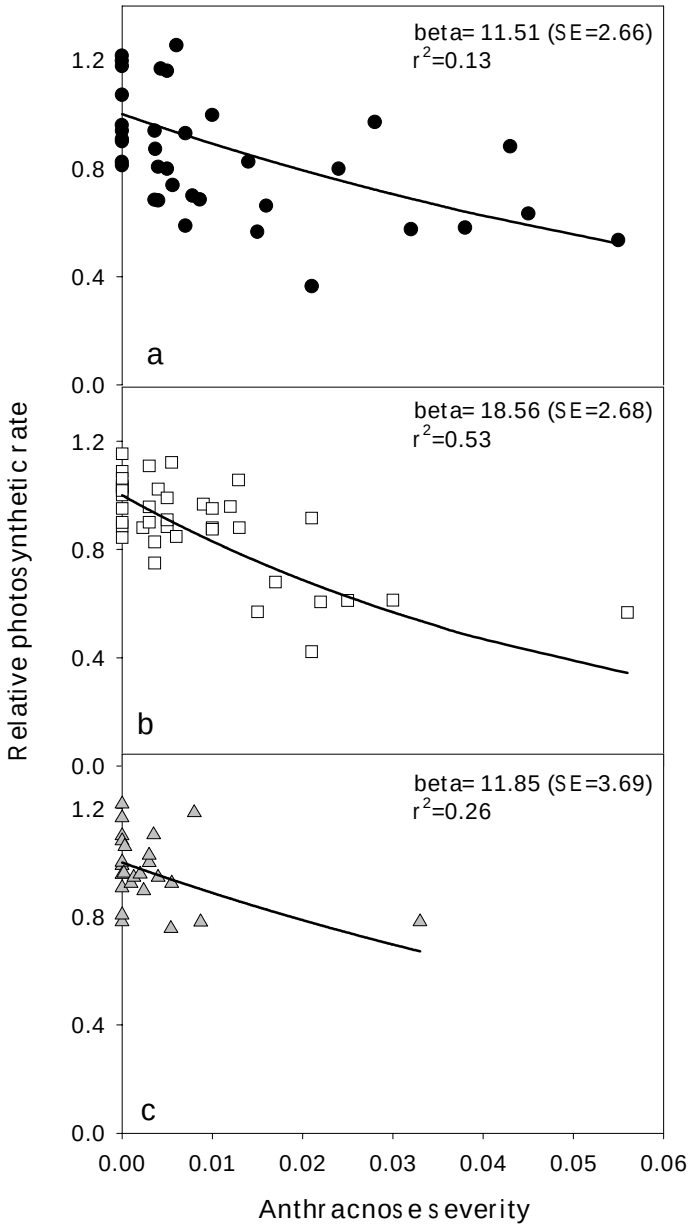

Figure 4.8. Effects of anthracnose severity (x) on the relative photosynthetic rate (P_x/P_0) of bean leaves with (a) no fertilizer, (b) 50% of the recommended fertilizer rate, and c) 100% fertilizer rate. The values of beta (β) were obtained with the model $P_x/P_0 = (1-x)^{\beta}$; the data from Experiments 4-3 and 3-4 were combined in these graphs.

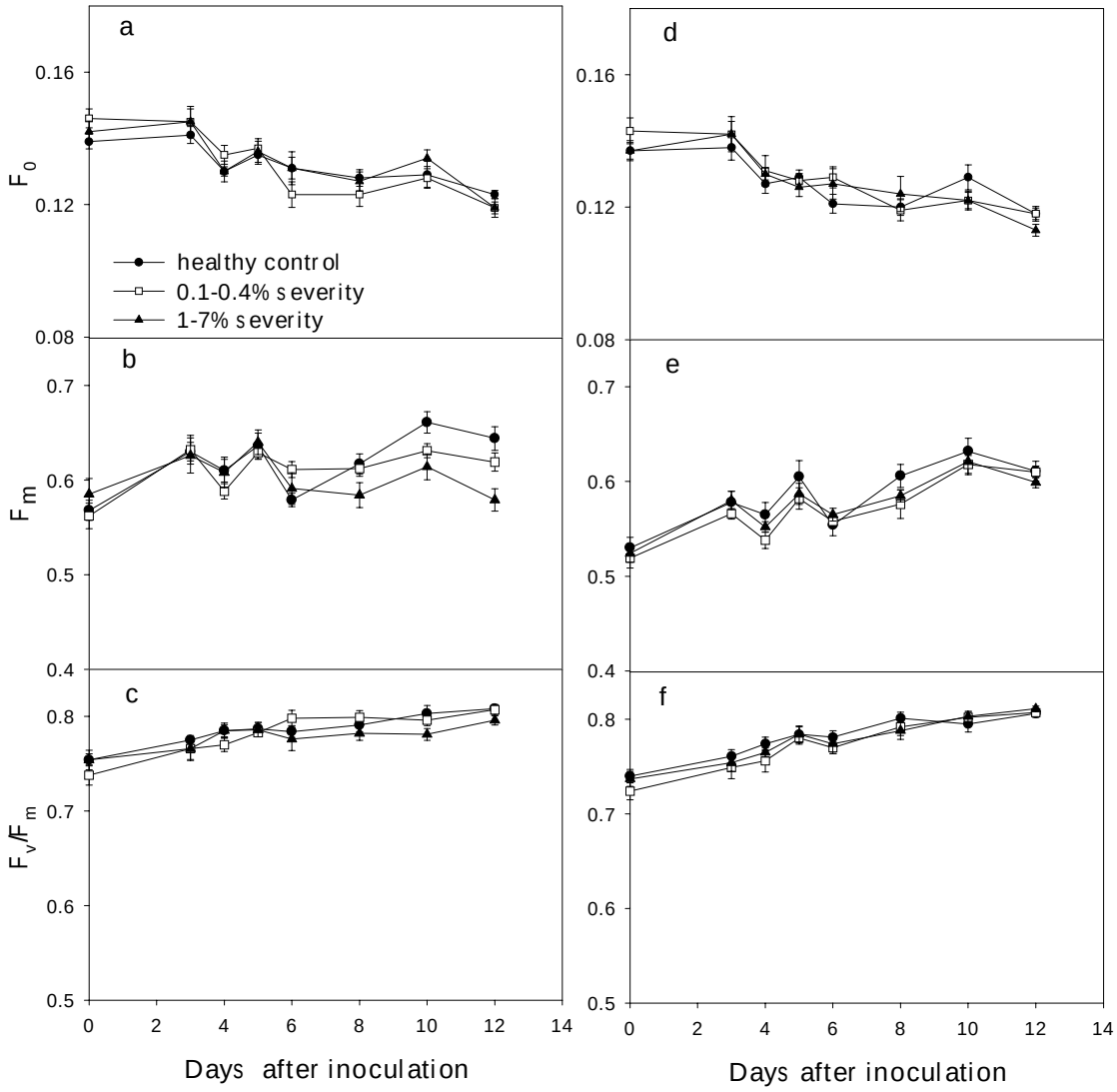

Figure 4.9. Minimal fluorescence (F_0), maximal fluorescence (F_m), and optimal quantum yield (F_v/F_m) during anthracnose development in non-necrotic areas of inoculated (a, b, c) and non-inoculated (d, e, f) areas of bean leaves with different anthracnose severities.

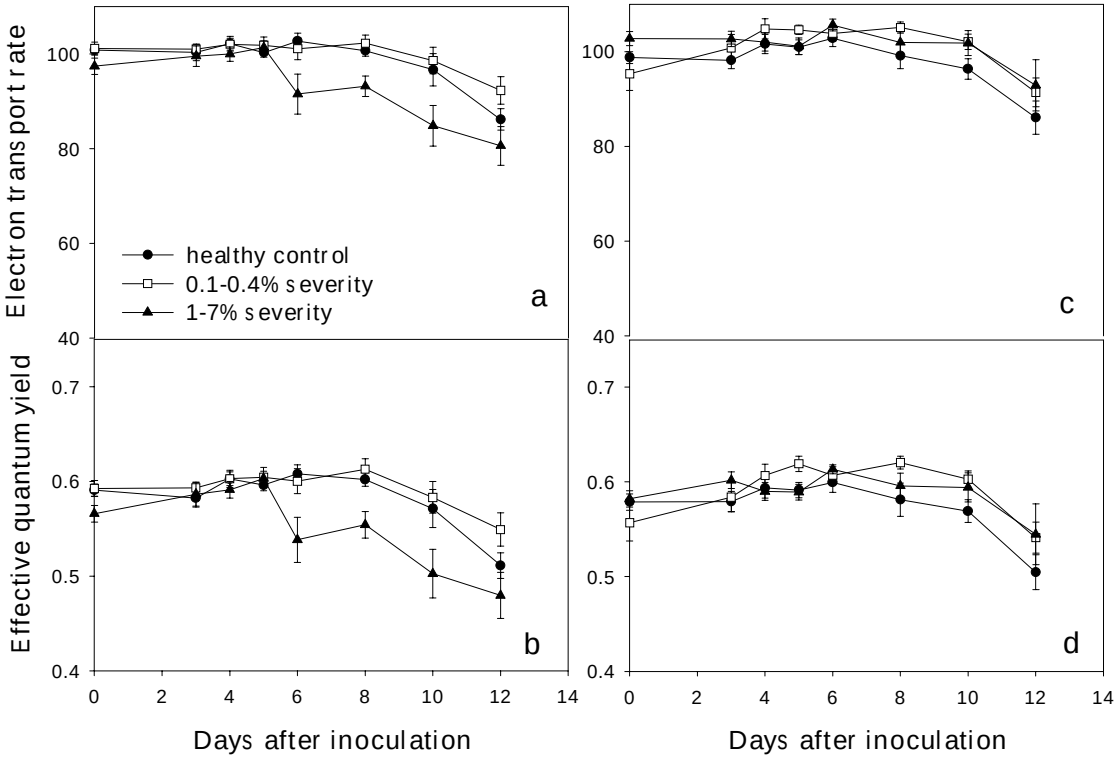

Figure 4.10. Electron transport rate (μmol $m^{-2}s^{-1}$) and effective quantum yield, in relative units, during anthracnose development in non-necrotic areas of inoculated (a, b) and non-inoculated (c, d) areas of bean leaves with different anthracnose severities.

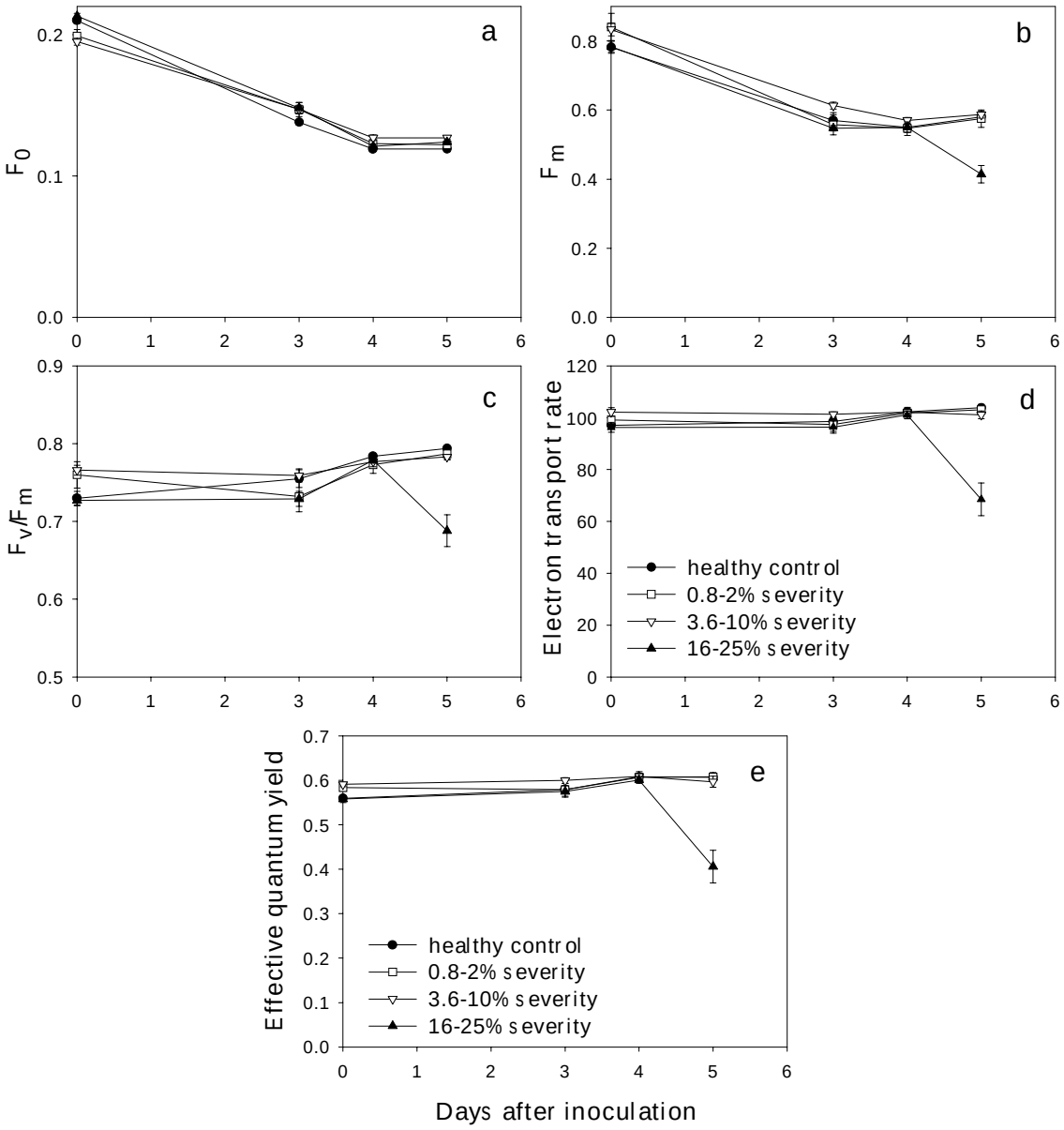

Figure 4.11. The effect of initial development of anthracnose in bean leaves on (a) minimal fluorescence (F_0), (b) maximal fluorescence (F_m), (c) optimal quantum yield (F_v/F_m), (d) effective quantum yield (all in relative units), and (e) electron transport rate (μmol $m^{-2}s^{-1}$).

leaves with 16-25% severity, as soon as the first symptoms of anthracnose were visible (5 days after inoculation) (Figures 4.11b, c, d, and e). Minimal fluorescence was not reduced for any of the levels of severity during disease development. Leaves with 0.8 to 2% or 3.6 to 10% severity did not differ from the healthy control leaves 5 days after inoculation. The fluorescence parameters of non-inoculated areas of inoculated leaves were not affected by the disease (data not shown).

Discussion

According to Luttrell's (1974) definition, *Colletotrichum 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, followed by a visibly destructive phase (Bailey et al., 1992; O'Connell and Bailey, 1991). The symptoms of the disease become visible when the pathogen switches to necrotrophic nutrition, and they are characterized by tissue maceration and water soaking. An increased activity of pectin lyases observed in infected tissues is associated with the switch from the transient biotrophic phase to the necrotrophic phase, in which extensive dissolution of cell walls occurs in advance of the pathogen (Wijesundera et al., 1989).

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 (Wong and Thrower, 1978a). Reduced photosynthetic and transpiration rates were reported for plants of *Phaseolus vulgaris* with different levels of anthracnose severity (Bassanezi et al., 1997). *Colletotrichum*

lindemuthianum had no significant effect on the translocation of current photosynthate from diseased leaves of *V. sesquipedalis* (Wong and Thrower, 1978b). The pathogen induced only a slight accumulation of photosynthates during the first days of pathogenesis.

The quantification of the impact of *C. lindemuthianum* on the photosynthesis of bean leaves is the first step to characterize the potential yield losses caused by anthracnose. The concept of virtual lesion, introduced by Bastiaans (1991), 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 by 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 photosynthesis in the remaining green leaf tissue. In the present study, the values of β obtained (8.46 and 12.18) are an indication that the virtual lesion induced by this pathogen is much larger than the visual lesion, which means that 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. (1997) for a different cultivar of common bean, 'Carioca Comum'. Since the same race of the pathogen, race kappa, was used in both studies, the difference in the β values could be due to a difference in the cultivar reaction to infection. However, this hypothesis should be investigated carefully, since differences in β values among cultivars may not imply genotypic differences in tolerance. Bastiaans and Roumen (1993) concluded that the β parameter was not a

suitable selection criterion in breeding rice for tolerance to leaf blast, as the β values were similar for three susceptible cultivars with different relative infection efficiencies.

The method of severity assessment also may have influenced the determination of the β values. The same diagrammatic scale of anthracnose symptoms (from Godoy et al., 1997) was used by Bassanezi et al. (1997) and in the present study (Experiment 4-1) to obtain the values of β of 7.24 and 8.46, respectively. For the experiment in the present study in which the value of 12.18 was obtained (Experiment 4-2), the actual severity was assessed by determining the ratio between the area of the lesions and the area of the leaf. In a preliminary experiment (Appendix E), conducted before Experiment 4-2, the ratings anthracnose severity present in the leaves obtained with the diagrammatic scale were overestimated, when compared to the actual values. If anthracnose severity were overestimated by the use of the scale, the resultant β value would tend to be lower than it would be with values of actual severity.

