WALDIR CINTRA DE JESUS JUNIOR

EFFECTS OF ANGULAR LEAF SPOT AND RUST ON PLANT GROWTH AND YIELD OF *Phaseolus vulgaris*

Thesis presented to the Universidade Federal de Viçosa, as a part of the requirements of the Plant Pathology graduate program, for the degree of "*Doctor Scientiae*".

VIÇOSA
MINAS GERAIS - BRASIL
2001

WALDIR CINTRA DE JESUS JUNIOR

EFFECTS OF ANGULAR LEAF SPOT AND RUST ON PLANT GROWTH AND YIELD OF *Phaseolus vulgaris*

Thesis presented to the Universidade Federal de Viçosa, as a part of the requirements of the Plant Pathology graduate program, for the degree of "*Doctor Scientiae*".

APPROVED: 09th March 2001.

Prof. Bernhard Hau	Prof. Laércio Zambolim (Committee Member)

Prof. Luiz Cláudio Costa
(Committee Member)

Prof. Armando Bergamin Filho

Prof. Francisco Xavier Ribeiro do Vale
(Adviser)

À DEUS, pela saúde, estímulo e pela presença em minha vida,

Aos meus queridos pais Waldir e Fanny,

Aos meus irmãos Otto e Tânia,

Aos meus sobrinho(a)s Otto, Igor, Thais e Thatiana,

A minha afilhada Anna Júlia,

Aos meus cunhado e cunhada, Clineu e Luzia.

AGRADECIMENTOS

À DEUS, em primeiro lugar, pela vida, motivação, proteção espiritual e por estar sempre iluminando os meus passos e caminhos.

À toda a minha família, especialmente aos meus queridos pais Waldir e Fanny, aos meus irmãos Otto e Tânia, aos meus sobrinhos Otto, Igor, Thais e Thatiana, aos meus cunhado(a) Clineu e Luzia, ao meu compadre Carlos Alberto e a minha afilhada Anna Júlia, pelo amor, compreensão, carinho, apoio e incentivo, manifestados durante toda minha vida.

À Tia Gessy, Dona Edna e Seu Diógenes, pelo carinho e incentivo durante toda minha vida.

Ao Professor Francisco Xavier Ribeiro do Vale, pela grande amizade, pelo constante incentivo e pela exemplar orientação, que não se resumiu somente neste trabalho, mas também durante a minha vida pessoal e profissional.

Aos Professores Laércio Zambolim, Luiz Cláudio Costa e Carlos Martinez, pela amizade, pela colaboração, pelas críticas e sugestões valiosas durante a realização deste trabalho.

Ao Professor Bernhard Hau, pela amizade, orientação e oportunidade de treinamento em seu laboratório na Universität Hannover, Alemanha.

Ao Prof. Armando Bergamin Filho e Prof. Lilian Amorim, pela colaboração, pelas sugestões e críticas durante a realização deste trabalho.

Aos meus primeiros mestres na Fitopatologia, Eliane Aparecida Benato, Prof. Nilton Luiz de Souza e Prof. Chukichi Kurozawa, sem os quais nada disso estaria acontecendo.

Aos verdadeiros amigos, Reginaldo Resende e Pierce Paul, pela valiosa amizade, pelo companheirismo, pelo estímulo constante nos momentos difíceis e alegria nos de descontração.

À Sonia Faria, pelo companheirismo e estímulo durante a realização de parte deste trabalho.

Aos amigos Trazilbo, Marô, Liz e Julia, pela amizade e ajuda durante o tempo que morei na Alemanha.

Aos amigos de "república", Giancarlo, Franscico, Juninho, Márcio, Lucas, Newton, pela amizade e pelo convívio agradável.

Aos estagiários e funcionários do laboratório, Paulo, Kelly, Cristian, Ênia, Macabeu, Sérgio, José Cláudio, pela amizade e ajuda na condução dos experimentos.

Aos amigos Eduardo Pezzopane, Marlon, João Wordell, José R. Liberato e Fabrício Rodrigues, pela amizade, auxílio e incentivo.

Ao Prof. Eduardo Mizubuti pelas sugestões e críticas durante a realização deste trabalho.

Aos colegas de curso, pelo companheirismo e convívio.

Ao Departamento de Fitopatologia da Universidade Federal de Viçosa, pela oportunidade de realizar os cursos de Mestrado e Doutorado.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES, pela cessão da bolsa de mestrado e doutorado.

À União Européia, FAPEMIG e FINEP pelo financiamento de parte deste projeto.

Aos Professores do Departamento de Fitopatologia da Universidade Federal de Viçosa, pela amizade e pelos conhecimentos transmitidos durante os cursos realizados.

A todos os funcionários do Departamento de Fitopatologia da Universidade Federal de Viçosa, especialmente Regina, Délio, Cecília, Braz e Jorge, sempre solícitos em ajudar.

A todos que colaboraram direta ou indiretamente para a realização deste trabalho.

BIOGRAPHY

The author was born on March 3rd, 1973, in Botucatu, SP, Brazil. His elementary education began at the Cardoso de Almeida State School and he finished high school at the La Salle School, in 1990. He began his university education at the UNESP/Faculdade de Ciências Agronômicas, Botucatu, in 1991. From 1991-1995 he did practical courses in the Plant Pathology Department, funded by FAPESP. He obtained his degree in Agronomic Engineering in 1995, receiving three awards (Diploma Faculdade de Ciências Agronômicas, Prêmio Instituto de Engenharia, and Diploma Horácio Passos) for being the best graduating student and the best student in the course of Agronomy at UNESP. He began his M.Sc. degree in Plant Pathology at the Universidade Federal de Viçosa, Viçosa, MG, in 1996, under the supervision of Professor Francisco X. R. do Vale. In 1997, because of his excellent performance at the M.Sc. level, he was transferred directly to the D.Sc. program (without having to present a M.Sc. thesis). During 1999-2000 he spent seven months at the Institut fur Pflanzenkrankheiten und Pflanzenschutz, Universitat Hannover, Hannover, Germany, under the supervision of Professor Bernhard Hau. During this stay in Germany, he conducted several experiments and did all of the analysis of his thesis data. On March 9th, 2001, he concluded his D.Sc. degree in Plant Pathology. He has since been approved in the 2001 job selection screening conducted by EMBRAPA.

CONTENTS

RESUMO

JESUS JUNIOR, Waldir Cintra de, D.S., Universidade Federal de Viçosa, Março de 2001. **Effects of angular leaf spot and rust on plant growth and yield of *Phaseolus vulgaris*.** Professor Orientador: Francisco Xavier Ribeiro do Vale. Professores Conselheiros: Laércio Zambolim e Luiz Cláudio Costa.

Este trabalho teve por objetivo estudar o efeito da mancha angular (*Phaeoisariopsis griseola*) e da ferrugem (*Uromyces appendiculatus*) sobre o crescimento e produção do feijoeiro (*Phaseolus vulgaris*).

A tese foi organizada em cinco capítulos. Os dois primeiros capítulos foram escritos utilizando-se dados coletados a partir de 3 experimentos de campo, em que foram efetuadas diferentes análises. Os demais capítulos referem-se a resultados obtidos em experimentos independentes.

Capítulo 1. No presente capítulo foram estudados os efeitos da mancha angular e da ferrugem, isoladas e conjuntas, sobre o crescimento (área abaixo da curva de progresso da área foliar, duração da área foliar sadia e absorção da área foliar sadia) e produção do feijoeiro. Em todos os experimentos não houve diferença estatística entre os tratamentos no tocante às variáveis área abaixo da curva de progresso da área foliar (AULAPC), duração da área foliar sadia (HAD) e absorção da área foliar sadia (HAA), entretanto todos os tratamentos inoculados diferiram do controle em severidade de doença e produção. Em geral, não obteve-se relação entre produção e doença, ao passo que a produção relacionou-se linearmente com as variáveis HAD e HAA. Concluiu-se que a mancha angular causa desfolha, enquanto que a ferrugem não afeta a área foliar. A ferrugem causou maior redução na produção se comparado à mancha angular, porém o decréscimo na fotossíntese causado pela mancha angular foi duas vezes maior.

Capítulo 2. Neste capítulo foram investigados os efeitos da mancha angular e da ferrugem, isoladas e conjuntas, sobre a troca gasosa (taxa fotossintética líquida, condutância estomática e transpiração) do feijoeiro. A inoculação das plantas com *P. griseola* (tratamento P), *U. appendiculatus* (tratamento U) e com ambos patógenos (tratamento P+U) causou significante redução na taxa fotossintética líquida e na produção. Os tratamentos P e P+U resultaram em redução significativa da condutância estomática. A partir da análise dos dados foi observado que os efeitos das doenças sobre a produção podem ser explicados por decréscimos na condutância estomática e na taxa fotossintética líquida.

Capítulo 3. No referido capítulo foram analisadas as relações entre mancha angular, área foliar sadia, área foliar efetiva e produção do feijoeiro. Não foi observada relação entre severidade visual ou virtual e produção, entretanto relação linear positiva foi obtida entre as variáveis duração da área foliar sadia (HAD), absorção da área foliar sadia (HAA), duração da área foliar efetiva (ELAD), absorção da área foliar efetiva (ELAA) e produção. Foi observado constância nos valores de inclinação (coeficiente angular), obtidos a partir da regressão linear entre produção e índice de área foliar sadio (HLAI), independentemente da data de plantio e estádio de crescimento do feijoeiro (de R6 a R8). HLAI é proposta como variável explanatória para um sistema transportável de manejo da doença, possibilitando recomendações precisas em nível de produtor.

Capítulo 4. Neste capítulo foram avaliadas estratégias de manejo da mancha angular do feijoeiro baseadas em aplicação de molibdênio e controle químico. Foi observado que a aplicação de molibdênio causou decréscimo na intensidade da doença, bem como promoveu incrementos na área foliar, na taxa fotossintética líquida e na produção do feijoeiro. O controle químico da mancha angular deve ser realizado durante a fase de florescimento, através de uma ou duas aplicações fungicidas.

Capítulo 5. No presente capítulo foi testado a aplicabilidade do equipamento LAI-2000 (Li-Cor) para estimar o índice de área foliar (LAI) do feijoeiro. Foi concluído que o equipamento pode ser empregado sem restrições na cultura do feijoeiro.

ABSTRACT

JESUS JUNIOR, Waldir Cintra de, D.S., Universidade Federal de Viçosa, March of 2001. **Effects of angular leaf spot and rust on plant growth and yield of *Phaseolus vulgaris*.** Adviser: Francisco Xavier Ribeiro do Vale. Committee Members: Laércio Zambolim and Luiz Cláudio Costa.

The main purpose of the thesis was to understand the effects of angular leaf spot (*Phaeoisariopsis griseola*) and rust (*Uromyces appendiculatus*) on the variables related to plant growth and yield of common bean (*Phaseolus vulgaris*).

This thesis was organized in to five chapters. In the first two chapters data from three field experiments were analyzed using different approaches. The last three chapters referred to independent experiments.

Chapter 1. The effects of angular leaf spot and rust, separately or combined, on host growth (expressed as area under leaf area progress curve - AULAPC, healthy leaf area duration - HAD, and healthy leaf area absorption - HAA) and yield of individual bean plants were investigated. All inoculated treatments had significantly more severe disease and less yield than the control treatment. In general, yield was not related to disease severity or area under disease progress curve. In contrast, the highest yields were always related to the highest values of HAD, and HAA. The relationship between yield and HAD, and HAA was linear. It was concluded that angular leaf spot reduced the leaf area because of defoliation while rust did not affect the leaf area. Rust reduced yield more than four times that of angular leaf spot, although the decrease in photosynthesis to angular leaf spot was twice that of rust.

Chapter 2. The effect of angular leaf spot and rust, separately or combined, on leaf gas exchange (net photosynthetc rate, stomatal conductance, and transpiration) of common bean was reported. The inoculation of plants with *P. griseola* (P), *U. appendiculatus* (U), and a combination of the two pathogens (P+U) caused a significant reduction in the net photosynthetic rate and yield. Treatments P and P+U resulted in a significant reduction of stomatal conductance. The interactive effects of the pathogens on yield could be explained in part by the decreases in stomatal conductance and in the net photosynthetic rate of diseased bean leaves.

Chapter 3. The relationships among angular leaf spot, healthy leaf area, effective leaf area, and pod yield of common bean were evaluated. Visual and virtual severity, and area under disease progress curve (AUDPC) showed no correlation with pod yield. However, healthy leaf area duration (HAD), healthy leaf area absorption (HAA), effective leaf area duration (ELAD), and effective leaf area absorption (ELAA) were significantly correlated with pod yield. The relationships between yield and HAD, HAA, ELAD, and ELAA were linear in each of the three trials. The slope of the yield-healthy leaf area index (HLAI) relationship proved to be stable, regardless of planting date and bean growth stage (from R6 to R8). HLAI is proposed as a key explanatory variable for a transportable system of disease management; it may be useful in producing precise recommendations at the farm level.

Chapter 4. The strategies to manage angular leaf spot on common bean based in molybdenum application and chemical control were studied. It was observed that the molybdenum treatments showed smaller severity of angular leaf spot, and higher leaf area, net photosynthetic rate and yield, than the treatments that had no Mo. To control angular leaf spot, it was important to spray fungicide once or twice during the flowering period, which takes place ca. 25 to 45 days after planting.

Chapter 5. The applicability of the equipment LAI-2000 (Li-Cor) to estimate leaf area index (LAI) was tested. It was concluded that the equipment could be used to estimate LAI on common bean.

GENERAL INTRODUCTION

Seeds of the common bean (*Phaseolus vulgaris* L.) are an important staple food in Brazil and in many countries of Central and South America, where animal protein is limited.

BRAZIL IS THE WORLD LEADING BEAN PRODUCER, HOWEVER THE AVERAGE PRODUCTIVITY IS ABOUT 665 KG/HA, WHICH IS CONSIDERED LOW COMPARED TO THE PRODUCTIVITY ACHIEVED IN DEVELOPED COUNTRIES. THE FACTORS THAT REDUCE BEAN YIELDS IN MOST DEVELOPING COUNTRIES CAN BE GROUPED INTO THREE MAJOR CATEGORIES: BIOLOGICAL (DISEASES, INSECTS, WEEDS), EDAPHIC (POOR FERTILIZATION, HIGH ALUMINUM SATURATION, ETC.), AND CLIMATIC (DROUGHT, HIGH TEMPERATURES). AMONG THESE FACTORS, DISEASES CAN BE CONSIDERED AS ONE OF THE LEADING CAUSES OF LOW BEAN YIELD IN MOST BEAN-PRODUCING REGIONS OF LATIN AMERICA. MORE THAN 200 PATHOGENS HAVE BEEN REPORTED ON BEANS; NEVERTHELESS, ONLY ABOUT A DOZEN OF THEM CAN CAUSE CONSIDERABLE ECONOMIC LOSSES.

In common beans, angular leaf spot (caused by *Phaeoisariopsis griseola* (Sacc.) Ferr.) and rust (caused by *Uromyces appendiculatus* (Pers.) Unger) are the most destructive foliar diseases in Brazil. *P. griseola* causes lesions on leaves, pods, branches, and petioles, that result in severe defoliation. The symptoms of *U. appendiculatus* are chlorotic, raised pustules on the surface of leaves, pods, and petioles without causing severe defoliation. In the absence of proper control measures, yield reductions of 70% and 36-45% in Brazil, and 80% and 22% in Colombia, have been reported for angular leaf spot and bean rust, respectively.

However, any of these levels of losses are difficult to predict, because it has been very difficult to relate yield loss to disease intensity on common bean, primarily because the highest possible yield (without disease, insects, weeds, etc.) is different for each field, region, and season due to differences in both environmental and edaphic factors.

Based on the fact that the occurrence of two or more pathogens simultaneously on the same host is frequent, particularly in tropical regions, the understanding of the interactions between *P. griseola* and *U. appendiculatus* will be fundamental to the management of these diseases. Interactions may be important because the expected benefit from the control of one pathogen depends on the level of the other pathogen and therefore multiple diseases can significantly alter economic decision criteria in comparison to single disease occurrence.

In several plant physiological publications, crop production has been shown to be closely related to the amount of insolation the plant is able to use. The variables healthy leaf area duration (HAD) and healthy leaf area absorption (HAA), have been found to be much better predictors of yield compared to diseases severity as a predictor of yield loss on common bean. The concepts of HAD and HAA can be utilized for decision making in IPM programs to assure that there will be sufficient healthy leaf area to achieve a satisfactory yield. Additionally, disease can be assessed and the general health status of the crop canopy can be determined by the modern techniques of remote sensing, analysis of photosynthesis, and color-video imagery. The applicability of these techniques in studies of common bean yield loss due to diseases need to be evaluated, once these techniques facilitate the approach of using HAD and HAA in studies of multiple pathogens and their integrated management. In this study, the equipment LAI-2000 (Li-Cor) that is used to estimate leaf area index (LAI) will be evaluated.

With its implementation, the system will help growers on the decision making process related to control measures, and also contribute to reduce fungicide usage.

CHAPTER 1

Effects of Angular Leaf Spot and Rust on Yield Loss of *Phaseolus vulgaris*

W. C. Jesus Junior, F. X. R. Vale, R. R. Coelho, B. Hau, L. Zambolim, L. C. Costa, and A. Bergamin Filho

FIRST, SECOND, THIRD, FIFTH, AND SIXTH AUTHORS: DEPARTMENTO DE FITOPATOLOGIA, UNIVERSIDADE FEDERAL DE VIÇOSA, 36571-000 VIÇOSA, MG, BRAZIL; FOURTH AUTHOR: INSTITUT FÜR PFLANZENKRANKHEITEN UND PFLANZENSCHUTZ, UNIVERSITÄT HANNOVER, 30419 HANNOVER, GERMANY; SEVENTH AUTHOR: DEPARTAMENTO DE ENTOMOLOGIA, FITOPATOLOGIA E ZOOLOGIA AGRÍCOLA, ESALQ/USP, 13418-900 PIRACICABA, SP, BRAZIL.

ACCEPTED FOR PUBLICATION

CORRESPONDING AUTHOR: FRANCISCO XAVIER RIBEIRO DO VALE; E-MAIL ADDRESS: DOVALE@MAIL.UFV.BR

PAPER SUBMITTED TO PHYTOPATHOLOGY.

ABSTRACT

Jesus Junior, W. C., Vale, F. X. R., Coelho, R. R., Hau, B., Zambolim, L., Costa, L. C., and Bergamin Filho, A. 2001. Effects of angular leaf spot and rust on yield loss of *Phaseolus vulgaris*. Phytopathology 91:000-000.

THREE FIELD EXPERIMENTS WERE CONDUCTED IN 1997, 1998 AND 1999 TO INVESTIGATE THE EFFECTS OF ANGULAR LEAF SPOT AND RUST, SEPARATELY OR COMBINED, ON HOST GROWTH AND YIELD OF INDIVIDUAL BEAN PLANTS (*PHASEOLUS VULGARIS*). IN EACH EXPERIMENT, THREE TREATMENTS WERE ESTABLISHED BY INOCULATING THE CULTIVAR CARIOCA WITH *PHAEOISARIOPSIS GRISEOLA,* OR *UROMYCES APPENDICULATUS,* OR WITH BOTH PATHOGENS. AN ADDITIONAL CONTROL TREATMENT WAS NOT INOCULATED, BUT SPRAYED WITH A FUNGICIDE. IN THE 1997 AND 1999 EXPERIMENTS, ANGULAR LEAF SPOT REACHED HIGHER DISEASE LEVELS THAN RUST, WHILE IN 1998, RUST WAS MORE SEVERE THAN ANGULAR LEAF SPOT. HOST GROWTH, EXPRESSED AS HEALTHY LEAF AREA DURATION (*HAD*), AND YIELD WERE THE HIGHEST IN 1997 AND LOWEST IN 1998. IN EACH EXPERIMENT, THE TREATMENTS DID NOT DIFFER SIGNIFICANTLY TO THE AREA UNDER LEAF AREA PROGRESS CURVE (*AULAPC*), HEALTHY LEAF AREA DURATION (*HAD*), AND HEALTHY LEAF AREA ABSORPTION (*HAA*). ALL INOCULATED TREATMENTS HAD SIGNIFICANTLY MORE SEVERE DISEASE AND LESS YIELD THAN THE CONTROL TREATMENT. BASED ON THE ANALYSIS OF THE 60 PLANTS IN EACH EXPERIMENT, YIELD WAS NOT RELATED TO THE AREAS UNDER DISEASE PROGRESS CURVE FOR EITHER OR BOTH DISEASES. IN 1997 AND 1999, YIELD WAS RELATED TO *HAD* (R^2 = 0.57 AND 0.43) AND *HAA* (R^2 = 0.60 AND 0.55). BASED ON THE COMBINED ANALYSIS OF ALL 36 PLOTS, ANGULAR LEAF SPOT REDUCED THE LEAF AREA BECAUSE OF DEFOLIATION WHILE RUST DID NOT AFFECT THE LEAF AREA. RUST REDUCED YIELD MORE THAN FOUR TIMES THAT OF ANGULAR LEAF SPOT, ALTHOUGH THE DECREASE IN PHOTOSYNTHESIS TO ANGULAR LEAF SPOT WAS TWICE THAT OF RUST.

ADDITIONAL KEYWORDS: DISEASE INTERACTION, DISEASE-YIELD RELATIONSHIP.

DETAILED INFORMATION ABOUT CROP LOSS IS THE BASIS FOR ANY VALID ECONOMIC ANALYSIS OF DISEASE-MANAGEMENT STRATEGIES. DISEASE CONTROL DOES NOT INCREASE YIELD BUT RATHER REDUCES CROP LOSS; THUS, INFORMATION ON THE RELATIONSHIPS BETWEEN DISEASE AND CONTROL MEASURES, AND DISEASE AND YIELD, ARE INDISPENSABLE TO MAKE DECISIONS IN THE MANAGEMENT OF PLANT DISEASES (35). SIMILARLY, ECONOMICAL CROP PRODUCTION REQUIRES QUANTITATIVE INFORMATION ABOUT HOW DISEASES AFFECT CROP PRODUCTIVITY (17).

THE OCCURRENCE OF TWO OR MORE PATHOGENS ON THE SAME HOST AT THE SAME TIME IS FREQUENT, ESPECIALLY ON TROPICAL CROPS (27,36). USUALLY THE EFFECTS OF A DISEASE COMPLEX ON YIELD ARE ESTIMATED BY ASSUMING THAT EACH DISEASE ACTS INDEPENDENTLY. HOWEVER, THE SIMULTANEOUS OCCURRENCE OF DISEASES CAN LEAD TO COMBINED EFFECTS ON CROP YIELD AND ON THE POPULATION DYNAMICS OF THE PATHOGENS. IN GENERAL, THE INTERACTIONS OF PATHOGENS COMPLICATE THE CONTROL OF DISEASES AND THE PARTITIONING OF THE PRIMARY CAUSES OF LOSS. THESE INTERACTIONS MAY ALTER THE OCCURRENCE AND SPEED OF EPIDEMICS AND CAN HAVE SIGNIFICANT IMPLICATIONS TO ASSESS CROP LOSSES, TO DIAGNOSE THE CAUSES OF THESE LOSSES, TO SELECT APPROPRIATE MANAGEMENT STRATEGIES, AND TO FORECAST, MODEL, AND SIMULATE EPIDEMICS (1,36). NEVERTHELESS ONLY A FEW INVESTIGATIONS OF CROP LOSSES AND POPULATION DYNAMICS OF INTERACTING PATHOGENS OR DISEASES HAVE BEEN PUBLISHED (18,19,20,25,37).

In common beans (*Phaseolus vulgaris* L.), angular leaf spot (caused by *Phaeoisariopsis griseola* (Sacc.) Ferr.) and rust (caused by *Uromyces appendiculatus* (Pers.) Unger) are the most destructive foliar diseases in Brazil. *P. griseola* causes lesions on leaves, pods, branches, and petioles, that result in severe defoliation. The symptoms of *U. appendiculatus* are chlorotic, raised pustules on the surface of leaves, pods, and petioles without causing severe defoliation. In the absence of adequate control measures, reductions in yield to angular leaf spot of 70% in Brazil (6) and 80% in Colombia(30). Some reported losses to rust were 36-45% in Brazil (24) and 22% in Colombia (34).

Reliable estimates of losses caused by these two important foliar diseases are a pre-requisite for the rational development of any bean protection program. Accordingly, the main objective of this study was to determine the effects of these two diseases, separately and combined, on crop growth and on loss in yield of bean plants.

MATERIALS AND METHODS

Field experiments. Three field experiments were conducted at the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil, from October to December of 1997, May to July of 1998, and February to May 1999, with the bean cultivar Carioca. All experiments were set in a randomized complete block design with four treatments and three replications. Each plot (16 m^2) consisted of eight 4-m long rows, spaced 0.5 m apart. There was 1 m between plots. To avoid across contamination among plots with different treatments, only the four central rows of each plot were used for assessment (0.5 m at each end of the row were omitted). Twelve seeds were sown and 10 plants were allowed to grow per linear meter of row. The plots were maintained with the conventional cultural practices used in commercial fields, that included planting, topdressing with fertilizer, insecticide sprays, weeding, and, when necessary, sprinkle irrigation. The treatments were 1) inoculation with *P. griseola*; 2) inoculation with *U. appendiculatus*; 3) inoculation with *P. griseola* + *U. appendiculatus*, and 4) control treatment (not inoculated). These treatments will be referred hereafter as P, U, P+U, and C, respectively. The plants were inoculated, always at nightfall, with *P. griseola* or *U. appendiculatus* with a spore suspension at a concentration of 10^4 spores per ml for each pathogen (2 L of spore suspension per plot). For the treatment P+U, the inoculation with each pathogen was made separately in the same plot. The first inoculation was made when the second trifoliolate leaf had expanded (approximately thirty days after planting), and the second inoculation followed ten days later. The control plots were sprayed with tebuconazole at 0.75 kg ha^{-1} (187.5 g a.i. ha^{-1}) the day before each inoculation.

Crop growth, disease severity, and yield. Crop growth and disease severity were evaluated in the four central rows of each plot. The single-plant approach was adopted (19), in which five plants in each plot were marked with plastic tape, and thus a total of 60 plants per trial were assessed weekly. The plants were marked after the appearance of the first trifoliolate leaf, and individual plants of similar height and vigor were selected.

The leaf area (LA, cm^2) of all leaves on each marked plant was assessed weekly, starting with the appearance of the first trifoliolate leaf. The maximum width of the central leaflet of each leaf (W, cm) was measured with a ruler and LA was estimated for each leaf from the empirical relationship (16): $LA = 2.1371W^{1.9642} - 2.7013$ with $R^2 = 0.95$.

The sum of the leaf areas of individual leaves represented the leaf area per plant (LAP, m^2).

The visual severities of angular leaf spot (X_A) and bean rust (X_R) were assessed simultaneously with the leaf area measurements by use of diagrammatic scales for each disease (13). The average severity (in percentage) of the three leaflets of all leaves on the marked plants was estimated. Both diseases were separately evaluated in all treatments, since natural infections occurred in the field. The total disease severity (X_T) was calculated as the sum of the two individual disease severities of $X_A + X_R$.

The concept of the virtual lesion (3) was adopted and, the virtual disease severities for rust and angular leaf spot were calculated as $X_{\beta R} = 100[1 - (1 - 0.01\ X_R)^{\beta R}]$ and $X_{\beta A} = 100[1 - (1 - 0.01\ X_A)^{\beta A}]$. The β-values applied here were $\beta_R = 2$ for bean rust (2) and $\beta_A = 4$ for angular leaf spot (2). The total virtual disease severity $X_{\beta T}$ was determined by employing the multiple infection transformation (14) for the individual diseases and for the overlap of lesions of the two diseases on the same leaf, that led to $X_{\beta T} = 100\ [1 - (1 - 0.01\ X_R)^{\beta R}\ (1 - 0.01\ X_A)^{\beta A}]$.

Defoliation was observed, but not quantified. The cultivar Carioca has an indeterminate, polybrachiate growth habit, which makes it difficult to identify specific leaves in sequential assessments. Thus, the total numbers, areas, and original positions of fallen leaves were not considered. At each evaluation, the stage of host growth was determined by the descriptive scale of van Schoonhoven and Pastor-Corrales (33).

In the first, second, and third experiment, beans were harvested at 88, 103, and 93 days after planting, respectively. Yield (Y) was determined for each marked plant (g/plant) and per plot (g m^{-2}) by weighing the seeds (with 12% moisture).

Integral variables. The area under disease progress curve ($AUDPC$) and the area under leaf area progress curve ($AULAPC$) for each plant were calculated by trapezoidal integration over the n observation dates. The numbers of observations were 7, 10, and 7, respectively, for the first, second, and third trial. For each disease, the $AUDPC$ was calculated as:

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{X_i + X_{i+1}}{2} \right) (t_{i+1} - t_i)$$

where X was the disease severity (in percentage), which was specified by a subscript R for bean rust, A for angular leaf spot, and T for the total of both disease severities. Similarly, the $AUDPC$ of a specific disease was indicated by a subscript.

Accordingly, $AULAPC$ (m^2 days^{-1}) was calculated using the leaf area per plant (LAP, m^2):

$$AULAPC = \sum_{i=1}^{n-1} \left(\frac{LAP_i + LAP_{i+1}}{2} \right) (t_{i+1} - t_i).$$

Under the assumption of 20 plants per m^2, the leaf area index (LAI) was calculated as $LAI = 20\ LAP$. The integration of LAI led to the leaf area index duration (LAD) which was defined as:

$$LAD = \sum_{i=1}^{n-1} \left(\frac{LAI_i + LAI_{i+1}}{2} \right) (t_{i+1} - t_i).$$

The healthy leaf area index ($HLAI$) was determined as $HLAI = LAI\ (1\text{-}0.01X_T)$. The integration of $HLAI$ over the season resulted in healthy leaf area duration (HAD, days).

