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Relationships between angular leaf spot, healthy leaf area, effective leaf area and yield of *Phaseolus vulgaris*

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Abstract

Three field experiments were carried out with 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 curves (*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 with fungicide at different stages of plant growth (before, during and after flowering). Visual and virtual severity and *AUDPC* showed no significant correlation with yield. However, *HAD*, *HAA*, *ELAD* and *ELAA* were significantly correlated with yield. Variables that considered the effective leaf area (*ELAD* and *ELAA*) provided similar or better coefficients of determination (R^2) than those that considered the remaining green leaf area only (*HAD* and *HAA*). Single-point models with *HLAI*, effective leaf area index (*ELAI*), intercepted radiation by healthy leaf area (*HRI*) and intercepted radiation by effective leaf area (*EHRI*) to estimate yield at various times during the crop season were developed. The slope of the relationship between yield and *HLAI*, *ELAI*, *HRI* and *EHRI* proved to be stable, regardless of planting date and bean growth stage (from R6 to R8).

Introduction

Angular leaf spot, caused by *Phaeoisariopsis griseola*, is one of the most destructive foliar diseases of common beans (*Phaseolus vulgaris*) 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% have been reported in Brazil (Brenes et al., 1983) and 80% in Colombia (Schwartz et al., 1981).

Information about crop loss is the basis for any valid economic analysis of disease management measures. To determine crop losses, plant pathologists have examined the relationship between crop yield and disease severity. The relationship is often disappointing. Waggoner and Berger (1987) discuss some of the reasons why this may be so. In contrast, crop production is often closely related to the amount of solar radiation utilized by the plant (Monteith and Elston, 1983). Healthy leaf area duration (*HAD*) and healthy leaf area absorption (*HAA*) have been shown to be better predictors of yield than disease severity (Waggoner and Berger, 1987). *HAD* is the integral of the healthy leaf area index over the growing season and *HAA* is the integral of the radiation intercepted by the healthy leaf area over the growing season (Waggoner and Berger, 1987). *HAD* and *HAA* have been used to study crop yield in many pathosystems (Bergamin Filho et al., 1997).

In field experiments, only the visual lesions of a disease can be assessed. However, the disease may also affect the apparently disease-free leaf area around the visual lesions, the so-called virtual lesions (Bastiaans, 1991). This may be the same as, or larger than, the visual lesion area. The relationship between visual lesion and effective loss of leaf area, given by the equation $y = 1-(1-x)^{\beta}$, is a way to describe the reduction in photosynthesis of the leaf due to a disease, where y represents the proportion of the foliage area with virtual lesions, x is the proportion of the foliage area with visual lesions and β is the parameter relating virtual and visual lesions. Knowing β, integral measures of leaf area or disease can be corrected for the invisible effect of disease. This leads to the concepts of effective leaf area duration (*ELAD*) and effective leaf area absorption (*ELAA*). These concepts may be useful in integrated pest management programs to ensure that there is sufficient healthy and effective leaf area to produce satisfactory yield.

The objectives of this study were to compare two different approaches used to assess yield – one based on the 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 test the usefulness of the virtual lesion concept for the bean/angular leaf spot pathosystem.

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, October to December 1998 and from March to May 1999 with the bean cultivar Carioca Comum, under conditions of natural infection. All trials were set in a randomized complete block design with four replicates in the first trial and three in the second and third trials. To establish treatments with varied amounts of disease, the plots were sprayed with the fungicide Thiabendazole at a rate of 0.75 kg per ha (187.5 g active ingredient per ha) at different stages of plant growth (before, during and after flowering). The schedules used were 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. Each plot (48 m^2) consisted of sixteen 6-m long rows, spaced 0.5 m apart. There was 1 m between plots. To minimize cross-contamination among plots with different treatments, only the eight central rows of each plot were used for assessment (0.5 m at each end of the row was 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, weed control and sprinkle irrigation.