Besides the fact that the lesions of anthracnose on the leaves are irregularly shaped, which makes the assessment of severity difficult, there are other characteristics of the symptomatology of this disease that further complicate the scenario. The necrotic symptoms of anthracnose are localized on the leaf veins, which most likely interferes with the transport of water and assimilates in the leaf. Moreover, in very susceptible cultivars, such as the one used in the present study, the leaf tissue adjacent to the lesions usually becomes water-soaked. On leaves with high disease severity, the water-soaked regions rapidly coalesce and large portions of the leaf can collapse in less than one day after necrotic symptoms are first observed. Also, lesions of the same size but in different positions on the leaf may have a different impact on the leaf physiology. Bastiaans and

Roumen (1993) observed that blast lesions on the central vein of rice leaves caused a marked reduction in the photosynthetic rate of the distal part of the leaf, while lesions on either side of the central vein caused only localized effects.

While the net photosynthetic rate of bean leaves, determined by gas-exchange measurements, was drastically reduced with increases in anthracnose severity, the effects on the chlorophyll fluorescence parameters were less severe. Similar to the results with bean rust reported in Chapter 3, there were no relative changes in the fluorescence parameter in non-inoculated areas of inoculated leaves in any of the levels of severity investigated.

Minimal fluorescence (F_0) represents the emission from molecules of chlorophyll a of the light harvesting antenna associated with PSII prior to the excitation energy being used for electron transport (Krause and Weis, 1984). It is therefore independent of photochemical events, but dependent upon the chlorophyll content of the tissue and on the ultrastructure of the thylakoid membrane. In this study, F_0 was not altered by anthracnose development, even at high severity levels (Figures 4.9a and 4.11a), which may be due to the lack of change in chlorophyll content in the early development of this disease (Experiment 4-2, Table 4.1).

Maximal fluorescence (F_m) is a measure of the oxidation-reduction status of the electron acceptors between PSII and PSI and a direct indicator of PSII activity (Scholes and Farrar, 1985). Environmental stresses that cause thylakoid damage, such as heat and freezing stresses, usually lower F_m. The F_m values of leaves with high anthracnose severity were reduced by 28%, in relation to the control leaves, as soon as the necrotic symptoms were visible (Figure 4.11b). In leaves with lower anthracnose severity, F_m was

reduced only a few days after the appearance of symptoms (Figure 4.9b). Maximal fluorescence was also reduced in plants infected by other pathogens, such as *Phaeoisariopsis griseola* on common bean (Bassanezi, 1995), *Uromyces muscari* on bluebell (Scholes and Farrar, 1985), *Taphrina deformans* on peach (Raggi, 1995), and tobacco mosaic virus on tobacco (Balachandran and Osmond, 1994).

The optimal quantum yield (F_v/F_m) provides information on the potential photosynthetic efficiency of PSII, and it is often discussed as a vitality index (Moll et al., 1995). It has a range of 0.75 to 0.85 for healthy leaves of many plant species and ecotypes, decreasing under stress (Bolhàr-Nordenkampf and Öquist, 1993). The calculation of this parameter depends on the values of F_0 and F_m. In the present study, F_v/F_m was unchanged in leaves with low severity (Figures 4.9c and 4.11c). The reduction in F_m for plants with 16-25% anthracnose severity induced a reduction of 13% in F_v/F_m, compared to the control leaves (Figure 4.11c). This reduction in F_v/F_m may be an indication that mechanisms of photon protection are being activated in response to the stress caused by the disease development. Photon protection is the result of a decrease in the efficiency of photosynthesis by means of dissipating the excess of photons as heat, before these photons can reach the reaction centers (Osmond, 1994).

Effective quantum yield is the quantum yield of the non-cyclic electron flow (Foyer, 1993; Genty et al., 1989). It is proportional to the concentration of the open PSII reaction centers, and to the efficiency with which these centers capture and use excitation energy. It is the basis for the calculation of the electron transport rate. Both effective quantum yield and electron transport rate suffered reductions, compared to the control leaves, on diseased leaves with 1-7% severity (Figure 4.10a and b) and 16-25% severity

(Figure 4.11d and e). The reduction in these parameters coincided with the appearance of necrotic symptoms, which occurred 5 to 6 days after the inoculation.

Chlorophyll fluorescence is a measure of the efficiency of light utilization on PSII. There is a ubiquitous curvilinear or biphasic relationship between the effective quantum yield of fluorescence and the apparent quantum yield of CO_2 assimilation (mol CO_2/mol quanta) (Cornic and Briantais, 1991; Genty et al., 1989; Seaton and Walker, 1990). This relationship may allow good estimates of photosynthetic rate through measurements of fluorescence, without recourse to analysis of gaseous exchange between leaf and environment. Inhibition of primary photochemistry, electron transport, or carbon metabolism caused by environmental stresses will affect the function of PSII and will be expressed as changes in fluorescence yield. These changes in fluorescence yield can provide quantitative information about plant responses to the duration and intensity of the stress (Bolhàr-Nordenkampf and Öquist, 1993). In the present study, there was a linear relationship between effective quantum yield and apparent quantum yield of CO_2 assimilation (Figure 4.3). However, the linear regression did not pass through the origin, which means that for bean leaves with anthracnose, the effective quantum yield of PSII is not zero when the efficiency of CO_2 assimilation is zero, as would be theoretically expected (Genty et al., 1989; Seaton and Walker, 1990).

There was a clear imbalance between the drastic inhibition in net photosynthetic rate and the moderate decreases in the photochemical reactions (F_v/F_m and electron transport rate) in bean leaves with anthracnose. Raggi (1995) observed a similar trend for peach leaves infected with *Taphrina deformans*. He suggested that the refixation of the CO_2 released by stimulated respiration could partially explain the disparity between gas-

exchange and fluorescence data. In the present study, this explanation also may apply, since respiration was highly stimulated by disease development at low severity levels (Figure 4.1).

The rate of respiration in the light is comparable to the rate of respiration in the dark (Azcón-Bieto and Osmond, 1983). Respiration rate contributes significantly to the total CO_2 exchange in illuminated leaves. Increased respiration was partially responsible for the reduced photosynthetic rates observed in bean leaves infected by *C. lindemuthianum*, because the rate obtained by gas-exchange measurements was a net photosynthetic rate, which included CO_2 effluxes and refixation, besides the CO_2 assimilation by the leaf tissue. Rates of dark respiration in the affected tissue were determined to be 26 times higher, in negative values, than in non-infected green tissue, according to Bastiaan's equation (Bastiaans, 1993). Wong and Thrower (1978a) also reported increased respiration rates at the onset of necrotic symptoms of anthracnose on *V. sesquipedalis*. Possible mechanisms of increased respiration in tissues infected by necrotrophic pathogens are related to regenerative activity of tissue physically damaged by the invading pathogen, increased activity of host enzymes involved in carbohydrate degradation and biosynthesis of secondary compounds, enhanced consumption of ATP and reductants through increased biosynthesis, increased levels of starch and soluble sugars that accumulate in the cells as a result of blocked translocation, loss of compartmentation of enzymes and key metabolites because of increased membrane permeability, and uncoupling of mitochondrial electron transport from ATP synthesis (Hutcheson and Buchanan, 1983). 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 non-specific 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* (Bailey et al., 1992).

The stomatal conductance of bean leaves decreased sharply with increases in anthracnose severity (Figure 4.2a). The simultaneous reduction in photosynthetic capacity and stomatal conductance of the diseased leaves was expected because these physiological processes are closely linked. Stomatal functioning is believed to be integrated with photosynthesis in such a way that it optimizes the use of water while only marginally limiting the photosynthetic process (Farquhar and Sharkey, 1982). 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 (Hall and Loomis, 1972; Schulze and Hall, 1982; Wong et al., 1979). A simultaneous reduction of carbon assimilation and transpiration rates 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 (Farquhar and Sharkey, 1982; Rabbinge et al., 1985). Carboxylation resistance is the resistance of the mesophyll to CO_2 diffusion. Stomatal resistance, which is the inverse of stomatal conductance, represents the degree of stomatal closure. The drop in the Ci/Ca ratio, which is related to the intercellular concentration of CO_2, was an indication that the flow of CO_2 from the stomatal cavities to the carboxylation sites was affected, and that stomatal closure was a major cause of reduced assimilation (Figure 4.2b).

Low availability of essential mineral elements for growth impairs the photosynthetic capacity of a plant. The question of whether or not there was an

interaction between level of plant nutrition and impact of anthracnose on leaf photosynthesis could not be answered conclusively by the experiments performed. Although the values of the β parameter were similar to the β values obtained in the previous experiments (Experiments 4-1 and 4-2), the model explained only a very small part of the variability of the data sets.

There was no significant loss of chlorophyll from bean leaves with up to 20% anthracnose severity (Experiment 4-2, Table 4.1; Figure 4.5a), which is an indication that factors other than chlorophyll content were responsible for the reductions in photosynthetic rate. Wong and Thrower (1978a) observed a drop in chlorophyll content on leaves of *V. sesquipedalis* with high anthracnose severity, but they also observed that the rate of photosynthesis declined more rapidly than the chlorophyll content. Plants with different levels of fertilizer used in Experiments 4-1 and 4-2 had leaves that differed in chlorophyll content (Figure 4.5a). Leaf color, expressed as the difference in the color of a leaf in relation to a healthy and well fertilized control standard, was also determined by the level of fertilizer applied to the plant (Figure 4.5b). The low levels of anthracnose severity observed in the experiments did not produce any change in the chlorophyll content and leaf color of the diseased leaves in any of the fertilizer levels. Nevertheless, leaf chlorophyll content and color had linear relationships with the variables net photosynthetic rate and electron transport rate, but these relationships were clearly a function of the nutritional status of the leaves.

From the measurement of various physiological variables in healthy and diseased bean leaves, it was concluded in the present study that anthracnose severely impairs the photosynthetic competence of bean leaves. The major factors involved in the reduction

of photosynthetic competence included increased respiration and stomatal closure. A reduced capacity in performing photosynthesis is likely to reduce the efficiency with which the intecepted radiation is utilized by the plant. Thus, accurate assessments of healthy leaf area duration and healthy leaf area absorption 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 (1991), could be incorporated in the calculation of HAA and HAD or in similar models used for yield predictions.

The quantitative determination of the effects of a disease on the physiology of individual leaves is the first step towards a broader understanding of crop losses (Bastiaans et al., 1994). The future steps would be to determine disease effects on the physiology of whole plants and, then, to integrate this information on plant performance over an entire season.

CHAPTER 5
THE PHOTOSYNTHETIC COMPETENCE OF BEAN LEAVES WITH RUST AND ANTHRACNOSE

Introduction

The attack of more than one pathogen in the same host is a common event under field conditions, especially in tropical areas (Waller and Bridge, 1984; Savary and Zadoks, 1992a; Zadoks and Schein, 1979). The simultaneous occurrence of different pathogens in the same plant greatly complicates the diagnosis of diseases, crop loss assessments, and decisions about control measures (Waller and Bridge, 1984). Although the importance of pathogen interaction has been recognized for many decades (Fawcett, 1931; Powell, 1979; Richardson and Doling, 1957), adequate progress in this area has been prevented due to the complexity of the subject and the lack of more objective methods to estimate the effects of multiple diseases and pests on crops (Kranz and Jörg, 1989; Teng, 1983).

The damage of multiple pathogens on the host may be classified in the following three categories (Waller and Bridge, 1984): absence of interaction, positive or synergistic interaction, and negative interaction. Absence of interaction occurs when the damage caused by one pathogen does not change that caused by another (Cole, 1982; Hyde, 1978; Jenkins and Jones, 1981). Where the damage is greater than that expected on a purely additive basis, there is a positive interaction (Datnoff and Sinclair, 1988; van der Wal et al. 1970; Rowe et al., 1985). Negative interactions occur where the damage is less than

that expected from the individual effects of each pathogen (Bassanezi et al., 1998; Johnson et al., 1986; Nelson and Campbell, 1992).

The effects of multiple pathogens on parameters of disease development may not reflect the effect of the interaction on yield (Simkin and Wheeler, 1974). Therefore, the effects of pathogen interactions on yield should be measured directly. 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 (Berger, 1988; Kranz and Jörg, 1989; Johnson and Teng, 1990; Savary and Zadoks, 1992a). However, some diseases may have an impact on the green leaf area beyond the symptomatic area (Bastiaans, 1991) and, in such cases, care should be taken to determine which portion of the tissue can be considered healthy.

The objective of this section was to determine the effects of the simultaneous infection of bean leaves with *Uromyces appendiculatus* and *Colletotrichum lindemuthianum* on leaf photosynthesis.

Material and Methods

Bean plants of cv. Rosinha were grown in 4-liter pots filled with Metromix under greenhouse conditions, where the temperature ranged from 19-31^{o}C in both runs of this experiment (Experiment 5-1 and Experiment 5-2). The plants were fertilized every other day with Peter's fertilizer (20-20-20, 1 g/l of water). 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.

The urediniospores of race 86 of *U. appendiculatus* were collected from pustules formed on 'Rosinha' plants, allowed to dry for 24 hours in a silica-gel container, and stored under -4^{o}C in glass vials submitted to vacuum. The viability of urediniospores in each sample was tested before each inoculation. A diluted suspension of urediniospores was plated on water-agar, and the percentage of germinated spores after a 12-hour 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 minutes. The concentration of spores was determined with a haemocytometer, and the suspension was then adjusted to the desired concentration by dilution.

Conidia of *C. lindemuthianum*, race kappa, were produced on Mathur's C modified medium (Balardin et al., 1997). A suspension of conidia was spread on the surface of the medium to obtain maximum sporulation. Ten- to fourteen-day-old cultures were rinsed with sterile distilled water to prepare a concentrated spore suspension, which was then diluted to obtain the desired concentrations. The viability of the conidia was determined prior to the preparation of the suspensions. A diluted suspension of conidia was spread on the surface of water-agar medium, and the percentage of germinated conidia was determined after 12 hours of incubation.

Concentrated spore suspensions of each fungus were prepared with sterile distilled water and 0.01% Tween-20. Nine spore suspensions were then prepared by dilution (Figure 5.1).

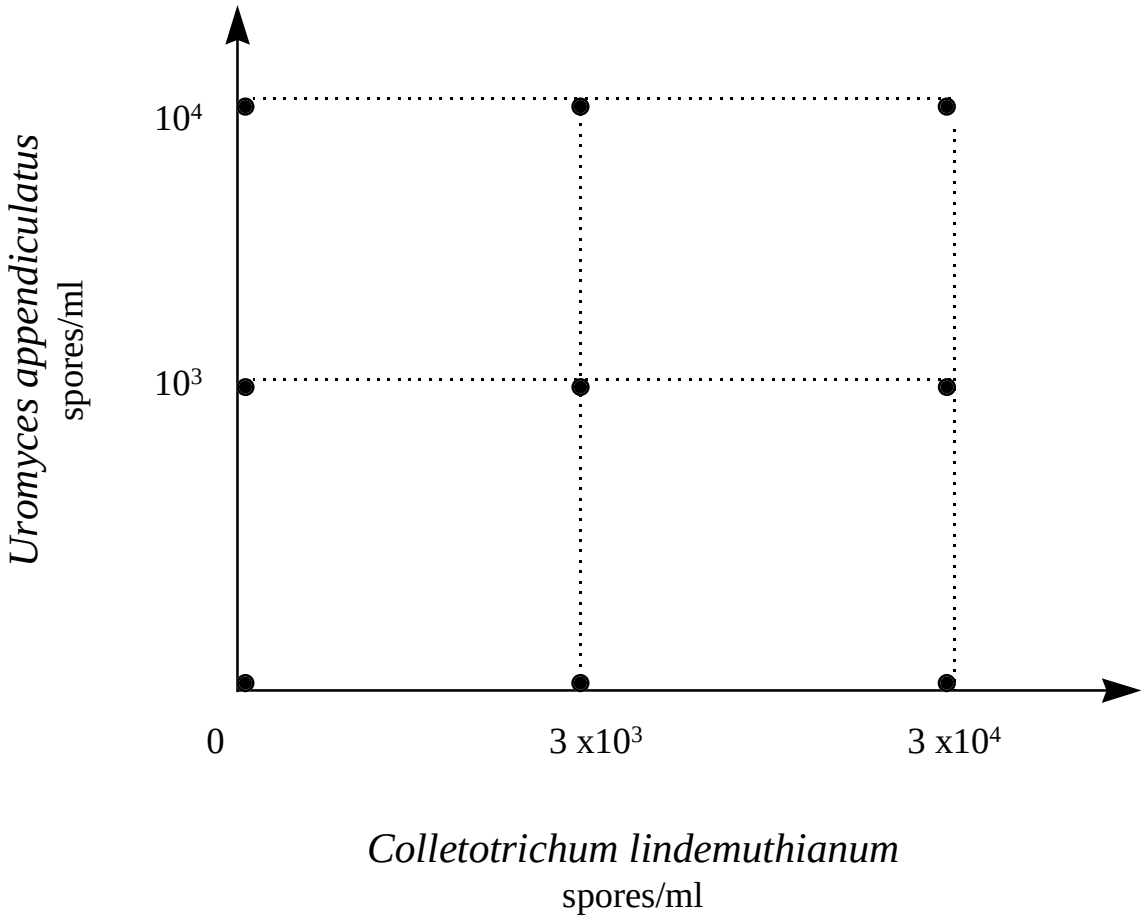

Figure 5.1. Single and combined spore suspensions prepared for inoculation. Each point in the graph represents the final concentration of one of the nine suspensions.

The suspensions were sprayed onto the underside of first and second trifoliate leaves for Experiment 5-1, and onto third and fourth trifoliate leaves for Experiment 5-2. The plants were then individually enclosed in transparent plastic bags for 16 hours. Since 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 to ensure that leaves with high severity of anthracnose would be available for the measurements.