The effective leaf area index ($ELAI$) (29) could then be calculated as $ELAI = LAI\ (1 - 0.01\ X_{\beta T})$. The integration over the season resulted in the effective leaf area index duration ($ELAD$). This is identical to the photosynthesizing leaf area index duration (PAD) of (2):

$$ELAD = \sum_{i=1}^{n-1} \left(\frac{ELAI_i + ELAI_{i+1}}{2} \right) (t_{i+1} - t_i).$$

The radiation intercepted RI_i (MJ.m^{-2}) was given by $RI_i = I_i\ [1 - \exp(-k\ LAI_i)]$ in which I_i was the average incident solar radiation (MJ m^{-2}) in the period ($t_{i+1} - t_i$) and k was the extinction coefficient. According to Miglioranza (23), the k-value for the cultivar Carioca is 0.7. The intercepted radiation of the healthy leaf area (HRI) in MJ m^{-2} for each assessment was determined as $HRI = RI\ (1 - X_T)$. The integration over the observation time resulted in the healthy leaf area absorption (HAA), measured in MJ m^{-2}:

$$HAA = \sum_{i=1}^{n-1}\left(\frac{(1-X_i)\left(1-e^{(-kLAI_i)}\right)+(1-X_{i+1})\left(1-e^{(-kLAI_{i+1})}\right)}{2}\right)(t_{i+1}-t_i).$$

The length of the observation period was shorter in the summer (40 days in 1997) and autumn seasons (42 days in 1999) than in the winter season (63 days in 1998). To enable a comparison among experiments, the integral variables were divided by the time ($t_n - t_1$) over which the integration (or summation) was carried out. All standardized variables were marked with *. Thus $AUDPC_R{}^*$ is the standardized area under the rust progress curve and can be interpreted as the mean daily rust severity during the whole season.

Combined analysis. To investigate the effects of both diseases on LAD^* in all 36 plots of the three experiments simultaneously, the following model was used:

$$LAD^* = (D_1\, LAD_1{}^* + D_2\, LAD_2{}^* + D_3\, LAD_3{}^*) \bullet$$
$$(1 - C_R\, AUDPC_R{}^* - C_A\, AUDPC_A{}^* - C_{AR}\, AUDPC_A{}^*\, AUDPC_R{}^*). \qquad (1)$$

In this equation, the d_j are dummy variables (0 or 1) to choose the year. The three parameters LAD^*_j represent the level of potential duration of the leaf area index assigned to each experiment. The coefficients c_R and c_A describe the reduction in leaf area for the individual diseases and c_{AR} for the interaction of both diseases.

UNDER THE ASSUMPTION THAT YIELD (Y) IS DETERMINED BY THE LEAF AREA INDEX DURATION (LAD^*), THE FOLLOWING EQUATION WAS FITTED TO THE DATA OF THE 36 PLOTS:

$$Y = (A_1 + A_2 LAD^*) \bullet (1 - B_R\, AUDPC_R{}^* - B_A\, AUDPC_A{}^* - B_{AR}\, AUDPC_A{}^*\, AUDPC_R{}^*) \quad (2),$$

where the coefficients b_R and b_A represent the effects of the individual diseases on yield, and the interaction term b_{AR} was the combined effect of both diseases.

DATA ANALYSIS. ANALYSIS OF VARIANCE WAS CONDUCTED USING THE SAS SYSTEM AND STATISTICAL DIFFERENCES BETWEEN THE MEANS OF THE TREATMENTS WERE DETERMINED WITH THE TUKEY TEST. LINEAR REGRESSION ANALYSES, PERFORMED BY THE STATISTICA SOFTWARE (STATSOFT, TULSA, OK), WERE USED TO QUANTIFY THE RELATIONSHIPS AMONG VARIABLES; FOR EXAMPLE, BETWEEN YIELD AND HAD^*.

RESULTS

Disease severities, crop growth, and yield in the 1997 experiment. In this experiment, carried out in the summer, the visual angular leaf spot severity on attached leaves was higher than the rust severity (Figs. 1, 2, and 4). The maximum severity value of angular leaf spot in the four treatments was nearly 4%, but only 0.3% for bean rust. In all plots, natural infections of both pathogens occurred. When *P. griseola* was inoculated in combination with *U. appendiculatus* (treatment P+U), $AUDPC_A*$ was not statistically different from the treatment in which *P. griseola* was inoculated alone (treatment P) (Fig. 4). The severity of rust was, in general, very low. Rust reached its highest severity in treatment U (Fig. 1). The progress curves of *HLAI* in the treatments C (control) and U were similar (Fig. 1). The maximum values of *HLAI* were lower for treatments P and P+U compared to the treatments U and C.

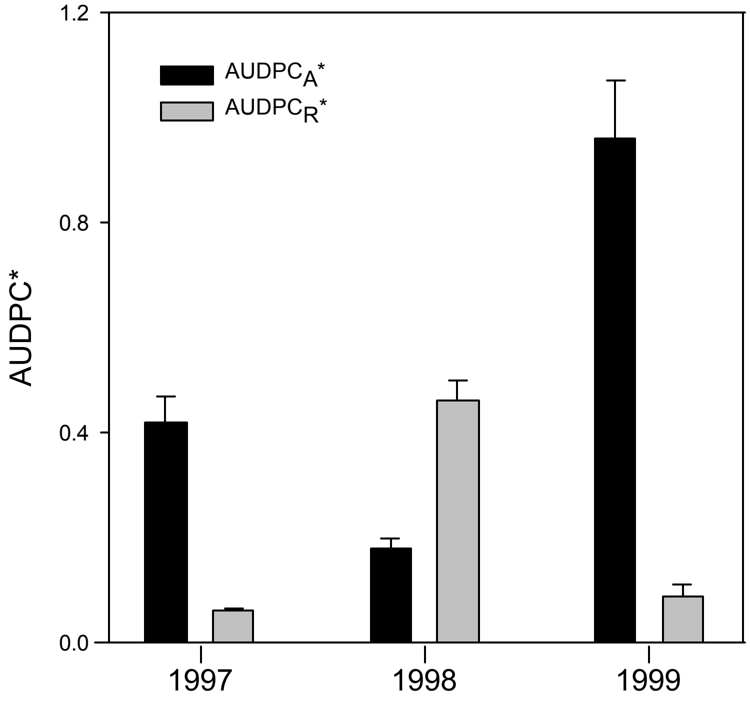
When virtual severity values were considered, the severity values were higher, especially for angular leaf spot (Fig. 3), but the shape of the progress curves and the relations among the treatments were not altered (Figs. 2 and 3). The values of *ELAI* were only slightly smaller than the corresponding *HLAI*-values (Figs. 2 and 3).

Larger areas under disease progress curve occurred for angular leaf spot ($AUDPC_A*$) than for rust ($AUDPC_R*$) (Fig. 1 and 4). There were no significant differences among the treatments for *AULAPC**, *HAD**, and *HAA** ($P > 0.05$). However, all inoculated treatments differed statistically from the control in disease severity and yield (Fig. 4).

Disease severities, crop growth, and yield in the 1998 experiment. In this experiment, carried out in the winter season with lower temperatures, the weather conditions were favorable for bean rust and this resulted in a significantly higher value for $AUDPC_R*$ compared to $AUDPC_A*$ (Figs. 1 and 4). Maximum visual severity of rust was 1.4% (treatment U), while angular leaf spot reached only 0.6% even in the plots inoculated with *P. griseola* (treatments P, P+U) (Fig. 2). Rust severity reached a maximum value of 0.9% in treatment P by natural infections. In general, the length of the epidemics in this season was longer than in the previous experiment, but the maximum values for *HLAI* were lower (Fig. 2). In the inoculated treatments, a similar pattern of the dynamics of *HLAI* (Fig. 2) was observed.

Again, the disease progress curves changed only slightly when the virtual disease severity was analyzed (Fig. 3). The progress curves for *HLAI* and *ELAI* were nearly identical (Figs. 2 and 3).

The differences in $AULAPC^*$, HAD^*, and HAA^* among all treatments (including the control) were not significant ($P > 0.05$), but the disease severity and yield of the inoculated treatments differed from the control. No significant difference in yield was obtained among the three inoculated treatments (Fig. 4).



1.2
0.8
0.4
0.0
AUDPC*
AUDPC$_A$*
AUDPC$_R$*
1997
1998
1999

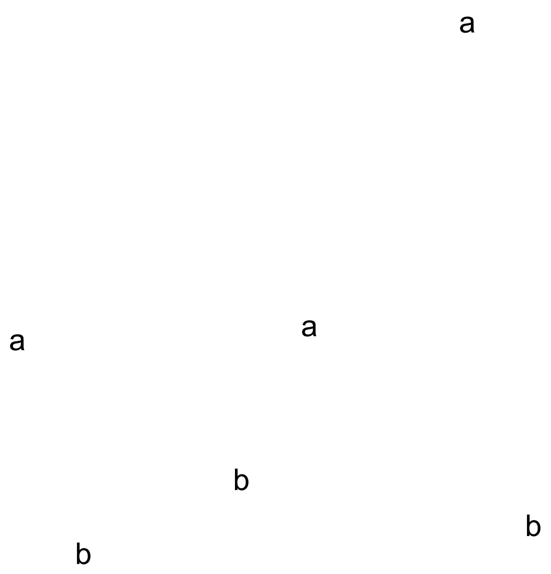
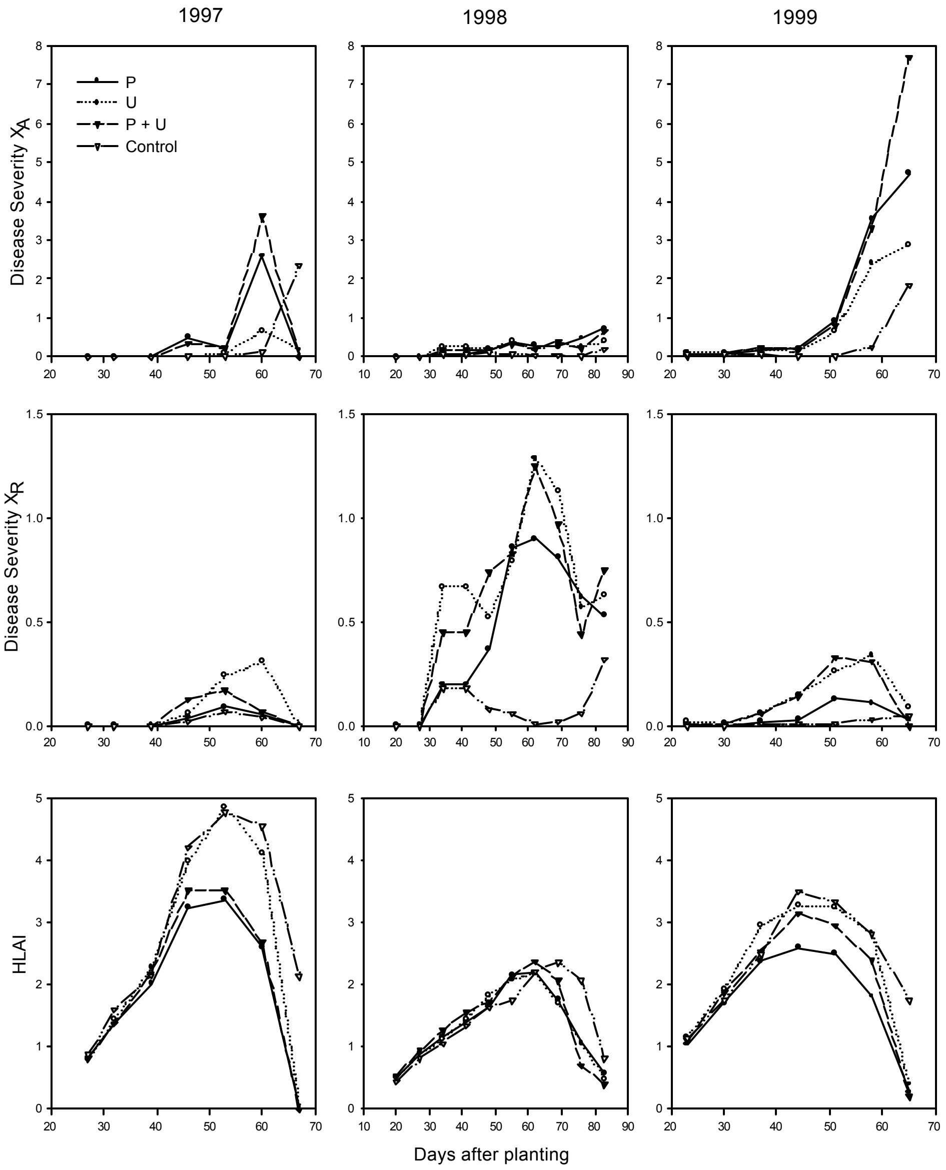


Fig. spot ($AUDPC_A$*) and of rust ($AUDPC_R$*), averaged over all treatments, from the experiments in 1997, 1998, and 1999. Vertical bars represent ± standard error.



ppendiculatus) (X_R), and healthy leaf area index (*HLAI*) versus time (days after planting) in 1997, 1998, and 1999, of the four treatments: P: inoculated *with P. griseola*; U: inoculated with *U. appendiculatus*; P+U: inoculated with *P. griseola* and *U. appendiculatus*; Control: not inoculated, but sprayed with tebuconazole.

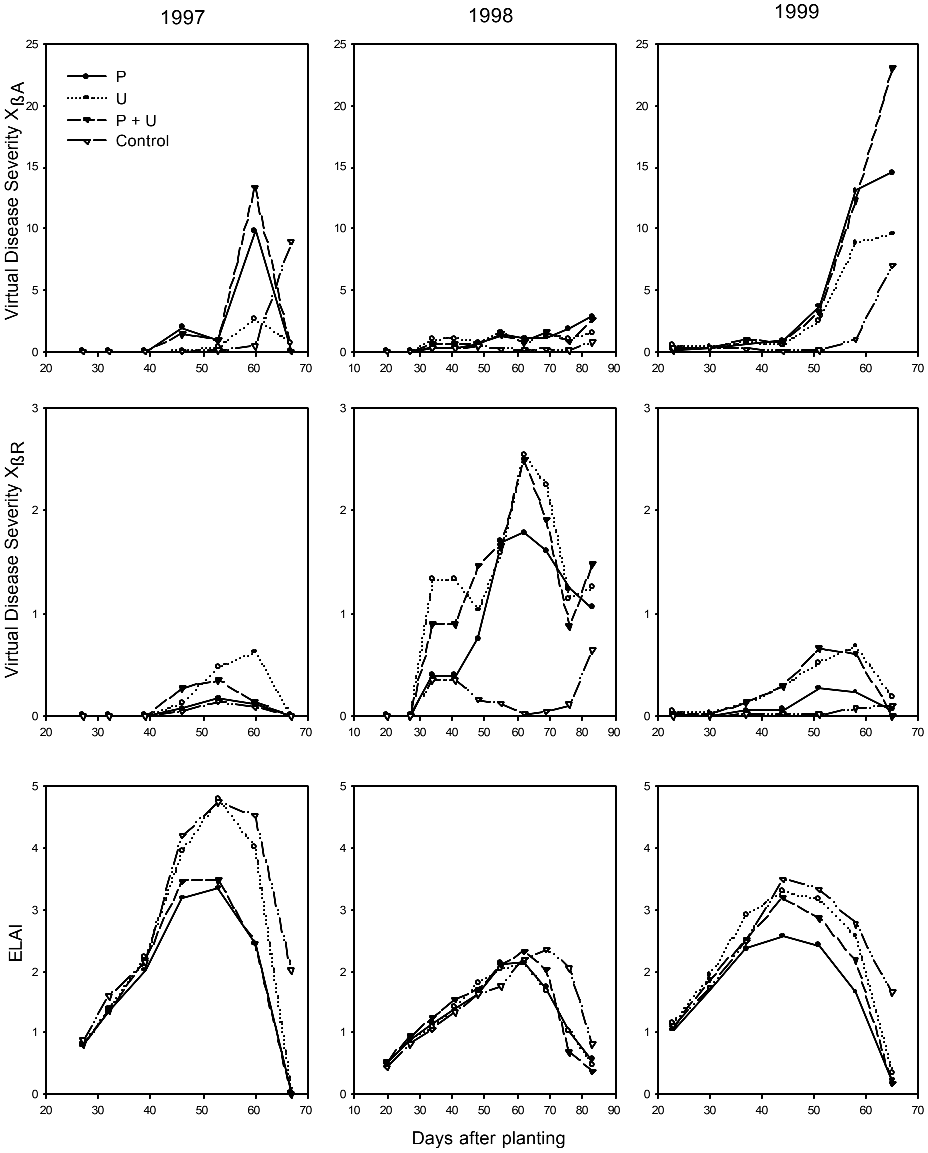

1997
1998
1999
P
U
P + U
Control
Virtual Disease Severity XßA
0
5
10
15
20
25
Virtual Disease Severity XßR
0
1
2
3
ELAI
0
1
2
3
4
5
20
30
40
50
60
70
10
20
30
40
50
60
70
80
90
Days after planting

Fig. 3. Percent of virtual severity of angular leaf spot (*P. griseola*) ($X_{\beta A}$) and rust (*U. appendiculatus*) ($X_{\beta R}$), and effective leaf area index (*ELAI*) versus time (days after planting) in 1997, 1998, and 1999, for the four treatments (see. Fig. 2).

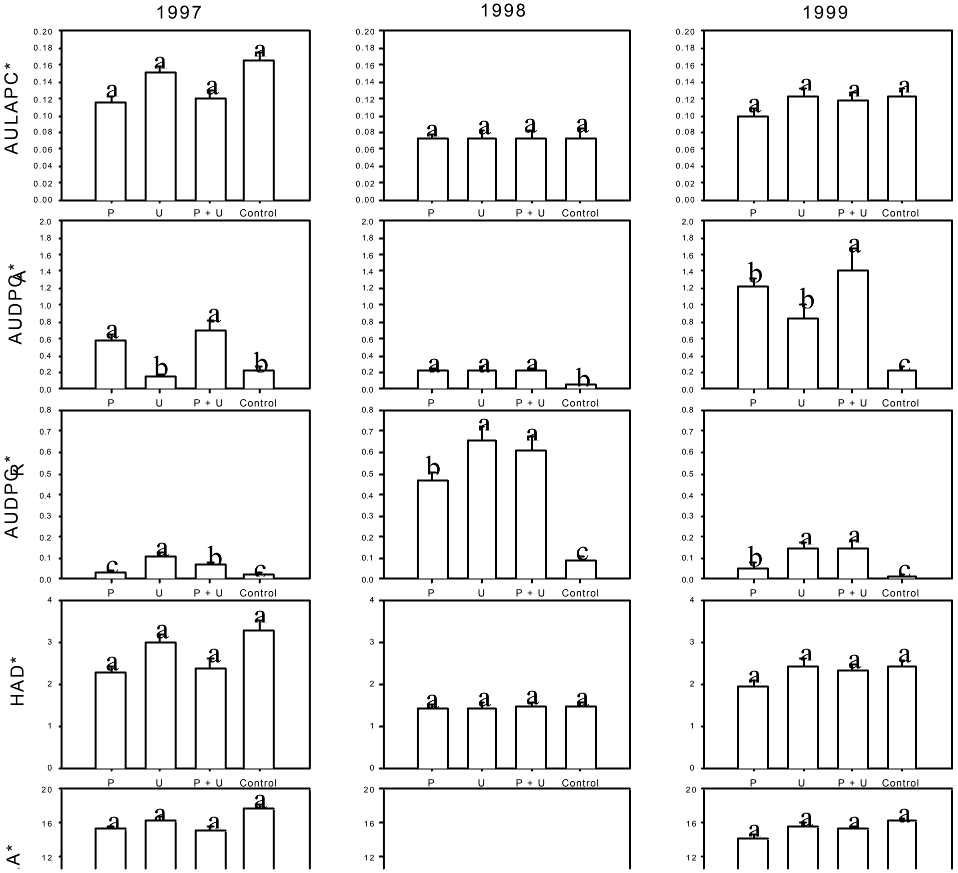
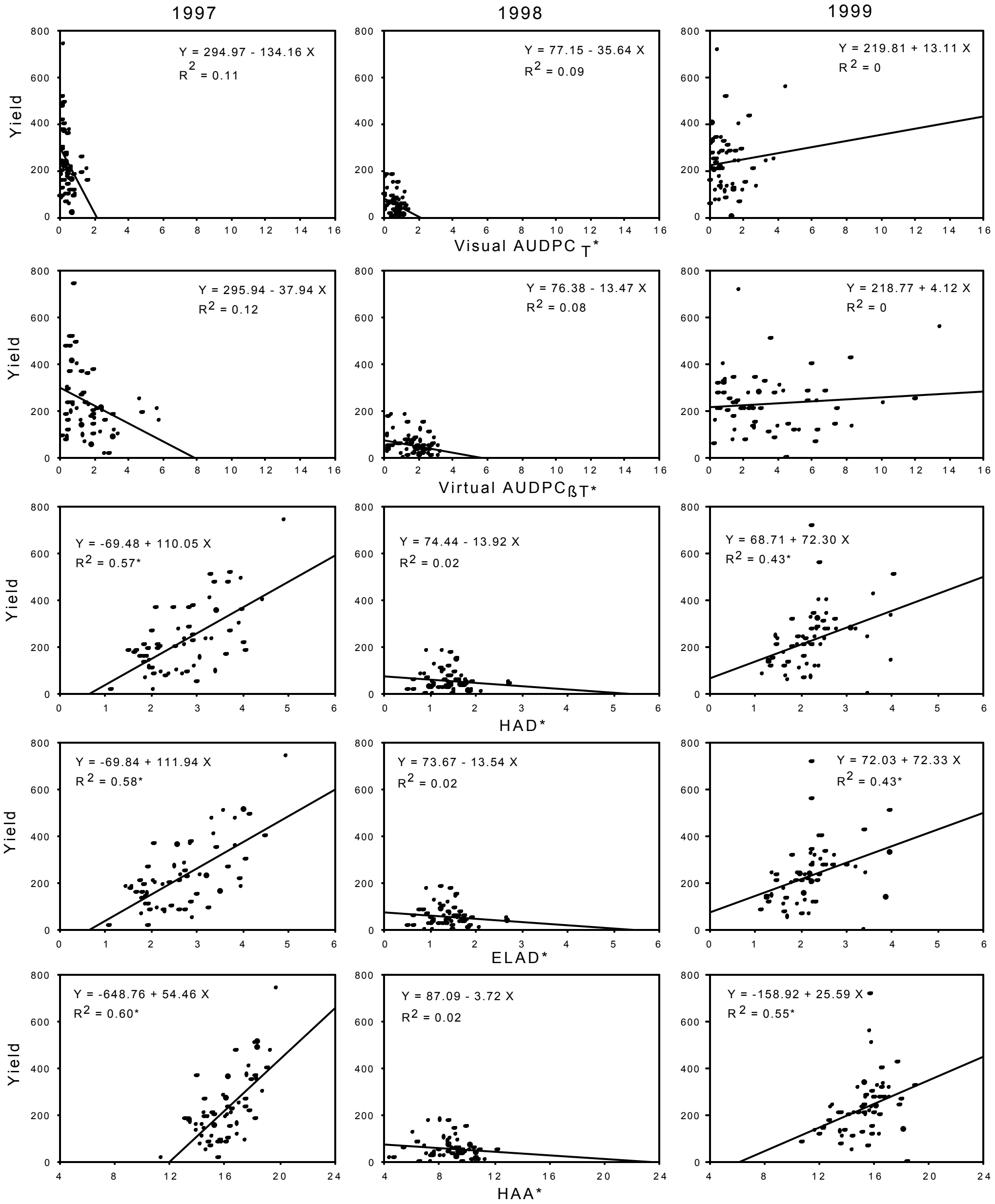


Fig. 4. Area under leaf area progress curve ($AULAPC^*$), area under visual disease progress curve of angular leaf spot (*P. griseola*) ($AUDPC_A^*$) and of rust (*U. appendiculatus*) ($AUDPC_R^*$), healthy leaf area duration (HAD^*), healthy leaf area absorption (HAA^*), and yield in 1997, 1998, and 1999, for the four treatments (see Fig. 2). Vertical bars represent ± standard error and * represents standardized variable. The means for each bar followed by the same letter are not significantly different ($P < 0.05$).



$UDPC_T$*), area under total virtual disease progress curves ($AUDPC_{\beta T}*$), healthy leaf area duration ($HAD*$), effective leaf area duration ($ELAD*$), and healthy leaf area absorption ($HAA*$) for individual bean plants (60 plants per trial) in 1997, 1998, and 1999. All integral variables were standardized (marked with *).

Disease severities, crop growth, and yield in the 1999 experiment. This experiment was conducted in the autumn with weather conditions favorable especially for angular leaf spot. Like in the 1997 experiment, angular leaf spot severity was higher than rust severity (Figs. 1, 2, and 4). For all treatments, a similar progress of *HLAI* was observed, but the leaf area remained at a high level for a longer time in the control treatment compared to the inoculated treatments (Fig. 2). The maximum level of *HLAI* in the control treatment was less than 3.5 and substantially lower than in the 1997 experiment (Fig. 2).

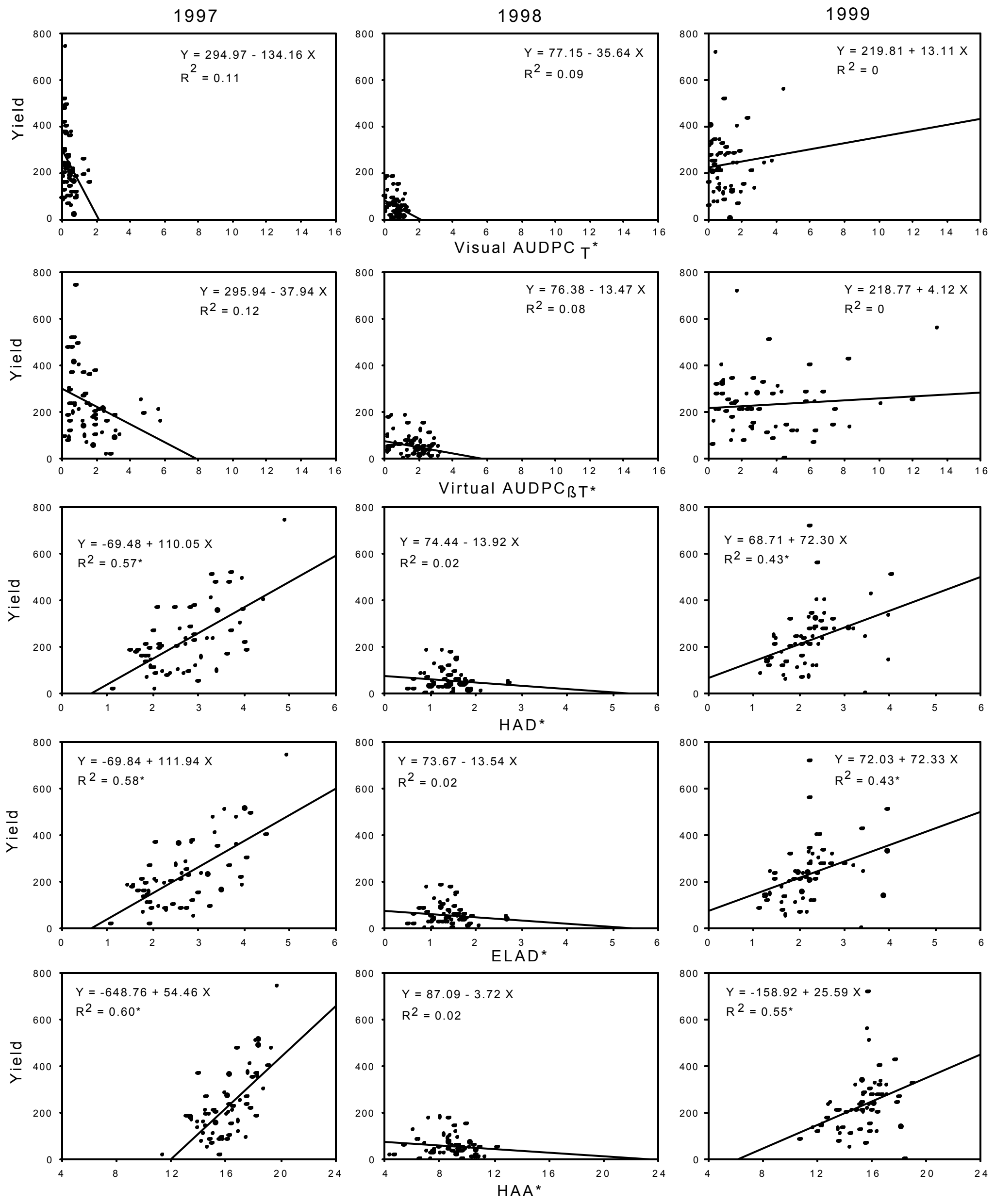
Values of virtual disease severities were higher than visual severities, especially for angular leaf spot (Fig. 3), but the patterns of disease progression did not change (Figs. 2 and 3). Also, the *ELAI* curves were similar to the *HLAI* curves (Figs. 2 and 3).

There were no significant differences among all treatments for *AULAPC**, *HAD**, and *HAA** ($P > 0.05$) (Fig. 4). However, disease severities and yields were statistically different in the three inoculated treatments compared to the control (Fig. 4).