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 on each day of observation, 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 with an area meter (Model LI-3100; Licor, Lincoln, NB, USA). Severity of angular leaf spot 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 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 (1987). Yield was determined for each plot ($g\,m^{-2}$) 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

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

in which n is number of assessments, X is the visual disease severity of angular leaf spot (%) 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*) was calculated as $LAI = 20\ LAP$, in which *LAP* is the leaf area per plant (m^2). 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 *HAD* (days), estimated as

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

For the concept of the virtual lesion (Bastiaans, 1991), the virtual disease severity of angular leaf spot was calculated as $X_\beta = 100[1 - (1 - 0.01X)^\beta]$. The β-value used for angular leaf spot was $\beta = 3.81$ (Bassanezi et al., 2001). Virtual disease severity was integrated over the season. This resulted in the area under virtual disease progress curve ($AUDPC_{\text{virtual}}$).

The effective leaf area index (*ELAI*) was calculated as $ELAI = LAI[1 - 0.01X_\beta]$, and then integrated over the season to give the *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 with the formula: $RI_i = I_i[1 - e^{(-kLAI_i)}]$, in which I_i was the average incident solar radiation (MJ m^{-2}) during the period (t_i, t_{i+1}) and k was 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 *HAA* for each plant, measured in MJ m^{-2}:

$$HAA = \sum_{i=1}^{n-1} I_i \Big(\big((1 - X_i)\big(1 - \mathrm{e}^{(-kLAI_i)}\big) + (1 - X_{i+1})\big(1 - \mathrm{e}^{(-kLAI_{i+1})}\big) \big)/2 \Big) (t_{i+1} - t_i)$$

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

The length of the observation period was 60, 47 and 69 days in the first, second and third experiments, respectively. 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 in this way were marked with *. Thus, $AUDPC^*_{\text{visual}}$ is the standardized area under visual disease progress curve and can be interpreted as the mean daily visual disease severity during the whole season.

Data analysis

The relationships between variables were performed by linear regression with STATISTICA (Statsoft, Tulsa, OK, USA). The relationship of yield to *HLAI*, *ELAI*, *HRI* and *EHRI* was examined by linear regression forced through the origin, also performed by STATISTICA. The relationship of the slope coefficients from the latter analyses and growth stages was examined by nonlinear regression (in the abscissa, V3 = 1, V4 = 2, R5 = 3, R6 = 4, R7 = 5, R8 = 6 and R9 = 7) with PlotIT for Windows (Scientific Programming Enterprises, Haslett, MI, USA), using the beta function (Hau and Kranz, 1990). When more than one assessment was performed in the same growth stage, the average slope was used. The same procedure was used by Bergamin Filho et al. (1997).

Results

Relationships between $\text{AUDPC}^*_{\text{visual}}$, $\text{AUDPC}^*_{\text{virtual}}$, HAD*, ELAD*, HAA*, ELAA* *and yield*

The different spray schedules resulted in different severities of angular leaf spot in each trial (Tables 1 and 2). The overall average of $AUDPC^*_{\text{visual}}$ and $AUDPC^*_{\text{virtual}}$ in all three experiments on a plant basis were rather low; $AUDPC^*_{\text{visual}}$ varied between 0.26 and 0.68 and $AUDPC^*_{\text{virtual}}$ from 1.01 to 2.62. The mean *HAD**, *HAA**, *ELAD** and *ELAA** values were similar in the three trials, ranging from 1.54 to 1.66, 9.65 to 9.93, 1.43 to 1.65 and 9.60 to 9.89 (Tables 1 and 2). Differences in mean yield were also small (between 53.7 and 66.5 g) (Table 1).