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 and the stomatal conductance of healthy and diseased leaves were determined with the LI-6200 Portable Photosynthesis System (LI-COR Inc.). Dark respiration rate was also measured in Experiment 5-2. Measurements were taken on both inoculated leaves of each plant at a range of light intensity of 700 to 1000 μmol photon $m^{-2} s^{-1}$, in the same way described in chapter 3. Electron transport rate and effective quantum yield were measured on healthy leaves and on non-symptomatic areas of diseased leaves using the PAM-2000 fluorometer (Walz, Germany).

After the 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 an area meter (LI-3000 Portable Area Meter, LI-COR). Rust severity was determined from the number of lesions (pustule + 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 plastic was then passed through the area meter and severity was calculated as the proportion of leaf area with lesions.

The data for photosynthetic rate, stomatal conductance, and electron transport rate were plotted against rust and anthracnose severity in a tridimensional space. The software Table Curve 3D (Jandel Scientific, version 1.0) was then used to find the response surfaces that would best fit the set of data points. The choice of the best fitted equation was based on the values of the coefficient of determination, number of parameters, and the value of the F statistic.

Results

The data of the two experiments were combined, since there was no significant variation in the average rates of the measured variables for the healthy control plants in both experimental runs. Net photosynthetic rates of bean leaves with only one disease or both rust and anthracnose were reduced when compared to healthy leaves (Figure 5.2). Similar increases in disease severity caused greater decreases in photosynthetic rate on leaves with only anthracnose than on leaves with only rust, as described in the previous chapters. Stomatal conductance and electron transport rate were also severely reduced on leaves with anthracnose, while slight reductions were observed on leaves with rust only (Figures 5.3 and 5.4). Low severities of rust, anthracnose, and the combination of the two diseases induced increases, in negative values, in the dark respiration rate of diseased leaves (Figure 5.5).

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, stomatal conductance, or electron transport rate (Figures 5.2c, 5.3c, and 5.4c). This weak correlation between green leaf tissue and the physiological variables was expected, since the impact of these diseases on the remaining green leaf area is different (Chapters 3 and 4).

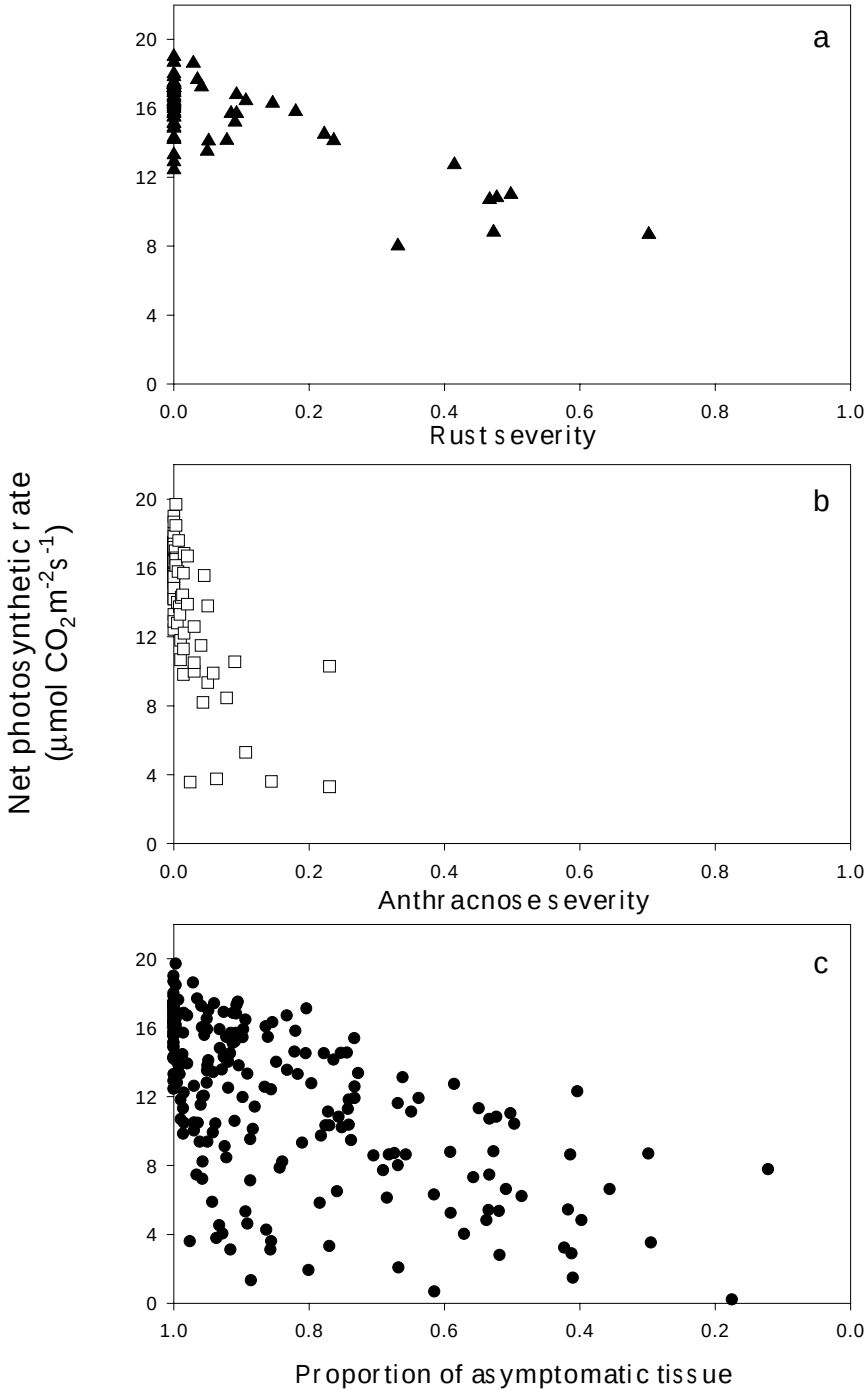

Figure 5.2. Net photosynthetic rate on healthy and diseased bean leaves. (a) Leaves with rust only; (b) leaves with anthracnose only; (c) leaves with rust, anthracnose or both diseases. The proportion of asymptomatic tissue was obtained by subtracting rust and/or anthracnose severities from one.

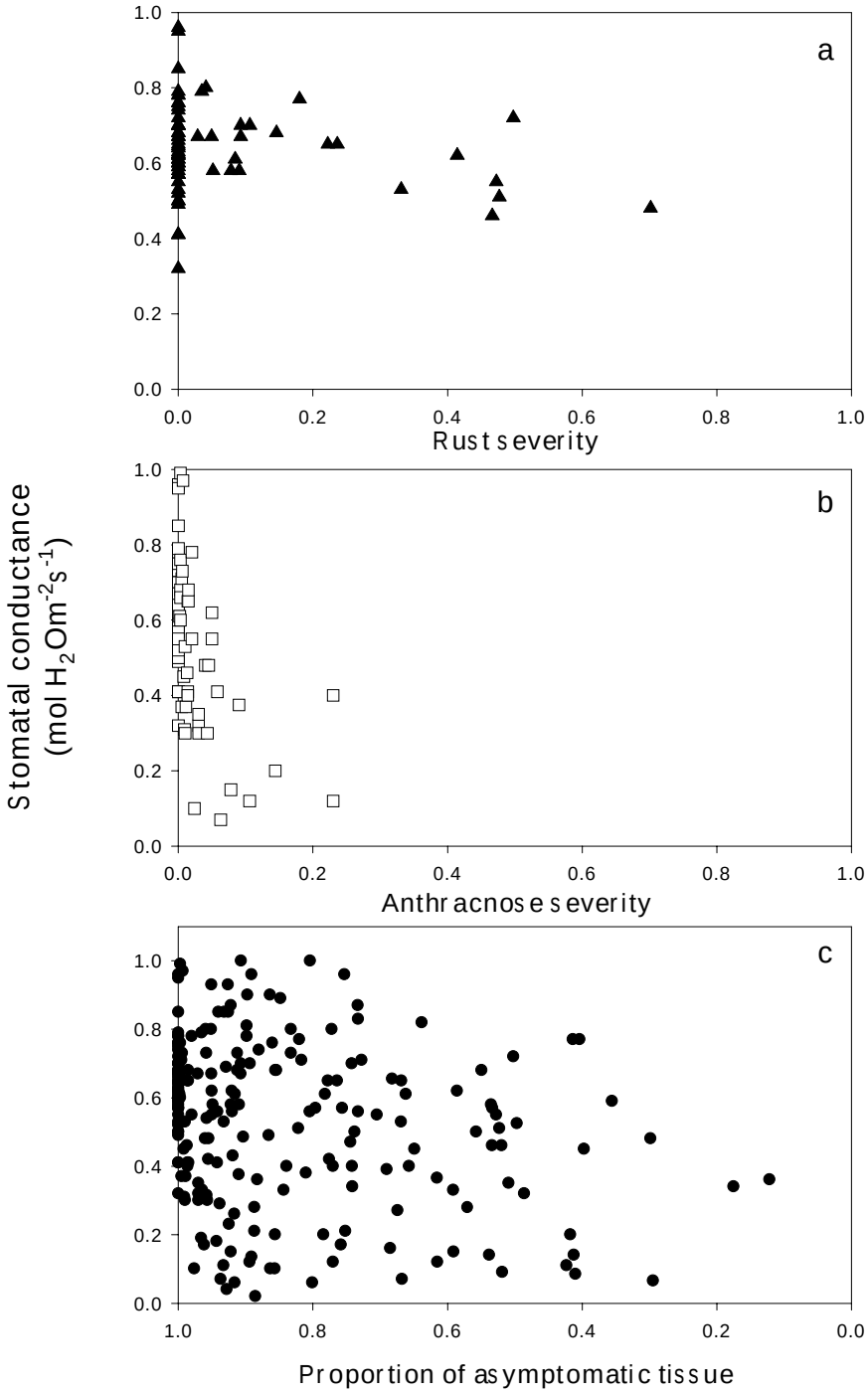

Figure 5.3. Stomatal conductance on healthy and diseased bean leaves. (a) Leaves with rust only; (b) leaves with anthracnose only; (c) leaves with rust, anthracnose or both diseases. The proportion of asymptomatic tissue was obtained by subtracting rust and/or anthracnose severities from one.

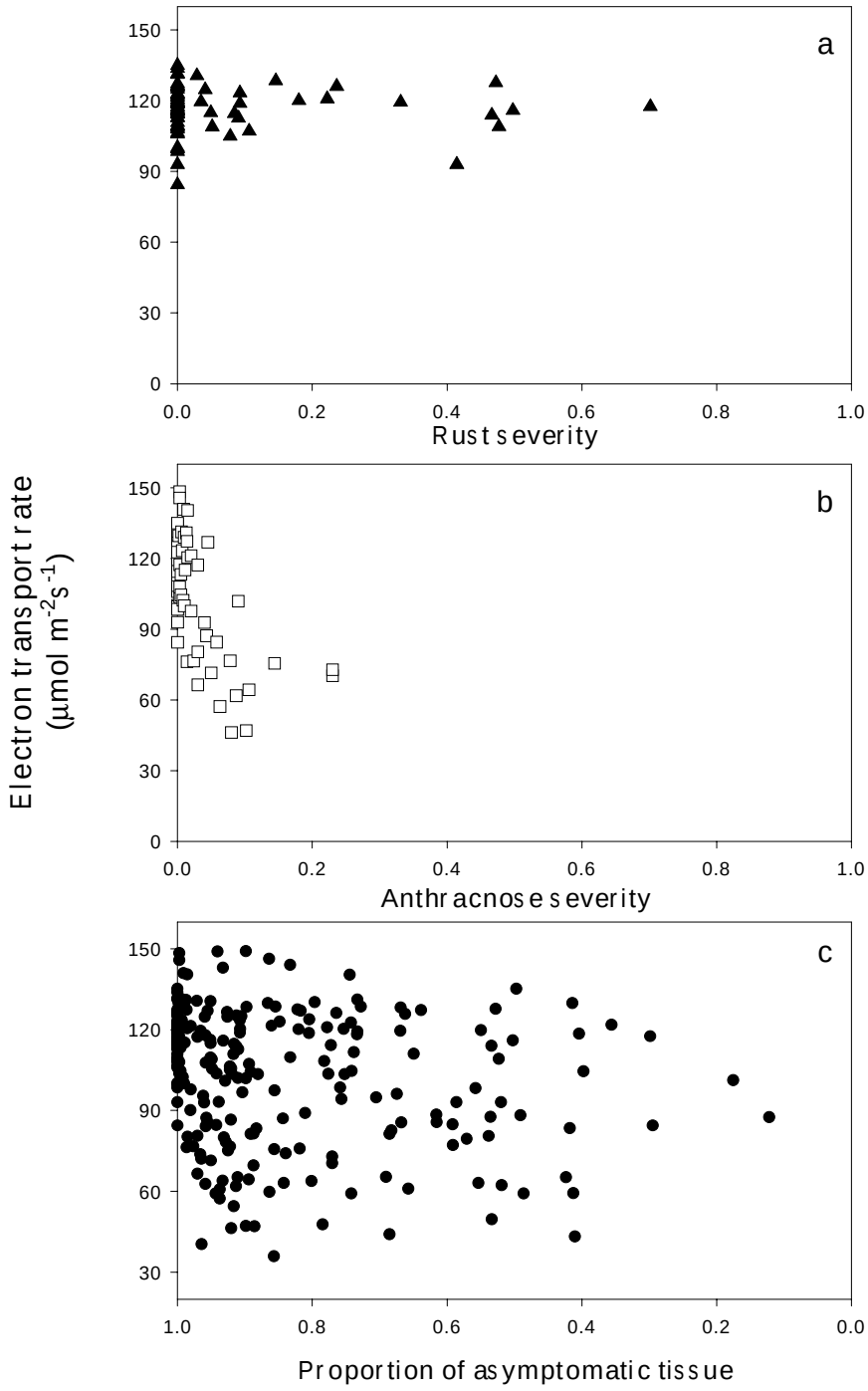

Figure 5.4. Electron transport rate on healthy and diseased bean leaves. (a) Leaves with rust only; (b) leaves with anthracnose only; (c) leaves with rust, anthracnose or both diseases. The proportion of asymptomatic tissue was obtained by subtracting rust and/or anthracnose severities from one.

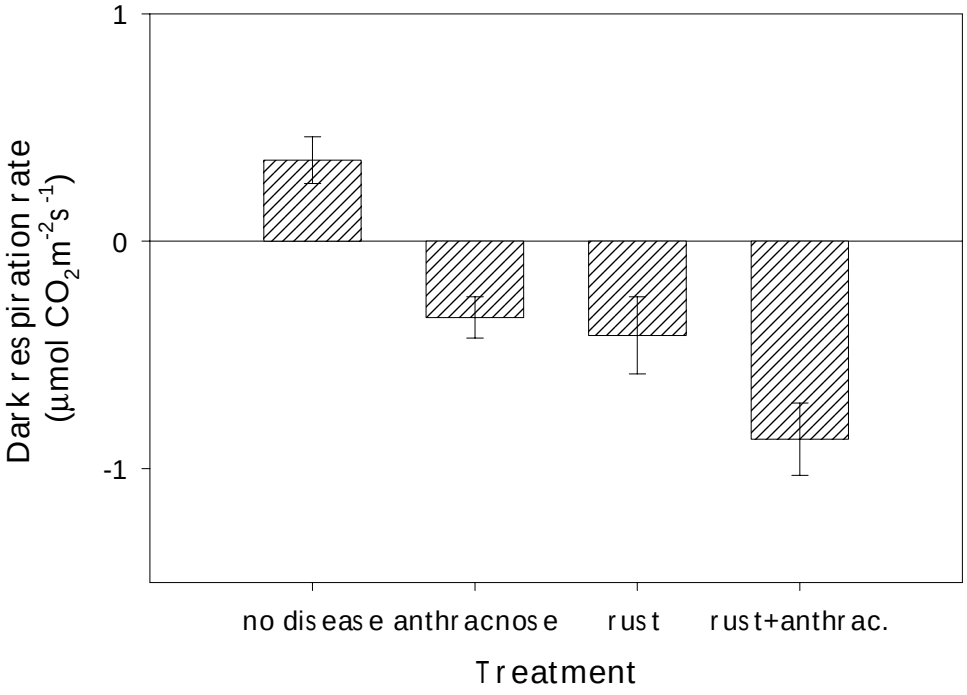

Figure 5.5. Dark respiration rate on healthy control leaves of bean, leaves with anthracnose (1-7%), leaves with rust (2-9% severity), and leaves with both diseases (3-14% rust severity, 0.6-2% anthracnose severity). The values of the columns are averages of five to seven leaves and the vertical bars represent the standard errors of the means.