Comparison of the three experiments. The three experiments conducted in three different seasons resulted in different levels of the two diseases. When the standardized areas under disease progress curves were considered, levels for both diseases were low in 1997, although angular leaf spot was more prevalent (Fig. 1). In 1998, bean rust predominated and reached the highest level of all three experiments. The conditions in 1999 were more favorable for angular leaf spot than for rust and this led to the highest level of angular leaf spot in all experiments (Fig. 1).

In 1997 and 1999, the conditions for the growth of bean plants were more favorable than in 1998. This can be seen in the lower values of *HAD** and *HAA** in the 1988 experiment. Also, the yield in the winter season in 1998 was lower than in 1997 (summer season) and in 1999 (autumn season).

Relationships between *AUDPC, *HAD**, *ELAD**, *HAA**, and yield.** The relationships between yield and the integral variables *AUDPC**, *HAD**, *ELAD**, and *HAA** for all marked plants were separately investigated in the three experiments (Fig. 5). The $AUDPC_T$* of the total visual disease was not related to yield (Fig. 5). With $AUDPC_{\beta T}$* used for the virtual disease, the relationships were not improved. In 1997 and 1999, yield increased with the increased *HAD** ($R^2 = 0.57$ and $R^2 = 0.43$) or *ELAD** ($R^2 = 0.58$ and R^2



1UDPC$_T$*), area under total virtual disease progress

curves ($AUDPC_{\beta T}*$), healthy leaf area duration ($HAD*$), effective leaf area duration ($ELAD*$), and healthy leaf area absorption ($HAA*$) for individual bean plants (60 plants per trial) in 1997,
1998, and 1999. All integral variables were standardized (marked with *).

= 0.43) (Fig. 5). This positive relationship was even clearer when $HAD*$ or $ELAD*$ was replaced by $HAA*$ (R^2 = 0.60 and R^2 = 0.55). In the 1998 experiment with low yield, the yield was not related significantly to $HAD*$, $ELAD*$, or $HAA*$.

When the plant data of the three years were combined, no significant relationship between $AUDPC_T*$ and $AUDPC_{\beta T}*$ existed, while $HAD*$, $ELAD*$, and $HAA*$ were significantly related to yield with R^2 values between 0.35 and 0.53. However, the relation between $HAA*$ and yield was not linear for the combined data (data not shown).

COMBINED ANALYSIS ON A PLOT BASIS. AS THE VARIATION IN ALL VARIABLES WAS QUITE HIGH ON A PLANT BASIS, ADDITIONAL ANALYSES WERE CARRIED OUT ON A PLOT BASIS WITH THE 36 PLOTS IN ALL EXPERIMENTS TOGETHER. FOR THIS ANALYSIS, THE DIFFERENT LEVELS OF $LAD*$ AND $HAD*$ IN THE THREE EXPERIMENTS (FIG. 4) WERE TAKEN INTO CONSIDERATION. TO ASCERTAIN THE EFFECTS OF BOTH DISEASES ON THE ACTUAL $LAD*$, EQUATION 1 WAS FITTED TO THE COMBINED DATA ON A PLOT BASIS. THE ESTIMATED COEFFICIENT C_R WAS NOT DIFFERENT FROM ZERO THAT MEANT THAT BEAN RUST HAD NO EFFECT ON THE LEAF AREA, AT LEAST NOT AT THE LOW DISEASE LEVEL OBSERVED. AFTER THE TERM WHICH CONTAINED C_R WAS REMOVED FROM EQUATION 1, THE EQUATION WAS FITTED AGAIN TO THE COMBINED DATA THAT RESULTED IN AN EQUATION WITH COEFFICIENTS SIGNIFICANTLY DIFFERENT FROM ZERO THAT EXPLAINED 68.3% OF THE VARIATION IN $LAD*$. THE ESTIMATED LEVELS FOR LAD_j* IN THE THREE YEARS WERE 2.92 (± 0.16), 1.37 (± 0.12), AND 2.65 (± 0.20), THAT CORRELATED WITH THE DIFFERENT FAVORABILITIES OF THE SEASONS FOR BEAN GROWTH. ANGULAR LEAF SPOT REDUCED $LAD*$ ($C_A = 0.28 \pm 0.075$), WHILE THE INTERACTION COEFFICIENT $C_{AR} = -1.12 \pm 0.36$ LED TO AN INCREASE IN LAD*. WHEN ALL DISEASE EFFECTS WERE REMOVED FROM

EQUATION 1, THE RESULTING EQUATION EXPLAINED ONLY 56.5% OF THE VARIATION IN *LAD**. THUS *LAD** WAS MAINLY DETERMINED BY THE CONDITIONS OF THE SEASONS. HOWEVER, ANGULAR LEAF SPOT HAD A CLEAR REDUCING EFFECT ON *LAD**, WHILE RUST HAD NO INFLUENCE ON THE LEAF AREA.

When model 2 was fitted to the yield data on a plot basis, the interaction term b_{AR} was not significantly different from zero. After this term was removed from the equation, all estimated coefficients were significantly different from zero: a_1 = -98.03±37.73, a_2 = 130.57 ± 16.04, b_A = 0.1917 ± 0.067, and b_R = 0.9177 ± 0.2598. An $AUDPC_R$* value of 0.2, i.e. a mean rust severity of 0.2% over the whole season, reduced yield by around 18% while the same disease level for angular leaf spot caused losses of less than 4%. This model explained 79% of the variation of yield. If yield was regressed only with *LAD** and the disease terms were neglected, the estimated parameters were a_1 = -113.69 ± 32.37 and a_2 = 118.73 ± 14.51, and the explained variation in yield was only 66.31%. Thus *LAD** was the most important factor for the formation of yield and this term explained 66% of the variation in yield, but the two diseases explained an additional 13% of the variation in yield.

DISCUSSION

To ascertain the effect of a disease on a crop, single-point, multiple-point, and integral models have been used to describe the relationship between disease severity and crop yield. Single-point and multiple-point models relate yield to disease severity at a single growth stage of the crop or at several growth stages, respectively. Integral models correlate yield with the area under the disease progress curve (*AUDPC*), calculated over the whole observation period. As the duration of the three epidemics varied in our experiments, a comparison of the integral variables was only possible after the standardization of the values by dividing with the length of the observation time. For *AUDPC*, this standardization resulted in the mean disease severity over the whole season. These full-season disease averages were used in this paper rather than weighting disease by host phenology. This approach was chosen because no knowledge was available about the vulnerability of bean plants to disease-induced yield loss in particular growth stages. If a critical growth stage could be identified, a single-point model could be constructed which would overcome the problem with the standardized *AUDPC* values. Moreover, if the effects of growth stages over the whole season are known for both diseases, it would be possible to weight the disease values by a function of the growth stages which may lead to stronger relationships to yield. To deal with angular leaf spot on beans, Bergamin Filho et al. (4) developed models to predict yield dependent on the growth stages.

In the three field experiments conducted in different seasons, a significant difference between the disease levels of angular leaf spot and rust, expressed as *AUDPC**, was observed. The experiments in the summer (1997) and autumn (1999) season had higher disease levels of angular leaf spot compared to rust, while in the winter season (1999) rust was the dominating disease. These observations agree with Coelho (9). The winter season had also a marked effect on bean growth which is shown in the lower values for *HAD** and *HAA** as well as for yield in the 1998 experiment compared to the two other years (see control treatment in Fig. 4). Within experiments, no significant differences among the treatments for the variables *AULAPC**, *HAD**, and *HAA** existed, although the yield in the control plots was significantly higher in all years, but significantly different from year to year. Thus, the base yield of the disease-free crop varied which is one of the reasons that hampers the transportability of disease-crop-loss models to other seasons and locations (4).

In all three trials, no relationships between *AUDPC** and yield were found (Fig. 5), which was also supported by other experiments (4,5,7,16). Usually, the absence of a relationship between *AUDPC* and yield is more common when data from different seasons are compared (11,12). *HAD**, *ELAD**, or *HAA** were closely related to yield, which is in accordance with several other investigations (4,26,28). Similar to our findings, in other experiments with bean diseases (4,8,16,31), *HAD** or *HAA** and yield were linearly related.

Visual lesions of both diseases were assessed, but also the virtual lesions (3) were considered. The relation between virtual and visual lesion, given by the parameter β, is a way to describe the decrease in leaf photosynthesis due to a disease. The β-values applied in this study were $\beta_R = 2$ for bean rust (2) and $\beta_A = 4$ for angular leaf spot (2). Both values were determined by the reduction of photosynthetic efficiency. The resulting *ELAD** (or *PAD** sensu Bassanezi et al. (2)) were slightly more related to yield than *HAD**, which is in accordance with previous results (2).

For our experiments, the single plant approach was adopted (19). Since individual marked plants were used, the variation in leaf area and yield was high among plants although the levels of diseases were rather low. On a plant basis, the natural variability in yield was higher than the effects of the diseases: a phenomenon that usually occurs in experiments with the single plant approach and low disease levels (15). To remove part of the variation, the analysis was carried out on a plot basis (five plants).

From our analyses, we found the different ways in which the two diseases reduced yield. As shown with equation 1, the effect of angular leaf spot was mainly due to defoliation that led to a reduction of the total leaf area. It is well known (8,10,31,32) that angular leaf spot causes defoliation of diseased leaves. According to our analysis, the reduction in LAD^* was related to the observed disease severities of the remaining plant area. This is in contradiction to the results of Bergamin Filho et al. (4) who reported that plants with a higher severity of angular leaf spot had larger leaf areas in four out of five experiments. It can be assumed that defoliation depends on the disease severity of angular leaf spot of the affected leaves. As defoliation was not determined in our study, this assumption cannot be validated. A second effect of angular leaf spot was detected with equation 2. This was the reduction in leaf photosynthesis, expressed in the β-value of about 4.

Bean rust did not cause defoliation (insignificant coefficient c_R in equation 1), but rust had a much stronger effect on loss in yield compared to angular leaf spot (b_R = 0.92 vs. b_A = 0.19), in equation 2. When the concept of the virtual lesion is considered, this result was rather unexpected, as both diseases reduced leaf photosynthesis, but the β-value of 4 for angular leaf spot (2) was twice the β-value of 2 assumed for rust (2). In the measurement of photosynthesis, Lopes & Berger (22) determined a β-value for rust near to one, which meant that the effect of this pathogen on the remaining green leaf area was minimal. In a previous study, Schuld (29) estimated β_R-values from the correlation between $ELAD$ and yield, that ranged from 4 to 13 in different experiments. This was an indication that the effect of rust on yield was stronger than one would expect from the reduction in photosynthesis. The severe effect of bean rust on yield may be connected with the biotrophic nature of this pathogen. The rust pustules act as sinks for carbohydrates (21,38) which then could not be allocated for production of yield.

With equation 1, no interaction of both diseases with respect to their combined effect on host growth could be shown. Contrarily, a combined effect of both diseases on yield was proven by the significant interaction term in equation 2. There seems to be an antagonistic effect of the diseases, as the sign of the interaction coefficient was negative.

Further research on yield loss caused by disease complexes is needed. Disease components must be studied separately from any interaction with other diseases, insects, weeds, and unusual physical conditions. Once a baseline for potential yield loss is determined for each pathogen, the effect of two or more pathogens on a host can be determined under different environmental conditions. In the future, such research will provide realistic information for the grower to decide what control measures to apply.

ACKNOWLEDGMENTS

This research was partially supported by the European Commission (project ERBIC18CT96-0037), FAPEMIG, and FINEP. First author was supported by CAPES. We thank R. D. Berger (University of Florida) for the review of this manuscript and suggestions for its improvement.

LITERATURE CITED

1. Bassanezi, R. B., Amorim, L., Bergamin Filho, A., 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 Pathol. 47:289-298.
2. Bassanezi, R. B., Amorim, L., Bergamin Filho, A., Hau, B. and Berger, R. D. 2001. Accounting for photosynthetic efficiency of bean leaves with rust, angular leaf spot and anthracnose to assess crop damage. Plant Pathol. 50: (in press).
3. 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.
4. Bergamin Filho, A., Carneiro, S. M. T. P. G., Godoy, C. V., Amorim, L., Berger, R. D., and Hau, B. 1997. Angular leaf spot of *Phaseolus* beans: Relationships between disease, healthy leaf area, and yield. Phytopathology 87:506-515.
5. Bissonnette, S. M., D´Arcy, C. J., and Pedersen, W. L. 1994. Yield loss in two spring oat cultivars due to *Puccinia coronata* f. sp. *avenae* in the presence or absence of barley dwarf virus. Phytopathology 84:363-371.

6. Brenes, B. M., Chaves, G. M., and Zambolim, L. 1983. Estimativas de perdas no rendimento do feijoeiro comum (*Phaseolus vulgaris*) causadas pela mancha angular (*Isariopsis griseola* Sacc.). Fitopatol. Bras. 8:599.
7. Bryson, R. J., Sylvester-Bradley, R., Scott, R. K., and Paveley, N. D. 1995. Reconciling the effects of yellow rust on yield of winter wheat through measurements of green leaf area and radiation interception. Pages 9-18 in: Physiological Responses of Plants to Pathogens. R. D. Walters et al. eds. Association of Applied Biologists, Wellesbourne, Warwick, England.
8. Carneiro, S. M. T. P. G., Amorim, L., and Bergamin Filho, A. 1997. Avaliação de dano provocado pela mancha angular em feijoeiro: relação entre severidade, área foliar e componentes de produção. Fitopatol. Bras. 22:427-431.
9. Coelho, R. R. 1999. Caracterização das condições climáticas que favorecem o desenvolvimento da mancha angular e ferrugem do feijoeiro. MS thesis, Universidade Federal de Viçosa, Viçosa, MG, Brasil.
10. Ferraz, S. 1980. La mancha foliar angular. Pages 55-64 in: Problemas de producción del fríjol: enfermedades, insectos, limitaciones edáficas e climáticas de *Phaseolus vulgaris*. H. P. Schwartz and G. E. Gálvez, eds. CIAT, Cali, Colombia.
11. Gaunt, R. E. 1995. The relationship between plant disease severity and yield. Annu. Rev. Phytopathol. 33:119-144.
12. Gaunt, R. E., and Bryson, R. J. 1995. Plant and crop yield potential and response to disease. Pages 1-7 in: Physiological Responses of Plants to Pathogens. R. D. WALTERS et al., eds. Association of Applied Biologists, Wellesbourne, Warwick, England.
13. Godoy, C. V., Carneiro, S. M. T. P. G., Iamauti, M. T., Dalla Pria, M., Amorim, L., Berger, R. D., and Bergamin Filho, A. 1997. Diagrammatic scales for bean diseases: development and validation. Z. Pflanzenkr. Pflanzenschutz 104: 336-345.
14. Gregory, P. H. 1948. The multiple-infection transformation. Ann. Appl. Biol. 35:412-417.
15. Hamelink, J., Hau, B., and Kranz, J. 1988. Untersuchungen zur Entwicklung von Befalls/Verlust-Relationen bei Schaderreger-Komplexen an Einzelpflanzen des Maises. Z. Pflanzenkr. Pflanzenschutz 95:258-269.
16. Iamauti, M. T. 1995. Avaliação dos danos causados por *Uromyces appendiculatus* no feijoeiro. Ph.D. Dissertation. Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, SP, Brasil.

17. Johnson, K. B. 1990. Assessing multiple pest population and their effects on crop yield. Pages 203-213 in: Crop Loss Assessment in Rice. P. S. Teng, ed. International Rice Research Institute, Manila, Philippines.
18. Johnson, K. B., Teng, P. S., and Radcliffe, E. B. 1987. Analysis of potato foliage losses caused by interacting infestations of early blight, Verticillium wilt, and potato leafhopper, and the relationship to yield. Z. Pflanzenkr. Pflanzenschutz 94:22-23.
19. Kranz, J., and Jörg, E. 1989. The synecological approach in plant disease epidemiology. Rev. Trop. Plant Pathol. 6:27-38.
20. Latch, G. C. M., and Potter, L. R. 1977. Interaction between crown rust (*Puccinia coronata*) and two viruses of ryegrass. Ann. Appl. Biol. 87:139-145.
21. Livne, A., and Daly, J. M. 1966. Translocation in healthy and rust affected beans. Phytopathology 56:170-175.
22. Lopes, D. B., and Berger, R. D. 2001. The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. Phytopathology 91:212-220.
23. Miglioranza, E. 1992. Modelo matemático-fisiológico para simular o crescimento e a produtividade da cultura do feijão (*Phaseolus vulgaris*). Ph.D. Dissertation, Universidade Federal de Viçosa, Viçosa, MG, Brasil.
24. Nasser, L. C. B. 1976. Efeito da ferrugem em diferentes estádios de desenvolvimento do feijoeiro e dispersão de esporos de *Uromyces phaseoli* var. *typica* Arth. MS Thesis, Universidade Federal de Viçosa, Viçosa, MG, Brasil.
25. Pinnschmidt, H. 1991. Effects of multiple pests on crop growth and yield. Philippine Phytopathol. 27:1-11.
26. Pinnschmidt, H., Teng, P. S., and Young, 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.
27. Savary, S., and Zadoks, J. C. 1991. Méthodes pour l'étude du domage dû à un ensemble de contraintes phytosanitaires. Le cas de l'arachide en Côte d'Ivoire. Pages 256-80 in: Approaches de la Pathologie des Cultures Tropicales. Example de l'Arachide en Afrique de l'Ouest. S. Savary, ed. Èditions de l'Orstom, Paris, France.
28. Savary, S., and Zadoks, J. C. 1992. Analysis of crop loss in the multiple pathosystem groundnut-rust-late leaf spot. I. Six experiments. Crop Prot. 11:99-109.

29. Schuld, P. 1996. Auswirkungen des Befalls durch drei Blattpathogene (*Uromyces appendiculatus, Phaeoisariopsis griseola* und *Colletotrichum lindemuthianum*) auf das Wachstum und den Ertrag von Phaseolus-Bohnen. Ph.D. thesis, Universität Hannover.
30. Schwartz, H. F., Correa, V. F., Pineda, D. P. A., Otoya, M. M., and Katherman, M. J. 1981. Dry bean yield losses caused by Ascochyta, angular, and white leaf spots in Colombia. Plant Dis. 65:494-496.
31. Silva, M. B., Vale, F. X. R. do, Zambolim, L., and Hau, B. 1998. Efeitos da ferrugem, da antracnose e da mancha angular na área foliar de plantas de feijoeiro em condições de campo. Fitopatol. Bras. 23:442-447.
32. Vale, F. X. R. do, Costa, H., and Zambolim, L. 1997. Feijão comum. Controle de doenças. Doenças da parte aérea causada por fungos. Pages 335-373 in: Controle de doenças de plantas. Grandes Culturas, v.1. F. X. R. do Vale and L. Zambolim, eds. Ministério da Agricultura e do Abastecimento, Brasília, Brasil.
33. Van Schoonhoven, A., and Pastor-Corrales, M. A. 1987. Sistema Estándar para la evaluación de germoplasma de frijol. CIAT, Cali, Colombia.
34. Vargas, E. 1980. La roya. Pages 17-36 in: Problemas de producción del fríjol: enfermedades, insectos, limitaciones edáficas e climáticas de *Phaseolus vulgaris*. H. P. Schwartz and G. E. Gálvez, eds. CIAT, Cali, Colombia.
35. Waibel, H. 1990. Requirements for an economic interpretation of crop losses. Pages 313-320 in: Crop Loss Assessment in Rice. P. S. Teng, ed. International Rice Research Institute, Manila, Philippines.
36. 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.H.S. Wood and G. J. Jellis, eds. Blackwell Scientific Publications. Oxford, London.
37. Weber, G. E., Gülec, S., and Kranz, J. 1994. Interactions between *Erysiphe graminis* and *Septoria nodorum* on wheat. Plant Pathol. 45:158-63.
38. Zaki, A. I., and Durbin, R. D. 1965. The effect of bean rust on the translocation of photosynthetic products from diseased leaves. Phytopathology 55:528-529.

CHAPTER 2

Effects of angular leaf spot and rust on leaf gas exchange and yield of common bean (*Phaseolus vulgaris*)

W.C. JESUS JUNIOR[*], F.X.R. VALE[*,*****], C.A. MARTINEZ[**], R.R. COELHO[*], L.C. COSTA[***], B. HAU[****] and L. ZAMBOLIM*

*Universidade Federal de Viçosa, Departamento de Fitopatologia, 36571-000 Viçosa, MG, Brazil**
*Universidade Federal de Viçosa, Departamento de Biologia Vegetal, 36571-000 Viçosa, MG, Brazil l***.*
*Universidade Federal de Viçosa, Departamento de Engenharia Agrícola, 36571-000 Viçosa, MG, Brazil ***.*
*Institut fur Pflanzenkrankheiten und Pflanzenschutz, Universitat Hannover, 30419 Hannover, Germany*****

*****Author for correspondence; fax: 55 31 3899-2240, e-mail: dovale@mail.ufv.br

Paper will be submitted to Photosynthetica.

Abstract

The effects of angular leaf spot (caused by *Phaeoisariopsis griseola*) and rust (caused by *Uromyces appendiculatus*), alone and in combination, on leaf gas exchange and yield were studied in common bean (*Phaseolus vulgaris* cv. Carioca) plants. Gas exchange was measured on 37, 44, 51 and 58 days after planting using a portable photosynthesis system. The inoculation of plants with *P. griseola* (P), *U. appendiculatus* (U), and the combination of both pathogens (P+U) caused a significant reduction in the photosynthetic rate (A) and yield. The decrease in stomatal conductance, photosynthesis, and yield was higher in the treatments inoculated with *P. griseola* (P and P+U) than in the treatment U. In treatment U, yield was stronger reduced than gas exchange parameters. In treatment P+U, the net photosynthetic rate A was reduced by 23%, but yield by 32%. In the other treatments, A and, consequently, yield were lowered to a less degree. However all inoculated treatments differed statistically from the control in disease severity and yield. There were no significant differences among treatments concerning area under leaf area progress curve (AULAPC), healthy leaf area duration (HAD) and healthy leaf area absorption (HAA). These results suggest that the interactive effects of *P. griseola* and *U. appendiculatus* on yield could be explained in part by the decrease in stomatal conductance and in the photosynthetic rate of diseased bean leaves.

Additional key words: angular leaf spot; *Phaeoisariopsis griseola*; photosynthesis; rust; stomatal conductance; *Uromyces appendiculatus.*

Abbreviations: A = net photosynthetic rate; A/E = photosynthetic water use efficiency; AULAPC = area under leaf area progress curve; E = transpiration rate; g_s = stomatal conductance; HAA = healthy leaf area index absorption; HAD = healthy leaf area index duration; HLAI = healthy leaf area index; P = treatment inoculated with *Phaeoisariopsis griseola*; P+U = treatment inoculated with *Phaeoisariopsis griseola* and *Uromyces appendiculatus;* R = treatment inoculated with *Uromyces appendiculatus.*

Introduction

Angular leaf spot (caused by the fungus *Phaeoisariopsis griseola* (Sacc. Ferr.)) and rust (caused by the fungus *Uromyces appendiculatus* (Pers. Unger)) are the most destructive foliar diseases in common beans (*Phaseolus vulgaris* L.) in Brazil and other tropical countries. Under inadequate methods of control, yield reductions of 70% to 80% and 22% to 45% due to angular leaf spot and rust, respectively, have been reported (Nasser 1976, Brenes *et al.* 1983, Schwartz *et al.* 1981).

Plants infected with fungi, bacteria, or virus usually exhibit a reduced photosynthetic rate (Lucas 1998). Abnormalities in form and function of chloroplasts of diseased tissues are commonly associated with declines in photosynthetic phosphorilation (Berghaus and Reisener 1985), photochemical reactions (Mathre 1968), and carbon dioxide assimilation (Lopes and Berger 2001). These changes are frequently associated with reductions in chlorophyll content (Berghaus and Reisener 1985), decrease in mesophyll conductance, reduced activity of ribulose-1,5-bisphosphate-carboxylase (Gordon and Duniway 1982) or increase in leaf carbohydrate concentration (Livne and Daly 1966). Recently, Moore *et al.* (1998, 1999) and Smeekens (2000) showed that excessive carbohydrate accumulation in leaves might inhibit photosynthesis. It suggests that reduction in photosynthesis in plants infected by rust, as observed by Livne and Daly (1966), may be explained by increase in leaf carbohydrate concentration.

Because the pattern of reduction in photosynthesis is apparently related to the trophic relationship between pathogens and plants (Shtienberg 1992), different effects of angular leaf spot caused by the facultative fungus *P. griseola* (hemibiotrophic and necrotrophic pathogen) and rust caused by the obligate fungus *U. appendiculatus* (biotrophic pathogen) can be expected. As biotrophic pathogens need the vitality of the plant for survival, they cause less effect on the host physiology during colonization and reproduction than hemibiotrophic pathogens, which are more aggressive and cause more drastic and destructive effects on host physiological processes. Plants affected by these pathogens can show considerable differences in terms of leaf growth and photosynthesis (Scholes 1992).

Different modes of action of bean foliar pathogens during the infection and colonization of the host were observed. Related to angular leaf spot, Bassanezi (1995), Bassanezi *et al.* (2000) and Stangarlin (1999) observed a reduction in chlorophyll content correlated with disease severity. The reduction on carbon assimilation rate was probably greater due to the reduction of Rubisco activity in the spot regions. Regarding rust, the main physiological effects observed during infection of bean plants were a loss of chlorophyll in the pustule area, a more than proportional reduction of the photosynthetic rate compared to the reduction of green leaf area, an increase in respiration, changes in the patterns of translocation of photosynthates and an increase in invertase activity (Bassanezi 1995, Bassanezi *et al.* 2000, Daly *et al.* 1961, Livne 1964, Livne and Daly 1966, Lopes and Berger 2001, Moll *et al.* 1995, Wagner and Boyle 1995). Recently, Lopes and Berger (2001) observed that stomatal conductance and photosynthetic capacity of leaves decreased with increasing severity of rust. Losses of chlorophyll from leaves affected with rust were proportional to the visually chlorotic tissue.

The objective of this study was to determine the effects of angular leaf spot and rust, occurring singly or simultaneously on the same leaf, on the gas exchange processes, crop growth and yield of common bean.

Materials and methods

Plant material and growth conditions: This study was conducted under field conditions at the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil. Bean (*Phaseolus vulgaris* L. cv. Carioca) plants were inoculated with *Phaeoisariopsis griseola*, *Uromyces appendiculatus*, and the combination of *P. griseola* + *U. appendiculatus*. Treatments will be referred hereafter as P, U, P+U and Control, respectively. Treatments were set in a randomized complete block design with four treatments and three replications. Plot size was 16 m^2. Twelve seeds were sown and 10 plants were allowed to grow per linear meter of row. The plots were maintained with the conventional cultural practices used in commercial fields. To establish the treatments, the plots were inoculated, always at nightfall, with *P. griseola* and/or *U. appendiculatus* at 10^4

spores per ml for each pathogen. The first inoculation was made when the second trifoliolate leaf had expanded (approximately thirty days after planting), and the second inoculation followed ten days later. The control plots were sprayed with tebuconazole at 0.75 kg per ha (187.5 g ai per ha) the day before each inoculation.

Evaluations of disease severity, growth, gas exchange and yield: Disease severity and gas exchange were evaluated in the four central rows of each plot. The single-plant approach was adopted. Individual plants of similar height and vigor were selected for evaluations after the appearance of the first trifoliate leaf. Assessment of the severity of angular leaf spot and rust was done with the aid of a diagrammatic scale (Godoy *et al.* 1997) for each disease. The average severity (in percentage) of the three leaflets of five leaves on the marked plants was estimated. Both diseases were separately evaluated in all treatments, since natural infections occurred in the field. The leaf area (LA, cm^2) from selected plants was weekly determined according the empirical relationship developed by Iamauti (1995).

Gas exchange was measured on 37, 44, 51 and 58 days after planting using a LI-6400 Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA). Net photosynthetic rate, A (μ mol m^{-2} s^{-1}), stomatal conductance, g_s (mol m^{-2} s^{-1}) and transpiration, E (mmol m^{-2} s^{-1}) were recorded. Measurements were taken between 8:00 and 11:00 h. Readings were done at the central leaflet of each leaf. Leaves with same physiological age were used. Instantaneous photosynthetic water use efficiency was calculated as A/E relationship. Yield was determined at the end of the crop cycle in terms of seed weight (12% moisture).

Integral variables: The area under disease progress curve (AUDPC) and the area under leaf area progress curve (AULAPC) for each plant were calculated by trapezoidal integration over the n observation dates, according to Campbell and Madden (1990). An index A or R is used to characterize AUDPC due to angular leaf spot and rust, respectively.

The variables healthy leaf area index (HLAI), healthy leaf area index duration (HAD), and healthy leaf area index absorption (HAA) were estimated according to Waggoner and Berger (1987) and Bergamin Filho *et al.* (1997).

The integral variables were divided by the time ($t_n - t_I$) over which the integration (or summation) was carried out. All standardized variables were marked with an asterisk (*). Thus, $AUDPC_R$* is the standardized area under the rust progress curve and can be interpreted as the mean daily rust severity during the whole season.

Data analysis: Analysis of variance was conducted using the SAS system (SAS Institute, Cary, NC) and statistical differences between the means of the treatments were determined with the Tukey test.

Results

Disease severity, crop growth and yield: The experiment was conducted in the autumn when weather conditions were favorable for bean rust and angular leaf spot, although the angular leaf spot reached higher disease levels than rust (Fig. 1A,B). In the different treatments mainly those diseases reached higher levels which were inoculated, but also natural infections occurred to a low extend. The severity of angular leaf spot, expressed as $AUDPC_A$*, was higher in the treatment P+U than in treatment P. The level of rust ($AUDPC_R$*) was the same in both treatments in which *U. appendiculatus* was inoculated (treatments U and P+U), but significantly higher than in the treatments with natural rust infections (treatment P and the control) (Table 1).