Although the variability in $AUDPC^*_{\text{visual}}$ and $AUDPC^*_{\text{virtual}}$ was rather low in all experiments, the yield values, even on a plot basis, varied over a wide range (Figure 1). The relationships between $AUDPC^*_{\text{visual}}$ or $AUDPC^*_{\text{virtual}}$ and plot yield were not

Table 1. Visual severity of angular leaf spot, $AUDPC^{*}_{visual}$, HAD^{*}, HAA^{*} and yield of beans for three trials conducted in Minas Gerais, Brazil, on a plant basis

Trial	Disease severity (%)		$AUDPC^{*}_{visual}$ (%)		HAD^{*}		HAA^{*} ($MJ\,m^{-2}$)		Yield ($g\,m^{-2}$)	
	Max[1]	Mean[2]	Max	Mean	Max	Mean	Max	Mean	Max	Mean
1	4.64	0.26 ± 0.01	1.26	0.26 ± 0.01	3.15	1.66 ± 0.03	12.18	9.65 ± 0.09	101.30	62.6 ± 3.40
2	14.04	0.81 ± 0.05	1.54	0.68 ± 0.03	2.23	1.54 ± 0.03	11.99	9.76 ± 0.09	87.93	53.7 ± 3.16
3	25.30	0.52 ± 0.04	1.85	0.49 ± 0.01	2.16	1.60 ± 0.02	11.36	9.93 ± 0.05	135.22	66.5 ± 6.15

[1]Maximum values and [2]means (±standard error) for 160 plants in the first trial and 120 plants in the second and third trials. Maxima and means were calculated across replicates for each trial.

Table 2. Virtual severity of angular leaf spot, $AUDPC^{*}_{virtual}$, $ELAD^{*}$ and $ELAA^{*}$ of beans for three trials conducted in Minas Gerais, Brazil, on a plant basis

Trial	Disease severity (%)		$AUDPC^{*}_{virtual}$ (%)		$ELAD^{*}$		$ELAA^{*}$ ($MJ\,m^{-2}$)	
	Max[1]	Mean[2]	Max	Mean	Max	Mean	Max	Mean
1	17.31	1.02 ± 0.06	4.79	1.01 ± 0.06	3.14	1.65 ± 0.03	12.17	9.60 ± 0.09
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
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

[1]Maximum values and [2]means (±standard error) for 160 plants in the first trial and 120 plants in the second and third trials. Maxima and means were calculated across replicates for each trial.

significant in experiments 1 and 3, but a significant correlation ($P < 0.05$) was observed in the second experiment (Figure 1). The relationship between yield and HAD^{*}, HAA^{*}, $ELAD^{*}$ or $ELAA^{*}$ was investigated separately for each of the three trials (Figure 1). In all cases, yield increased linearly with HAD^{*}, HAA^{*}, $ELAD^{*}$ 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 (Figure 1; Tables 3 and 4).

The effect of the use of parameter β

The parameter β increases the proportion of leaf area considered to be diseased. This, consequently, leads 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 virtual disease progress curve ($AUDPC^{*}_{virtual}$) still did not explain the variations in bean yield (Figure 1; Tables 3 and 4). 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 (Figure 1). The residual plots for $ELAD^{*}$ and $ELAA^{*}$ fulfilled better the requirements for a random distribution of the residuals than those for HAD^{*} and HAA^{*} (data not shown).

The effect of growth stage

The relationships between yield and *HLAI*, *ELAI*, *HRI* and *EHRI* values for the main bean growth stages were determined for the three trials. The regression coefficients (slopes) for all the relationships decreased with the growth stage up to R8 in most trials. 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. The slopes of these regression lines also decreased with the growth stage up R8 (Figure 2).