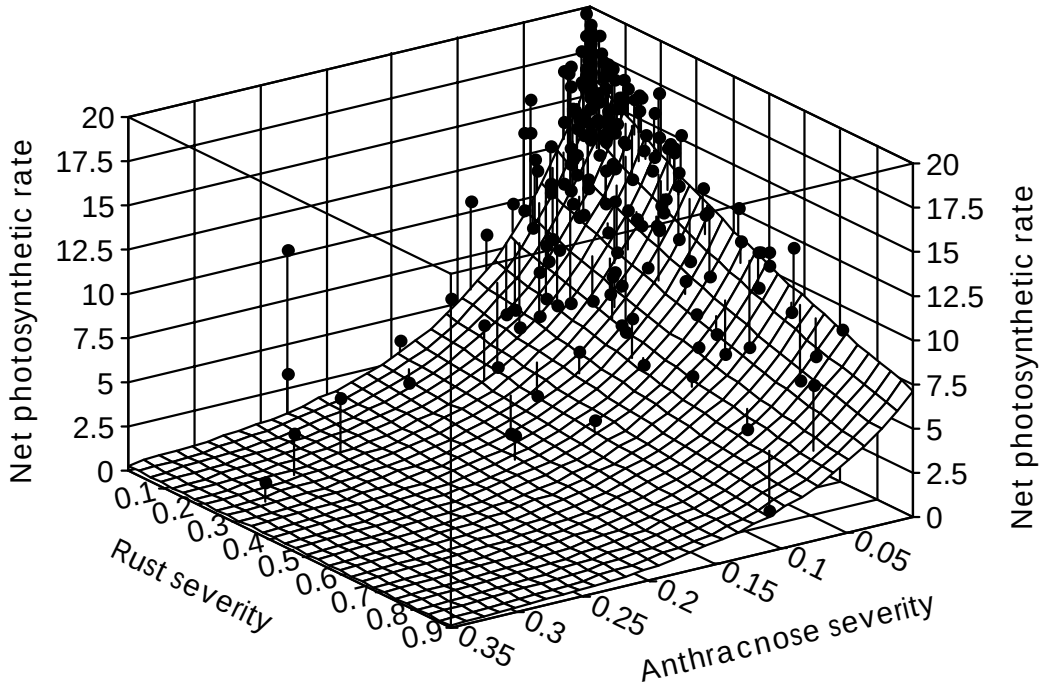

Figure 5.6. Effects of anthracnose (x) and rust (y) severities on the net photosynthetic rate (z, μmol CO_2 $m^{-2}s^{-1}$) of bean leaves. The equation for the response surface is z=16.28exp(-(x/0.085))exp(-(y/1.1)) (r^2=0.70)

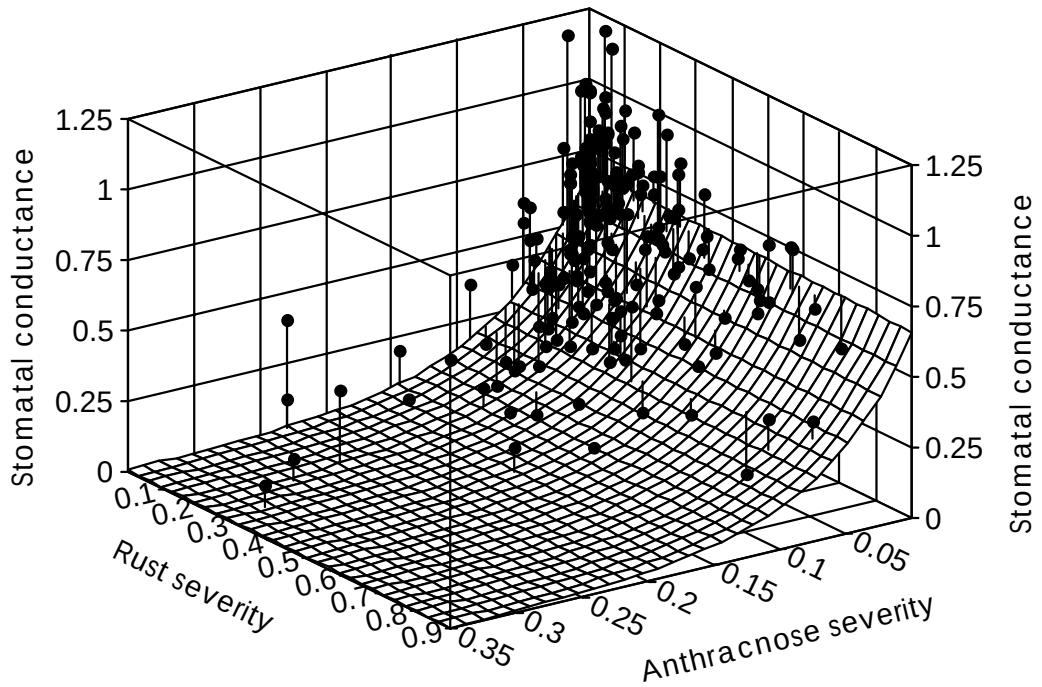

Figure 5.7. Effects of anthracnose (x) and rust (y) severities on stomatal conductance (z, mol H_2O $m^{-2}s^{-1}$) of bean leaves. The equation for the response surface is $z=0.68\exp(-(x/0.067))\exp(-(y/29.62))$ ($r^2=0.46$)

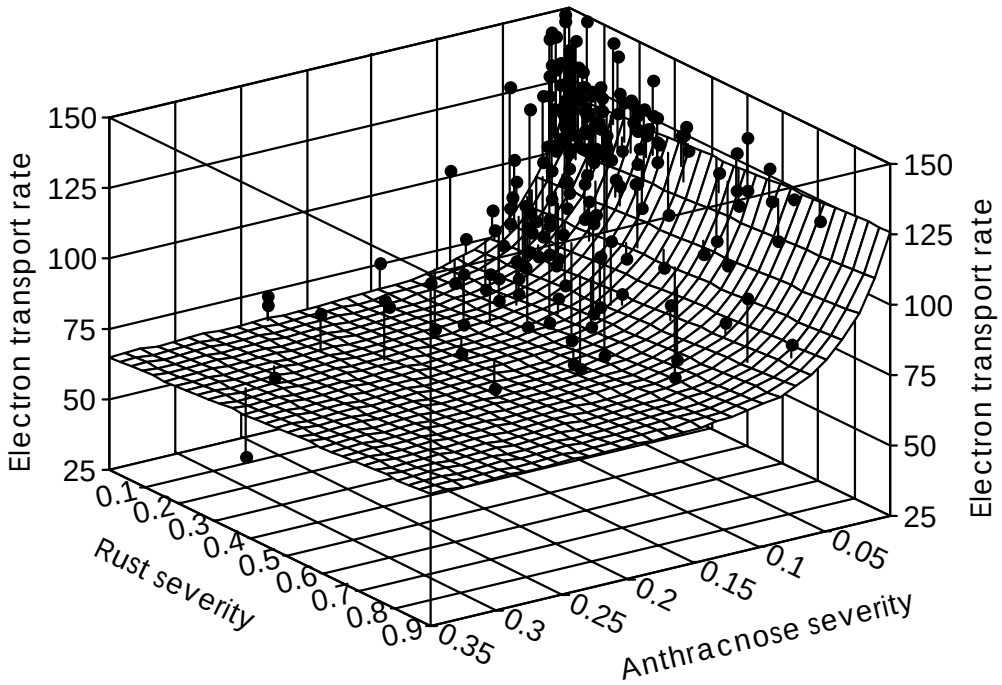

Figure 5.8. Effects of anthracnose (x) and rust (y) severities on the electron transport rate (z, μmol $m^{-2}s^{-1}$) of bean leaves. The equation for the response surface is $z=64.77+7.93y+54.32\exp(-(x/0.036))$ ($r^2=0.53$)

From preliminary experiments on the effects of the interaction of rust and anthracnose on the monocyclic parameters of both diseases (Appendix F), it was concluded that there was no clear evidence of synergism or antagonism between these two pathogens, when present in the same leaf. Similarly, there was no indication of a significant interaction between rust and anthracnose in relation to the effects of both diseases on net photosynthetic rate, stomatal conductance, or electron transport rate (Figures 5.6, 5.7, and 5.8). The reductions in these three variables on leaves with both rust and anthracnose were determined largely by the proportion of leaf tissue affected by anthracnose. While net photosynthetic rate and stomatal conductance were measured in areas of the leaf that included a mixture of green and symptomatic tissues, electron transport rate was determined only on non-symptomatic tissue and was a measure of the photosynthetic efficiency of this portion of the leaf.

Discussion

Under the conditions of the present study, there was no apparent interaction between rust and anthracnose in relation to their effects on leaf photosynthesis. When co-inoculated on the same leaf at the same time or after a few days, the pathogens that cause rust and anthracnose had an impact on photosynthesis similar to the added effects induced by single inoculations. This was evident from the steep reduction in photosynthetic rate in response to increased severities of anthracnose, and from the mild reduction of the photosynthetic rate with increased severity of rust (Figure 5.6). These different patterns of impact on leaf physiology are probably related to pathogenicity events associated with the mode of nutrition of each pathogen.

The absence of interaction under the conditions of the experiments conducted in the present study may not be indicative of what happens 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 (Waller and Bridge, 1984; Weber et al., 1994). 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 to competition for plant resources.

Simkin and Wheeler (1974) reported that pre-inoculation of barley with either *Blumeria graminis* or *Puccinia hordei* reduced the development of the other pathogen. Larger reductions on lesion density of either disease was related to increased intervals between inoculations, which could be due to induced resistance. Systemic resistance to *U. appendiculatus* and *C. lindemuthianum* was observed in trifoliate leaves of bean plants when unifoliate leaves were inoculated with *C. lindemuthianum* 7 or 12 days earlier (Dann and Deverall, 1995). Similar experiments should be conducted with rust and anthracnose on beans to clarify further the effects of dual inoculations on monocyclic parameters of both diseases and on plant photosynthesis.

Foliar pathogens occupy the same ecological niche and tend to compete for the same resources (Nelson and Campbell, 1992). However the niche overlap of species may have generated different spatial or temporal patterns of occurrence. For example, pathogens that may occur in the same area or field may not occur together at a plant or

leaf level due to exclusion, competition, or different environmental conditions for infection. The study of the effects of pathogen interactions on plant photosynthesis should consider the spatial distributions of the infections of each pathogen within a plant. If the occurrence of both pathogens in the same leaf is not significant, a study of the interaction at different layers of the canopy or at the whole plant level should be considered.

Although 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 (Berger, 1988; Kranz and Jörg, 1989; Johnson and Teng, 1990; Savary and Zadoks, 1992a), some diseases have unproportionally high physiological effects on crop yield, which may confuse the assessment of the healthy area (Kranz and Jörg, 1989). 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 does, the severities of each disease must be assessed separately. The size of the virtual lesion (Bastiaans, 1991) 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 (Savary and Zadoks, 1992; Teng and Gaunt, 1980). Savary and Zadoks (1992) 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 the present 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.

CHAPTER 6
GENERAL CONCLUSIONS

Foliar pathogens that cause defoliation or necrotic lesions interfere with the amount of radiation intercepted by the plant. There is evidence that some pathogens can also interfere with the efficiency of use of the radiation intercepted. The impact of this latter group of pathogens on photosynthesis, the most important physiological process in yield formation, can be assessed accurately only through direct measurements of physiological parameters.

The effects of two important diseases of common bean (*Phaseolus vulgaris*) on leaf photosynthesis were studied under controlled conditions. Rust, caused by *Uromyces appendiculatus,* and anthracnose, caused by *Colletotrichum lindemuthianum,* were inoculated separately or together on the same leaf, and several levels of disease intensity were considered in the present study.

The concept of a virtual lesion (Bastiaans, 1991), an area of the diseased leaf where the photosynthetic rate is zero, was used extensively in the present study to quantify the effects of rust and anthracnose on leaf photosynthesis. The virtual lesion can be smaller, equal, or bigger in size than the visual lesion. Based on the ratio of virtual lesion to visual lesion sizes, or the β parameter, I concluded that although both rust and anthracnose reduced the photosynthetic rates of diseased leaves in comparison to healthy leaves, the magnitude of the reduction was very different. The photosynthetic rate of leaves with rust was reduced mainly within the area of the lesions (pustule + chlorotic

halo). The effect of this disease on the remaining green area of the leaf was insignificant in most of the experiments. On the other hand, photosynthesis in the green area beyond the necrotic symptoms of anthracnose was severely impaired shortly after the appearance of the symptoms. The usefulness of the β parameter for the quantification of disease impact on photosynthesis under various conditions should be explored. In the present study, the effect of nutritional stress had little impact on the β values of leaves with rust or anthracnose. The impact of disease on photosynthesis may be different in the presence of other abiotic stresses, in different cultivars, or with different races of a specific pathogen.

Increased rates of respiration and loss of chlorophyll from the leaf tissue were apparently the major factors responsible for the reduction of photosynthetic rates on leaves with rust. Even higher rates of respiration were observed on leaves with anthracnose. The stomatal conductance of leaves with anthracnose decreased sharply with increases in severity and probably limited carbon assimilation.

The optimal quantum yield and the electron transport rate, which are parameters of chlorophyll fluorescence related to the efficiency of the photosynthetic apparatus, were reduced in leaves with high rust severity (65-85% severity) after the appearance of the fleck symptoms. The same parameters were also reduced in relation to the healthy control leaves on non-symptomatic areas of leaves with 16-25% severity of anthracnose as soon as the necrotic symptoms were visible. Chlorophyll fluorescence measurements are a measurement of the efficiency of photosynthetic reactions, while gas exchange measurements include both photosynthesis and respiration. The effective quantum yield of the photosystem II was significantly correlated to the quantum yield of carbon

assimilation on leaves with both diseases. The use of fluorescence to estimate photosynthetic carbon assimilation of diseased leaves without recourse to gas exchange analysis should be investigated. Moreover, in-depth analysis of fluorescence with measurements of photochemical and non-photochemical quenching of fluorescence could elucidate the mechanisms through which disease development reduces photosynthetic efficiency.

Both chlorophyll content and color of leaves with several levels of rust severity and different nutritional status were well correlated to relative photosynthetic rates. If this correlation can be validated under field conditions, leaf color, and chlorophyll content could then be used as predictors of leaf photosynthetic rate for the bean rust pathosystem. These variables were not as strongly correlated to relative photosynthetic rate on leaves with anthracnose and their usefulness to estimate photosynthesis in this case would be questionable.

The different effects of the infections by *U. apppendiculatus* and *C. lindemuthianum* on leaf photosynthesis are probably related to the different modes of nutrition of these foliar pathogens. The rust fungus is a biotroph and its nutrition is dependent on a gentle relationship between the infection structures and the host cells. It is believed that biotrophic fungi alter the source/sink relations on the leaf and compete with other host sinks, such as roots, for nutrient acquisition (Farrar, 1995; Scholes, 1992). Production of diffusible toxins that can alter host membrane permeability has not been associated with biotrophy. *Colletotrichum lindemuthianum*, on the other hand, has a highly aggressive and destructive necrotrophic phase, which coincides with the production of visible symptoms. Extensive degradation of cell walls and death of cells,

often well in advance of the hyphae, are reported for this pathosystem (Bailey et al., 1992). The ulcer-like necrotic lesion on the leaf veins most probably interferes with water and nutrient transport in the leaf. Although many aspects of the process of infection by this fungus at the cellular level have been elucidated recently (O'Connell et al., 1985; O'Connell and Bailey, 1991), there is a lack of hystological studies on this system.

No obvious interaction was observed between the rust and anthracnose pathogens when both were simultaneously inoculated in the same leaf. The photosynthetic rate and the electron transport rate of leaves with both diseases were determined largely by the proportion of leaf tissue with anthracnose. Further experiments on coinoculation of these two pathogens should consider the interval between inoculations with each pathogen and the density of infection. Observations on the occurrence of both rust and anthracnose under field conditions may clarify the spatial and temporal distribution of the disease symptoms in the plants. These observations could then serve as a guide for further investigations on changes in monocyclic parameters and in photosynthetic efficiency under controlled environmental conditions.

The impact of rust and anthracnose on bean leaf photosynthesis should be considered in assessments of the proportion of healthy tissue on 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. However, true understanding of the impact of a disease on plant photosynthesis require measurements not only at the leaf level but also at the whole plant level. Spatial distribution of the disease in the canopy and the impact of the presence of

disease in one or more leaves on the rest of the plant may elucidate some aspects of canopy photosynthesis on diseased plants.

The next step towards a holistic approach to predict yield reductions due to diseases would be to couple pathogens to crop growth simulators (Bastiaans et al., 1994; Boote et al., 1983; Luo et al., 1997; Rouse, 1988). In this kind of ecophysiological approach, the effects of disease on basic plant growth processes are introduced in a crop growth model to estimate the total effect of the disease on yield. If the resulting model is validated under natural conditions, it can be a powerful tool to estimate yield reduction for various epidemics and under variable environmental conditions.

APPENDIX A
EFFECT OF THE REMOVAL OF THE GROWING POINT OF BEAN PLANTS ON THE PHOTOSYNTHESIS OF DISEASED LEAVES

The bean cultivar 'Rosinha' used in the experiments of the present study has indeterminate growth. This kind of growth makes the handling of plants difficult at some times because the growing points of the plants tend to intermingle. The removal of the growing point of bean plants was necessary for some of the experiments performed to avoid shading of the inoculated leaves and to facilitate the handling of the plants. The objective of this preliminary experiment was to determine whether the removal of the growing point of the healthy and diseased plants would interfere with their photosynthetic rates.

The experiment was conducted under greenhouse conditions with plants grown in 4-liter pots, and one plant per pot. A factorial experiment was designed with two factors: (a) removing or not removing the growing point of the plants, and (b) levels of rust severity (no disease, low rust severity, and high rust severity). The experimental unit was a bean plant, and four replicates were randomly assigned to each treatment. The first trifoliate leaf of each plant was sprayed with inoculum suspensions containing 0, 10^3, or 10^4 viable urediniospores/ml to generate the desired levels of rust severity. The growing point of plants in each severity group was removed 7 days after inoculation, right above the second trifoliate leaf.

The photosynthetic rate of all plants was measured 12 days after inoculation using the LI-6200 Portable Photosynthesis System. Readings were taken at light saturation (800-1100 μmol photon $m^{-2}s^{-1}$). The measured leaves were then detached from the plants and rust severity was determined using the number of lesions per leaf and an estimate of average lesion size. Plants assigned to the low severity level had 25 to 30% severity and plants in the high severity level had 65 to 73% severity. The results were analyzed with the general linear models procedure of the SAS program.

The photosynthetic rate was significantly reduced in plants with low and high rust severity. Plants that had their growing points removed had significantly higher photosynthetic rates than intact plants (P=0.0029). However, there was no interaction between removal of the growing point and level of disease severity. Thus, although the removal of the growing point of bean plants induced an increase in the rate of photosynthesis, it did not influence the effect of rust severity on photosynthetic rate.