There were no significant differences among all treatments in terms of the plant growth parameters AULAPC*, HAD* and HAA* (P = 0.05). However, all inoculated treatments differed statistically from the control in $AUDPC_A$*, $AUDPC_R$* and in yield. In the inoculated treatments (P, U, and P+U) yield was reduced by 21%, 20% and 32% (Table 1). The progress of the healthy leaf area index (HLAI) was similar in all treatments, but in the control treatment the leaf area remained on a high level for a longer time compared to inoculated treatments. The maximum level of HLAI in the control was less than 3.5 (Fig. 1C).

Gas exchange parameters: The inoculation of bean plants with *P. griseola*, *U. appendiculatus,* and the combination of both *P. griseola* + *U. appendiculatus* caused a significant (P = 0.05) reduction on the net photosynthetic rate, A (Fig. 1D, Table 1). Besides A, the transpiration (E) and photosynthetic water use efficiency (A/E) were also significantly reduced in the inoculated treatments. In addition, the stomatal conductance (g_s) was significantly lower in the treatments with inoculation of *P. griseola* (treatments P and P+U) compared to treatment U and the control (Table 1). However, the intercellular (Ci) to ambient (Ca) CO_2 concentration ratio (Ci/Ca) increased in the treatments P and U, but not in P+U (Table1).

In Figure 2, the reduction in *A*, g_s, *A/E* and yield of the inoculated treatments compared to the control are shown. In treatment P, the 21% reduction in yield was associated with 17% reduction in *A*, 21% reduction in g_s and 14% reduction in *A/E*. In treatment P+U, the corresponding reductions were 32% in yield, 23% in A and g_s and 14% in A/E. However, in treatment U, the 20% reduction in yield was associated only with 11% reduction in A, 14% reduction in g_s and 10% reduction on *A/E* (Figure 2).

In all treatments g_s and *E* were closely (Fig. 3A, B, C, D), but g_s and *A* only weakly correlated (Fig. 3E, F, G, H).

Table 1. Area under leaf area progress curves (AULAPC), area under visual disease progress curves of angular leaf spot ($AUDPC_A$) and rust ($AUDPC_R$), healthy leaf area index duration (HAD), healthy leaf area absorption (HAA), net photosynthetic rate (A), stomatal conductance (g_s), transpiration (E), photosynthetic water use efficiency (A/E), and ratio of intercellular (Ci) to ambient (Ca) CO_2 concentration (Ci/Ca), and yield of bean plants inoculated with *P. griseola* (P), *U. appendiculatus* (U), and *P. griseola* + *U. appendiculatus* (P+U).

Treatment	AULAPC* (m^2)	$AUDPC_A$* (%)	$AUDPC_R$* (%)	HAD* (days)	HAA* ($MJ.m^{-2}$)	A ($\mu mol\ m^{-2} s^{-1}$)	g_s ($mol\ m^{-2} s^{-1}$)	E ($mmol\ m^{-2} s^{-1}$)	A/E	Ci/Ca	Yield ($g.\ m^{-2}$)
P	0.09^1 a	1.22^1 a	0.05^1 a	1.94^1 a	14.29^1 a	14.77^2 c	$0,32^2$ b	3.01^2 c	0.0049^2 b	0.69^2 b	123.7^1 a
U	0.11 a	0.83 a	0.15 b	2.49 a	15.61 a	16.99 b	0.36 a	3.28 b	0.0051 b	0.69 b	126.1 a
P+U	0.10 a	1.42 b	0.14 b	2.26 a	15.04 a	15.84 b	0.33 b	3.23 b	0.0049 b	0.67 a	107.1 a
Control	0.12 a	0.23 c	0.01 c	2.55 a	16.29 a	19.22 a	0.42 a	3.36 a	0.0057 a	0.66 a	158.1 b

*Variables calculated by trapezoidal integration over 7 observations divided by the time ($t_n - t_1$) over which the integration was carried out. [1]Means from 15 plants per treatment. [2] Data are means from 4 evaluations. Values in a column followed by the same letters are not significantly (*P* = 0.05) different according to ANOVA, by Tukey's test.

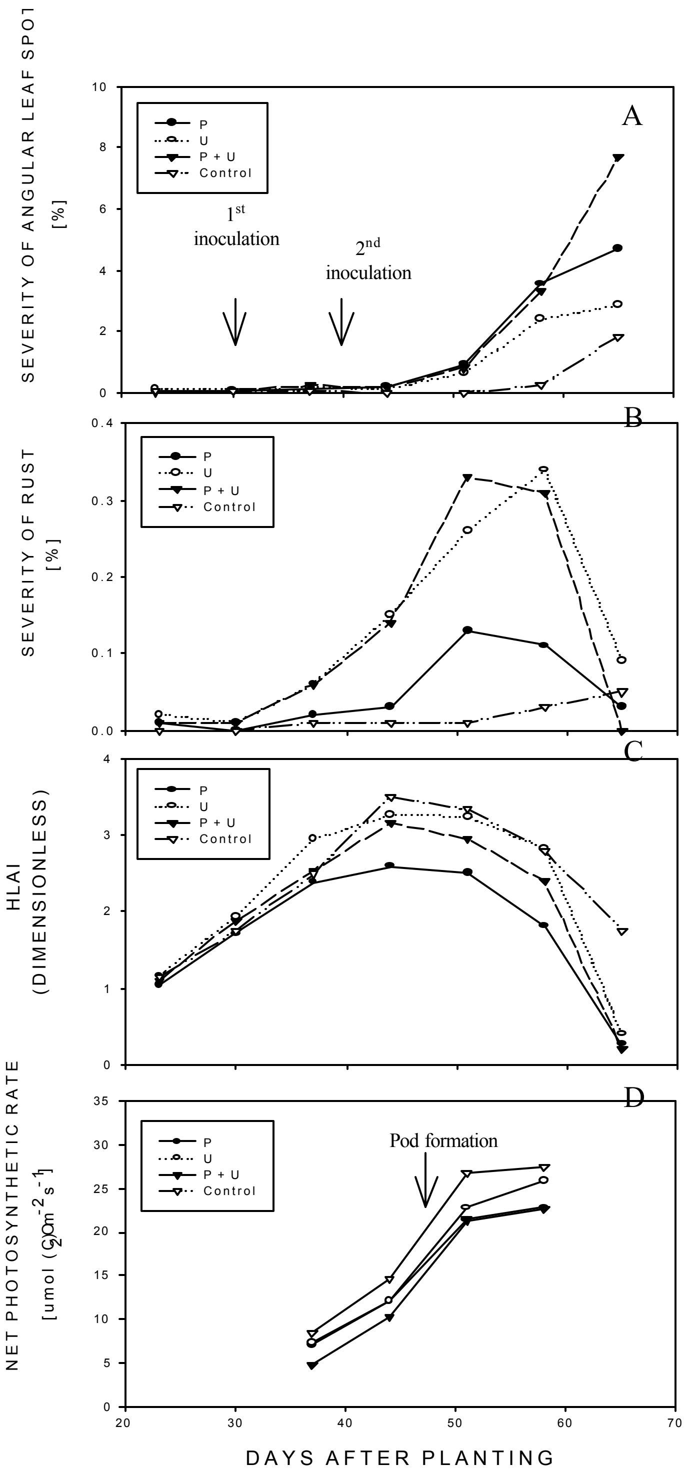


Figure 1. area index, HLAI (C), and net photosynthetic rate (D) of bean plants inoculated with *Phaeoisariopsis griseola* (P), *Uromyces appendiculatus* (U) and *P. griseola* + *U.*

appendiculatus (P+U) during the experiment. Pathogens were inoculated on 30 and 40 days after planting.

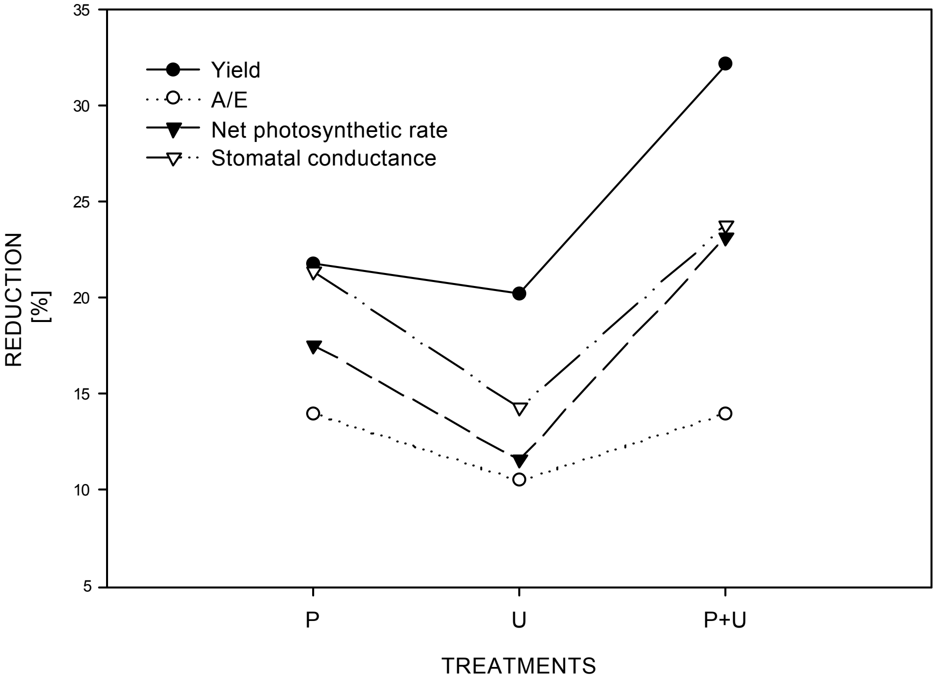


Figure 2. Reductions in yield, net photosynthetic rate, stomatal conductance and the photosynthetic water use efficiency (A/E) on bean plants inoculated with *Phaeoisariopsis griseola* (P), *Uromyces appendiculatus* (U) and *P. griseola* + *U. appendiculatus* (P+U). Data from 4 evaluations, expressed as percent of the healthy plants.

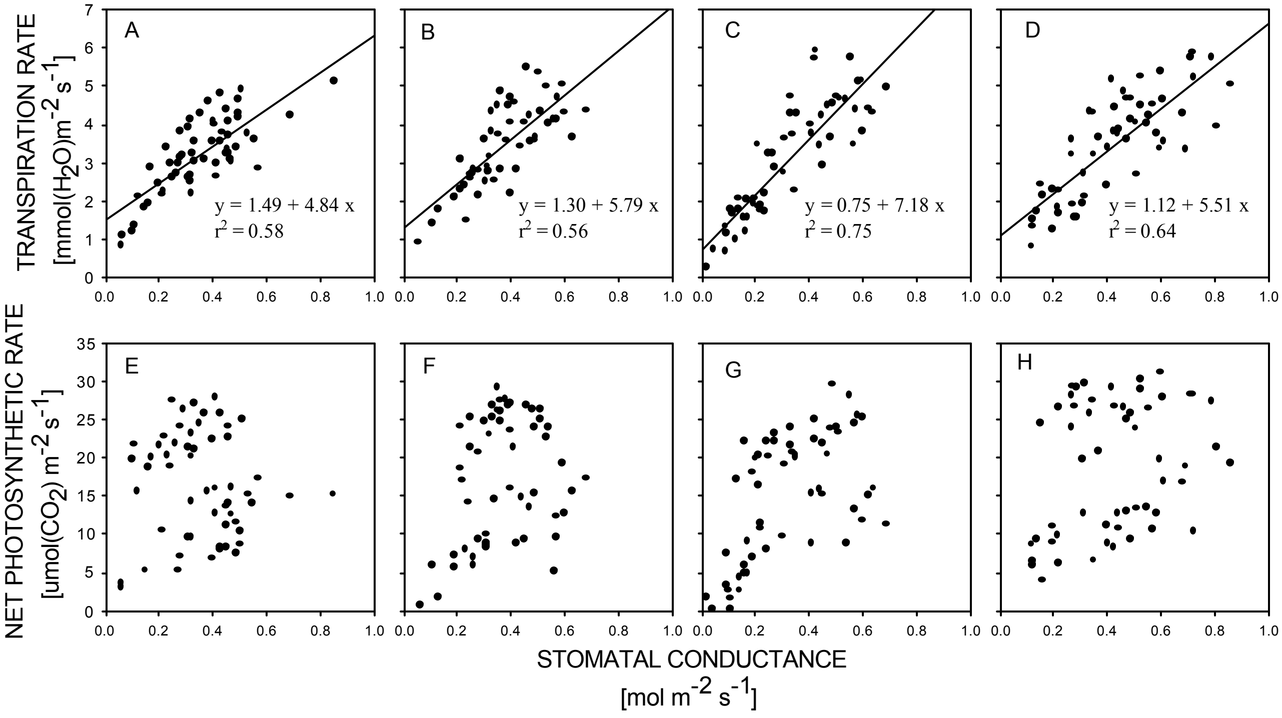


Figure 3. Relationships between stomatal conductance and transpiration rate (A,B,C,D) and between stomatal conductance and net photosynthetic rate (E,F,G,H) of bean plants inoculated with *Phaeoisariopsis griseola* (A, E), *Uromyces appendiculatus* (B, F), *P. griseola + U. appendiculatus* (C, G) and Control (D, H). Data from 4 evaluations.

Discussion

As the common bean is one of the most important crops in developing countries, reliable estimates of the effects caused by foliar diseases on the physiological processes are important to understand yield loss. The direct assessment of photosynthesis of infected compared to healthy leaves allows the quantification of the impact of one or more diseases on the remaining green area of diseased leaves. In our experiment, the severity of angular leaf spot and rust in bean plants differed significantly. Although no significant differences among the treatments on plant growth parameters (AULAPC*, HAD* and HAA*) were observed, there was a significant effect of both diseases on yield. The reductions of stomatal conductance and photosynthesis were higher in the treatments P and P+U compared to the treatment R. In treatment R, the reduction in yield was higher than the reductions on gas exchange parameters (Fig. 2). However, in the treatment P+U, the transpiration of the plant continued, but the photosynthesis suffered a decrease. The high correlation between g_s and E and the low one between g_s and A (Fig. 3) suggest that the transpiration rate is highly influenced by the stomatal aperture, but the photosynthetic process is probably more reduced due to a non-stomatal effect of the diseases than because of stomatal closure induced by the pathogens. Probably, others metabolic effects of pathogens on the biochemical or photochemical process of photosynthesis, besides stomatal closure, could explain the observed reduction in A.

Because biotrophic pathogens as *U. appendiculatus* have little influence on the stomata physiology, the resistance to carboxylation, caused by metabolic alterations on chloroplasts, could explain the reduction in net photosynthesis in leaves infected with rust as observed on barley (Owera *et al.* 1981). Our study demonstrated that rust and angular leaf spot reduced the carbon assimilation, however, we did not specifically examine the relative contributions of biochemical limitations on photosynthesis by effects of pathogens. In rust, little reductions in photosynthesis, associated with reduced green leaf area, were observed by Shtienberg (1992). Some studies have been carried out to determine the effects of angular leaf spot on leaf area reduction (Bergamin Filho *et al*. 1997, Silva *et al*. 1998). When conditions were favorable to *U. appendiculatus*, no significant defoliation was observed. In that case, the yield decrease probably occurred because the rusted leaves are sinks of carbohydrates (Livne and Daly 1966, Zaki and

Durbin 1965) and the remaining leaves cannot compensate for the carbon requirement needed for growth.

In addition to the reduction in leaf area and thus in the amount of intercepted radiation, some pathogens can also affect the radiation use efficiency of the plants that may also lead to a reduction in yield. Under rust infection, probably other metabolic processes as the increase of the resistance to carboxilation and reduction of chlorophyll content could be affected (Bassanezi 1995, Bassanezi *et al.* 2000, Lopes and Berger 2001, Owera *et al.* 1981, Rabbinge *et al.* 1985).

Healthy primary leaves can export about 50% of photosynthates to the roots and shoots, but rusted leaves only less than 2% (Livne and Daly 1966). The reduction in the net assimilation of CO_2 in presence of a pathogen could be associated with the use of photosynthates by the pathogen. On the other hand, accumulated carbohydrates in leaves might cause inhibition of photosynthesis (Moore *et al.* 1999, Smeekens 2000). It could limit the availability of photosynthates to growing roots and seeds (Livne and Daly 1966). It could explain why the reduction in net assimilation of CO_2 in leaves infected by rust appears to be higher than the reduction of loss in the green leaf area estimated by the disease severity (Bassanezi 1995).

Stomatal functioning is 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). Positive linear relationships between conductance and carbon assimilation were 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). If stomatal closure would be the cause of the reduced assimilation, a reduction in the intercellular CO_2 would be observed. The slight increase in the Ci/Ca ratio in the case of treatments infected with rust is an indication that the flow of CO_2 from the stomatal cavities to the carboxylation sites was not affected (Table 1). Consequently, it can be assumed that mesophyll resistance to carboxylation could increase in leaves infected with rust, which is in agreement with Lopes and Berger (2001) and Bassanezi *et al.* (2000).

The comparison of the effect of the inoculation with *P. griseola*+*U. appendiculatus* (treatment P+U) on the reductions in yield and A with the effects of the other treatments, separately (Fig. 2), suggests an antagonistic (less-than-additive) interaction of both diseases acting together on bean plants. This interaction may result from competition for plant resources between pathogen populations or from stimulation of active defense mechanisms in the plant (Waller and Bridge 1984).

Acknowledgements. This research was partially supported by the European Commission (project ERBIC18CT96-0037), FAPEMIG and FINEP. CAPES support first author.

References

Bassanezi RB, Amorim L, Bergamin Filho A. (2000) Análise das trocas gasosas em feijoeiro com ferrugem, mancha angular e antracnose. Fitopatol. Bras. 25(4):643-650

Bassanezi RB (1995) Interações entre o mosaico-em-desenho do feijoeiro e duas doenças fúngicas, ferrugem e mancha angular, em plantas de feijoeiro. M.S. Dissertation – ESALQ/Brazil

Bergamin Filho, A., Carneiro, S. M. T. P. G., Godoy, C. V., Amorim, L., Berger, R. D., and Hau, B. 1997. Angular leaf spot of *Phaseolus* beans: Relationships between disease, healthy leaf area, and yield. Phytopathol. 87:506-515.

Berghaus R, Reisener HJ (1985) Changes in photosynthesis of wheat plants infected with wheat stem rust (*Puccinia graminis* f.sp. *tritici*). Phytopathol. Z 112:165-172

Brenes BM, Chaves GM, Zambolim L (1983) Estimativas de perdas no rendimento do feijoeiro comum (*Phaseolus vulgaris*) causadas pela mancha angular (*Isariopsis griseola* Sacc.). Fitopatol. Bras. 8:599

Campbell Madden L. 1990. Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York

Daly JM, Bell AA, Krupka LR (1961) Respiratory changes during development of rust diseases. Phytopathol. 51:461-471

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. 33:317-345

Godoy CV, Carneiro SMTPG, Iamauti MT, Dalla Pria M, Amorim L, Bergamin Filho A (1997) Diagrammatic scales for bean diseases: development and validation. Z. fur Pflanzenkrankheiten und Pflanzenschutz 104: 336-45

Gordon TR, Duniway JM (1982) Effects of powdery mildew infection on the efficiency of CO_2 fixation and light utilization by sugar beet leaves. Plant Physiol. 69:139-142

Hall AE, Loomis RS (1972) Na explanation for the difference in photosynthetic capabilities of healthy and beet yellows virus-infected sugar beets (*Beta vulgaris* L.). Plant Physiol. 50:576-580

Iamauti MT (1995) Avaliação dos danos causados por *Uromyces appendiculatus* no feijoeiro. Ph.D. Thesis – ESALQ/Brazil

Livne A (1964) Photosynthesis in healthy and rust affected plants. Plant Physiol. 39:614-621

Livne A, Daly JM (1966) Translocation in healthy and rust-affected beans. Phytopathol. 56:170-175

Lopes DB, Berger RD (2001) The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. Phytopathol. 91:212-220

Lucas JA (1998) Plant Pathology and Plant Pathogens. 3rd ed. Oxford. Blackwell Science.

Mathre DE (1968) Photosynthetic activities of cotton plants infected with *Verticillium albo-atrum*. Phytopathol. 58:137-141

Moerschbacher BM, Vander P, Springer C, Noll U, Schmittmann G (1994) Photosynthesis in stem rust-infected, resistant and susceptible near-isogenic wheat leaves. Can. J. Bot. 72:990-997

Moll S, Serrano P, Boyle C (1995) In vivo chlorophyll fluorescence in rust-infected bean plants. Angew. Bot. 69:163-168.

Moore BD, Cheng SH, Rice J, Seeman JR (1998) Sucrose cycling, Rubisco expression, and predictions of photosynthetic acclimation to elevated CO_2. Plant Cell and Environment 21:905-916

Moore BD, Cheng SH, Sims DA, Seeman JR (1999) The biochemical and molecular basis for photosynthetic acclimation to elevated CO_2. Plant Cell and Environment 22:567-582

Nasser LCB (1976) Efeito da ferrugem em diferentes estádios de desenvolvimento do feijoeiro e dispersão de esporos de *Uromyces phaseoli* var. *typica* Arth. M.S. Dissertation – UFV/Brazil

Owera SAP, Farrar JF, Whitebread R (1981) Growth and photosynthesis in barley infected with brown rust. Plant Physiol. 18:79-90

Rabbinge R, Jorritsma ITM, Schans J (1985) Damage components to powdery mildew in winter wheat. Neth. J. Plant Pathol. 91:235-247

Scholes JD (1992) Photosynthesis: cellular and tissue aspects in diseased leaves. In: Ayres PG (ed) Pest and pathogens: plant responses to foliar attack. Oxford Bios Scientific Publisher, London, pp85-106

Schulze ED, Hall AE (1982) Stomatal responses, water loss and CO2 assimilation rates of plants in contrasting environments. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (ed) Physiological plant ecology. Vol 2. Encyclopedia of plant physiology. Springer-Verlag, New York, pp.181-230

SCHWARTZ HF, CORREA V, PINEDA DPA, OTOYA MM, KATHERMAN MJ (1981) DRY BEAN YIELD LOSSES CAUSED BY ASCOCHYTA, ANGULAR, AND WHITE LEAF SPOTS IN COLOMBIA. PLANT DIS. 65:494-496

Shtienberg D (1992) Effects of foliar diseases on gas exchange processes: a comparative study. Phytopathol. 760-765

Silva MB, do Vale FXR, Zambolim L, Hau B (1998) Efeitos da ferrugem, da antracnose e da mancha angular na área foliar de plantas de feijoeiro em condições de campo. Fitopatol. Bras. 23:442-447

Smeekens S (2000) Sugar-induced signal transduction in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51:49-81

Stangarlin JR (1999) Atividades de ribulose-1,5-bifosfato carboxilase (Rubisco), clorofilase, b-1,3-glucanase e quitinase e conteúdo de clorofila em cultivares de feijoeiro (*Phaseolus vulgaris*) infectados com *Uromyces appendiculatus* ou *Phaeoisariopsis griseola*. Ph.D. Thesis – ESALQ/Brazil

WAGGONER PE, BERGER RD (1987) DEFOLIATION, DISEASE, AND GROWTH. PHYTOPATHOL. 77, 393-398.

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

Waller JM, Bridge J (1984) Effects of pathogen interactions on tropical crop production. In: Wood RHS, Jellis GJ (ed) Plant diseases: infection, damage and loss. Blackwell Scientific Publications, Oxford, pp 311-320

Wong SC, Cowan IR, Farquhar GD (1979) Stomatal conductance correlates with photosynthetic capacity. Nature 282:424-426

Zaki AI, Durbin RD (1965) The effect of bean rust on the translocation of photosynthetic products from diseased leaves. Phytopathol. 55:528-529

CHAPTER 3

RELATIONSHIPS AMONG ANGULAR LEAF SPOT, HEALTHY LEAF AREA, EFFECTIVE LEAF AREA AND POD YIELD OF PHASEOLUS VULGARIS

W. C. Jesus Junior [a], F. X. R. Vale [a], R. R. Coelho [a], P. A. Paul [b], B. Hau [c], L. C. Costa [d], L. Zambolim [a] and A. Bergamin Filho [e]

[A]DEPARTAMENTO DE FITOPATOLOGIA, UNIVERSIDADE FEDERAL DE VIÇOSA, 36571-000 VIÇOSA, MG, BRAZIL; [B]DEPARTMENT OF PLANT PATHOLOGY, IOWA STATE UNIVERSITY, 50011-1020 AMES, IOWA; [C]INSTITUT FÜR PFLANZENKRANKHEITEN UND PFLANZENSCHUTZ, UNIVERSITÄT HANNOVER, 30419 HANNOVER, GERMANY; [D]DEPARTAMENTO DE ENGENHARIA AGRÍCOLA, UNIVERSIDADE FEDERAL DE VIÇOSA; [E]DEPARTAMENTO DE ENTOMOLOGIA, FITOPATOLOGIA E ZOOLOGIA AGRÍCOLA, ESALQ/USP, 13418-900 PIRACICABA, SP, BRAZIL.

ACCEPTED FOR PUBLICATION

AUTHOR FOR CORRESPONDENCE: FRANCISCO XAVIER RIBEIRO DO VALE (DOVALE@MAIL.UFV.BR)

*PAPER SUBMITTED TO PLANT PATHOLOGY.

SUMMARY

THREE FIELD EXPERIMENTS WERE CARRIED OUT, USING THE BEAN CULTIVAR CARIOCA COMUM, TO INVESTIGATE THE RELATIONSHIPS AMONG VISUAL AND VIRTUAL SEVERITY OF ANGULAR LEAF SPOT (CAUSED BY *PHAEOISARIOPSIS GRISEOLA*), AREA UNDER VISUAL AND VIRTUAL DISEASE PROGRESS CURVE (AUDPC*), HEALTHY LEAF AREA INDEX ON ANY GIVEN DAY (HLAI), HEALTHY LEAF AREA DURATION (HAD*), HEALTHY LEAF AREA ABSORPTION (HAA*), EFFECTIVE LEAF AREA DURATION (ELAD*), EFFECTIVE LEAF AREA ABSORPTION (ELAA*), AND YIELD OF *PHASEOLUS VULGARIS*. TO OBTAIN A WIDE RANGE OF DISEASE SEVERITIES, THE PLOTS WERE SPRAYED AT DIFFERENT STAGES OF PLANT GROWTH (BEFORE, DURING AND AFTER FLOWERING). VISUAL AND VIRTUAL SEVERITY, AND AUDPC* SHOWED NO CORRELATION WITH POD YIELD. HOWEVER, HAD*, HAA*, ELAD*, AND ELAA* WERE SIGNIFICANTLY CORRELATED WITH THE POD YIELD. THE RELATIONSHIPS BETWEEN YIELD AND HAD*, HAA*, ELAD*, AND ELAA* WERE LINEAR IN EACH OF THE THREE TRIALS ($21 < R^2 < 54\%$, $17 < R^2 < 53\%$, $22 < R^2 < 54\%$, $17 < R^2 < 54\%$, $P < 0.05$, RESPECTIVELY). THE MAIN CONSEQUENCE OF INCORPORATING THE PARAMETER β, WHICH CONSIDERS THE EFFECT OF VIRTUAL LESION, WAS AN INCREASE IN THE PROPORTION OF LEAF AREA CONSIDERED TO BE DISEASED. THIS, CONSEQUENTLY, LEAD TO A REDUCTION OF THE VARIABLES USED TO MEASURE HEALTHY LEAF AREA (ELAD < HAD AND ELAA < HAA). HOWEVER, EVEN AFTER THE INCORPORATION OF THE PARAMETER β, THE AREA UNDER THE VIRTUAL DISEASE PROGRESS CURVE ($\text{AUDPC}_{\text{VIRTUAL}}$*) STILL WAS NOT ENOUGH TO EXPLAIN THE VARIATIONS IN BEAN YIELD. WHEN ELAD AND ELAA WERE REGRESSED AGAINST YIELD, THE R^2 VALUES WERE EQUAL TO OR GREATER THAN THOSE OBTAINED FOR THE RELATIONSHIPS BETWEEN HAD AND HAA, AND YIELD. SINGLE-POINT MODELS USING HLAI TO ESTIMATE YIELD AT VARIOUS TIMES DURING THE CROP SEASON WERE DEVELOPED. THE SLOPE OF THE YIELD-HLAI RELATIONSHIP PROVED TO BE STABLE,

REGARDLESS OF PLANTING DATE AND BEAN GROWTH STAGE (FROM R6 TO R8). HLAI IS PROPOSED AS A KEY EXPLANATORY VARIABLE FOR A TRANSPORTABLE SYSTEM OF DISEASE MANAGEMENT; IT MAY BE USEFUL IN PRODUCING PRECISE RECOMMENDATIONS AT THE FARM LEVEL.

KEYWORDS: DISEASE ASSESSMENT, *PHAEOISARIOPSIS GRISEOLA*, *PHASEOLUS VULGARIS*, YIELD LOSS

INTRODUCTION

Angular leaf spot, caused by *Phaeoisariopsis griseola* (Sacc.) Ferr., is one of the most destructive foliar diseases of common beans (*Phaseolus vulgaris* L.) in Brazil. The pathogen causes lesions on leaves, pods, branches, and petioles and may cause severe defoliation. Without adequate disease control, yield reductions of up to 70% and 80% have been reported in Brazil (Brenes *et al.*, 1983) and Colombia (Schwartz *et al.*, 1981), respectively.