The beta function fitted well to the average of the slopes (intercepts forced through the origin) between yield and *HLAI* or *ELAI* for the three trials in the different growth stages: $S = 84.2639 * (GS - 0.71835)^{-0.55006}(7.00001 - GS)^{-0.10007}$, with $R^2 = 66\%$ and $S = 84.1999 * (GS - 0.72321)^{-0.55004}(7.00000 - GS)^{-0.10004}$, with $R^2 = 64\%$ (Figure 2), in which S is the slope of the yield-*HLAI* and yield-*ELAI* linear relationships, and GS is the growth stage (V3 = 1, V4 = 2, R5 = 3, R6 = 4, R7 = 5, R8 = 6 and R9 = 7). The linear relationships (intercepts forced through the origin) between yield and *HRI* or *EHRI* were also fitted well with the beta function for the three trials in the different growth stages: $S = 8.0003 * (GS - 0.72024)^{-0.40019}$ $(7.00009 - GS)^{-0.09888}$, with $R^2 = 57\%$ and

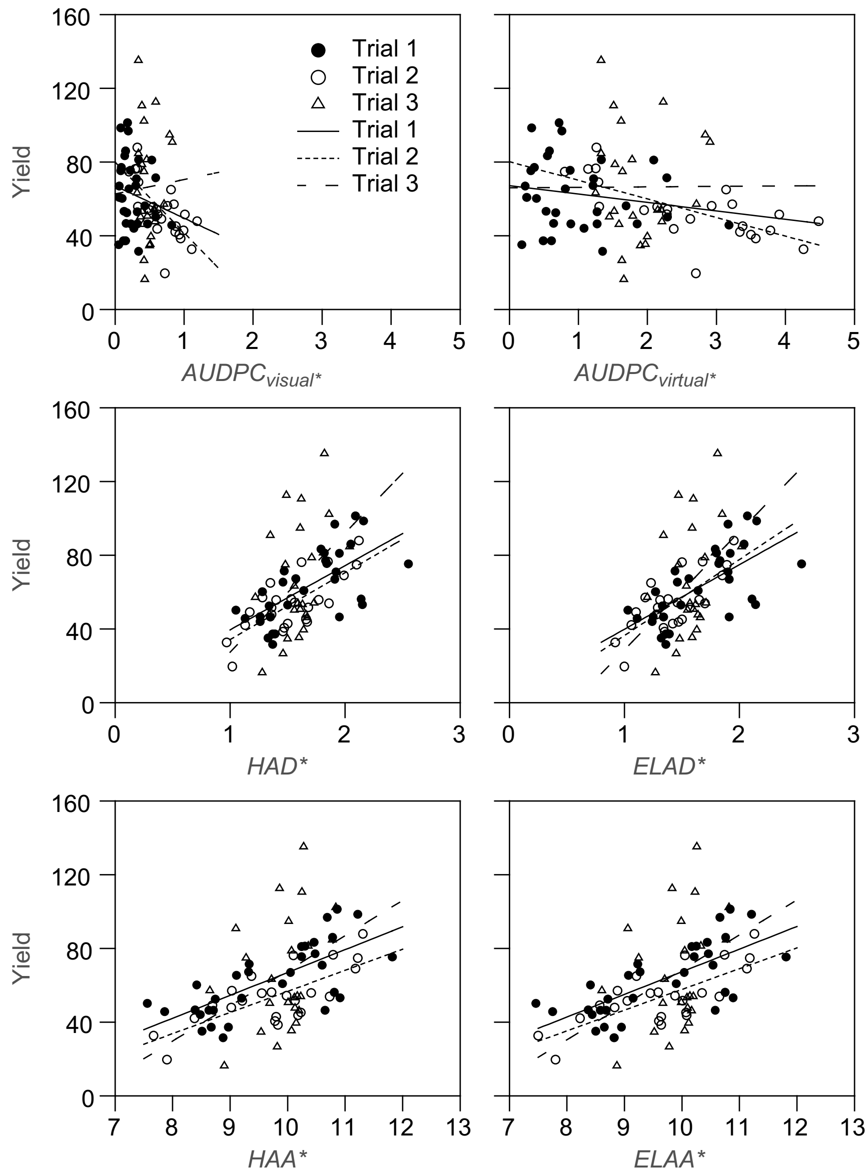

Figure 1. Relation between yield ($g\,m^{-2}$) and $AUDPC^{*}_{visual}$ of angular leaf spot, $AUDPC^{*}_{virtual}$ of angular leaf spot, HAD^{*}, $ELAD^{*}$, HAA^{*} and $ELAA^{*}$ for individual plots.