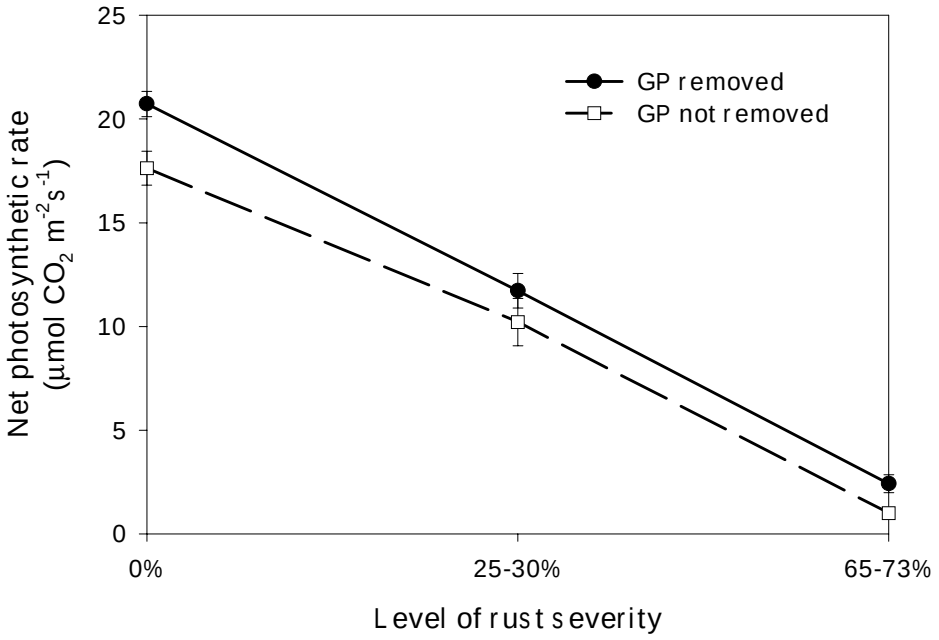

Figure A.1. Effect of the removal of the growing points (GP) of bean plants on the net photosynthetic rate of healthy leaves and leaves with two levels of rust severity.

APPENDIX B
SAS PROGRAM

The following SAS statements were used to fit a linearized form of the model proposed by Bastiaans (1991), which relates disease severity to relative photosynthetic rate (P_x/P_0). This program enabled the comparison among the curves obtained from different experiments. The program was also used to compare the curves obtained from the experiments with plants that received different fertilizer treatments.

```
data one;
infile 'path and name of the file' firstobs=2;
input time severity pxpo;
log_1_sv=log(1-severity);
logpxpo=log(pxpo);
if time=2 then slope2=log_1_sv;else slope2=0;
if time=3 then slope3=log_1_sv;else slope3=0;
cards;

proc print;
run;

proc glm;
model logpspo=log_1_sv/noint solution;

proc glm;
model logpspo=log_1_sv slope2 slope3/noint solution;
contrast 'any diff' slope2 1, slope3 1;
estimate 'Slope of 1' log_1_sv 1;
estimate 'Slope of 2' log_1_sv 1 slope2 1;
estimate 'Slope of 3' log_1_sv 1 slope3 1;
estimate 'S1-S2' slope2 -1;
estimate 'S1-S3' slope3 -1;
estimate 'S2-S3' slope2 1 slope3 -1;
run;
```

APPENDIX C
COMPARISON OF TWO METHODS TO ESTIMATE LEAF CHLOROPHYLL CONTENT

The chlorophyll content of bean leaves collected from plants with different nutritional status and different levels of rust severity was estimated using a chlorophyll meter and a colorimetric method. The SPAD-502 Chlorophyll Meter (Minolta Co., Ltd.) is a non-destructive, dual-wavelength meter. Its measuring head includes both a red and an infrared light-emitting diodes, which emit light in sequence through the leaf. The equipment measures peak chlorophyll absorbance at 650 nm and non-chlorophyll absorbance at 940 nm. A microprocessor calculates a SPAD value, which is proportional to the relative optical density, based on the ratio of absorbancies of the two wavelengths (Minolta, 1989). Lower values of SPAD represent less chlorophyll in the tissue and more yellowing. The area measured by the equipment is very small (6 mm^2), thus a minimum of 10 readings were taken randomly on each leaf and then averaged.

The same leaves sampled with the SPAD-502 were then destructively sampled for *in vitro* chlorophyll determination using the method described by Arnon (1949). An area of 10.18 cm^2 of each leaf was soaked and ground in a small known volume of 80% acetone. The extracted samples were maintained on ice, in the dark, while all samples were being prepared. The extracted samples were then centrifuged for 10 minutes at 2000 rpm. The absorbance of the resultant extract was measured in a spectrophotometer

at 645, 652, and 663nm. Total chlorophyll concentration, in mg/cm^2, was calculated using the equations of Arnon (1949).

The SPAD values were plotted against the data on chlorophyll concentration obtained through colorimetry. A quadratic equation provided the best correlation between the two variables (Figure B.1). Curvilinear relationships between SPAD values and chlorophyll concentration were also found for data sets including different plant species and genotypes (Markwell et al., 1995; Monge and Bugbee, 1992). A linear relationship between *in vivo* and *in vitro* measurements would be expected, according to Beer's Law, if absorbance were solely dependent on pigment concentration. However, the transmission of light through a leaf is determined not only by pigment concentration, but also by light scattering, reflectivity of the leaf surface, and distribution of pigment in the leaf. A combination of these factors probably explains the curvilinear relationship obtained in Figure C.1.

Leaf chlorophyll content is often well correlated with leaf nitrogen status, Rubisco activity, and photosynthetic activity (Evans, 1983; Seeman et al., 1987; Thompson et al., 1996). Also, the amount of leaf chlorophyll is believed to be a sensitive indicator of many types of plant stress . In the last few years, the SPAD-502 has been used to monitor nitrogen status and schedule fertigation in corn fields (Fox and Piekielek, 1997), to estimate specific leaf weight on breeding lines of soybean (Thompson et al., 1996), and to assess disease severity in a hydroponic seedling assay for resistance to Cephalosporium stripe of wheat (Cowger and Mundt, 1998), just to cite a few examples. In the present study, the hand-held SPAD-502 Chlorophyll Meter provided rapid and non-destructive

estimates of chlorophyll content on healthy and diseased leaves with various levels of nutrition.

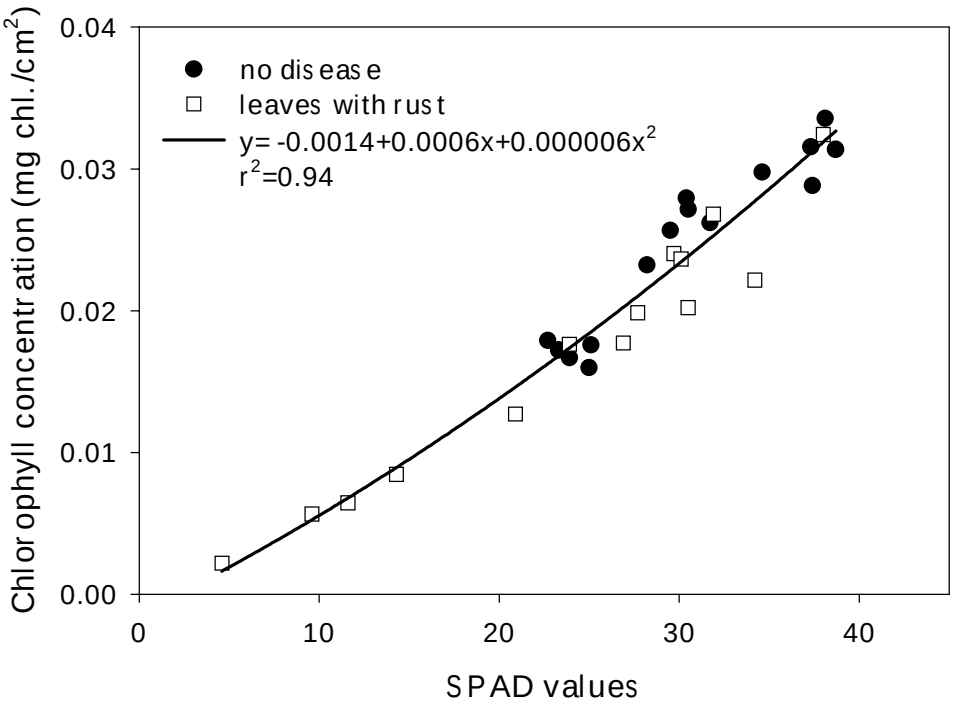

Figure C.1. Correlation between the estimates of chlorophyll content of bean leaves obtained with the SPAD-502 and chlorophyll concentration determined by colorimetry. Leaves of different nutritional status and with several levels of rust severity are represented.

APPENDIX D
RESULTS FROM EXPERIMENT 3-6, CHAPTER 3

Minimal fluorescence (F_0), maximal fluorescence (F_m), and optimal quantum yield (F_v/F_m) were measured at frequent intervals for 16 days after inoculation in healthy leaves and leaves with two levels of rust severity. Leaves with 16 to 23% severity constituted the low rust severity group, and leaves with 80 to 90% severity were the high severity group.

Minimal fluorescence was not significantly reduced in leaves with high severity until 15 days after inoculation (Figure D.1). This parameter was reduced in Experiment 3-5 (Figure 3.8; Chapter 3) 10 days after inoculation on leaves with 65 to 85% rust severity. Significant reductions on maximal fluorescence and optimal quantum yield in leaves with high severity were first observed at the end of the fleck stage (8 days after inoculation). At the end of both experiment 3-5 and 3-6, there were similar reductions in maximal fluorescence and optimal quantum yield in leaves with high severity. Also, in both experiments, the fluorescence parameters were not altered in non-inoculated areas of inoculated leaves.

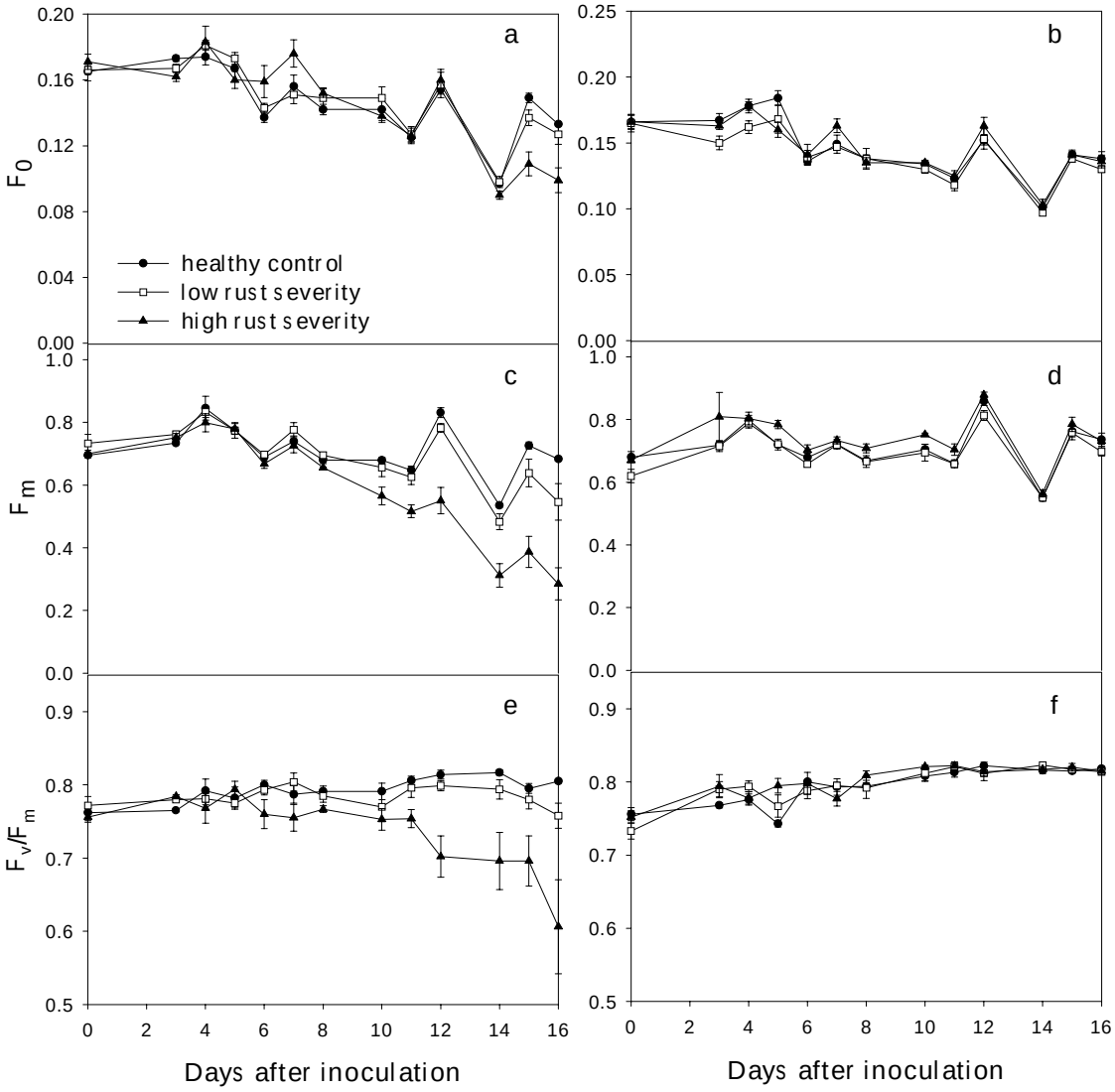

Figure D.1. Minimal fluorescence (F_0), maximal fluorescence (F_m), and optimal quantum yield (F_v/F_m) during rust development, in inoculated (a, c, e) and non-inoculated (b, d, f) areas of bean leaves with different rust severities (Experiment 3-6). The fluorescence parameters are expressed in relative units. The percentage of area affected by rust was 16-23% in leaves with low severity, and 80-90% in leaves with high severity.

APPENDIX E
ASSESSMENT OF ANTHRACNOSE SEVERITY WITH A DIAGRAMMATIC SCALE OF SYMPTOMS

The leaf area with symptoms of anthracnose was determined by tracing the necrotic lesions and any adjacent area with chlorosis onto transparent plastic. The plastic, as well as the actual leaf from which the lesions were traced, was then passed through an area meter (LI-3000 Portable Area Meter, LI-COR). The proportion of diseased area, or actual severity, was determined as the ratio of the area with symptoms to the total leaf area. The actual severities of several leaves were then compared to the estimates of severity obtained with a diagrammatic scale of symptoms (Godoy et al., 1997) (Figure E.1). It is clear that the estimates obtained with the diagrammatic scale overestimated the actual severity.

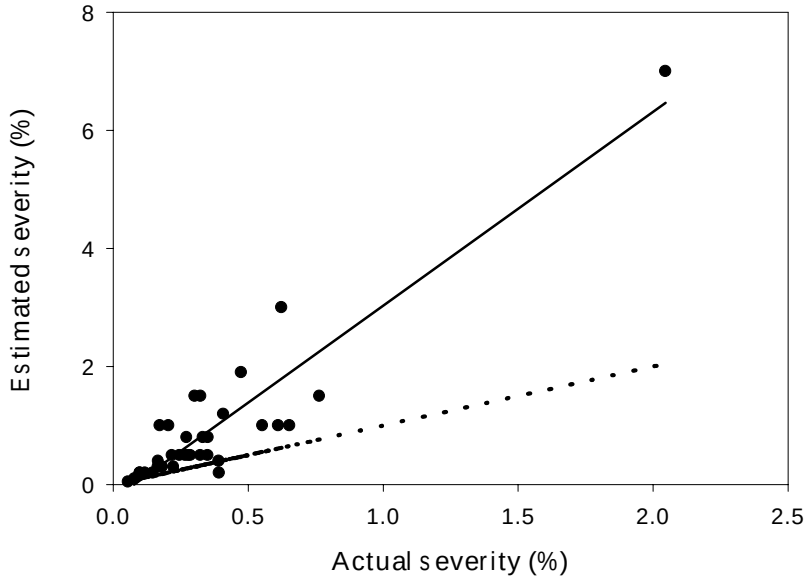

Figure E.1. Correlation between anthracnose severity on bean leaves estimated with a diagrammatic scale and actual severity. The equation for the linear regression (solid line) is y= -0.25+3.28x (r^2=0.84). The dotted line represents the ideal situation, with estimates identical to reality.

APPENDIX F
EFFECTS OF DUAL INFECTIONS OF *Uromyces appendiculatus* AND *Colletotrichum lindemuthianum* ON THE MONOCYCLIC PARAMETERS OF BEAN RUST AND BEAN ANTHRACNOSE

Introduction

The damage of multiple pathogens on the host may be classified in three categories (Waller and Bridge, 1984): absence of interaction, positive or synergistic interaction, and negative interaction. Absence of interaction occurs when the damage caused by one pathogen does not change that caused by another (Cole, 1982; Hyde, 1978; Jenkins and Jones, 1981). Where the damage is greater than that expected on a purely additive basis, there is a positive interaction (Datnoff and Sinclair, 1988; Rowe et al., 1985; van der Wal et al., 1970). Negative interactions occur where the damage is less than that expected from the individual effects of each pathogen (Bassanezi et al., 1998; Johnson et al., 1986; Nelson and Campbell, 1992).

The objective of the present study was to investigate the effect of simultaneous inoculation of bean leaves with *U. appendiculatus* and *C. lindemuthianum* on the development of rust and anthracnose.