DETAILED INFORMATION ABOUT CROP LOSS IS THE BASIS FOR ANY VALID ECONOMIC ANALYSIS OF DISEASE MANAGEMENT MEASURES. SINCE DISEASE CONTROL INPUTS DO NOT INCREASE YIELDS BUT RATHER REDUCE CROP LOSSES, INFORMATION ON THE RELATIONSHIPS BETWEEN CONTROL MEASURE AND DISEASE, AND DISEASE AND YIELD, ARE INDISPENSABLE FOR DECISION-MAKING AIMED AT DISEASE MANAGEMENT (WAIBEL, 1990). IN ADDITION, ECONOMICAL CROP PRODUCTION REQUIRES QUANTITATIVE INFORMATION ABOUT HOW DISEASES AFFECT CROP PRODUCTIVITY (JOHNSON, 1990).

To determine crop losses, plant pathologists usually examine the relationship between crop yield and disease severity, using single-point, multiple-point and integral models. The relationship is often disappointing because the logic of the same is uncertain; the relative effect of disease severity is different early and late in the growing season, and defoliation is often not included in severity assessments (Waggoner & Berger, 1987). The lack of transportability of the models to other seasons and locations is due primarily to the great variation in the base yield of a disease-free crop and to the weak or indirect relationship between yield and disease (Waggoner & Berger, 1987).

IN SEVERAL PLANT PHYSIOLOGICAL PUBLICATIONS, CROP PRODUCTION IS CLOSELY RELATED TO THE AMOUNT OF SOLAR RADIATION UTILIZED BY THE PLANT (WATSON, 1947; GALLAGHER & BISCOE, 1978; CHARLES-EDWARDS, 1982; MONTEITH & ELSTON, 1983). THE AMOUNT OF RADIATION CAPTURED BY THE PLANT IS NOT ALWAYS RELATED TO DISEASE SEVERITY. HEALTHY LEAF AREA DURATION (HAD) AND HEALTHY LEAF AREA ABSORPTION (HAA) HAVE

BEEN SHOWN TO BE MUCH BETTER PREDICTORS OF YIELD THAN DISEASE SEVERITY (WAGGONER & BERGER, 1987). HAD AND HAA HAVE BEEN USED TO STUDY CROP LOSSES IN MANY PATHOSYSTEMS (AQUINO *ET AL.*, 1992; FERRANDINO & ELMER, 1992; JOHNSON, 1992; ROSSING *ET AL.*, 1992; WRIGHT & GAUNT, 1992; BASTIAANS, 1993; BASTIAANS & KROPFF, 1993; BASTIAANS *ET AL.*, 1994; LOPES *ET AL.*, 1994; MADEIRA & CLARK, 1994; MADEIRA *ET AL.*, 1994; PINNSCHMIDT *ET AL.*, 1994; BERGAMIN FILHO *ET AL.*, 1995; BRYSON *ET AL.*, 1995; GAUNT, 1995; GAUNT & BRYSON, 1995; MADDEN & NUTTER, 1995; MADEIRA & CLARK, 1995; BERGAMIN FILHO & AMORIM, 1996; NUTTER & LITTRELL, 1996; BERGAMIN FILHO *ET AL.*, 1997; CARNEIRO *ET AL.*, 1997; BIANCHINI, 1998; CANTERI, 1998; SILVA *ET AL.*, 1998; GIANASI, 1999; CARNEIRO, 2000).

In field experiments only the visual lesions of a disease are assessed. However, the disease may also be affecting the apparently disease-free leaf area around the visual lesions, the so-called virtual lesions (Bastiaans, 1991). The relation between virtual and visual lesion, given by the parameter ß, is a way of describing the reduction in photosynthesis of the leaf due to a disease. Using the concept of virtual disease, it is possible to incorporate the effect of angular leaf spot on the photosynthetic efficiency of the remaining green leaf area into the calculation of the area under the visual disease progress curve ($AUDPC_{visual}$), healthy leaf area duration (HAD), and healthy leaf area absorption (HAA). This is done by substituting the proportion of the leaf area in which the photosynthetic activity is null (Bastiaans, 1991) for the proportion of the leaf area with visual symptoms of the disease by incorporating the parameter ß = 4 for angular leaf spot (Bassanezi, 2000). Once the substitution is done, it is possible to calculate the area under the virtual angular leaf spot progress curve ($AUDPC_{virtual}$), effective leaf area duration (ELAD) and effective leaf area absorption (ELAA).

THE CONCEPTS OF HAD, HAA, ELAD, AND ELAA CAN BE UTILIZED FOR DECISION MAKING IN IPM PROGRAMS TO ENSURE THAT THERE IS SUFFICIENT HEALTHY LEAF AREA TO PRODUCE SATISFACTORY YIELD. ADDITIONALLY, DISEASES CAN BE ASSESSED AND THE GENERAL HEALTH STATUS OF THE CROP CANOPY CAN BE DETERMINED BY USING NEW TECHNIQUES SUCH AS COLOR-VIDEO IMAGERY, ANALYSIS OF PHOTOSYNTHESIS, AND REMOTE SENSING OF REFLECTED

RADIATION. WITH THESE TECHNIQUES, IT IS POSSIBLE TO APPLY THE APPROACH OF HEALTHY LEAF AREA IN STUDIES INVOLVING MULTIPLE PATHOSYSTEMS AND PESTS, AND THEIR INTEGRATED MANAGEMENT.

The objectives of this study were to compare two different approaches used to assess yield loss - one based on relationship between the radiation captured by healthy leaf area (on a given day or integrated over the cropping season) and yield, and the other on the relationship between disease severity and yield - and to verify the effect of the incorporation of the parameter ß to establish crop loss relationships on common beans.

Materials and Methods

Field experiments

Three field experiments were carried out at the Federal University of Viçosa, Viçosa, Minas Gerais State, Brazil, from April to July 1998 (first experiment), October to December 1998 (second experiment) and from March to May 1999 (third experiment) using the bean cultivar Carioca, under conditions of natural infection. All trials followed the same experimental design with four replicates in the first trial and three in the second and third trials. Each plot (48 m^2) consisted of sixteen rows, 6-m long, spaced 0.5 m apart. Twelve seeds were sown and 10 plants were allowed to grow per meter of row. The plots were maintained using the conventional cultural practices used in commercial fields, including planting and topdressing fertilization, insecticide sprays, weed control, and, when necessary, sprinkle irrigation.

To establish several treatments varying in the amount of disease, the plots were sprayed with the fungicide Thiabendazole at a rate 0.75 kg per ha (187.5 g ai per ha) at different stages of plant growth (before, during, and after flowering). The schedule was as follows: 1) No spray; 2) Spray on the 25th day after planting (DAP); 3) Spray on the 35th DAP; 4) Spray on the 45th DAP; 5) Spray on the 25th and 35th DAP; 6) Spray on the

25th and 45th DAP; 7) Spray on the 35th and 45th DAP, and 8) Spray on the 25th, 35th and 45th DAP.

Crop growth, disease severity, and yield assessments

Crop growth and disease severity were evaluated weekly in the eight central rows of each plot (disregarding the 0.5 m at each end of the row). Starting from the appearance of the first trifoliolate leaf, five randomly chosen plants were removed from each plot at each observation day, giving a total of 160, 120, and 120 plants per week for the first, second, and third trials, respectively. The total leaf area (LA, cm^2) of the five plants was determined using an area meter (Model LI- 3100, LI-COR). Angular leaf spot severity was assessed with the aid of a standard area diagram (Godoy *et al.*, 1997), and the average severity (%) for the three leaflets of each leaf on all removed plants was estimated. Even though defoliation was observed, it was not quantified. The cultivar Carioca has an indeterminate, polybrachiate growth habit, and it was difficult to identify specific leaves in sequential assessments. Thus, the number, area, and original position of fallen leaves were not determined. At each evaluation, the host growth stage was determined using a descriptive scale (van Schoonhoven and Pastor-Corrales, 1987).

Yield was determined for each plot ($g.m^{-2}$) at the end of the crop cycle by weighing the seeds (with 12% moisture).

Integral variables

The area under visual disease progress curve ($AUDPC_{visual}$) value was estimated using the trapezoidal integration method as

$$AUDPC_{Visual} = \sum_{i=1}^{n-1} \left(\frac{X_i + X_{i+1}}{2} \right) (t_{i+1} - t_i)$$

in which n is number of assessments, X is the visual disease severity of angular leaf spot (percent), and $(t_{i+1} - t_i)$ is the interval between two consecutive assessments.

Assuming that there were 20 plants per m^2, the leaf area index (LAI) was calculated by multiplying LA (cm^2) by 0.002. The healthy leaf area index (HLAI) was estimated as $HLAI(t_i) = LAI(t_i)\ [1 - 0.01X(t_i)]$ in which X is angular leaf spot severity. HLAI was then integrated over time, giving the healthy leaf area duration (HAD-days), estimated as

$$HAD = \sum_{i=1}^{n-1} \left(\frac{HLAI_i + HLAI_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Adopting the concept of the virtual lesion (Bastiaans, 1991), the virtual disease severity of angular leaf spot was calculated as $X_{\beta A} = 100[1- (1-0.01\ X_A)^{\beta A}]$. The β-value used for angular leaf spot was $\beta_A = 4$ (Bassanezi, 2000). Virtual disease severity was integrated over the season, resulting in the area under the virtual disease progress curve ($AUDPC_{virtual}$).

The effective leaf area index (ELAI) was then be calculated as ELAI = LAI $[1 - 0.01\ X_{\beta A}]$, and then integrated over the season to give the effective leaf area duration (ELAD - days), estimated as

$$ELAD = \sum_{i=1}^{n-1}\left(\frac{ELAI_i + ELAI_{i+1}}{2}\right)(t_{i+1} - t_i)$$

The intercepted radiation RI_i (MJ.m^{-2}) was calculated using the formula: $RI_i = I_i\ [1 - e^{(-k\ LAIi)}]$, in which I_i is the average incident solar radiation (MJ.m^{-2}) during the period ($t_{i+1} - t_i$) and *k* is the extinction coefficient. According to Miglioranza (1992), the *k-value* for the cultivar Carioca is 0.7. The intercepted radiation of the healthy leaf area (*HRI*) (MJ.m^{-2}) for each assessment was determined as HRI = RI (1-*X*). The integration over the observation time results in the healthy leaf area absorption (HAA) for each plant, measured in MJ.m^{-2}:

$$HAA = \sum_{i=1}^{n-1}\left(\frac{(1 - X_i)\left(1 - e^{(-kLAI_i)}\right) + (1 - X_{i+1})\left(1 - e^{(-kLAI_{i+1})}\right)}{2}\right)(t_{i+1} - t_i)$$

The intercepted radiation of the effective healthy leaf area (*EHRI*) was calculated as EHRI = RI ($1-X_{\beta A}$) and integrated over the season, giving the effective leaf area absorption (ELAA, MJ.m^{-2}).

The length of the observation period was 60 days in the first experiment, 47 days in the second, and 69 days in the third experiment. In order to make comparisons among seasons, the integral variables were standardized by divided each AUDPC value by the observation time ($t_n - t_1$) over which the integration (or summation) was carried out. All variables standardized in this way were marked with *. Thus, $AUDPC_{visual}$* means $AUDPC_{visual}$ divided by ($t_n - t_1$), and can be interpreted as the mean visual disease severity during the whole season.

DATA ANALYSIS

THE RELATIONSHIPS AMONG VARIABLES WERE EXAMINED USING LINEAR REGRESSION PERFORMED BY STATISTICA SOFTWARE (STATSOFT, TULSA, OK).

RESULTS

RELATIONSHIPS BETWEEN AUDPC$_{VISUAL}$*, HAD*, HAA* AND YIELD

THE DIFFERENT SPRAY SCHEDULES RESULTED IN DIFFERENT EPIDEMICS OF ANGULAR LEAF SPOT IN EACH TRIAL (TABLE 1). THE OVERALL AVERAGE AUDPC$_{VISUAL}$* IN ALL THREE EXPERIMENTS ON A PLANT BASIS WAS RATHER LOW, VARYING BETWEEN 0.26 AND 0.68. THE MEAN HAD* AND HAA* VALUES WERE SIMILAR IN THE THREE TRIALS, RANGING FROM 1.54 TO 1.66 AND FROM 9.65 TO 9.93, RESPECTIVELY. DIFFERENCES IN MEAN YIELD WERE ALSO SMALL (BETWEEN 53.7 AND 66.5 G) (TABLE 1).

Although the variability in AUDPC$_{visual}$* was rather low in all experiments, the yield values, even on a plot basis, varied over a wide range (Fig. 1). The relationships between AUDPC$_{visual}$* and plot yield were not significant in experiments 1 and 3, but a significant correlation ($P < 0.05$) was observed in the second experiment (Table 3). The relationship between yield and HAD* or HAA*, for all removed plants, was investigated separately for each of the three trials (Fig. 1 and Table 3). In all cases, yield increased linearly with an increase in HAD* or HAA*. The coefficients of determination (R^2) were high in the first and the second trial, but much lower, though significant ($P < 0.05$), in the third trial.

RELATIONSHIPS BETWEEN AUDPC$_{VIRTUAL}$*, ELAD*, ELAA* AND YIELD

THE SPRAY SCHEDULES RESULTED IN DIFFERENT EPIDEMICS OF ANGULAR LEAF SPOT IN EACH TRIAL (TABLE 2). THE AVERAGE AUDPC$_{VIRTUAL}$* IN ALL THREE EXPERIMENTS ON A PLANT BASIS WAS RATHER LOW AND VARIED FROM 1.01 TO 2.62. THE MEAN VALUES OF

ELAD* AND ELAA* IN THE THREE TRIALS WERE SIMILAR, RANGING FROM 1.43 TO 1.65 AND FROM 9.60 TO 9.89, RESPECTIVELY (TABLE 2).

The relationships between $AUDPC_{virtual}$* and plot yield were not significant in the experiments 1 and 3, but a significant correlation ($P < 0.05$) was observed in the second experiment (Table 4). The relationship between yield and ELAD* or ELAA*, for all removed plants, was investigated in each of the three trials separately (Fig. 1 and Table 4). In all cases, yield increased linearly with an increase in ELAD* or ELAA*. The coefficients of determination (R^2) were high in the first and the second trial, but much lower, though significant ($P < 0.05$), in the third trial.

Healthiest versus most-diseased plants

To get more insight into the effects of angular leaf spot on yield, the relationship between visual and virtual disease severity, HLAI, HAD*, ELAD*, HAA*, ELAA*, and yield was investigated by comparing the 5 healthiest plots (lowest AUDPC*) with the 5 most diseased plots (highest AUDPC*) in each of the three trials (Fig. 2 and Tables 5 and 6). As the disease pressure was in general low in all three seasons, the average $AUDPC_{visual}$* and $AUDPC_{virtual}$* ranged from 0.07 to 0.35 and from 0.26 to 1.37, respectively, in the healthiest plots, while the mean visual and virtual disease level was between 0.60 and 1.05 and between 2.34 and 4.00 in the most diseased plots, respectively. The variation in HAD* and ELAD* was between 1.72 and 1.92 and between 1.71 and 1.91, respectively, in the healthiest plots, and from 1.33 to 1.51 in the most diseased ones. On the other hand, the variation in HAA* and ELAA* was between 10.14 and 10.77 in the healthiest plots, and from 9.10 to 9.55 in the most diseased ones. Yield in the healthy plots was higher (67.4 – 88.9 g) than in the diseased plots (42.7 – 63.2 g).

Table 1. Visual severity of angular leaf spot, area under the visual disease progress curve ($\text{AUDPC}_{\text{visual}}$*), healthy leaf area duration (HAD*), healthy leaf area absorption (HAA*) and yield of beans for three trials conducted in Minas Gerais, Brazil, on a plant basis.

Trial	Disease severity (%)		$\text{AUDPC}_{\text{visual}}$* (%)		HAD*		HAA*		Yield (g.m^{-2})	
	Máx	Mean ± SE	Máx	Mean ± SE	Máx	Mean ± SE	Máx	Mean ± SE	Máx	Mean ± SE
1	4.64[a]	0.26±0.01[b]	0.82	0.26±0.01	2.55	1.66±0.03	11.82	9.65±0.09	101.30	62.6±3.40
2	14.04	0.81±0.05	1.19	0.68±0.03	2.12	1.54±0.03	11.31	9.76±0.09	87.93	53.7±3.16
3	25.30	0.52±0.04	0.83	0.49±0.01	2.04	1.60±0.02	10.84	9.93±0.05	135.22	66.5±6.15

[a] Maximum value, [b] means (± standard error) for 160 plants in the first trial and 120 plants in the second and third trials, and *standardized variables

Table 2. Virtual severity of angular leaf spot, area under the virtual disease progress curve ($\text{AUDPC}_{\text{virtual}}$*), effective leaf area duration (ELAD*), effective leaf area absorption (ELAA*) and yield of beans for three trials conducted in Minas Gerais, Brazil, on a plant basis.

Trial	Disease severity (%)		$\text{AUDPC}_{\text{virtual}}$* (%)		ELAD*		ELAA*		Yield (g.m^{-2})	
	Máx	Mean ± SE	Máx	Mean ± SE	Máx	Mean ± SE	Máx	Mean ± SE	Máx	Mean ± SE
1	17.31[a]	1.02±0.06[b]	4.79	1.01±0.06	3.14	1.65±0.03	12.17	9.60±0.09	101.30	62.6±3.40
2	45.40	3.06±0.18	5.77	2.62±0.12	2.22	1.43±0.03	11.79	9.66±0.10	87.93	53.7±3.16
3	68.86	1.97±0.12	5.70	1.87±0.06	2.12	1.59±0.02	11.34	9.89±0.05	135.22	66.5±6.15

[a] Maximum value, [b] means (± standard error) for 160 plants in the first trial and 120 plants in the second and third trials, and *standardized variables

Table 3. Intercepts (b_0) and slope (b_1) of regression lines between yield ($g.m^{-2}$) and area under the visual disease progress curve ($AUDPC_{visual}$*) and between yield and healthy leaf area duration (HAD*) and healthy leaf area absorption (HAA*) for beans with angular leaf spot in three trials on a plot basis.

Trial	$AUDPC_{visual}$* (%)			HAD*			HAA*		
	b_o	b_1	R^2	b_o	b_1	R^2	b_o	b_1	R^2
1 (n^1 = 32)	67.09	-0.29	0.02	4.89	0.58	0.44	-57.17	12.41	0.50
2 (n = 24)	79.93	-0.81	0.50	-1.95	0.77	0.54	-58.20	11.47	0.53
3 (n = 24)	62.55	0.12	0	-36.42	0.93	0.21	-123.02	19.09	0.17

[1] number of plots per trial, and *standardized variables

Table 4. **Intercepts (b_o) and slope (b_1) of regression lines between yield ($g.m^{-2}$) and area under the virtual disease progress curve ($AUDPC_{virtual}$*) and between yield and effective leaf area duration (ELAD*) and effective leaf area absorption (ELAA*) for beans with angular leaf spot in three trials on a plot basis.**

Trial	$AUDPC_{virtual}$* (%)			ELAD*			ELAA*		
	b_o	b_1	R^2	b_o	b_1	R^2	b_o	b_1	R^2
1 (n^1 = 32)	67.19	-4.55	0.02	4.80	35.04	0.44	-55.46	12.28	0.50
2 (n = 24)	80.17	-10.09	0.49	-4.90	41.28	0.54	-54.74	11.25	0.54
3 (n = 24)	66.02	0.25	0	-35.54	64.18	0.22	-121.44	18.98	0.17

[1] number of plots per trial, and *standardized variables

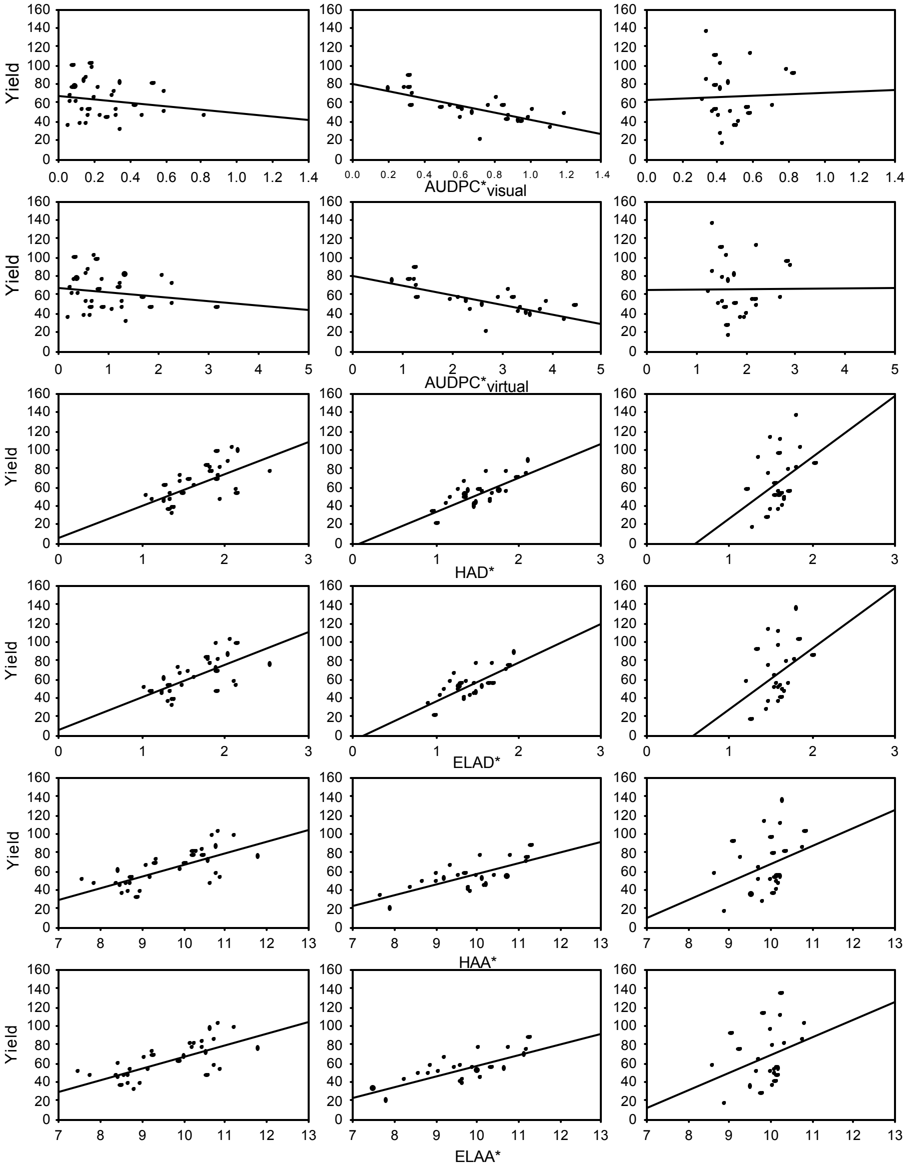


Figure 1. Yield (g m^{-2}) versus area under the visual disease progress curve (AUDPC$_{visual}$*) of angular leaf spot, area under the virtual disease progress curve (AUDPC$_{virtual}$*) of angular leaf spot, healthy leaf area duration (HAD*), effective leaf area duration (ELAD*), healthy leaf area absorption (HAA*) and effective leaf area absorption (ELAA*) for individual plots (average of 5 plants per plot): 32 plots in the first (left column) and 24 plots for the second (middle column) and third trial (right column). All integral variables were standardized (marked with *).

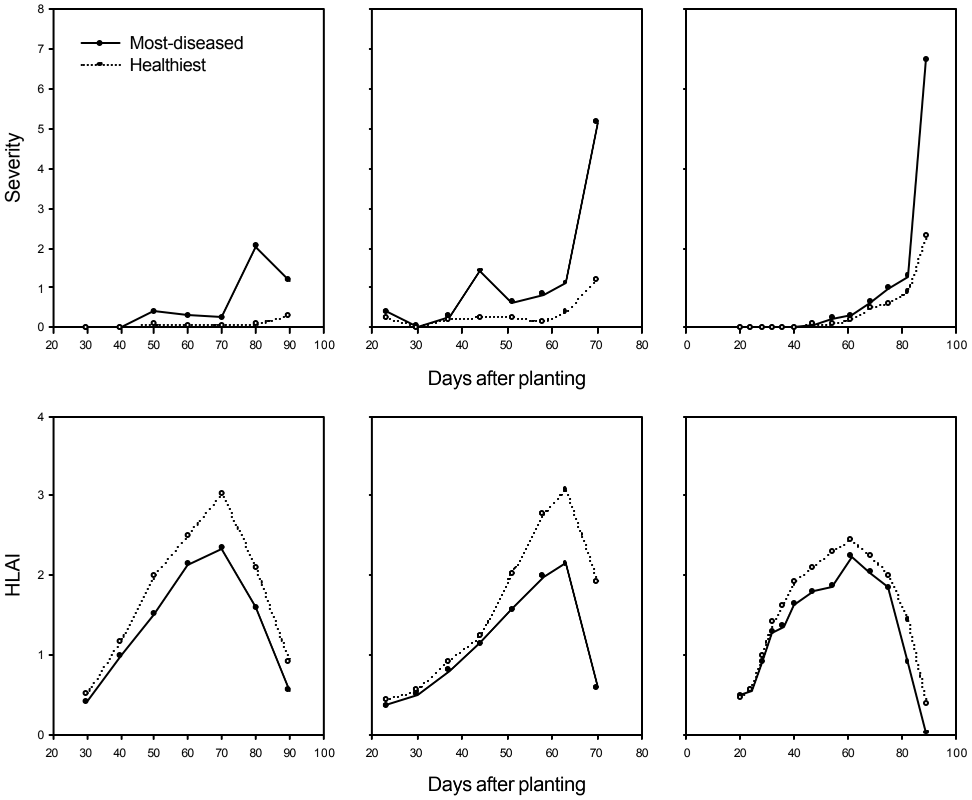


Figure 2. Visual severity (percentage) of angular leaf spot on beans and healthy leaf area index (HLAI) versus time (days after planting) in three trials conducted in Minas Gerais, Brazil. Mean values of the 5 healthiest plots and of the 5 most diseased plots in trial 1 (left column), trial 2 (middle column) and trial 3 (right column).

TABLE 5. AREA UNDER THE VISUAL DISEASE PROGRESS CURVE (AUDPC$_{\text{VISUAL}}$*), HEALTHY LEAF AREA DURATION (HAD*), HEALTHY LEAF AREA ABSORPTION (HAA*) AND YIELD FOR BEANS WITH ANGULAR LEAF SPOT CONSIDERING THE 5 HEALTHIEST PLOTS (H) VERSUS THE 5 MOST DISEASED PLOTS (D)^{A} IN THREE TRIALS CONDUCTED IN MINAS GERAIS, BRAZIL.

Trial	AUDPC$_{\text{visual}}$* (%)		HAD*		HAA*		Yield (g·m^{-2})	
	H	D	H	D	H	D	H	D
1	0.07±0.01[a]	0.60±0.03	1.92±0.09	1.51±0.09	10.33±0.26	9.13±0.28	67.39±4.60	59.0±3.24
2	0.29±0.01	1.05±0.02	1.90±0.04	1.33±0.04	10.77±0.10	9.10±0.18	74.28±2.32	42.7±1.49
3	0.35±0.01	0.70±0.02	1.72±0.04	1.48±0.04	10.14±0.09	9.55±0.13	88.85±6.91	63.2±5.44

[a] Means (± standard error) for 5 plots (each plot with 5 plants), and *standardized variables

TABLE 6. AREA UNDER THE VIRTUAL DISEASE PROGRESS CURVE (AUDPC$_{\text{VIRTUAL}}$*), EFFECTIVE LEAF AREA DURATION (ELAD*), EFFECTIVE LEAF AREA ABSORPTION (ELAA*) AND YIELD FOR BEANS WITH ANGULAR LEAF SPOT CONSIDERING THE 5 HEALTHIEST PLOTS (H) VERSUS THE 5 MOST DISEASED PLOTS (D)^{A} IN THREE TRIALS CONDUCTED IN MINAS GERAIS, BRAZIL.

Trial	AUDPC$_{\text{virtual}}$* (%)		ELAD*		ELAA*		Yield (g·m^{-2})	
	H	D	H	D	H	D	H	D
1	0.26±0.01[a]	2.34±0.10	1.91±0.09	1.51±0.09	10.33±0.26	9.13±0.28	67.39±4.60	59.0±3.24
2	1.15±0.04	4.00±0.07	1.74±0.04	1.33±0.04	10.77±0.10	9.10±0.18	74.28±2.32	42.7±1.49
3	1.37±0.02	2.58±0.07	1.71±0.04	1.48±0.04	10.14±0.09	9.55±0.13	88.85±6.91	63.2±5.44

[a] Means (± standard error) for 5 plots (each plot with 5 plants), and *standardized variables.

THE EFFECT OF THE USE OF THE PARAMETER β

THE MAIN EFFECT OF INCORPORATING THE PARAMETER β, WHICH CONSIDERS THE EFFECT OF VIRTUAL LESION, WAS AN INCREASE IN THE PROPORTION OF LEAF AREA CONSIDERED TO BE DISEASED. THIS, CONSEQUENTLY, LEAD TO A REDUCTION OF THE VARIABLES USED TO MEASURE HEALTHY LEAF AREA (ELAD < HAD AND ELAA < HAA). HOWEVER, EVEN AFTER THE INCORPORATION OF THE PARAMETER β, THE AREA UNDER THE VIRTUAL DISEASE PROGRESS CURVE ($AUDPC_{VIRTUAL}$*) STILL WAS NOT ENOUGH TO EXPLAIN THE VARIATIONS IN BEAN YIELD. WHEN ELAD AND ELAA WERE REGRESSED AGAINST YIELD, HOWEVER, THE R^2 VALUES WERE EQUAL TO OR GREATER THAN THOSE OBTAINED FOR THE RELATIONSHIPS BETWEEN HAD AND HAA, AND YIELD (FIG. 1 AND TABLES 1 TO 6).

Effect of growth stage

Models which predict yield independent of integral variables like HAD* or HAA* can not always be applied, since they lack information about the total observation period and as such can only be applied after the season. Since decision-making has to be done during the season, single point models at a specific time or growth stage, or integral models using data collected during the entire season are more useful. Consequently, the relationships between yield and HLAI values for the main bean growth stages were determined for the three trials (Table 7). The regression coefficients (slopes) for this relationship decreased with the growth stage up to R7 (trial 1) and R8 (trial 2 and 3), then increased again towards the latter growth stages (Fig. 3). The intercept, however, remained the same at the earlier growth stages, but increased towards the latter stages. Most intercepts were not significantly different from 0 ($P<0.01$), so that in a second step the regression lines were forced through the origin (Table 8). The slopes of these regression lines decreased with the growth stage up to R7 (trial 1 and 3) and R8 (trial 2).

The beta function fitted well to the average of the slopes of the three trials in the different growth stages: $s = 84.2639*(GS - 0.71835)^{-0.55006} (7.00001 - GS)^{-0.10007}$, with $R^2 = 66\%$ (Fig. 3), in which s is the slope of the yield-HLAI linear relationship, and GS is the growth stage (V3 = 1, V4 = 2, R5 = 3, R6 = 4, R7 = 5, R8 = 6, and R9 = 7).

Table 7. Intercepts (b_o) and slopes (b_1) of regression lines between yield (g.m^{-2}) and healthy leaf area index (HLAI) at different growth stages (GS) in three trials on a plot basis.

GS	HLAI								
	Trial 1 (n[1] = 32)			Trial 2 (n = 24)			Trial 3 (n = 24)		
	b_o	b_1	R^2	b_o	b_1	R^2	b_o	b_1	R^2
V3[2]	11.9	112.9	0.28	1.93	128.3	0.39	3.43	110.0	0.36
V4	1.11	58.38	0.28	-2.54	112.7	0.55	-6.38	55.9	0.31
R5	28.12	18.92	0.20	-2.55	67.01	0.52	-9.19	43.81	0.35
R6	8.53	23.48	0.45	2.69	41.89	0.42	-14.39	42.4	0.35
R7	17.71	17.75	0.39	-1.02	32.42	0.59	10.48	26.32	0.15
R8	28.85	18.28	0.53	6.28	19.77	0.69	36.21	24.77	0.20
R9	39.16	33.33	0.51	29.42	18.51	0.50	52.99	74.8	0.27

[1]number of plots per trial, and [2]Growth stages: V3 = first trifoliolate leaf; V4 = third trifoliolate leaf; R5 = pre-flowering; R6 = flowering; R7 = pod formation; R8 = pod filling; and R9 = physiological maturity.

Table 8. **Slopes (b_1) (± standard error) of regression lines (intercepts forced through the origin) between yield (g·m^{-2}) and healthy leaf area index (HLAI) at different growth stages (GS) in three trials on a plot basis.**

GS	HLAI					
	Trial 1 (n[1] = 32)		Trial 2 (n = 24)		Trial 3 (n = 24)	
	b_1	R^2	b_1	R^2	b_1	R^2
V3[2]	139.20±7.37	0.37	133.08±7.34	0.40	146.16±11.08	0.37
V4	59.41±2.87	0.39	107.75±5.04	0.53	51.12±4.15	0.28
R5	33.92±2.05	0.42	64.03±3.09	0.50	38.64±3.05	0.29
R6	27.04±1.23	0.52	44.07±2.37	0.44	35.02±2.78	0.26
R7	24.40±1.22	0.53	31.84±1.41	0.58	31.23±2.88	0.20
R8	31.61±1.78	0.67	22.24±0.87	0.73	50.39±5.34	0.46
R9	73.37±6.08	0.64	36.87±2.89	0.65	185.29±40.21	0.53

[1]number of plots per trial, and [2]Growth stages: V3 = first trifoliolate leaf; V4 = third trifoliolate leaf; R5 = pre-flowering; R6 = flowering; R7 = pod formation; R8 = pod filling; and R9 = physiological maturity.

$$s = 84.2639*(GS - 0.71835)^{-0.55006} (7.00001 - GS)^{-0.10007}$$

R^2=0 66

Figure 3. Slopes of lines obtained by linear regression (intercepts forced through the origin) of yield ($g \cdot m^{-2}$) and healthy leaf area index (HLAI) measured at different growth stages of *Phaseolus vulgaris* with angular leaf spot in three trials. One beta function was adjusted to all the data. Growth stages: V3 = first trifoliolate leaf; V4 = third trifoliolate leaf; R5 = pre-flowering; R6 = flowering; R7 = pod formation; R8 = pod filling; and R9 = physiological maturity.

Discussion

In all three trials described in this paper, no relationship between yield and AUDPC* was found (Fig. 1). Although data similar to these are not rare in the literature (Bissonnette *et al.*, 1994; Bryson *et al.*, 1995; Godoy, 1995; Iamauti, 1995; Bergamin Filho *et al.*, 1997; Carneiro *et al.*, 1997; Silva *et al.*, 1998), the absence of a relationship between yield and AUDPC is more common when data from different seasons are compared (Lopes *et al.*, 1994; Gaunt, 1995). The lack of a relationship between yield and AUDPC* in all of the three trials in this study, even when data from each trial were analyzed individually, was probably due to three main reasons: (i) intense defoliation caused by the pathogen, (ii) the lack of an estimate of defoliation in our disease assessment method, and (iii) the indeterminate growth habit of the host plant.

The results of the comparison of the healthiest plots with the most-diseased plots supports the hypothesis that the three above-mentioned reasons might have played a role in determining the relationships between the aforementioned variables and yield (Fig. 1 and Fig. 2). Models based on disease severity can succeed only when leaf area is the same in the treatments that are being compared. The same proportion of disease severity (X) on different leaf areas is unlikely to have the same impact on yield. This is especially true for pathosystems in which defoliation is a major part of the disease syndrome, and for hosts with an indeterminate growth habit (Bergamin Filho *et al.*, 1997). The yield of the group of 5 plots analyzed (the healthiest versus the most-diseased plots) (Fig. 2) was always positively correlated with the variable derived from HLAI (HAD or ELAD) (Tables 5 and 6).

The effect of disease severity on yield could be demonstrated by the analysis of the healthiest versus the most-diseased plots (Fig. 2). In all three trials, the most-diseased plots had lower values of HLAI, HAD*, ELAD*, HAA* and ELAA*, and consequently lower yield (Fig. 2 and Tables 5 and 6). Defoliation is the main consequence of angular leaf spot. As a result, disease quantification based only on the assessment of disease severity on the remaining leaves was inadequate. A similar situation is likely to occur in many other pathosystems, a condition that has been underestimated (Lim & Gaunt, 1981; Johnson *et al.*, 1987; Waggoner & Berger, 1987; Waggoner, 1990) in many pathosystems.

The linear relationship between yield and HAD* or HAA* in all trials described in this paper contrasted with the curvilinear relationship (the Gompertz curve) originally proposed by Waggoner and Berger (1987) for the peanut-*Cercosporidium personatum* pathosystem. Aquino *et al.* (1992) confirmed the curvilinear relationship for the same pathosystem, and this implied an asymptotic level for yield. In the case of cultivar Carioca, yield increased linearly and non-asymptotically with increased leaf area (at least to HLAI = 3). This relationship was probably due to characteristics features of this cultivar, such as pod production at the base of each leaf and indeterminate growth habit. Recently Bergamin Filho *et al.* (1997), Carneiro *et al.* (1997) and Silva *et al.* (1998) observed a linear relationship between yield and HAD or HAA in the pathosystem studied here. These results are consistent with the results obtained in this study.

Because of the variation in yield, the use of a single-point or a multiple-point model to make a reasonably accurate prediction of yield or yield loss of common bean from disease severity would be worthless. As Waggoner & Berger (1987) have shown mathematically, both single-point and multiple-point models are successful only when the leaf area is the same in the treatments being compared (e.g., healthy crops vs. diseased crop, season$_1$ vs. season$_2$, etc.). The same proportion of disease severity on different leaf areas would not have the same impact on yield. In addition, the use of models to determine yield (or yield loss) based on disease severity is more troublesome for those pathosystems in which defoliation is a major part of the disease syndrome because of the difficulties associated with assessing defoliation (Johnson, 1987). The use of HAD model avoids these problems, because yield is estimated using the true determinant of yield, the duration of the healthy leaf tissue.

The results of this study were highly consistent with the data presented in the literature (Bergamin Filho *et al.*, 1997; Carneiro *et al.*, 1997; Silva *et al.*, 1998). Yield cannot be accurately predicted using disease variables, such as disease severity, alone. Rather, parameters of host growth, such as the healthy leaf area duration (HAD) and healthy leaf area absorption (HAA), have to be considered.

Regarding the use of the parameter β, which considers the effect of virtual lesion, the main effect of incorporating this parameter was an increase in the proportion of leaf area considered to be diseased. This, consequently, lead to a reduction of the variables used to measure healthy leaf area (ELAD < HAD and ELAA < HAA). However, even after the incorporation of the parameter β, the area under the virtual disease progress curve ($AUDPC_{virtual}*$) still was not enough to explain the variations in bean yield. When ELAD and ELAA were regressed against yield, however, the R^2 values were equal to or greater than those obtained for the relationships between HAD and HAA, and yield. The improvement of the relationship between yield, and ELAD and ELAA was greatest in the trials with the highest levels of disease severity throughout the epidemic. In these cases, disease intensity could be expressed, not only as the proportion of diseased leaf area, but also as the proportion of the leaf area on which the photosynthetic activity is affected by the activity of the pathogen. The use of visual disease severity alone may lead to an underestimation of the effect of the disease on yield. The use of parameter β, however, allows for correction of the visual severity, taking into consideration the photosynthetic activity of the diseased leaf.

The use of β to correct visual disease severity, however, is certainly not enough to establish a relationship between disease severity and yield. Although β incorporates the effect of the pathogen on the radiation use efficiency (RUE) of the diseased leaves, the effects of compensation and the reduction of the photosynthetic capacity of the healthy leaves, not included in β, may still be influencing pod (Bassanezzi, 2000). In our study it was observed that the main consequence of incorporating the parameter β was an increase in the proportion of leaf area considered to be diseased. This, consequently, lead to a reduction of the variables used to measure healthy leaf area (ELAD < HAD and ELAA < HAA). However, even after the incorporation of the parameter β, the area under the virtual disease progress curve ($AUDPC_{virtual}$*) still was not enough to explain the variations in bean yield.

For decision-making, integral variables (such as HAD, HAA, ELAD, and ELAA) are not the most appropriate (Madden & Nutter, 1995). It would be better to develop more empirical single-point models using HLAI to estimate yield at various times during a cropping season, rather than automatically integrate this variable. The results shown in Fig. 3 and Tables 7 and 8 are promising. The slope of the yield-HLAI was shown to be stable (36.11±2.85), regardless of planting time and bean growth stage (from R6 to R8). This stable level is in contrast with the instability of the original damage threshold based on pest or pathogen population size (Stem *et al.*, 1959), which compelled Zadoks (1985) to coin the terms *sliding*, *fluctuating*, and *dynamic thresholds.* Thus, HLAI seems to incorporate the ideal conditions to be the key explanatory variable of a transportable disease management system, a system that can be used to produce precise recommendations at the farm level. For this, Lopes *et al.* (1994) proposed the adoption, for each production situation, of a disease-free "control plots". Each production situation should be the most homogeneous possible with regard to factors influencing yield, such as soil, microclimate, cultural practices, and cultivar. Based on the yield-HLAI relationship and using information on the efficiency of the possible control measures and on the costs involved, a conceptually new damage threshold is determined. The threshold is no longer expressed in insects per plant or severity of visible disease, both of uncertain relationship with yield (Waggoner and Berger, 1987), but in units of HLAI. Thus, control measures will only be recommended when the difference between the HLAI of the control plot and the HLAI of the crop is equal, or higher than, the damage threshold (Lopes *et al.*, 1994).

To satisfy the requirements for decision making based on the HLAI approach, new demands are placed on the scientists and on the farmer in the assessment of the crop during the season. They must assess, as usual, the proportion of total leaf area that is affected by disease. The assessment of disease is facilitated by pictorial and diagrammatic keys. In addition, the leaf area index (LAI) must be determined at frequent intervals. To assess LAI, recently, several electronic instruments, such as sun-fleck ceptometer (Chason et al., 1991), multispectral radiometer (Nilsson, 1995; Nutter and Littrell, 1996), and the equipment LAI-2000 (Jesus Junior *et al.*, 2001), have been tested to estimate leaf area and LAI, with promising results for both research studies and precision farming systems (Gaunt and Bryson, 1995).

In order to be better able to understand and explain the effect of angular leaf spot on the yield of common bean, further studies addressing the effects of defoliation and the effect of the pathogen on RUE of the remaining healthy leaves on diseased plants and the redistribution of photosynthates need to be conducted.

Acknowledgements

This research was partially supported by the European Commission (project ERBIC18CT96-0037), FAPEMIG and FINEP. CAPES supported the first author.

References

Aquino VM, Shokes FK, Berger RD, Gorbet DW, Kucharek TA, 1992. Relationships among late leafspot, healthy leaf area duration, canopy reflectance, and pod yield of peanut. *Phytopathology* **82**, 546-552.

BASSANEZI RB, 2000. *EFEITO DE DOENÇAS FOLIARES NA EFICIÊNCIA FOTOSSINTÉTICA DO FEIJOEIRO (PHASEOLUS VULGARIS L.) COMO CONTRIBUIÇÃO NA AVALIAÇÃO DE DANOS*. PIRACICABA, BRAZIL: ESCOLA SUPERIOR DE AGRICULTURA "LUIZ DE QUEIROZ", PHD THESIS.

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 growth and production of a rice crop. 2. Analysis of the reduction in dry matter production, using two models with different complexity. *Netherlands Journal of Plant Pathology* **99**, 19-28.

Bastiaans L, Kropff MJ, 1993. Effects of leaf blast on photosynthesis of rice. 2. Canopy photosynthesis. *Netherlands Journal of Plant Pathology* **99**, 205-207.

Bastiaans L, Rabbinge R, Zadoks JC, 1994. Understanding and modeling leaf blast effects on crop physiology and yield. In: Zeigler RS, Leong SA, Teng PS, eds. *Rice Blast Disease*. Wallingford, England: CAB International, 357-380.

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

Bergamin Filho A, Carneiro SMTPG, Godoy CV, Amorim L, Berger RD, Hau B, 1997. Angular leaf spot of *Phaseolus* beans: relationships between disease, healthy leaf area, and yield. *Phytopathology* **87**, 506-515.

Bergamin Filho A, Lopes DB, Amorim L, Godoy CV, Berger RD, 1995. Avaliação de danos causados por doenças de plantas. *Revisão Anual de Patologia de Plantas* **3**, 133-184.

Bianchini A, 1998. *Mosaico dourado do feijoeiro: crescimento do hospedeiro, progresso da doença e produção.* Piracicaba, Brazil: Escola Superior de Agricultura "Luiz de Queiroz", PhD thesis.

Bissonette SM, D´arcy CJ, Pedersen WL, 1994. Yield loss in two spring oat cultivars due to *Puccinia coronata* f. sp. *avenae* in the presence or absence of barley dwarf virus. *Phytopathology* **84**, 363-371.

BRENES BM, CHAVES GM, ZAMBOLIM L, 1983. ESTIMATIVAS DE PERDAS NO RENDIMENTO DO FEIJOEIRO COMUM (*PHASEOLUS VULGARIS*) CAUSADAS PELA MANCHA ANGULAR (*ISARIOPSIS GRISEOLA* SACC.). *FITOPATOLOGIA BRASILEIRA* **8**, 599.

BRYSON RJ, SYLVESTER-BRADLEY R, SCOTT RK, PAVELEY ND, 1995. RECONCILING THE EFFECTS OF YELLOW RUST ON YIELD OF WINTER WHEAT THROUGH MEASUREMENTS OF GREEN LEAF AREA AND RADIATION INTERCEPTION. IN: WALTERS RD, SCHOLES JD, BRYSON RJ, PAUL ND, MCROBERTS N, EDS. *PHYSIOLOGICAL RESPONSES OF PLANTS TO PATHOGENS*. WELLESBOURNE, WARWICK, ENGLAND: ASSOCIATION OF APPLIED BIOLOGISTS, 9-18.

CANTERI MG, 1998. *USO DE MEDIDAS DA ÁREA FOLIAR SADIA E REFLETÂNCIA NO MANEJO DA MANCHA ANGULAR DO FEIJOEIRO*. PIRACICABA, BRAZIL: ESCOLA SUPERIOR DE AGRICULTURA "LUIZ DE QUEIROZ", PHD THESIS.

CARNEIRO SMTPG, 2000. *EFEITO DA INFECÇÃO CONJUNTA DE* PHAEOISARIOPSIS GRISEOLA *E* COLLETOTRICHUM LINDEMUTHIANUM *NOS COMPONENTES DE PRODUÇÃO E NAS VARIÁVEIS DE ÁREA FOLIAR DO FEIJOEIRO*. PIRACICABA, BRAZIL: ESCOLA SUPERIOR DE AGRICULTURA "LUIZ DE QUEIROZ", PHD THESIS.

CARNEIRO SMTPG, AMORIM L, BERGAMIN FILHO A, 1997. AVALIAÇÃO DE DANO PELA MANCHA ANGULAR EM FEIJOEIRO: RELAÇÃO ENTRE SEVERIDADE, ÁREA FOLIAR E COMPONENTES DE PRODUÇÃO. *FITOPATOLOGIA BRASILEIRA* **22**, 427-431.

Charles-Edwards DA, 1982. *Physiological Determinants of Crop Growth.* Austrália, Sydney: Academic Press.

Chason JW, Baldochi DD, Huston MA, 1991. A comparison of direct and indirect methods for estimating forest canopy leaf area. *Agric. For. Meteorol.* **57**, 107-128.

Ferrandino FJ, Elmer WH, 1992. Reduction in tomato yield due to *Septoria* leaf spot. *Plant Disease* **76**, 208-211.

Gallagher JN, Biscoe PV, 1978. Radiation absorption, growth and yield of cereals. *Journal of Agricultural Science* **91**, 47-60.

Gaunt RE, 1995. The relationship between plant disease severity and yield. *Annual Review of Phytopathology* **33**, 119-144.

GAUNT RE, BRYSON RJ, 1995. PLANT AND CROP YIELD POTENTIAL AND RESPONSE TO DISEASE. IN: WALTERS RD, SCHOLES JD, BRYSON RJ, PAUL ND, MCROBERTS N, EDS. *PHYSIOLOGICAL RESPONSES OF PLANTS TO PATHOGENS*. WELLESBOURNE, WARWICK, ENGLAND: ASSOCIATION OF APPLIED BIOLOGISTS, 1-7.

GIANASI L, 1999. *ANTRACNOSE DO FEIJOEIRO: QUANTIFICAÇÃO DE DANOS E EFEITO DO TRIFENIL ACETATO DE ESTANHO NO CRESCIMENTO DO HOSPEDEIRO E NO PROGRESSO DA DOENÇA.* PIRACICABA, BRAZIL: ESCOLA SUPERIOR DE AGRICULTURA "LUIZ DE QUEIROZ", PHD THESIS.

GODOY CV, 1995. *DANOS CAUSADOS PELA MANCHA ANGULAR EM FEIJOEIRO, NO MUNICÍPIO DE PIRACICABA*. PIRACICABA, BRAZIL: ESCOLA SUPERIOR DE AGRICULTURA "LUIZ DE QUEIROZ", MS DISSERTATION.

GODOY CV, CARNEIRO SMTPG, IAMAUTI MT, DALLA PRIA M, AMORIM L, BERGAMIN FILHO A, 1997. DIAGRAMMATIC SCALES FOR BEAN DISEASES: DEVELOPMENT AND VALIDATION. *ZEITSCHRIFT FUR PFLANZENKRANKHEITEN UND PFLANZENSCHUTZ* **104**, 336-345.

IAMAUTI MT, 1995. *AVALIAÇÃO DOS DANOS CAUSADOS POR* UROMYCES APPENDICULATUS *NO FEIJOEIRO*. PIRACICABA, BRAZIL: ESCOLA SUPERIOR DE AGRICULTURA "LUIZ DE QUEIROZ", PHD THESIS.

Jesus Junior WC de, Vale FXR do, Coelho RR, Costa LC, 2001. Comparison of two methods for estimating leaf area index on common bean. *Agronomy Journal* 91(5) **(in press).**

JOHNSON KB, 1987. DEFOLIATION, DISEASE, AND GROWTH: A REPLY. *PHYTOPATHOLOGY* **77**, 1495-1497.

JOHNSON KB, 1990. ASSESSING MULTIPLE PEST POPULATION AND THEIR EFFECTS ON CROP YIELD. IN: TENG PS, ED. *CROP LOSS ASSESSMENT IN RICE*. MANILA, PHILIPPINES: INTERNATIONAL RICE RESEARCH INSTITUTE, 203-213.

JOHNSON KB, 1992. EVALUATION OF A MECHANISTIC MODEL THAT DESCRIBES POTATO CROP LOSSES CAUSED BY MULTIPLE PESTS. *PHYTOPATHOLOGY* **82**, 363-369.

JOHNSON KB, TENG PS, RADCLIFFE EB, 1987. ANALYSIS OF POTATO FOLIAGE LOSSES CAUSED BY INTERACTING INFESTATIONS OF EARLY BLIGHT, VERTICILLIUM WILT, AND POTATO LEAFHOPPER, AND THE RELATIONSHIP TO YIELD. *ZEITCHRIFT FUR PFLANZEKRANKHEITEN UND PFLANFENSCHUTZ* **94**, 22-23.

Lim LG, Gaunt RE, 1981. Leaf area as a factor in disease assessment. *Journal of Agricultural Science* **97**, 481-483.

LOPES DB, BERGER RD, BERGAMIN FILHO A, 1994. ABSORÇÃO DA ÁREA FOLIAR SADIA (HAA): UMA NOVA ABORDAGEM PARA A QUANTIFICAÇÃO DE DANO E PARA O MANEJO INTEGRADO DE DOENÇA. *SUMMA PHYTOPATHOLOGICA* **20**, 143-151.

Madden LV, Nutter Jr FW, 1995. Modeling crop losses at the field scale. *Canadian Journal of Plant Pathology* **17**, 124-137.

Madeira AC, Clark JA, 1994. Disease and resource capture. In: Monteith JL, Scott RK, Unsworth MH, eds. *Resource Capture by Crops*. Loughborough, Leicestershire, England: Nottingham University Press, 167-188.

Madeira AC, Clark JA, 1995. The principles of resource capture in relation to necrotrophic infection. In: Walters RD, Scholes JD, Bryson RJ, Paul ND, McRoberts N, eds. *Physiological Responses of Plants to Pathogens*. Wellesbourne, Warwick, England: Association of Applied Biologists, 19-31.

Madeira AC, Clark JA, Rossall S, 1994. Growth and light interception in field bean (*Vicia faba*) infected by *Ascochyta fabae*. *Journal of Agricultural Science* **123**, 225-232.

Miglioranza E, 1992. *Modelo matemático-fisiológico para simular o crescimento e a produtividade da cultura do feijão* (Phaseolus vulgaris). Viçosa, Brazil: Universidade Federal de Viçosa, PhD thesis.

Monteith JL, Elston J, 1983. Performance and productivity in the field. In: Dale JE, Milthorpe FL, ed. *The growth and functioning of leaves.* Cambridge, UK: University Press, 499-518.

Nilsson HE, 1995. Remote sensing and image analysis in plant pathology. *Can. J. Plant Pathol.* **17**, 154-166.

Nutter Jr FW, Littrell RH, 1996. Relationships between defoliation, canopy reflectance and pod yield in the peanut-late leafspot pathosystem. *Crop Protection* **15**, 135-142.

Pinnschmidt HO, Teng PS, Young L, 1994. Methodology for quantifying rice yield effects of blast. In: Zeigler RS, Leong SA, Teng PS, eds. *Rice Blast Disease*. Wallingford, England: CAB International, 381-408.

Rossing WAH, Van Oijen M, van der Werf W, Bastiaans L, Rabbinge R, 1992. Modeling the effects of foliar pests and pathogens on light interception, photosynthesis, growth rate and yield of field crops. In: Ayres PG, ed. *Pest and Pathogens. Plant responses to Foliar Attack*. Oxford, UK: Bios Scientific Publishers, 161-180.

SCHWARTZ HF, CORREA V, PINEDA DPA, OTOYA MM, KATHERMAN MJ, 1981. DRY BEAN YIELD LOSSES CAUSED BY ASCOCHYTA, ANGULAR, AND WHITE LEAF SPOTS IN COLOMBIA. *PLANT DISEASE* **65**, 494-496.

SILVA MB, VALE FXR, ZAMBOLIM L, HAU B, 1998. EFEITOS DA FERRUGEM, DA ANTRACNOSE E DA MANCHA ANGULAR NA ÁREA FOLIAR DE PLANTAS DE FEIJOEIRO EM CONDIÇÕES DE CAMPO. *FITOPATOLOGIA BRASILEIRA* **23**, 442-447.

STERN VM, SMITH RF, VAN DEN BOSCH R, HAGEN KS, 1959. THE INTEGRATED CONTROL CONCEPT. *HILGARDIA* **28**, 81-101.

VAN SCHOONHOVEN A, PASTOR-CORRALES MA, 1987. *SISTEMA ESTÁNDAR PARA LA EVALUACIÓN DE GERMOPLASMA DE FRIJOL.* CALI, COLOMBIA: CIAT.

Waggoner PE, 1990. Assembling and using models of epidemics. In: Kranz J, ed. *Epidemics of Plant Diseases: Mathematical Analysis and Modeling.* Berlin, Germany: Springer, 230-260.

WAGGONER PE, BERGER RD, 1987. DEFOLIATION, DISEASE, AND GROWTH. *PHYTOPATHOLOGY* **77**, 393-398.

WAIBEL H, 1990. REQUIREMENTS FOR AN ECONOMIC INTERPRETATION OF CROP LOSSES. IN: TENG PS, ED. *CROP LOSS ASSESSMENT IN RICE*. MANILA, PHILIPPINES: INTERNATIONAL RICE RESEARCH INSTITUTE, 313-320.

WATSON DJ, 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.

WRIGHT AC, GAUNT RE, 1992. DISEASE-YIELD RELATIONSHIP IN BARLEY. I. YIELD, DRY MATTER ACCUMULATION AND YIELD LOSS MODELS. *PLANT PATHOLOGY* **41**, 676-687.

Zadoks JC, 1985. On the conceptual basis of crop loss assessment: The threshold theory. *Ann. Rev. Phytopathol.* **23**, 455-473.

CHAPTER 4

Strategies to manage angular leaf spot on *Phaseolus vulgaris* based in molybdenum application and chemical control

W. C. JESUS JUNIOR[1], F. X. R. VALE[1], L. ZAMBOLIM[1], R. R. COELHO[1], L. C. COSTA[2] AND B. HAU[3]
[1] DEPARTAMENTO DE FITOPATOLOGIA, UNIVERSIDADE FEDERAL DE VIÇOSA, 36571-000 VIÇOSA, MG, BRAZIL
[2] DEPARTAMENTO DE ENGENHARIA AGRÍCOLA, UNIVERSIDADE FEDERAL DE VIÇOSA, 36571-000 VIÇOSA, MG, BRAZIL
[3] INSTITUT FUR PFLANZENKRANKHEITEN UND PFLANZENSCHUTZ, UNIVERSITAT HANNOVER, HERRENHAUSER STR. 2, 30419 HANNOVER, GERMANY

*Paper will be submitted to Journal of Plant Diseases and Protection.

Summary

Although common bean (*Phaseolus vulgaris* L.) has a good potential for N_2 fixation, poor nodulation, principally under field conditions, has led to increased nitrogen (N) fertilizer in this crop. Previous results showed that application of molybdenum (Mo) to common bean foliage improve crop growth. Two field experiments were conducted to study the influences of molybdenum spray of the bean foliage on the disease progress of angular leaf spot (*Phaeoisariopsis griseola*) as well as on the plant growth (leaf area and photosynthesis) and yield. Significant differences were found for the area under disease progress curve (AUDPC), area under leaf area progress curve (AULAPC), photosynthesis and yield for treatments that received Mo when compared with those without Mo. The molybdenum treatments showed smaller AUDPC, and higher AULAPC, photosynthesis and yield, than the treatments that had no Mo. To control angular leaf spot, it was found important to spray once or twice a fungicide close to the bean flowering period, which takes place ca. 25 to 45 days after planting.

Key words: *Phaeoisariopsis griseola*, *Phaseolus vulgaris*, molybdenum, fungicide.

Zusammenfassung

1 Introduction

Although common bean (*Phaseolus vulgaris* L.) has a good potential for N_2 fixation, poor nodulation, principally under field conditions, has led to increased nitrogen (N) fertilizer in this crop. Utilization of mineral N is expensive, and besides, entails some environmental risks when used continuously (STIKA et al. 1993). In the face of the negative environmental effects of N fertilizer, alternative methods have been studied to minimize the amount to be applied. In this sense, foliar application of molybdenum (Mo) has been cited as a promising method. Several papers (ROBITAILLE 1975; JUNQUEIRA NETTO et al. 1977; VIEIRA et al. 1992; AMANE et al. 1994; RODRIGUES et al. 1996) showed that high bean yields (1.500 - 2.500 $Kg.ha^{-1}$) may be obtained in the southeastern region of Brazil, where either application of N as side dressing or Mo spray 25 days after plant emergence, at negligible cost. VIEIRA et al. (1998 a, b) showed that foliar application of Mo resulted in increases of acetylene reduction activity and nitrate reductase activity, and in a greater N remobilization during pod-filling stage. The combination of all these activated processes resulted in higher grain yields. However these effect were not observed in all region of Brazil (ANDRADE et al., 1999; ARAÚJO et al., 1999; BASSAN et al., 1999; SORATTO et al., 1999a, b; VIEIRA et al., 1999), due to the soil characteristics. Additionally, Mo could act in disease reduction.