Material and Methods

Bean plants of cv. Rosinha were grown in 4-liter pots under greenhouse conditions, in which the temperature ranged from 18-33^{o}C in both runs of this experiment

(Experiment F-1 and Experiment F-2). The plants were fertilized once at the unfolding of the first trifoliate leaf with Osmocote (2g/pot) and watered daily.

The urediniospores of *U. appendiculatus* race 86 were collected from pustules formed on 'Rosinha' plants, allowed to dry for 24 hours in a closed silica gel container, and stored under -4^{o}C in glass vials submitted to vacuum. The viability of each sample was tested before each inoculation. A diluted suspension of urediniospores was plated on water-agar, and the percentage of germinated spores after a 12-hour 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 minutes. The concentration of spores was determined with a haemocytometer, and the suspension was then adjusted to the desired concentration by dilution.

Conidia of *C. lindemuthianum*, race kappa, were produced in Mathur's C modified medium (Balardin et al., 1997). A suspension of conidia was spread on the surface of the medium to obtain maximum sporulation. Ten- to fourteen-day-old cultures were rinsed with sterile distilled water to prepare the concentrated spore suspension, which was then diluted to obtain the desired concentrations. The viability of the conidia was determined prior to the preparation of the suspensions. A diluted suspension of conidia was spread on the surface of water-agar medium, and the percentage of germinated conidia was determined after 12 hours of incubation.

Spore suspensions of both fungi were prepared with sterile water and 0.01% Tween-20. A combined spore suspension was then obtained by mixing the two suspensions. The concentrations of spores of *C. lindemuthianum* and *U. appendiculatus* in this combined suspension were the same, 1.25 x 10^3 viable spores/ ml of suspension in

Experiment F-1, and 10^4 viable spores/ml of suspension in Experiment F-2. Sterile water was added to the suspension of each fungus to obtain the same spore concentration of the combined suspension.

The central leaflet of the first trifoliate leaf was inoculated when the leaf was fully expanded. The spore suspensions were sprayed onto the abaxial surface of the leaflet, according to the inoculation treatment (Table F.1). The sprayer nozzle was held at a fixed distance from the leaflet and one spray of the suspension was applied to each leaflet. The same procedure was used to spray the surface of water-agar plates, which were then used to assess the number of spores deposited per unit area. After inoculation, the plants were placed inside a dew chamber at 21°C for 24 hours and then returned to the greenhouse. Plants inoculated with only one pathogen, on both days, were considered the controls.

Table F.1 - Inoculation scheme of bean plants, using single or combined spore suspensions of *U. appendiculatus* and *C. lindemuthianum*

Treatment	**day 0**		**day 2**	
	Uromyces	***Colletotrichum***	***Uromyces***	***Colletotrichum***
1	[a]			
2				
3				
4				
5				
6				
7				
8				

[a] - Marked squares indicate an inoculation with the specified pathogen in the specified day.

Central leaflet width, number of rust pustules, and number of anthracnose lesions were assessed daily, from the appearance of the first symptoms until there was no further increase in the number of lesions per leaflet. Total leaf area was estimated from central leaflet width using the equation $y=2.445x^{1.94}$ (Bassanezi, 1995). At the end of each experiment, the leaflets were passed through a leaf area meter and the ratio of central leaflet area to total leaf area was calculated. This ratio was used to determine the central leaflet area for each day during the assessment period. The final severity was also determined after the leaflets were detached from the plant, by counting the number of pustules and estimating their average size in the case of rust, or by using a diagrammatic scale of symptoms for the assessment of anthracnose severity.

From these assessments, the variables of maximum lesion density, incubation period, and infection efficiency were calculated. Disease progress curves of lesion density (y, number of lesions/cm^2 of leaflet) as a function of time (t, in days) were determined for both diseases in each replication, using the following monomolecular model (Bassanezi et al., 1998; Campbell and Madden, 1990):

$$y= b_1-(1-b_2\exp(-b_3t)) \qquad (1)$$

The parameters b_1, b_2, and b_3 were determined by non-linear regression analysis, using the software STATISTICA. The parameter b_1 is the asymptotic value of the monomolecular function and, in the present study, represents the maximum lesion density for each leaflet. The incubation period (t_{ip}), the time required for the appearance of 50%

of the lesions or the time required to reach 50% of the b_1 value, was calculated from equation 1:

$$t_{ip} = (\ln(2/b_1)-\ln(1-b_2))/b_3 \quad (2)$$

Infection efficiency was calculated by dividing the maximum lesion density (b_1) by the number of spores deposited/cm^2 of leaf, which was estimated from the assessment of the water-agar plates sprayed at the time of the inoculation of the plants.

The values of maximum lesion density, incubation period, infection efficiency, and maximum severity for each disease were averaged in each treatment and their means were compared using pre-planned contrasts.

Results and Discussion

The ranges of rust and anthracnose severity were different for the two experiments (Tables F.2, F.3, F.4, and F.5). The values of severity for individual leaflets in Experiment F-1 were higher for rust than the values of anthracnose severity (2.5 to 19% for rust and 0.1 to 3.5% for anthracnose). In Experiment F-2, the opposite was true: higher values were observed for anthracnose than for rust (0.6 to 5% for rust, and 0.1 to 15% for anthracnose). Since the variables analyzed are clearly related to each other, these differences in severity may explain the inconsistencies between the two experiments. The maximum lesion density for treatments with anthracnose was not affected by the presence of rust, in both experiments. The lesion density for treatments with rust was not affected by the presence of anthracnose in Experiment F-1. In Experiment F-2, the rust

lesion density was higher in leaflets that were pre-inoculated with *Colletotrichum* than in leaflets that were not pre-inoculated with the anthracnose pathogen (Table F.3).

For Experiment F-1, the incubation period was approximately 1 day longer for both diseases in leaflets that were pre-inoculated with the other pathogen 2 days earlier than in the other treatments (Tables F.2 and F.4). No changes in the incubation period of each disease were noticed in Experiment F-2.

The infection efficiency of the rust pathogen increased significantly with the age of the leaflet in Experiment F-1, but not in Experiment F-2 (Tables F.2 and F.3). In the second experiment, the infection efficiency of the rust pathogen was highest when *C. lindemuthianum* had been already present in the leaf. The infection efficiency of the anthracnose pathogen did not change significantly with leaflet age (Tables F.4 and F.5), but there was a tendency for reduction in the efficiency in more mature leaflets. In the first experiment, the infection efficiency of *C. lindemuthianum* was higher when both pathogens were inoculated simultaneously compared to when *C. lindemuthianum* was inoculated first (Table F.4). However, the opposite occurred in experiment F-2 (Table F.5).

Anthracnose severity was not affected by the presence of the rust pathogen in either experiment (Table F.4 and F.5). The presence of the anthracnose prior to the inoculation of the rust pathogen reduced rust severity in Experiment F-1 (Table F.2), but not in Experiment F-2 (Table F.3). In some leaflets inoculated with both pathogens, there was a different type of symptom; sporulating rust pustules were surrounded by a necrotic halo. This was interpreted as a combination of the symptoms of the two diseases.

Table F.2. Effects of inoculations of bean leaves with *Uromyces appendiculatus* and *Colletotrichum lindemuthianum* on the monocyclic parameters of rust (Experiment F-1).

Treatments[a]	Lesion density (lesions/cm^2)	Incubation period (days)	Infection efficiency (no. lesions/no. spores)	Rust severity (%)
1. *Uromyces* (day 0)	3.10±0.50[b]	4.92±0.36	0.32±0.05	10.63±1.61
2. *Colletotrichum* (day 0)	-	-	-	-
3. *Uromyces* + *Colletotrichum* (day 0)	2.25±0.43	4.80±0.412	0.31±0.06	7.04±1.29
4. *Uromyces* (day 0) + *Colletotrichum* (day 2)	3.46±0.44	5.87±0.08	0.36±0.05	13.07±1.56
5. *Colletotrichum* (day 0) + *Uromyces* (day 2)	2.46±0.53	5.49±0.37	0.59±0.13	5.77±1.71
6. *Uromyces* (day 2)	2.98±0.27	4.75±0.25	0.72±0.06	10.68±2.35
7. *Colletotrichum* (day 2)	-	-	-	-
8. *Uromyces* + *Colletotrichum* (day 2)	2.67±0.47	4.67±0.37	0.34±0.06	9.03±1.07
Contrasts[c]				
1 x 3	NS[d]	NS	NS	NS
1 x 4	NS	NS	NS	NS
1 x 6	NS	NS	0.0008	NS
3 x 4	NS	0.0291	NS	0.0166
3 x 8	NS	NS	NS	NS
5 x 6	NS	NS	NS	0.0468
6 x 8	NS	NS	0.0012	NS

[a] Inoculation treatments - see Table F.1 for explanation.

[b] Variable mean ± standard error of the mean; means of four replicates.

[c] Pre-planned contrasts; the numbers correspond to the treatment number.

[d] Non-significant at the 5% probability level; the numbers in other columns are significance levels.

Table F.3. Effects of inoculations of bean leaves with *Uromyces appendiculatus* and *Colletotrichum lindemuthianum* on the monocyclic parameters of rust (Experiment F-2).

Treatments	Lesion density (lesions/cm^2)	Incubation period (days)	Infection efficiency (no. lesions/no. spores)	Rust severity (%)
1. *Uromyces* (day 0)	0.91±0.20	5.06±0.33	0.10±0.02	2.75±0.58
2. *Colletotrichum* (day 0)	-	-	-	-
3. *Uromyces* + *Colletotrichum* (day 0)	1.60±0.15	4.62±0.70	0.19±0.01	5.04±0.46
4. *Uromyces* (day 0) + *Colletotrichum* (day 2)	1.81±0.34	4.84±0.70	0.20±0.04	2.16±0.58
5. *Colletotrichum* (day 0) + *Uromyces* (day 2)	3.10±0.73	5.83±0.31	0.55±0.13	2.17±0.60
6. *Uromyces* (day 2)	1.02±0.35	6.07±0.09	0.18±0.03	1.50±0.34
7. *Colletotrichum* (day 2)	-	-	-	-
8. *Uromyces* + *Colletotrichum* (day 2)	1.32±0.29	5.16±0.33	0.2±0.02	0.95±0.14
Contrasts				
1 x 3	NS	NS	NS	0.0037
1 x 4	NS	NS	NS	NS
1 x 6	NS	NS	NS	NS
3 x 4	NS	NS	NS	0.0005
3 x 8	NS	NS	NS	0.0001
5 x 6	0.0007	NS	0.0003	NS
6 x 8	NS	NS	NS	NS

[a] Inoculation treatments - see Table F.1 for explanation.

[b] Variable mean ± standard error of the mean; means of four replicates.

[c] Pre-planned contrasts; the numbers correspond to the treatment number.

[d] Non-significant at the 5% probability level; the numbers in other columns are significance levels.

Table F.4. Effects of inoculations of bean leaves with *Uromyces appendiculatus* and *Colletotrichum lindemuthianum* on the monocyclic parameters of anthracnose (Experiment F-1).

Treatments	Lesion density (lesions/cm^2)	Incubation period (days)	Infection efficiency (no. lesions/no. spores)	Anthracnose severity (%)
1. *Uromyces* (day 0)	-	-	-	-
2. *Colletotrichum* (day 0)	0.037±0.010	5.46±0.48	0.087±0.023	0.50±0.06
3. *Uromyces* + *Colletotrichum* (day 0)	0.059±0.014	5.20±0.46	0.19±0.045	0.84±0.24
4. *Uromyces* (day 0) + *Colletotrichum* (day 2)	0.053±0.025	5.50±0.23	0.047±0.022	0.85±0.56
5. *Colletotrichum* (day 0) + *Uromyces* (day 2)	0.049±0.004	6.36±0.28	0.114±0.009	0.64±0.07
6. *Uromyces* (day 2)	-	-	-	
7. *Colletotrichum* (day 2)	0.029±0.027	5.87±0.55	0.022±0.012	0.43±0.14
8. *Uromyces* + *Colletotrichum* (day 2)	0.070±0.029	5.31±0.21	0.038±0.016	1.08±0.61
Contrasts				
2 x 3	NS	NS	0.0165	NS
2 x 5	NS	NS	NS	NS
2 x 7	NS	NS	NS	NS
3 x 5	NS	0.0299	0.0379	NS
3 x 8	NS	NS	0.0002	NS
4 x 7	NS	NS	NS	NS
7 x 8	NS	NS	NS	NS

[a] Inoculation treatments - see Table F.1 for explanation.

[b] Variable mean ± standard error of the mean; means of four replicates.

[c] Pre-planned contrasts; the numbers correspond to the treatment number.

[d] Non-significant at the 5% probability level; the numbers in other columns are significance levels.

Table F.5. Effects of inoculations of bean leaves with *Uromyces appendiculatus* and *Colletotrichum lindemuthianum* on the monocyclic parameters of anthracnose (Experiment F-2).

Treatments	Lesion density (lesions/cm^2)	Incubation period (days)	Infection efficiency (no. lesions/no. spores)	Anthracnose severity (%)
1. *Uromyces* (day 0)	-	-	-	-
2. *Colletotrichum* (day 0)	0.362± 0.131	4.99± 0.06	0.102± 0.039	3.52± 1.30
3. *Uromyces* + *Colletotrichum* (day 0)	0.337± 0.131	5.07± 0.06	0.070± 0.026	3.45± 1.68
4. *Uromyces* (day 0) + *Colletotrichum* (day 2)	1.047± 0.421	5.01± 0.02	0.097± 0.041	8.37± 2.41
5. *Colletotrichum* (day 0) + *Uromyces* (day 2)	0.765± 0.175	5.00± 0.10	0.217± 0.050	4.75± 1.54
6. *Uromyces* (day 2)	-	-	-	-
7. *Colletotrichum* (day 2)	0.567± 0.136	5.03± 0.13	0.050± 0.013	4.87± 0.87
8. *Uromyces* + *Colletotrichum* (day 2)	0.280± 0.042	5.05± 0.04	0.062± 0.009	3.12± 1.00
Contrasts				
2 x 3	NS	NS	NS	NS
2 x 5	NS	NS	0.026	NS
2 x 7	NS	NS	NS	NS
3 x 5	NS	NS	0.006	NS
3 x 8	NS	NS	NS	NS
4 x 7	NS	NS	NS	NS
7 x 8	NS	NS	NS	NS

[a] Inoculation treatments - see Table F.1 for explanation.

[b] Variable mean ± standard error of the mean; means of four replicates.

[c] Pre-planned contrasts; the numbers correspond to the treatment number.

[d] Non-significant at the 5% probability level; the numbers in other columns are significance levels.

Although this symptom was not widespread in the present study, it may be worthy of consideration in experiments with higher severities of both diseases, when it is more likely to be present. Zaiter et al. (1990) reported that the coinoculation of *U. appendiculatus* and *Xanthomonas campestris* pv. phaseoli on common bean leaves induced bacterial-rust necrosis symptoms that were different from those caused by the bacterial infection alone.

The presence of a pathogenic infection in a plant reportedly reduces the development of later infections caused by different pathogens (Bassanezi et al., 1998; Omar et al., 1986; Simkin and Wheeler, 1974; Weber et al., 1994). Barley plants pre-inoculated with *Puccinia hordei* had fewer colonies of *Blumeria graminis* and vice-versa (Simkin and Wheeler, 1974). Infection of wheat plants with *Septoria nodorum* reduced the disease severity of *B. graminis* in field trials (Weber et al., 1994). However, the severity of *S. nodorum* was increased when *B. graminis* was already present in the plants.

There are a few reported cases where the interaction between pathogens did not significantly affect the amount or rate of development of each pathogen involved. Potter (1982) did not observe significant reductions in the number of pustules or latent period of *Puccinia hordei* on barley pre-infected with barley yellow dwarf virus. Similarly, corn plants infected with the maize streak virus were not less susceptible to *Peronosclerospora sorghi* or *P. philippinensis* (Damsteegt et al., 1993).

The concomitant presence of rust and anthracnose on bean leaves, under the conditions of the two experiments conducted, had little effect on maximum lesion density, incubation period, and severity of both diseases. There was a greater effect of the interaction on infection efficiency in the first test, but not in the second one; and, thus, no

conclusions could be drawn from the results of both experiments. Therefore, no obvious positive or negative interactions between these two pathogens could be observed in the present study. Further studies on this subject should include various intervals between inoculations with the different pathogens and various disease intensities.

REFERENCES

Agrios, G. N. 1997. Plant Pathology. Academic Press, San Diego, CA.

Ahmad, I., Farrar, J. F., and Whitbread, R. 1983. Photosynthesis and chloroplast functioning in leaves of barley infected with brown rust. Physiological Plant Pathology 23: 411-419.

Almeida, A. M. R., Chaves, G. M., and Zambolim, L. 1977. Influência da época de ataque de *Uromyces phaseoli typica* Arth. sobre o rendimento de duas variedades de feijoeiro (*Phaseolus vulgaris* L.) em casa-de-vegetação. Fitopatologia Brasileira 2: 17-21.

Amorim, L., Berger, R. D., Bergamin, A. F., Hau, B., Weber, G. E., Bacchi, L. M. A., Vale, F. X. R., and Silva, M. B. 1995. A simulation model to describe epidemics of rust of Phaseolus beans. II. Validation. Phytopathology 85: 722-727.

Aquino, V. M., Shokes, F. M., Berger, R. D., Gorbet, D. W., and Kucharek, T. A. 1992. Relationships among late leafspot, healthy leaf area duration, canopy reflectance, and pod yield of peanut. Phytopathology 82: 546-552.

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology 14: 1-15.

Azcón-Bieto, J., and Osmond, C. B. 1983. Relationship between photosynthesis and respiration. Plant Physiology 71: 574-581.