There have been few reports associating Mo with response of plants to disease (GRAHAM 1983) and no reports have been found that specifically address the effects of Mo deficiency. However, DUTTA and BREMMER (1981) and MILLER and BECKER (1983) demonstrated that Mo applied to tomato roots reduced the symptoms of *Verticillium* wilt. Molybdenum had a direct effect by reducing the production of the roridin E, a toxin produced by *Myrothecium roridum* (FERNANDO et al. 1986), and in slightly inhibiting zoosporangia formation by *Phytophthora cinnamomi* and *P. dreschleri* (HALSALL 1977). Soil application of Mo decreased nematode populations (HAQUE and MUKHOPADHYAYA 1983).

It is not know whether Mo within the host plays any specific role in protecting plants from disease and, because of the requirement for Mo by the enzymes nitrogenase and nitrate reductase (SHKOLNIK 1984; MARSCHNER 1986), any effect of Mo deficiency on pathogenesis may be indirect through an effect on N metabolism.

Angular leaf spot of beans (*Phaseolus vulgaris* L.), caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris, is a disease of world-wide occurrence (CORREA-VICTORIA et al. 1989). The disease has recently become one of the main problems of bean crops in Brazil (VALE et al. 1997). The pathogen causes lesions on the leaves, pods, branches, and petioles and may cause severe defoliation. In the absence of adequate control measures, yield reductions of 70% in Brazil (BRENES et al. 1983) and 80% in Colombia (SCHWARTZ et al. 1981) have been reported.

Due to the potential to improve crop growth and on disease control with the application of molybdenum, two experiments were carried out with the aim to study the influences of molybdenum spray of the bean foliage on the disease progress of angular leaf spot as well as on the plant growth and yield.

Considering that the common bean is one of the most important crop in the Americas, **costless** management system of angular leaf spot, that could cause less pollution to the environment, is urgently needed in Latin America to obtain a sustainability of bean crop.

2 Materials and methods

Field experiments. Under natural field conditions, two field experiments were carried out at the Viçosa Federal University, Viçosa, Minas Gerais State, Brazil, from October to December 1998 and from March to May 1999, with the bean cultivar Carioca, to study how molybdenum (Mo) sprayed on the bean foliage influences plant growth and progress of angular leaf spot. The effect of Mo was evaluated on the disease development (area under disease progress curves, AUDPC), plant growth (area under leaf area progress curves, AULAPC), photosynthesis and their relationships to yield.

A split-plot design was employed with eight treatments and three replications, in which a group of eight treatments received Mo and another group of eight treatments did not receive it, for a total of 48 plots. The experimental blocks consisted of eight six-meter long rows, and 0.5 m was kept between two successive rows ($24m^2$). Twelve seeds were sown and 10 plants were allowed to grow per meter of row. The plots were maintained with the conventional cultural practices used in commercial fields, including planting and topdressing fertilization, insecticide sprays, weeding and, when necessary, sprinkle irrigation. A backpack mistblower was used to apply sodium molybdate (20 g Mo/ha) in solution on the bean foliage 25 days after seeding (BERGER et al. 1996). In addition, a chemical control system was established to test strategies to control angular leaf spot adjusted to different stages of plant growth. To study the disease effects at different plant growth stages, tebuconazole was sprayed on bean plants at 0.75 kg per ha (187.5 ai per ha) before flowering, during and after flowering.

Treatments for fungicide spraying, with and without molybdenum were: 1) No spray; 2) Spray on the 25th day after planting (DAP); 3) Spray on the 35th DAP; 4) Spray on the 45th DAP; 5) Spray on the 25th and 35th DAP; 6) Spray on the 25th and 45th DAP; 7) Spray on the 35th and 45th DAP, and 8) Spray on the 25th, 35th and 45th DAP.

The soil analysis results for the first and second trial was: pH in water (1:25) 5.6 and 6.5; P disponible[1] (mg/dm^3) 4.3 and 4.8; K disponible[1] (mg/dm^3) 62.0 and 70.0; Ca^{2+} exchange ($cmol_c/dm^3$) 1.4 and 1.8; Mg^{2+} exchange ($cmol_c/dm^3$) 0.6 and 0.7; and Al^{+3} exchange ($cmol_c/dm^3$) 0.0 and 0.0, respectively for the first and second trial.

Mo content in this soil was low, as determined in previous experiments in this area.

Crop growth, disease severity, photosynthesis and yield. Crop growth and disease severity were evaluated in the eight central rows of each plot (disregarding 0.5 m at each end of the row). The leaf area (LA, cm^2) of all leaves of the five plants, chosen randomly per plot, was estimated using an area meter, starting from the appearance of the first trifoliolate leaf. Assessment of the severity of angular leaf spot was done with the aid of a diagrammatic scale (GODOY et al. 1997), and the average severity (percent) for the three leaflets of all leaves on all collected plants was estimated. Even though defoliation was observed, it was not quantified. The cultivar Carioca has indeterminate, polybrachiate growth habit, and it was difficult to identify specific leaves in sequential assessments. Thus, the number, area and original position of fallen leaves were not determined. At each evaluation, the host growth stage was determined, according to the descriptive scale of VAN SCHOONHOVEN and PASTOR-CORRALES (1987).

A LI-6400 Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA), with a leaf chamber, was used to measure CO_2 uptake, air temperature and relative humidity. Two leaves at the same physiological rate were sampled per plant, 5 replications per plot. Measurements were done between 8 and 11 a.m., one for each leaf, at the central area of the main leaflet, using a PAR of the 650 $\mu mol\ m^{-2}\ s^{-1}$. Results were expressed as net photosynthetic rate (A, $\mu mol\ m^{-2}\ s^{-1}$). All evaluations were made weekly and on days under clear weather conditions, with occasional clouds and slight haze.

To estimate yield (Kg plot^{-1}), all seeds (12% moisture) from each plot were weighed at the end of the crop cycle .

Integral variables. The AUDPC and AULAPC values for each plant were calculated by trapezoidal integration:

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{X_i + X_{i+1}}{2} \right) (t_{i+1} - t_i)$$

$$AULAPC = \sum_{i=1}^{n-1} \left(\frac{LA_i + LA_{i+1}}{2} \right) (t_{i+1} - t_i)$$

In which *n* is number of assessments, X is the disease severity of angular leaf spot (percent), LA is the leaf area (cm^2) and ($t_{i+1} - t_i$) is the interval between two consecutive assessments.

Under the assumption of 20 plants per m^2, the leaf area index (LAI) can be calculated by multiplying LA (cm^2) by 0.002. The healthy leaf area index (HLAI) can then be determined as HLAI = LAI [(1-0.01(X)].

Data analysis. **Analysis of variance were conducted using the SAS system.**

3 Results and discussion

Significant differences were found for the area under the disease progress curve (AUDPC), area under the leaf area progress curve (AULAPC), photosynthesis and yield for treatments that received Mo when compared with those without Mo. The molybdenum treatments showed smaller AUDPC, and higher AULAPC, photosynthesis, and yield, than the treatments that had no Mo.These results corroborate with other authors concerning the increase in leaf area and yield (ROBITAILLE 1975; JUNQUEIRA NETTO et al. 1977; VIEIRA et al. 1992; AMANE et al. 1994; RODRIGUES et al. 1996) and concerning the decrease in disease (HALSALL 1977; DUTTA and BREMMER 1981; GRAHAM 1983; HAQUE and MUKHOPADHYAYA 1983; MILLER and BECKER 1983; FERNANDO et al. 1986).

Treatments 2, 3, 6, 7 and 8 had less AUDPC in relation to the other treatments (Figure 1). These treatments received a spray between 25 and/or 35 days after planting, showing that it was important to spray one or two times the bean foliage during the interval of 25 to 45 days after planting to control angular leaf spot. It is also important to note that the flowering stage occurs during this interval (25 to 45 days after planting); so the protection of common bean foliage against diseases during this period is important to maintain the yield. The analysis of the AULAPC (Figure 2) and photosynthesis (Figure 3) showed that best treatments in terms of disease control were those with higher leaf area and photosynthesis. The reduction in leaf area observed in the treatments that had more disease could be explained because the great effect of angular leaf spot is defoliation (CARNEIRO et al. 1997; VALE et al. 1997; SILVA et al. 1998). In addition, this disease causes a lesion in the leaf, with necrotic and yellowing areas, in which the photosynthesis is lower than a healthy area (STANGARLIN 1999; BASSANEZI et al. 2000).

The treatments sprayed at 25 and/or 35 days after planting, resulted in yield increase compared to the control and to the treatment that was sprayed only at 25 days after planting (Figure 4). Similar result was obtained by BARROS et al. (1992), which demonstrated that the best control of angular leaf spot was obtained spraying at 20, 35 and 50 days after plant emergence.

The difference observed in all characteristics evaluated in terms of application or not of Mo probably occur because the function of this micronutrient: molybdenum ions are components of several enzymes, including nitrate reductase and nitrogenase. Nitrate reductase catalyzes the reduction of nitrate to nitrite during its assimilation by the plant cell; nitrogenase converts nitrogen gas to ammonia in nitrogen-fixing microorganisms. Although plants require only small amounts of molybdenum, some soils supply inadequate levels. Small additions of molybdenum to such soils or application of molybdenum directly to the foliage can greatly enhance crop or forage growth at negligible cost (TAIZ and ZEIGER 1998). Probably this micronutrient could induce resistance of the plant against pathogens. Several chemicals, that are not directly antimicrobial, have also been reported to induce resistance to pathogens when applied to plants. However more studies will be necessary to elucidate the function of this micronutrient in terms of control of plant disease.

The most important result obtained with these experiments was that Mo application on bean foliage could result in an increase of plant growth and it could improve disease control. These results indicated the potential of using molybdenum spray in an angular leaf spot management system, without increase production costs, because of the low price of the salt (sodium molibdate).

It is argued that adequate micronutrient nutrition should be viewed as an essential component of any integrated crop protection program because, by virtue of the small amounts needed, micronutrients in general are the cheapest agricultural chemicals on the market. Although disease remission is rarely complete, micronutrient fertilization may reduce the disease to an acceptable level, or at least to a level at which further control by other cultural practices or conventional organic biocides are more successful and less expensive.

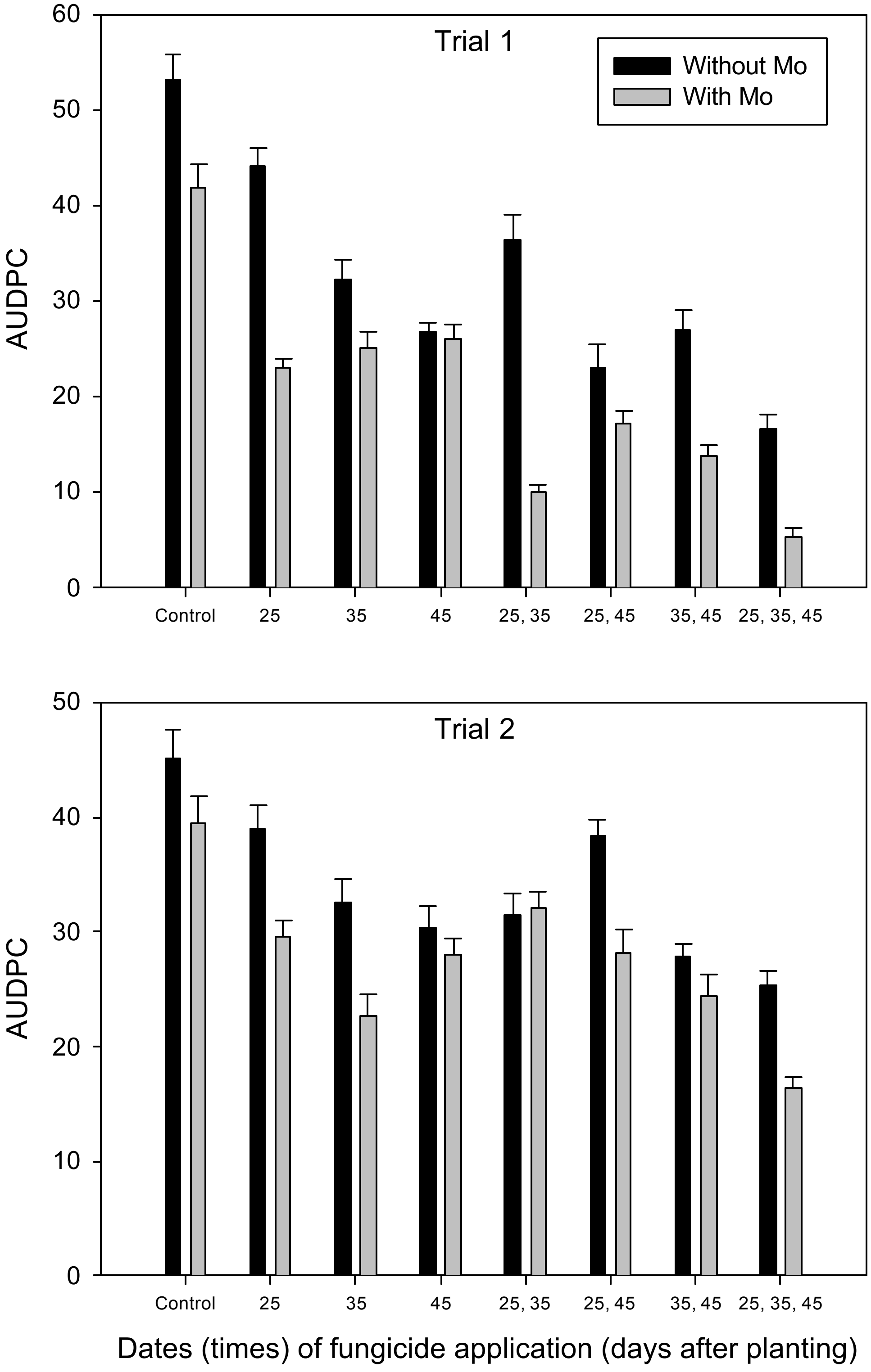

Trial 1
Without Mo
With Mo
AUDPC
60
50
40
30
20
10
0
Control
25
35
45
25, 35
25, 45
35, 45
25, 35, 45
Trial 2
AUDPC
50
40
30
20
10
0
Control
25
35
45
25, 35
25, 45
35, 45
25, 35, 45
Dates (times) of fungicide application (days after planting)

Figure 1. Area under angular leaf spot progress curves (AUDPC) for the eight treatments, with and without molybdenum application. Vertical bars represent ± standard error.

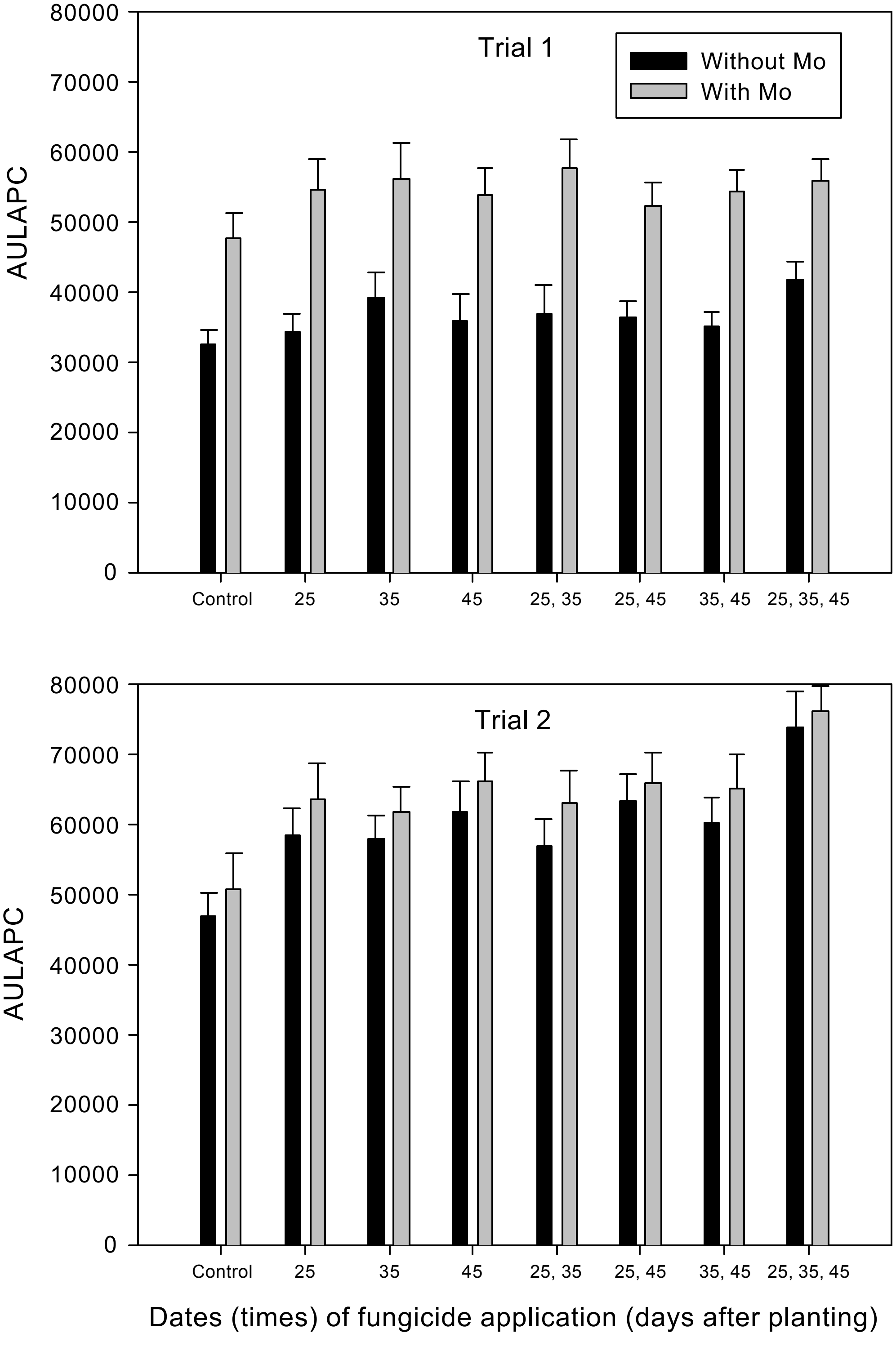


Figure 2. Area under leaf area progress curves (AULAPC) for the eight treatments, with and without molybdenum application. Vertical bars represent ± standard error.

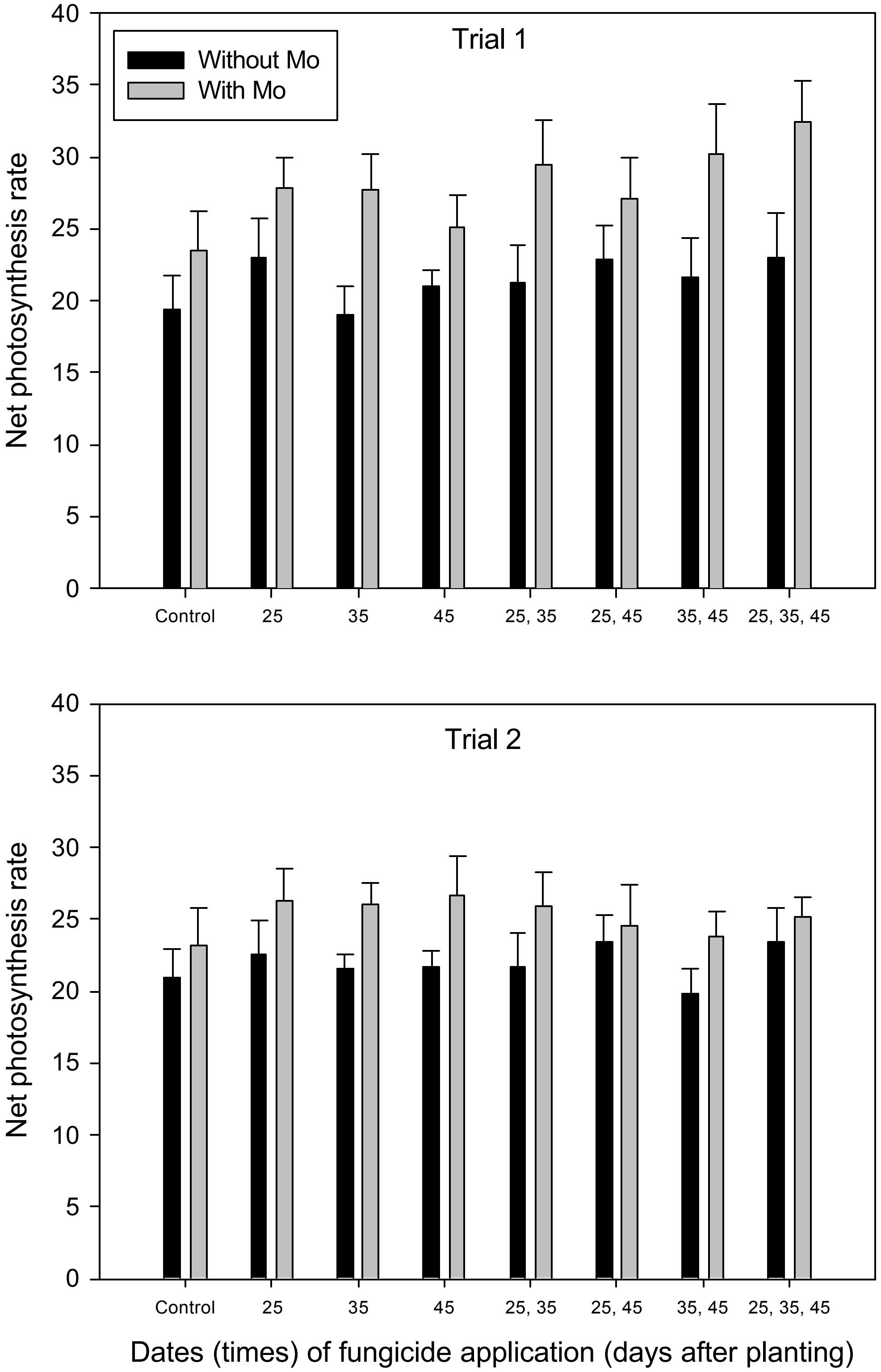


Figure 3. Net photosynthesis rate (µmol CO_2. m^{-2}. s^{-1}) for the eight treatments, with and without molybdenum application. Vertical bars represent ± standard error.

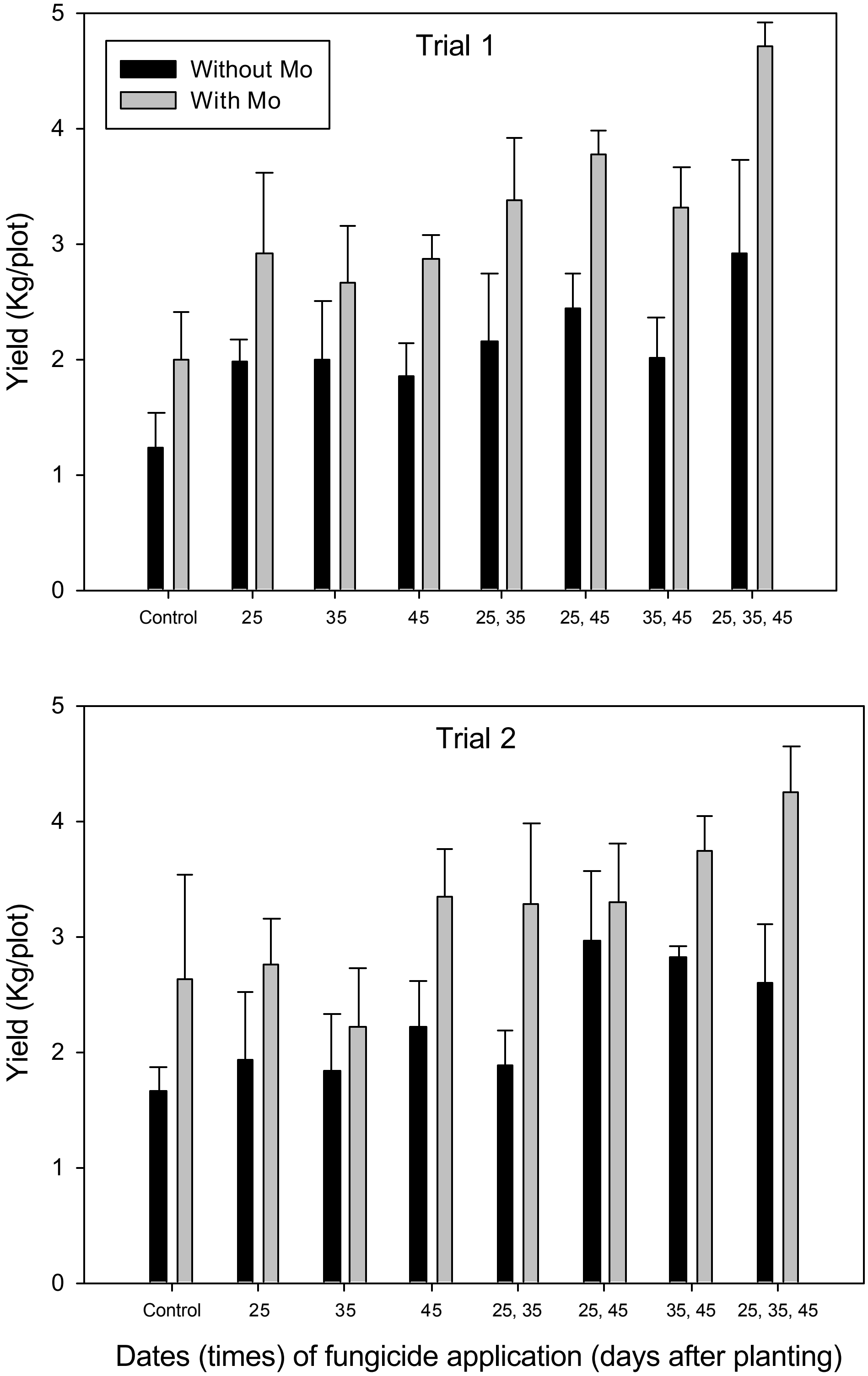


Figure 4. Yield (Kg/plot) for the eight treatments, with and without molybdenum application. Vertical bars represent ± standard error.

Acknowledgements

This research was partially supported by the European Commission (project ERBIC18CT96-0037), FINEP, and FAPEMIG. First author was supported by CAPES.

Literature

AMANE, M. I. V., C. VIEIRA, A. A. CARDOSO, G. A. A. ARAÚJO: RESPOSTA DE CULTIVARES DE FEIJÃO (*PHASEOLUS VULGARIS* L.) ÀS ADUBAÇÕES NITROGENADA E MOLÍBDICA. - REVISTA CERES **41**, 202-16, 1994.

ANDRADE, C. A. B., J. C. PINTRO, C. A. SCAPIM, E. B. dos SANTOS: Efeitos de doses de molibdênio no rendimento e nutrição de duas cultivares de feijão. - RENAFE, 801-804, 1999.

ARAÚJO, P. R. A., L. R. FERREIRA, G. A. A. ARAÚJO, J. M. GOMES: Eficácia da aplicação de molibdênio em mistura no tanque com herbicidas pós-emergentes sobre o feijoeiro. - RENAFE, 480-483, 1999.

BARROS, B. C., J. L. CASTRO, S. H. F. OLIVEIRA, D. A. OLIVEIRA: Efeito do número de aplicações de mancozeb em diferentes épocas no controle de doenças e no rendimento do feijoeiro (*Phaseolus vulgaris*). - Summa Phytopathologica **18**, 276-282, 1992.

BASSAN, D. A. Z., O ARF, S. BUZETTI, N. C. B. dos SANTOS, M. A. C. de CARVALHO: Efeito da inoculação de sementes e aplicação de nitrogênio e molibdênio sobre a produção de feijão (Phaseolus vulgaris L.) no período de inverno. - RENAFE, 785-787, 1999.

BASSANEZI, R. B., L. AMORIM, A. BERGAMIN FILHO: Análise das trocas gasosas em feijoeiro com ferrugem, mancha angular e antracnose. - Fitoptol Bras **25**, 643-650, 2000.

BERGER, P. G., C. VIEIRA, G. A. de ARAÚJO: Efeitos de doses e épocas de aplicação do molibdênio sobre a cultura do feijão. - Pesq. Agropec. Bras. **31**, 473-80, 1996.

BRENES, B. M., G. M. CHAVES, L. ZAMBOLIM: ESTIMATIVAS DE PERDAS NO RENDIMENTO DO FEIJOEIRO COMUM (*PHASEOLUS VULGARIS*) CAUSADAS PELA MANCHA ANGULAR (*ISARIOPSIS GRISEOLA* SACC.). - FITOPATOLOGIA BRASILEIRA **8**, 599, 1983.

CARNEIRO, S. M. T. P. G., L. AMORIM, A. BERGAMIN FILHO: Avaliação de dano provocado pela mancha angular em feijoeiro: relação entre severidade, área foliar e componentes de produção. - Fitopatologia Brasileira **22**, 427-431, 1997.

CORREA-VICTORIA, F. J., M. A. PASTOR-CORRALES, A. W. SAETTLER: Angular leaf spot. - In: SCHWARTZ, H. F., M. A. PASTOR-CORRALES (eds.): Bean Production Problems in the Tropics, 2^{nd}. - CIAT (International Center of Tropical Agriculture), pp. 59-75, Cali, Colômbia, 1989.

DUTTA, B. K., E. BREMMER: Trace elements as plant chemotherapeutants to control *Verticillium* wilt. - Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz **88**, 405-412, 1981.

FERNANDO, T., B. B. JARVIS, G. BEAN: Effects of micro-elements on production of roridin E by *Mirothecium roridum*, a strain pathogenic to muskmelon (*Cucumis melo*). - Trans. Br. Mycol. Soc. **86**, 273-277, 1986.

GODOY, C. V., S. M. T. P. G. CARNEIRO, M. T. IAMAUTI, M. DALLA PRIA, L. AMORIM, A. BERGAMIN FILHO: DIAGRAMMATIC SCALES FOR BEAN DISEASES: DEVELOPMENT AND VALIDATION. - ZEITSCHRIFT FUR PFLANZENKRANKHEITEN UND PFLANZENSCHUTZ **104,** 336-45, 1997.

GRAHAM, R. D.: Effect of nutrient stress on susceptibility of plants to disease with particular reference to the trace elements. - Adv. Bot. Res. **10**, 221-276, 1983.

HALSALL, D. M.: Effects of certain cations on the formation and infectivity of *Phytophthora* zoospores. 2. Effects of copper, boron, cobalt, manganese, molybdenum, and zinc ions. - Can. J. Microbiol. **23,** 1002-1010, 1977.

HAQUE, M. S., M. C. MUKHOPADHYAYA: Influence of some micro-nutrients on *Rotylenchus reniformis*. - Indian J. Nematol. **13,** 115-116, 1983.

JUNQUEIRA NETTO, A., O. S. DOS SANTOS, H. AIDAR, C. VIEIRA: ENSAIOS PRELIMINARES SOBRE A APLICAÇÃO DE MOLIBDÊNIO E DE COBALTO NA CULTURA DO FEIJÃO (*PHASEOLUS VULGARIS*). REV. CERES **24**, 628-33, 1977.

MARSCHNER, H.: Mineral Nutrition of Higher Plants. Academic Press, London, 1986.

MILLER, V. R., Z. E. BECKER: The role of microelements in cotton resistance to *Verticillium* wilt. - Sel'skokhoz. Biol. **11,** 54-56, 1983.

ROBITAILLE, H. A.: Effect of foliar molybdenum sprays on nitrogen fixation in *Phaseolus vulgaris* L. - Annu. Rep. Bean Improvement Coop. **18**, 65, 1975.

RODRIGUES, J. R. DE M., M. J. B. DE ANDRADE, J. G DE CARVALHO: RESPOSTA DE CULTIVARES DE FEIJÃO (*PHASEOLUS VULGARIS*) A DOSES DE MOLIBDÊNIO APLICADAS VIA FOLIAR. CIENC. AGROTEC. **20**, 323-33, 1996.

SCHWARTZ, H. F., V. CORREA, D. P. A. PINEDA, M. M. OTOYA, M. J. KATHERMAN: DRY BEAN YIELD LOSSES CAUSED BY ASCOCHYTA, ANGULAR AND WHITE LEAF SPOTS IN COLOMBIA. PLANT DIS. **65**, 494-496, 1981.

SHKOLNIK, M. Y.: Trace Elements in Plants. Elsevier, Amsterdam, 1984.

SILVA, M. B., F. X. R. do VALE, L. ZAMBOLIM, B. HAU: Efeitos da ferrugem, da antracnose e da mancha angular na área foliar de plantas de feijoeiro em condições de campo. - Fitopatologia Brasileira **23,** 442-447, 1998.

SORATTO, R. P., T. R. B. da SILVA, S. N. CHIDI, O. ARF, M. E. de SÁ: Resposta do feijoeiro (*Phaseolus vulgaris* L.) à aplicação de nitrogênio em cobertura e molibdênio via foliar. II - Qualidade fisiológica das sementes. - RENAFE, 595-598, 1999b.

SORATTO, R. P., T. R. B. da SILVA, S. N. CHIDI, O. ARF, S. BUZETTI: Resposta do feijoeiro (*Phaseolus vulgaris* L.) à aplicação de nitrogênio em cobertura e molibdênio via foliar. I - Características agronômicas. - RENAFE, 854-857, 1999a.

STANGARLIN, J. R.: Atividades de ribulose-1,5-bifosfato carboxilase (Rubisco), clorofilase, b-1,3-glucanase e quitinase e conteúdo de clorofila em cultivares de feijoeiro (*Phaseolus vulgaris*) infectados com *Uromyces appendiculatus* ou *Phaeoisariopsis griseola*. Ph.D. Thesis – ESALQ/USP, Brazil, 1999.

STIKA, U., K. A. SMITH, D. FOWLER: NITRIFICATION AND DENITRIFICATION AS SOURCES OF NITRIC OXIDE IN A SANDY LOAM SOIL. - SOIL BIOL. BIOCHEM. **25**, 1527-1536, 1993.

TAIZ, L., E. ZEIGER: PLANT PHYSIOLOGY. SECOND EDITION, 792P. 1998.

VALE, F.X.R DO, H. COSTA, L. ZAMBOLIM: FEIJÃO COMUM: DOENÇAS DA PARTE AÉREA CAUSADAS POR FUNGOS. - IN: VALE, F.X.R DO, L. ZAMBOLIM (EDS.): CONTROLE DE DOENÇAS DE PLANTAS: GRANDES CULTURAS. SUPREMA GRÁFICA, PP. 341-350, BRASÍLIA, BRASIL, 1997.

VAN SCHOONHOVEN, A., M. A. PASTOR-CORRALES: SISTEMA ESTÁNDAR PARA LA EVALUACIÓN DE GERMOPLASMA DE FRIJOL. CIAT (INTERNATIONAL CENTRE OF TROPICAL AGRICULTURE), CALI, COLÔMBIA, 1987.

VIEIRA, C., A. O. NOGUEIRA, A. DE ARAÚJO: ADUBAÇÃO NITROGENADA E MOLÍBDICA NA CULTURA DO FEIJÃO. - REV. AGRIC. **67,** 117-24, 1992.

VIEIRA, R. F., C. VIEIRA, E. J. B. N. CARDOSO, P. R. MOSQUIM: FOLIAR APPLICATION OF MOLYBDENUM IN COMMON BEAN. II. NITROGENASE AND NITRATE REDUCTASE ACTIVITIES IN A SOIL OF LOW FERTILITY. - JOURNAL OF PLANT NUTRITION **21**, 2141-2151, 1998B.

VIEIRA, R. F., E. J. B. N. CARDOSO, C. VIEIRA, S. T. A. CASSINI: FOLIAR APPLICATION OF MOLYBDENUM IN COMMON BEANS. I. NITROGENASE AND NITRATE REDUCTASE ACTIVITIES IN A SOIL OF HIGH FERTILITY. - JOURNAL OF PLANT NUTRITION **21**, 169-180, 1998A.

VIEIRA, S. M., P. RONZELLI JÚNIOR, H. S. KOEHLER, B. M. S. PREVEDELLO: Nitrogênio, molibdênio e inoculante, isolados e associados para duas variedades de feijoeiro-comum. - RENAFE, 835-838, 1999.

CHAPTER 5

COMPARISON OF TWO METHODS FOR ESTIMATING LEAF AREA INDEX ON COMMON BEAN

Waldir Cintra de Jesus Junior[1], Francisco Xavier Ribeiro do Vale[1*], Reginaldo Resende Coelho[1] and Luiz Claúdio Costa[2]

Departamentos de [1] Fitopatologia e [2] Engenharia Agrícola, Universidade Federal de Viçosa, 36571-000 Viçosa, Minas Gerais State, Brazil.

Received ---

***Author for correspondence: Francisco X. R. do Vale (E-mail: dovale@mail.ufv.br)**

ACKNOWLEDGMENTS

This research was partially supported by the European Commission (Project ERBIC18CT96-0037), FAPEMIG, and FINEP. First author was supported by CAPES. We thank J. Marois (University of Florida) for the review of this paper and suggestions for its improvement.

This paper has been approved for publication in Agronomy Journal and is scheduled to appear in the Sept.-Oct. 2001 (Vol. 93, No. 5) issue.

ABSTRACT

Measurement of leaf area index (LAI) is important in studies of plant growth. Based on this concept, the analysis of crop growth has moved from the classical growth analysis to the mechanistic models, via the functional growth analysis. LAI is a major variable in mechanistic crop growth models, and in models that attempt to simulate loss caused by pathogens. A field experiment was carried out during the months of May to August 1998, using common bean (*Phaseolus vulgaris* L.) cv. Carioca, in a randomized complete block design, with two methods of evaluation of LAI and three replications. The objective of this study was to compare the applicability of the LAI-2000 Plant Canopy Analyzer with the central leaflet width method used to estimate LAI in common bean. The high correlation ($r^2 = 0.97$) observed between the two methods used to estimate LAI shows agreement between the two methods. The results of this study indicated that the LAI-2000 can be used to estimate the LAI of common bean.

The quantitative effects of environmental factors on plant growth have been studied for more than 80 yr. The basic component of this study, relative growth rate, was introduced by Blackman (1919). Watson (1947) was the first to recognize that the analysis of crop growth should be based on unit of field area rather than per plant, and introduced the concept of leaf area index (LAI), the plan area of leaves per unit area of ground beneath them. From then on, the analysis of crop growth has moved from classical growth analysis to mechanistic models, via functional growth analysis (Radford, 1967; Monteith, 1977; Jones and Kiniry, 1986; Tei et al., 1996; Costa et.al.,1997). The concept of LAI is now a major variable in mechanistic crop growth models (Goudriaan and van Laar, 1994) and in models that attempt to simulate loss caused by pathogens (Waggoner and Berger, 1987).

The leaf area of a crop is a determinant factor in mechanisms such as radiation interception and water and energy exchange. Therefore, accurate measurements of LAI are essential to understand the interaction between crop growth and environment.

The determination of LAI in phytopathological studies has become indispensable in calculating healthy leaf area duration (HAD, days) and healthy leaf area absorption (HAA, $MJ.m^{-2}$) in crop loss assessment (Waggoner and Berger, 1987). Several papers have been published considering the relationships among disease, healthy LAI, and yield (Bergamin Filho et al., 1997; Carneiro et al., 1997; Silva et al., 1998).

Direct measurement of LAI in crops is commonly carried out by measuring manually, or with equipment, the total leaf area of the plant in relation to the soil area covered by these leaves. This evaluation is difficult and time consuming, as demonstrated by Daughtry and Hollinger (1984) in an experiment that examined the trade-off between labor costs and measurement accuracy of several direct methods for measuring LAI in corn (Zea mays L.). Direct measurements are generally collected at the end of the growing season, and are prone to very large errors and that may lead to inconclusive results in crop growth analysis (Monteith, 1981; Costa et al., 1997).

Indirect techniques, which are based on the close coupling between radiation penetration and canopy structure, are a good alternative to the direct LAI measurement techniques. The measurement of canopy gap fractions (the fraction of sky visible through the canopy) at various angles is a particularly powerful approach. Gap fractions have been measured using fisheye photographs (Anderson, 1971), by traversing a sunward-pointed sensor beneath the canopy (Ross, 1981; Lang and Yuequin, 1985; Perry et al., 1988), by linear light sensors (Walker et al., 1988), and by pushing metal probes through the canopy (Wilson and Reeve, 1959). A more detailed discussion of various direct and indirect techniques can be found in Goel and Norman (1990).

In studies of common bean, LAI may also be estimated using an empirical relationship between the maximum width of the central leaflet and total leaf area using a regression equation (Iamauti, 1995). This method is accurate (Iamauti, 1995) but also very time consuming, laborious, and difficult to carry out in the field. These surveys might be done more efficiently using other techniques. Fast and accurate alternatives for LAI assessment would improve parameter estimation under field conditions.

The objective of this study was to compare estimates of LAI by the LAI-2000 Plant Canopy Analyzer (LI-COR, 1990) with those obtained by central leaflet width method in common bean.

MATERIALS AND METHODS

The experiment was carried out at the Vila Gianete experimental field of the Federal University of Viçosa, Minas Gerais State, Brazil from May through August 1998, using common bean cultivar. Carioca. A randomized complete block design was used with two treatments and three replications. Each plot (16m^2) consisted of eight rows, 4 m long, spaced 0.5 m apart. Twelve seeds were sown, and 10 plants were allowed to grow per linear meter of row. The plots were maintained with conventional cultural practices used in commercial fields, including planting and topdressing fertilization, insecticide sprays, weeding, and, when necessary, overhead irrigation. The methods evaluated were (i) estimation of LAI using the central leaflet width method and (ii) estimation of LAI using the LAI-2000. In the central leaflet width method, the leaf area of all leaves on each marked plant was estimated weekly, starting from the appearance of the first trifoliolate leaf. For this, the maximum width of the central leaflet of each leaf was measured with a ruler. The leaf area was estimated for each plant from the empirical relationship (Iamauti, 1995):

$$LA = 2.1371 * (L^{1.9642}) - 2.7013$$

Were LA is leaf area (cm^2) and L is the maximum width of the central leaflet of each leaf (cm). The LAI values were obtained by dividing the total leaf area of each plant on each day of assessment by the area of the soil occupied per plant (0.05 m^2).

Estimation of LAI with the LAI-2000 Plant Canopy Analyzer was made using the recommendations made by LI-COR (1990). During the morning (0800-1000h), a single above-canopy radiation measurement with five below-canopy readings in an equally spaced (20 cm) transect across the row and within the quadrant, with five subsamples per plot, was used to compute the LAI. A 90^0 view restrictor was used in all measurements to prevent direct sunlight from reaching the sensor and to occlude the operator from the field of view.

The results obtained were analyzed by plotting the data and determining the correlation between the two methods.

RESULTS AND DISCUSSION

THE HIGH POSITIVE CORRELATION (R^2 = 0.97) OBSERVED BETWEEN THE TWO METHODS USED TO EVALUATE THE GROWTH OF THE HOST PLANT THROUGH THE LAI, SHOWS GREAT ASSOCIATION BETWEEN THEM (FIGURE 1A AND 1B). THE MACHINE RESULTED IN GREATER LAIS THAN THE MANUAL METHOD, ESPECIALLY FOR LAIS NEAR 1. AT 73 D AFTER PLANTING, IT WAS OBSERVED THAT THE PLANTS WERE IN SENESCENCE AND THE LEAF AREA WAS LOW (FIGURE 1A). SO WE HYPOTHESIZE THAT THE BRANCH AREA TO THE INDIRECT ESTIMATE OF LAIS AT THAT TIME CONTRIBUTED TO THEIR OVERESTIMATION. LOWER LAI VALUES NEAR 1 ESTIMATED BY THE MACHINE COULD RESULT IN AN IDEAL 1:1 LINE. THESE RESULTS CONFORM WITH GOWER AND NORMAN (1991), WELLES AND NORMAN (1991), DEBLONDE ET AL. (1994), SMOLANDER AND STENBERG (1996), STENBERG (1996), WELLES AND COHEN (1996), AND HERBERT AND FOWNES (1997), WHICH DEMONSTRATED APPLICABILITY OF THE LAI-2000 PLANT CANOPY ANALYZER FOR ESTIMATING LAI IN CONIFEROUS TREES.

THE LAI-2000 PLANT CANOPY ANALYZER HAS SEVERAL ADVANTAGES OVER OTHER INSTRUMENTS THAT INDIRECTLY DETERMINE LAI. THE EQUIPMENT APPEARS TO PROVIDE ACCURATE ESTIMATES OVER A BROAD RANGE OF LAIS (GOWER AND NORMAN, 1991).

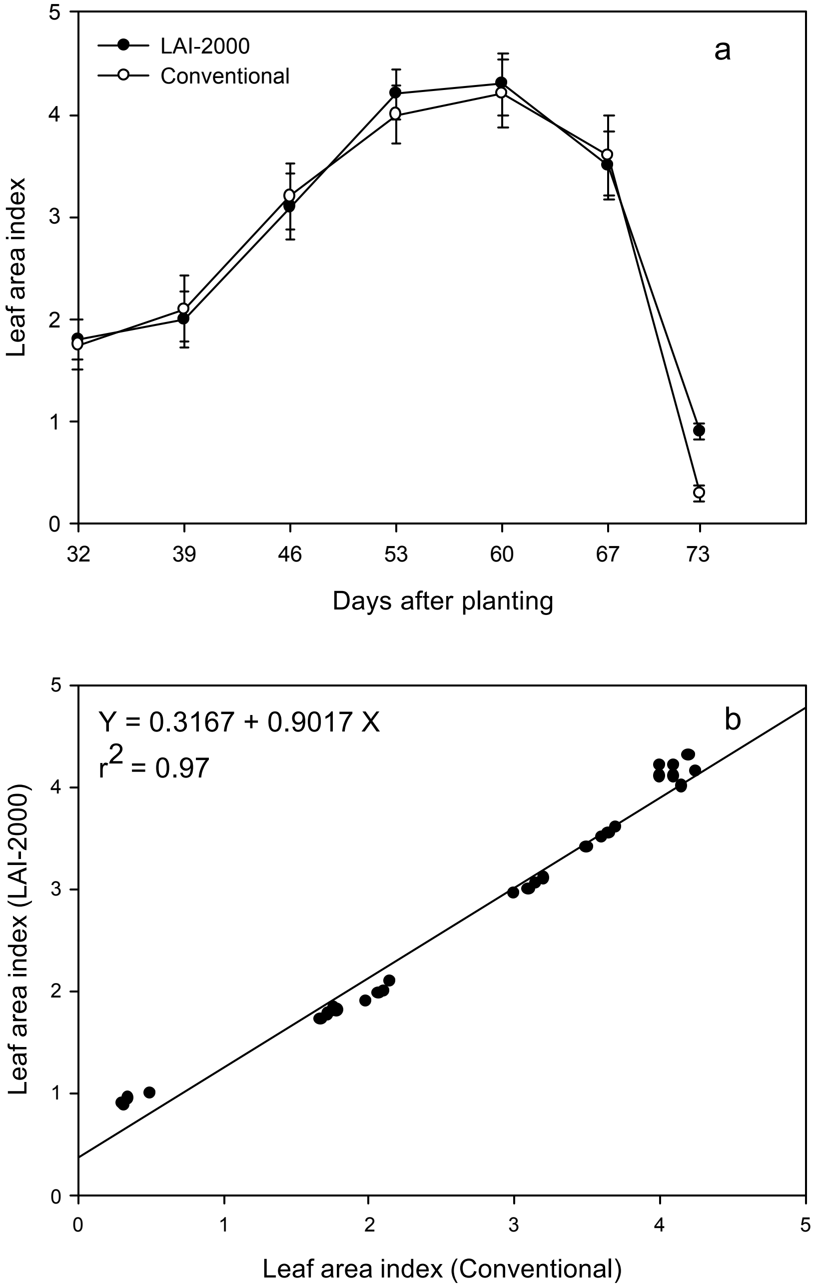


FIGURE 1. (A) LEAF AREA INDEX (LAI) PROGRESS CURVES (± STANDARD DEVIATION) FOR THE DIFFERENT ESTIMATION METHODS, AND (B) COMPARISON OF CENTRAL LEAFLET WIDTH METHOD OF ESTIMATING LAI (CONVENTIONAL) VALUES WITH INDIRECT ESTIMATES OBTAINED USING THE LAI-2000 (B)

THE RESULTS OBTAINED IN THIS STUDY SHOW THAT THE EQUIPMENT, LAI-2000, CAN BE USED TO ESTIMATE THE LAI OF COMMON

BEAN. THE USE OF THIS EQUIPMENT TO CALCULATE LAI SAVES TIME (GOWER AND NORMAN, 1991) AND MAY PERMIT EXTRAPOLATION TO HAD AND HAA IN THE QUANTIFICATION OF YIELD LOSS DUE TO COMMON BEAN DISEASES.

SUMMARY

THIS STUDY ATTEMPTED TO IDENTIFY THE APPLICABILITY THAT MAY BE EXPECTED WHEN ESTIMATING COMMON BEAN LAI WITH THE LAI-2000. THE ADVANTAGE OF THE EQUIPMENT IS THAT IT PERMITS NONDESTRUCTIVE AND RAPID IN SITU ESTIMATES OF LAI. THIS ALLOWS FOR DAY-TO-DAY ESTIMATES OF LAI TO BE OBTAINED THROUGHOUT THE GROWING SEASON ON THE SAME PLANTS WITHOUT NEED FOR EXTENSIVE FIELD PLOTS AND/OR LABOR-INTENSIVE LEAF AREA HARVESTING AND SAMPLING. IT ALSO REMOVES THE POTENTIAL ERROR ASSOCIATED WITH CANOPY LAI VARIABILITY BY ALLOWING FOR FLAGGING OF SAMPLING LOCATIONS.

IT APPEARS THAT THE LAI-2000 CAN ESTIMATE COMMON BEAN LAI. THE USE OF THIS EQUIPMENT TO CALCULATE LAI SAVES TIME AND MAY AID IN CALCULATION OF HAD AND HAA FOR QUANTIFYING YIELD LOSS DUE TO COMMON BEAN DISEASES.

REFERENCES

ANDERSON, M.C. 1971. RADIATION AND CROP STRUCTURE. P.412-466. *IN* Z. SESTAK, J. CATSKY, AND P.G. JARVIS (ED.) PLANT PHOTOSYNTHESIS PRODUCTION. MANUAL OF METHODS. JUNK, THE HAGUE, THE NETHERLANDS.

Bergamin Filho, A., S.M.T.P.G. Carneiro, C.V. Godoy, L. Amorim, R.D. Berger, and B. Hau. 1997. Angular leaf spot of *Phaseolus* beans: Relationships between disease, healthy leaf area, and yield. Phytopathol. 87:506-515.

Blackman, V.H. 1919. The compound interest law and plant growth. Annals of Bot. 33:353-360.

CARNEIRO, S.M.T.P.G., L. AMORIM, AND A. BERGAMIN FILHO. 1997. AVALIAÇÃO DE DANO PELA MANCHA ANGULAR EM FEIJOEIRO: RELAÇÃO ENTRE SEVERIDADE, ÁREA FOLIAR E COMPONENTES DE PRODUÇÃO. FITOPATOL. BRAS. 22:427-431.

Costa, L.C., J. Morison, and M. Dennett. 1997. Effects of the weather on growth and radiation intercepted by faba bean. Pesqu. Agropecu. Bras. 32:277-281.

DAUGHTRY, C.S.T., AND S.E. HOLLINGER. 1984. COSTS OF MEASURING LEAF AREA INDEX OF CORN. AGRON. J. 76:836-841.

Deblonde, G., M. Penner, and A. Royer. 1994. Measuring leaf area index with the LI-COR LAI-2000 in pine stands. Ecology 75:1507-1511.

Goel, N.S., and J.M. Norman. 1990. Instrumentation for studying vegetation canopies for remote sensing in optical and thermal infrared regions. Harwood Academic Publ. GmbH, London, England.

Goudriaan, J., and H.H. van Laar. 1994. Modelling Potential Crop Growth Processes. Kluwer Academic Publishers, London.

Gower, S.T., and J.M. Norman. 1991. Rapid estimation of leaf area index in conifer and broad-leaf plantations. Ecology 72:1896-1900.

Herbert, D. A., and J.H. Fownes. 1997. Effects of leaf aggregation in a broad-leaf canopy on estimates of leaf area index by gap-fraction method. For. Ecol. and Management, 97:277-282.

IAMAUTI, M.T. 1995. AVALIAÇÃO DOS DANOS CAUSADOS POR *UROMYCES APPENDICULATUS* NO FEIJOEIRO. PH.D. DISS. ESCOLA SUPERIOR DE AGRICULTURA “LUIZ DE QUEIROZ”, PIRACICABA, SP, BRASIL.

Jones, C.A., and J.R. Kiniry. 1986. CERES-MAIZE. A simulation model of maize growth and development. Texas A and M Press, College Station, Texas.

Lang, A.R.G., and Xiang Yuequin. 1985. Crop structure and the penetration of direct sunlight. Agric. For. Meteorol. 35:83-101.

LI-COR. 1990. LAI-2000 plant canopy analyzer. Instruction Manual. LI-COR, Lincoln, Nebraska, USA.

Monteith, J.L. 1977. Climate and efficiency of crop production in Britain. Philos. Trans. R. Soc. London, Ser. B, 281:277-294.

Monteith, J.L. 1981. Climatic variation and the growth of crops. Q. J. R Meteorol. Soc. 107: 749-74.

Perry, S.G., A.B. Fraser, D.W. Thompson, and J.M. Norman. 1988. Indirect sensing of plant canopy structure with simple radiation measurements. Agric. For. Meteorol. 42:255-278.

Radford, P.J. 1967. Growth analysis formulae: Their use and abuse. Crop Sci. 7:171-175.

Ross, J. 1981. The radiation regime and architecture of plant stands. Junk, the Hague, the Netherlands.

SILVA, M.B., F.X.R. VALE, L. ZAMBOLIM, B. HAU, AND A. BERGAMIN FILHO. 1998. RELAÇÃO ENTRE SEVERIDADE DE DOENÇA, ÁREA FOLIAR SADIA, ABSORÇÃO DA ÁREA FOLIAR SADIA E PRODUÇÃO, NA CULTURA DO FEIJOEIRO. SUMMA PHYTOPATHOL. 24:226-231.

Smolander, H., and P. Stenberg. 1996. Response of LAI-2000 estimates to changes in plant surface area index in a Scots pine stand. Tree Physiol. 16:345-349.

Stenberg, P. 1996. Correcting LAI-2000 estimates for the clumping of needles in shoots of conifers. Agric. For. Meteorol. 79:1-8.

TEI, F., A. SCAIFE, AND D.P. AIKMAN. 1996. GROWTH OF LETTUCE, ONION, AND RED BEET: II. GROWTH MODELLING, LIGHT INTERCEPTION, AND RADIATION USE EFFICIENCY. ANNALS OF BOT. 78: 645-652.

WAGGONER, P.E., AND R.D. BERGER. 1987. DEFOLIATION, DISEASE, AND GROWTH. PHYTOPATHOL. 77:393-8.

WALKER, G.K., R.E. BLACKSHAW, AND J. DEKKER. 1988. LEAF AREA AND COMPETITION OF LIGHT BETWEEN PLANT SPECIES USING DIRECT TRANSMISSION. WEED TECHNOL. 2:159-165.

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 BOT., 11:41-76.

Welles, J.M., and S. Cohen. 1996. Canopy structure measurement by gap fraction analysis using commercial instrumentation. J. Exp. Bot. 47:1335-1342.

Welles, J.M., and J.M. Norman. 1991. Instrument for indirect measurement of canopy architecture. Agron. J. 83:818-825.

Wilson, J. W., and J.E. Reeve. 1959. Inclined point quadrates. New Phytol. 59:1-8.

GENERAL CONCLUSION

Chapter 1.

An antagonistic interaction between *Phaeoisariopsis griseola* and *Uromyces appendiculatus* could be observed in the present study.

The two diseases caused yield loss in different ways: angular leaf spot caused defoliation and rusted leaves probably acted as sinks for carbohydrates.

Rust had a much stronger effect on loss in yield compared to angular leaf spot.

Chapter 2.

Pathogens caused a reduction in the net photosynthetic rate, in the stomatal conductance, in the transpiration and in the photosynthetic water use efficiency.

The effects of diseases on yield could be explained by the decreases in stomatal conductance and in the net photosynthetic rate.

Chapter 3.

Visual and virtual severities could not explain pod yield.

Pod yield could be explained by variables that consider healthy leaf area and effective leaf area.

The main consequence of incorporating the parameter β, which considers the effect of virtual lesion, was an increase in the proportion of leaf area considered to be diseased. The incorporation of this parameter was not enough to explain the variation in bean yield in function of the area under disease progress curve.

Healthy leaf area index (*HLAI*) is proposed as a key explanatory variable for a transportable system of disease management.

Chapter 4.

Foliar molybdenum application is recommended as one component of angular leaf spot management.

One or two fungicide sprays during the interval of 25 to 45 days after planting improved the control of angular leaf spot.

Chapter 5.

The equipment LAI-2000 can be used to estimate leaf area index (LAI) on common bean.

CONCLUSÕES GERAIS

Capítulo 1.

Foi observada interação antagonística entre *Phaeoisariopsis griseola* e *Uromyces appendiculatus*.

As duas doenças causaram redução na produção do feijoeiro de diferentes maneiras: a mancha angular causa desfolha e a ferrugem atua como dreno de carboidratos.

Os danos causados pela ferrugem são maiores que os da mancha angular.

Capítulo 2.

Os patógenos causaram redução na taxa fotossintética líquida, na condutância estomática, na transpiração e no uso eficiente da água fotossintética.

Os efeitos das doenças sobre a produção do feijoeiro podem ser explicados por decréscimos na condutância estomática e na taxa fotossintética líquida.

Capítulo 3.

As severidades visual e virtual não explicaram a produção do feijoeiro.

A produção pode ser explicada através das variáveis que consideram a área foliar sadia e efetiva.

A principal conseqüência da incorporação do parâmetro β, que considera o efeito da lesão virtual, foi um aumento na proporção da área foliar considerada doente. A incorporação não foi suficiente para explicar as variações na produção do feijoeiro em função da doença.

Sugere-se a utilização da variável HLAI em sistemas de manejo integrado.

Capítulo 4.

A aplicação foliar de molibdênio é recomendada no manejo da mancha angular do feijoeiro.

O controle químico da mancha angular deve ser feito durante o período de florescimento (25 a 45 dias após o plantio), por meio de uma ou duas pulverizações.

Capítulo 5.

O equipamento LAI-2000 pode ser utilizado para estimar o índice de área foliar (LAI) do feijoeiro.