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.

Baker, N. R. 1993. Light-use efficiency and photoinhibition of photosynthesis in plants under environmental stress. Pages 221-235 in: Water Deficits. Plant responses from cell to community. J. A. C. Smith and H. Griffiths, eds. Bios Scientific Publishers, Oxford.

Balachandran, S., Daley, P. F., and Osmond, C. B. 1992. Imaging photoinhibition following infection with tobacco mosaic virus, strain (PV230). Pages 37-40 in: Research in Photosynthesis, vol II. N. Murata, ed. Kluwer Academic Publishers, Dordrecht.

Balachandran, S., and Osmond, C. B. 1994. Susceptibility of tobacco leaves to photoinhibition following infection with two strains of tobacco mosaic virus under different light and nitrogen nutrition regimes. Plant Physiology 104: 1051-1057.

Balachandran, S., Osmond, C. B., and Daley, P. F. 1994a. Diagnosis of the earliest strain-specific interactions between tobacco mosaic virus and chloroplasts of tobacco leaves *in vivo* by means of chlorophyll fluorescence imaging. Plant Physiology 104: 1059-1065.

Balachandran, S., Osmond, C. B., and Makino, A. 1994b. Effects of two strains of tobacco mosaic virus on photosynthetic characteristics and nitrogen partitioning in leaves of *Nicotiana tabacum* cv. Xanthi during photoacclimation under two nitrogen nutrition regimes. Plant Physiology 104: 1043-1050.

Balardin, R., Jarosz, A., and Kelly, J. D. 1997. Virulence and molecular diversity in *Colletotrichum lindemuthianum* from South, Central, and North America. Phytopathology 87: 1184-1191.

Bassanezi, R. B. 1995. Interações entre o mosaico-em-desenho do feijoeiro e duas doenças fúngicas, ferrugem e mancha angular, em plantas de feijoeiro. Ms. thesis. Escola de Agricultura Luiz de Queiroz - USP, Piracicaba.

Bassanezi, R. B., Bergamin Filho, A., Amorim, L., and Hau, B. 1998. Effects of bean line pattern mosaic virus on the monocyclic components of rust and angular leaf spot of *Phaseolus* bean at different temperatures. Plant Pathology 47: 289-298.

Bassanezi, R. B., Martins, M. C., Godoy, C. V., Amorim, L., and Bergamin Filho, A. 1997. Efeito da antracnose na eficiência fotossintética do feijoeiro. Fitopatologia Brasileira 22: 520-524.

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.

Bastiaans, L. 1993. Effects of leaf blast on photosynthesis of rice. 1. Leaf photosynthesis. Netherlands Journal of Plant Pathology 99: 197-203.

Bastiaans, L., and Kropff, M. J. 1993. Effects of leaf blast on photosynthesis of rice. 2. Canopy photosynthesis. Netherlands Journal of Plant Pathology 99: 205-217.

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.

Bastiaans, L., and Roumen, E. C. 1993. Effect on leaf photosynthetic rate by leaf blast for rice cultivars with different types and levels of resistance. Euphytica 66: 81-87.

Bergamin Filho, A., and Amorim, L. 1996. Doenças de Plantas Tropicais: Epidemiologia e Controle Econômico. Editora Agronômica Ceres, São Paulo.

Bergamin Filho, A., Carneiro, S. M. T. P., 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.

Berger, R. D. 1988. Measuring disease intensity. Biological and Cultural Tests 3: 1-4.

Berger, R. D., Hau, B., Weber, G. E., Bacchi, L. M. A., Bergamin Filho, A., and Amorim, L. 1995. A simulation model to describe epidemics of rust of *Phaseolus* beans. I. Development of the model and sensitivity analysis. Phytopathology 85: 715-721.

Beyschlag, W. R., and Ryel, R. J. 1998. Modelling leaf/canopy photosynthesis. Pages 305-319 in: Photosynthesis: a comprehensive treatise. A. S. Raghavendra, ed. Cambridge University Press, Cambridge.

Bolhàr-Nordenkampf, H. R., and Lechner, E. 1988. Winter stress and chlorophyll fluorescence in Norway spruce. Pages 173-180 in: Applications of Chlorophyll Fluorescence in Photosynthesis Research, Stress Physiology, Hydrobiology and Remote Sensing. H. K. Lichtenthaler, ed. Kluwer Academic Publishers, Dordrecht.

Bolhàr-Nordenkampf, H. R., and Öquist, G. 1993. Chlorophyll fluorescence as a tool in photosynthesis research. Pages 193-206 in: Photosynthesis and production in a changing environment. D. O. Hall, J. M. O. Scurlock, H. R. Bolhàr-Nordenkampf, R. C. Leegood, and S. P. Long, eds. Chapman & Hall, London.

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.

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. Agronomy Journal 72: 247-252.

Bourgeois, G., and Boote, K. J. 1992. Leaflet and canopy photosynthesis of peanut affected by late leaf spot. Agronomy Journal 84: 359-366.

Campbell, C. L., and Madden, L. V. 1990. Introduction to Plant Disease Epidemiology. John Wiley and Sons, Inc., New York.

Charles-Edwards, D. A. 1982. Physiological Determinants of Crop Growth. Academic Press Australia, Sidney.

Chaves, M. 1991. Effects of water deficits on carbon assimilation. Journal of Experimental Botany 42: 1-16.

Cheeseman, J. M., and Lexa, M. 1996. Gas exchange: models and measurements. Pages 223-240 in: Photosynthesis and the Environment. Advances in Photosynthesis. Vol.5. N. R. Baker, ed. Kluwer Academic Publishers, Dordrecht.

Chou, H. M., Rolfe, S. A., and Scholes, J. D. 1995. The effect of *Albugo candida* on the photosynthetic and carbohydrate metabolism of *Arabidopsis thaliana*. Aspects of Applied Biology 42: 149-153.

Cole, D. L. 1982. Interactions between *Cercospora arachidicola* and *Phoma arachidicola*, and their effects on defoliation and kernel yield of groundnut. Plant Pathology 31: 355-362.

Cornic, G. 1994. Drought stress and high light effects on leaf photosynthesis. Pages 297-313 in: Photoinhibition of Photosynthesis. From molecular mechanisms to the field. N. R. Baker and J. R. Bowyer, eds. Bios Scientific Publishers, Oxford.

Cornic, G., and Briantais, J. M. 1991. Partitioning of photosynthetic electron flow between CO_2 and O_2 in a C_3 leaf (*Phaseolus vulgaris* L.) at different CO_2 concentrations and during drought stress. Planta 183: 178-184.

Cowger, C., and Mundt, C. C. 1998. A hydroponic seedling assay for resistance to Cephalosporium stripe of wheat. Plant Disease 82: 1126-1131.

Daamen, R. A., and Jorritsma, I. T. M. 1990. Effects of powdery mildew and weather on winter wheat yield. 2. Effects of mildew epidemics. Netherlands Journal of Plant Pathology 96: 35-46.

Daley, P. F., Raschke, K., Ball, J. T., and Berry, J. A. 1989. Topography of photosynthetic activity of leaves obtained from video images of chlorophyll fluorescence. Plant Physiology 90: 1233-1238.

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.

Daly, J. M., Bell, A. A., and Krupka, L. R. 1961. Respiratory changes during development of rust diseases. Phytopathology 51: 461-471.

Damsteegt, V. D., Bonde, M. R., and Hewings, A. D. 1993. Interactions between maize streak virus and downy mildew fungi in susceptible maize cultivars. Plant Disease 77: 390-392.

Dann, E. K., and Deverall, B. J. 1995. Effectiveness of systemic resistance in bean against foliar and soilborne pathogens as induced by biological and chemical means. Plant Pathology 44: 458-466.

Datnoff, L. E., and Sinclair, J. B. 1988. The interaction of *Fusarium oxysporum* and *Rhizoctonia solani* in causing root rot of soybeans. Phytopathology 78: 771-777.

Evans, J. T. 1983. Nitrogen and photosynthesis in the flag leaf of wheat. Plant Physiology 72: 297-302.

Farquhar, G. D., and Sharkey, T. D. 1982. Stomatal conductance and photosynthesis. Annual Review of Plant Physiology 33: 317-345.

Farquhar, G. D., and von Caemmerer, S. 1982. Modeling of photosynthetic response to environmental conditions. Pages 549-587 in: Physiological Plant Ecology II. O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, eds. Springer-Verlag, Berlin.

Farquhar, G. D., von Caemmerer, S., and Berry, J. A. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta. 149: 78-90.

Farrar, J. F. 1995. Just another sink? Sources of assimilate for foliar pathogens. Aspects of Applied Biology 42: 81-89.

Fawcett, H. S. 1931. The importance of investigations on the effects of known mixtures of microorganisms. Phytopathology 21: 545-550.

Fox, R. H., and Piekielek, W. P. 1997. Chlorophyll. Pages 56-58 in: McGraw-Hill Yearbook of Science and Technology 1998. McGraw-Hill Co., New York.

Foyer, C. 1993. Chlorophyll-a fluorescence as a probe for photosynthesis leaf metabolism and plant vitality. Pages 419-425 in: Crop Structure and light microclimate. Characterization and applications. C. Varlet-Grancher, R. Bonhomme, and H. Sinoquet, eds. Editions INRA, Paris.

Gallagher, J. N., and Biscoe, P. V. 1978. Radiation absorption, growth and yield of cereals. Journal of Agricultural Sciences 91: 47-60.

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.

Gay, J. L. 1984. Mechanisms of biotrophy in fungal pathogens. Pages 49-59 in: Plant Diseases. Infection, Damage, and Loss. R. K. S. Wood and G. J. Jellis, eds. Blackwell Scientific Publications, Oxford.

Genty, B., Briantais, J. M., and Baker, N. R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta 990: 87-92.

Genty, B., and Meyer, S. 1994. Quantitative mapping of leaf photosynthesis using chlorophyll fluorescence imaging. Australian Journal of Plant Physiology 22: 277-284.

Godoy, C. V., Carneiro, S. M. T. P., Iamauti, M. T., Dalla Pria, M., Amorim, L., and Bergamin Filho, A. 1997. Diagrammatic scales for bean diseases: development and validation. Journal of Plant Diseases and Protection 104: 336-345.

Goodwin, P. H. 1992. Effect of common bacterial blight on leaf photosynthesis of bean. Canadian Journal of Plant Pathology 14: 203-206.

Gordon, T. R., and Duniway, J. M. 1982. Effects of powdery mildew infection on the efficiency of CO_2 fixation and light utilization by sugar beet leaves. Plant Physiology 69: 139-142.

Guy, C. L. 1994. Low temperature and crop yield. Pages 417-424 in: Physiology and Determination of Crop Yield. K. J. Boote, J. M. Bennett, T. R. Sinclair, and G. M. Paulsen, eds. ASA, CSSA, SSSA, Madison.

Guzman, P., Donado, M. R., and Galvez, G. E. 1979. Pérdidas económicas causadas por la antracnosis del frijol (*Phaseolus vulgaris* L.) en Colombia. Turrialba 29: 65-67.

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 Physiology 50: 576-580.

Hall, D. O., and Rao, K. K. 1994. Photosynthesis. Cambridge University Press, Cambridge.

Hampton, R. E., Wullschleger, S. D., and Oosterhuis, D. M. 1990. Impact of verticillium wilt on net photosynthesis, respiration and photorespiration in field-grown cotton (*Gossypium hirsutum* L.). Physiological and Molecular Plant Pathology 37: 271-280.

Haverkort, A. J., and Bicamumpaka, M. 1986. Correlation between intercepted radiation and yield of potato crops infested by *Phytophthora infestans* in central Africa. Netherlands Journal of Plant Pathology 92: 239-247.

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.

Hyde, P. M. 1978. A study of the effects on wheat of inoculation with *Puccinia recondita* and *Leptosphaeria nodorum*, with respect to possible interactions. Phytopathologische Zeitschrift 92: 12-24.

Iamauti, M. T. 1995. Avaliação de danos provocados pela ferrugem na cultura do feijoeiro. Ph.D. Dissertation. ESALQ/USP, Piracicaba.

Jenkins, P. D., and Jones, D. G. 1981. The effects of dual inoculation of wheat cultivars with *Septoria tritici* and *Septoria nodorum*. Phytopathologische Zeitschrift 101: 210-221.

Johnson, K. B. 1987. Defoliation, disease, and growth: a reply. Phytopathology 77: 1495-1497.

Johnson, K. B. 1992. Evaluation of a mechanistic model that describes potato crop losses caused by multiple pests. Phytopathology 82: 363-369.

Johnson, K. B., Radcliffe, E. B., and Teng, P. S. 1986. Effects of interacting populations of *Alternaria solani*, *Verticillium dahliae*, and the potato leafhopper (*Empoasca fabae*) on potato yield. Phytopathology 76: 1046-1052.

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..

Kautsky, H., and Franck, U. 1943. Chlorophyll fluoreszenz und Kohlensaure assimilation. Biochem Zeitschrift 315: 139-232.

Kramer, D. M., and Crofts, A. R. 1996. Control and measurement of photosynthetic electron transport in vivo. Pages 25-66 in: Photosynthesis and the Environment. N. R. Baker, ed. Kluwer Academic Publishers, Dordrecht.

Kranz, J., and Jörg, E. 1989. The synecological approach in plant disease epidemiology. Review of Tropical Plant Pathology 6: 27-38.

Krause, G. H. 1988. Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiologia Plantarum 74: 566-574.

Krause, G. H., and Weis, E. 1984. Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosynthesis Research 5: 139-157.

Krupa, Z., Öquist, G., and Huner, N. P. A. 1993. The effects of cadmium on photosynthesis of *Phaseolus vulgaris* - a fluorescence analysis. Physiologia Plantarum 88: 626-630.

Last, F. T. 1955. Effect of powdery mildew on the yield of spring sown barley. Plant Pathology 4: 22-24.

Lichtenthaler, H. K. 1996. Vegetation Stress: and introduction to the stress concept in plants. Journal of Plant Physiology 148: 4-14.

Lim, L. G., and Gaunt, R. E. 1981. Leaf area as a factor in disease assessment. Journal of Agricultural Sciences 97: 481-483.

Lim, L. G., and Gaunt, R. E. 1986a. The effect of powdery mildew (*Erysiphe graminis* f. sp. *hordei*) and leaf rust (*Puccinia hordei*) on spring barley in New Zealand. I. Epidemic development, green leaf area and yield. Plant Pathology 35: 44-53.

Lim, L. G., and Gaunt, R. E. 1986b. The effect of powdery mildew (*Erysiphe graminis* f. sp. *hordei*) and leaf rust (*Puccinia hordei*) on spring barley in New Zealand. II. Apical development and yield potential. Plant Pathology 35: 54-60.

Livne, A. 1964. Photosynthesis in healthy and rust-affected plants. Plant Physiology 39: 614-621.

Livne, A., and Daly, J. M. 1966. Translocation in healthy and rust-affected beans. Phytopathology 56: 170-175.

Long, S. P., and Hallgren, J. E. 1993. Measurement of CO_2 assimilation by plants in the field and the laboratory. Pages 129-166 in: Photosynthesis and Production in a Changing Environment: a Field and Laboratory Manual. D. O. Hall, H. R. Bolhàr-Nordenkampf, R. C. Leegood, and P. S. Long, eds. Chapman & Hall, London.

Loomis, R. S., and Adams, S. S. 1983. Integrative analysis of host-pathogen relations. Annual Review of Phytopathology 21: 341-362.

Loomis, R. S., Rabbinge, R., and Ng, E. 1979. Explanatory models in crop physiology. Annual Review of Plant Physiology 30: 339-367.

Luo, Y., Teng, P. S., Fabellar, N. G., and TeBeest, D. O. 1997. A rice-leaf blast combined model for simulation of epidemics and yield loss. Agricultural Systems 53: 27-39.

Luttrell, E. S. 1974. Parasitism of fungi on vascular plants. Mycologia 66: 1-15.

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*. Annals of Applied Biology 112: 585-595.

Magyarosy, A. C., and Malkin, R. 1978. Effect of powdery mildew infection of sugar beet on the content of electron carriers in chloroplasts. Physiological Plant Pathology 13: 183-188.

Magyarosy, A. C., Schurmann, P., and Buchanan, B. B. 1976. Effect of powdery mildew infection on photosynthesis by leaves and chloroplasts of sugar beets. Plant Physiology 57: 486-489.

Markwell, J., Osterman, J. C., and Mitchell, J. L. 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosynthesis Research 46: 467-472.

Martin, P. J. 1986. Gaseous exchange studies of barley leaves infected with *Rhynchosporium secalis* (Oudem) J.J. Davis. Physiological and Molecular Plant Pathology 28: 3-14.

McGrath, M. T., and Pennypacker, S. P. 1990. Alteration of physiological processes in wheat flag leaves caused by stem rust and leaf rust. Phytopathology 80: 677-686.

Menezes, J. R., and Dianese, J. C. 1988. Race characterization of Brazilian isolates of *Colletotrichum lindemuthianum* and detection of resistance to anthracnose in *Phaseolus vulgaris*. Phytopathology 78: 650-655.

Minolta. 1989. SPAD-502 Owner's manual. Industrial meter division. Minolta Corp., Ramsey, N.J.

Moll, S., Serrano, P., and Boyle, C. 1995. *In vivo* chlorophyll fluorescence in rust-infected bean plants. Angewandte Botanik 69: 163-168.

Monge, O. A., and Bugbee, B. 1992. Inherent limitations of nondestructive chlorophyll meters: a comparison of two types of meters. HortScience 27: 69-71.

Montalbini, P., and Buchanan, B. B. 1974. Effect of a rust infection on photophosphorylation by isolated chloroplasts. Physiological Plant Pathology 4: 191-196.

Monteith, J. L. 1972. Solar radiation and productivity in tropical ecosystems. Journal of Applied Ecology 9: 746-747.

Monteith, J. L. 1977. Climate and the efficiency of crop production in Britain. Philosophical Transactions of the Royal Society of London 281: 277-294.

Monteith, J. L. 1981. Does light limit crop production? Pages 23-38 in: Physiological processes limiting plant production. O. B. Johnson, ed. Butterworths, London.

Nelson, S. C., and Campbell, C. L. 1992. Incidence and patterns of association of pathogens in a leaf spot disease complex on white clover in the Piedmont region of North Carolina. Phytopathology 82: 1013-1021.

Nunes, W. M. C., and Bergamin Filho, A. 1996. Avaliação dos danos causados pela antracnose (*Colletotrichum lindemuthianum*) do feijoeiro. Fitopatologia Brasileira 22: 520-524.

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.

O'Connell, R. J., Bailey, J. A., and Richmond, D. V. 1985. Cytology and physiology of infection of *Phaseolus vulgaris* by *Colletotrichum lindemuthianum*. Physiological Plant Pathology 27: 75-98.

Omar, S. A. M., Bailiss, K. W., Chapman, G. P., and Mansfield, J. W. 1986. Effects of virus infection of faba bean on subsequent infection by *Uromyces viciae-fabae*. Plant Pathology 35: 535-543.

Osmond, C. B. 1994. What is photoinhibition? Some insights from comparisons of shade and sun plants. Pages 1-24 in: Photoinhibition of Photosynthesis. From Molecular Mechanisms to the Field. N. R. Baker and J. R. Bowyer, eds. Bios Scientific Publishers, Oxford.

Owera, S. A. P., Farrar, J. F., and Whitbread, R. 1981. Growth and photosynthesis in barley infected with brown rust. Physiological Plant Pathology 18: 79-90.

Öquist, G., and Ogren, E. 1985. Effects of winter stress on photosynthetic electron transport and energy distribution between the two photosystems of pine as assayed by cholorophyll fluorescence kinetics. Photosynthesis Research 7: 19-30.

Paulsen, G. M. 1994. High temperature responses of crop plants. Pages 365-390 in: Physiology and Determination of Crop Yield. K. J. Boote, J. M. Bennett, T. R. Sinclair, and G. M. Paulsen, eds. ASA, CSSA, SSSA, Madison.

Pennypacker, B. W., Knievel, D. P., Leath, K. T., Pell, E. J., and Hill, Jr. R. R. 1990. Analysis of photosynthesis in resistant and susceptible alfalfa clones infected with *Verticillium albo-atrum*. Phytopathology 80: 1300-1306.

Peterson, R. B., and Aylor, D. E. 1995. Chlorophyll fluorescence induction in leaves of *Phaseolus vulgaris* infected with bean rust (*Uromyces appendiculatus*). Plant Physiology 108: 163-171.

Pinnschmidt, H. O., and Teng, P. S. 1993. Empirical versus mechanistic approaches to adjust yield expectations for multiple pest damage on rice. International Congress of Plant Pathology VI, 116. Abstract.

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, UK.

Potter, L. R. 1982. Interaction between barley yellow dwarf virus and rust in wheat, barley and oats, and the effects on grain yield and quality. Annals of Applied Biology 100: 321-329.

Powell, N. T. 1979. Internal synergisms among organisms inducing disease. Pages 113-134 in: Plant Disease: an advanced treatise. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York.

Rabbinge, R., Jorritsma, I. T. M., and Schans, J. 1985. Damage components of powdery mildew in winter wheat. Netherlands Journal of Plant Pathology 91: 235-247.

Rabbinge, R., and Rijsdijk, F. H. 1981. Disease and crop physiology: a modeller's point of view. Pages 201-220 in: Effects of Disease on the Physiology of the Growing Plant. P. G. Ayres, ed. Cambridge University Press, Cambridge.

Raggi, V. 1980. Correlation of CO_2 compensation point with photosynthesis and respiration and CO_2-sensitive compensation point in rust-affected bean leaves. Physiological Plant Pathology 16: 19-24.

Raggi, V. 1995. CO_2 assimilation, respiration and chlorophyll fluorescence in peach leaves infected by *Taphrina deformans*. Physiologia Plantarum 93: 540-544.

Richardson, D. E., and Doling, D. A. 1957. Potato blight and leaf-roll virus. Nature 180: 866-867.

Roberts, A. M., and Walters, D. R. 1988. Photosynthesis in discrete regions of leek leaves infected with the rust, *Puccinia allii* Rud. New Phytologist 110: 371-376.

Rolfe, S. A., and Scholes, J. D. 1995. Quantitative imaging of chlorophyll fluorescence. New Phytologist 131: 69-79.

Rooney, J. M. 1989. Biomass production and gas exchange of winter wheat as affected by flag leaf inoculations with *Septoria nodorum*. New Phytologist 112: 229-234.

Rotem, J., Bashi, E., and Kranz, J. 1983b. Studies of crop loss in potato late blight caused by *Phytophthora infestans*. Plant Pathology 32: 117-122.

Rotem, J., Kranz, J., and Bashi, E. 1983a. Measurement of healthy and diseased haulm area for assessing late blight epidemics in potatoes. Plant Pathology 32: 109-115.

Rouse, D. I. 1988. Use of crop growth-models to predict the effects of disease. Annual Review of Phytopathology 26: 183-201.

Rowe, R. C., Riedel, R. M., and Martin, M. J. 1985. Synergistic interactions between *Verticillium dahliae* and *Pratylenchus penetrans* in potato early dying disease. Phytopathology 75: 412-418.

Savary, S., Bosc, J.-P., Noirot, M., and Zadoks, J. C. 1988. Peanut rust in West Africa: a new component in a multiple pathosystem. Plant Disease 72: 1001-1009.

Savary, S., and Zadoks, J. C. 1991. Méthodes pour l'étude du dommage dû à um ensemble de contraintes phytosanitaires. Le cas de l'arachide en Côte d'Ivoire. Pages 256-280 in: Approches de la Pathologie des Cultures Tropicales. Exemple de l'Arachide en Afrique de l'Ouest. S. Savary, ed. Éditions de l'Orstom, Paris.

Savary, S., and Zadoks, J. C. 1992a. Analysis of crop loss in the multiple pathosystem groundnut-rust-late leaf spot. I. Six experiments. Crop Protection 11: 99-109.

Savary, S., and Zadoks, J. C. 1992b. 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 Protection 11: 110-120.

Scheuermann, R., Biehler, K., Stuhlfauth, T., and Fock, H. P. 1991. Simultaneous gas exchange and fluorescence measurements indicate differences in the response of sunflower, bean and maize to water stress. Photosynthesis Research 27: 189-197.

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, UK.

Scholes, J. D., and Farrar, J. F. 1985. Photosynthesis and chloroplast functioning within individual pustules of *Uromyces muscari* on bluebell leaves. Physiological Plant Pathology 27: 387-400.

Scholes, J. D., and Horton, P. 1993. Photosynthesis and chlorophyll fluorescence: simultaneous measurements. Pages 130-135 in: Methods in Comparative Plant Ecology. G. A. F. Hendry and J. P. Grime, eds. Chapman & Hall, London.

Scholes, J. D., Lee, P., Horton, P., and Lewis, D. H. 1990. Photosynthetic metabolism in leaves infected with powdery mildew. Pages 219-222 in: Current Research in Photosynthesis. Vol.4. M. Baltscheffsky, ed. Kluwer, Dordrecht.

Scholes, J. D., Lee, P. J., Horton, P., and Lewis, D. H. 1994. Invertase: understanding changes in the photosynthetic and carbohydrate metabolism of barley leaves infected with powdery mildew. New Phytologist 126: 213-222.

Scholes, J. D., and Rolfe, S. A. 1995. How do biotrophic pathogens affect the photosynthetic metabolism of their hosts? Aspects of Applied Biology 42: 91-99.

Scholes, J. D., and Rolfe, S. A. 1996. Photosynthesis in localised regions of oat leaves infected with crown rust (*Puccinia coronata*): quantitative imaging of chlorophyll fluorescence. Planta 199: 573-582.

Schreiber, U., and Bilger, W. 1993. Progress in chlorophyll fluorescence research: major developments during the past years in retrospect. Progress in Botany 54: 151-173.

Schreiber, U., Bilger, W., Hormann, H., and Neubauer, C. 1998. Chlorophyll fluorescence as a diagnostic tool: basics and some aspects of practical relevance. Pages 320-336 in: Photosynthesis: a Comprehensive Treatise. A. S. Raghavendra, ed. Cambridge University Press, Cambridge..

Schreiber, U., Neubauer, C., and Schliwa, U. 1993. PAM fluorometer based on medium-frequency pulsed Xe-flash measuring light: a highly sensitive new tool in basic and applied photosynthesis research. Photosynthesis Research 36: 65-72.

Schreiber, U., Schliwa, U., and Bilger, W. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynthesis Research 10: 51-62.

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 II. Encyclopedia of Plant Physiology NS. vol12B. O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, eds. Springer-Verlag, New York.

Seaton, G. R., and Walker, D. A. 1990. Chlorophyll fluorescence as a measure of photosynthetic carbon assimilation. Proceedings of the Royal Society of London B 242: 29-35.

Seeman, J. R., Sharkey, T. D., Wang, J., and Osmond, C. B. 1987. Environmental effects on photosynthesis, nitrogen-use efficiency, and metabolite pools in leaves of sun and shade plants. Plant Physiology 84: 796-802.

Shao, F., and Teri, J. M. 1985. Yield losses in *Phaseolus* beans induced by anthracnose in Tanzania. Tropical Pest Management 31: 60-62.

Shtienberg, D. 1992. Effects of foliar diseases on gas exchange processes: a comparative study. Phytopathology 82: 760-765.

Simkin, M. B., and Wheeler, B. E. J. 1974. Effects of dual infections of *Puccinia hordei* and *Erysiphe graminis* on barley, cv. Zephyr. Annals of Applied Biology 78: 237-250.

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, Palo Alto.

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. Netherlands Journal of Plant Pathology. 96: 3-15.

Stavely, J. R. 1984. Pathogenic specialization in *Uromyces phaseoli* in the United States and rust resistance in beans. Plant Disease 68: 95-99.

Stavely, J. R. 1991. Rust. Pages 24-25 in: Compendium of Bean Diseases. R. Hall, ed. APS Press, St. Paul.

Stavely, J. R., and Pastor-Corrales, M. A. 1989. Rust. Pages 159-194 in: Bean Production Problems in the Tropics. H. F. Schwartz and M. A. Pastor-Corrales, eds. CIAT, Cali.

Tang, X., Rolfe, S. A., and Scholes, J. D. 1996. The effect of *Albugo candida* (white blister rust) on the photosynthetic and carbohydrate metabolism of leaves of *Arabidopsis thaliana*. Plant, Cell and Environment 19: 967-975.

Teng, P. S. 1983. Estimating and interpreting disease intensity and loss in commercial fields. Phytopathology 73: 1587-1590.

Teng, P. S., and Gaunt, R. E. 1980. Modeling systems of disease and yield loss in cereals. Agricultural Systems 6: 131-154.

Thompson, J. A., Schweitzer, L. E., and Nelson, R. L. 1996. Association of specific leaf weight, an estimate of chlorophyll, and chlorophyll concentration with apparent photosynthesis in soybean. Photosynthesis Research 49: 1-10.

Townsend, G. R. 1939. Diseases of beans in Southern Florida. Florida Agriculture Experimental Station Bulletin, no. 336.

Tu, J. C. 1992. Bean anthracnose. Pages 1-17 in: Plant Diseases of International Importance: Diseases of Vegetables and Oil Seed Crops. H. S. Chaube, A. N. Mukhopadhyay, and J. Kumar, eds. Prentice Hall, New Jersey.

van der Wal, A. F., and Cowan, M. C. 1974. An ecophysiological approach to crop losses exemplified in the system wheat, leaf rust and gume blotch. II. Development, growth, and transpiration of uninfected and infected plants with *Puccinia recondita* f. sp. *triticina* and/or *Septoria nodorum* in a climate chamber. Netherlands Journal of Plant Pathology. 80: 192-214.

van der Wal, A. F., Smeitink, H., and Maan, G. C. 1975. An ecophysiological approach to crop losses exemplified in the system wheat, leaf rust and glume blotch. III. Effects of soil-water potential on development, growth, transpiration, symptoms, and spore production of leaf rust-infected wheat. Netherlands Journal of Plant Pathology. 81: 1-13.

van der Wal, A. F., Shearer, B. L., and Zadoks, J. C. 1970. Interaction between *Puccinia recondita* f. sp. *triticina* and *Septoria nodorum* on wheat, and its effects on yield. Netherlands Journal of Plant Pathology 76: 261-263.

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: Proceedings of the International Symposium on Biotic Stress of Barley in Arid and Semi-arid Environments. Bozeman, Montana, Montana State University.

van Kooten, O., and Snel, J. F. H. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynthesis Research 25: 147-150.

van Oijen, M. 1990. Photosynthesis is not impaired in healthy tissue of blighted potato plants. Netherlands Journal of Plant Pathology 96: 55-63..

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. Netherlands Journal of Plant Pathology 96: 17-28.

Venette, J. R., and Jones, D. A. 1982. Yield losses associated with severity of bean rust (*Uromyces phaseoli*) on pinto beans (*Phaseolus vulgaris* UI-114). Phytopathology 72: 974. Abstract.

Waggoner, P. E., and Berger, R. D. 1987. Defoliation, disease and growth. Phytopathology 77: 393-398.

Wagner, S., and Boyle, C. 1995. Changes in carbohydrate, protein and chlorophyll content, and enzyme activity durign the switch from uredinio- to teliospore sporulation in the bean-rust fungus *Uromyces appendiculatus* (Pers.) Link. Journal of Phytopathology 143: 633-638.

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.

Walters, D. R., and Ayres, P. G. 1984. Ribulose bisphosphate carboxylase protein and enzymes of CO_2 assimilation in barley infected by powdery mildew (*Erysiphe graminins hordei*). Phytopathologische Zeitschrift 109: 208-218.

Watson, D. J. 1947. Comparative physiological studies on the growth of field crops. I. Variation in net assimilation rate and leaf area between species and varieties, and within and between years. Annals of Botany 11: 41-76.

Watson, D. J. 1952. The physiological basis of variation in yield. Advances in Agronomy 4: 101-145.

Weber, G. E., Gulec, S., and Kranz, J. 1994. Interaction between *Erysiphe graminis* and *Septoria nodorum* on wheat. Plant Pathology 43: 158-163.

Wijesundera, R. L. C., Bailey, J. A., Byrde, R. J. W., and Fielding, A. H. 1989. Cell wall degrading enzymes of *Colletotrichum lindemuthianum*: their role in the development of bean anthracnose. Physiological and Molecular Plant Pathology 34: 403-413.

Wong, P. Y. O., and Thrower, L. B. 1978a. Effect of *Colletotrichum lindemuthianum* on photosynthesis and respiration of *Vigna sesquipedalis*. Phytopathologische Zeitschrift 92: 88-94.

Wong, P. Y. O., and Thrower, L. B. 1978b. Sugar metabolism and translocation in *Vigna sesquipedalis* infected by *Colletotrichum lindemuthianum*. Phytopathologische Zeitschrift 92: 102-112.

Wong, S. C., Cowan, I. R., and Farquhar, G. D. 1979. Stomatal conductance correlates with photosynthetic capacity. Nature 282: 424-426.

Zadoks, J. C., and Schein, R. D. 1979. Epidemiology and Plant Disease Management. Oxford University Press, New York.

Zaiter, H. Z., Coyne, D. P., and Steadman, J. R. 1990. Coinoculation effects of the pathogens causing common bacterial blight, rust, and bean common mosaic in *Phaseolus vulgaris*. Journal of the American Society of Horticultural Sciences 115: 319-323.

BIOGRAPHICAL SKETCH

Daniela Biaggioni Lopes was born on January 24, 1969, in São Paulo-S.P., Brazil. She received her bachelor's degree in agronomic engineering in 1990, from the Escola Superior de Agricultura 'Luiz de Queiroz', ESALQ, a branch campus of the Universidade de São Paulo in the city of Piracicaba-S.P. As an undergraduate, Ms. Lopes developed her interest in plant pathology while working with Dr. Hiroshi Kimati on the biological control of soilborne pathogens.

Dr. Kimati encouraged Ms. Lopes to continue the work in the department of plant pathology after her graduation, as a master's student. Ms. Lopes received her M.Sc. degree in plant pathology in 1994, with a thesis entitled "*In vitro* and growth chamber screening of fungicides and antagonistic microorganisms for the control of onion white rot."

While a student in the department of plant pathology at ESALQ/USP, Ms. Lopes had the opportunity to meet Dr. Richard Berger, who invited her to work in his program at the University of Florida. Ms. Lopes came to Gainesville in the fall of 1994, when she began to work on her Ph.D. project on the effects of bean diseases on leaf photosynthesis. During the period spent at the plant pathology department at the University of Florida., Ms. Lopes worked as an assistant to Dr. F.W. Zettler, teaching the laboratory portion of the course 'Fundamentals in Plant Pathology.' Also, she was part of the team of graduate students that launched the departmental newsletter, PLP News. Ms. Lopes was also very

involved in the integration of the Brazilian community in Gainesville. She was one of the founders and the secretary of the Brazilian Student Association at UF (BRASA) in its first year of existence.

Upon completing of her Ph.D. program, Ms. Lopes will return to her home country, where she plans to pursue a career in plant pathology focusing on the epidemiological and physiological aspects of crop losses due to diseases.