Table 3. Intercepts (b_0) and slope (b_1) of regression lines between yield ($g\,m^{-2}$) and $AUDPC^{*}_{visual}$, HAD^{*} and HAA^{*} for beans with angular leaf spot in three trials on a plot basis

Trial	$AUDPC^{*}_{visual}$ (%)			HAD^{*}			HAA^{*}		
	b_0	b_1	R^2	b_0	b_1	R^2	b_0	b_1	R^2
1	67.07	−17.54	0.03	4.66	34.85	0.44	−57.17	12.41	0.50
2	79.96	−38.35	0.50	−2.06	36.30	0.54	−58.02	11.47	0.53
3	62.67	7.81	0.00	−37.40	64.76	0.21	−123.02	19.09	0.11

Table 4. Intercepts (b_0) and slope (b_1) of regression lines between yield (g m^{-2}) and $AUDPC^*_{virtual}$, $ELAD^*$ and $ELAA^*$ for beans with angular leaf spot in three trials on a plot basis

Trial	$AUDPC^*_{virtual}$ (%)			$ELAD^*$			$ELAA^*$		
	b_0	b_1	R^2	b_0	b_1	R^2	b_0	b_1	R^2
1	67.18	−4.56	0.02	4.80	35.04	0.46	−55.46	12.28	0.50
2	80.17	−10.09	0.49	−4.90	41.28	0.54	−54.74	11.25	0.54
3	66.02	0.25	0.00	−35.54	64.18	0.22	−121.44	18.98	0.11

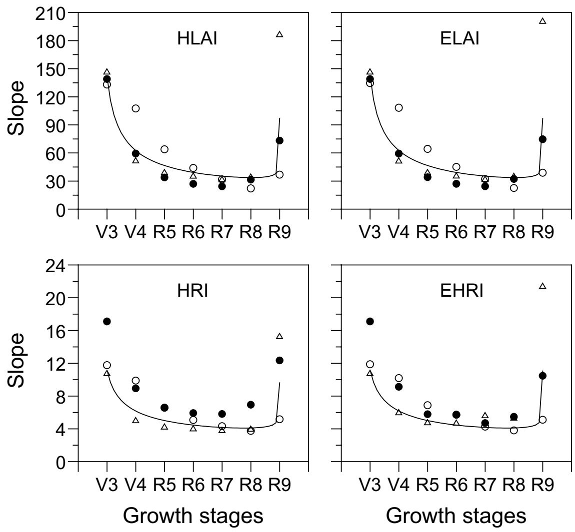

Figure 2. Slope of lines obtained by linear regression (intercepts forced through the origin) of yield (g m^{-2}) and *HLAI*, *ELAI*, *HRI* and *EHRI* measured at different growth stages of *Phaseolus vulgaris* in three trials. Growth stages: V3 = first trifoliolate leaf; V4 = third trifoliolate leaf; R5 = pre-flowering; R6 = flowering; R7 = pod formation; R8 = pod filling; and R9 = physiological maturity.

$S = 8.0004 * (GS - 0.72028)^{-0.40037}(7.00002 - GS)^{-0.09799}$, with $R^2 = 56\%$ (Figure 2).

Discussion

In the first and third trials described in this paper, no relationship between yield and $AUDPC^*$ was found (Figure 1). Although data similar to these are not rare in the literature (Bergamin Filho et al., 1997; Carneiro et al., 1997; Silva et al., 1998; Jesus Junior et al., 2001a), 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 absence of a linear relationship at a 5% level between yield and $AUDPC^*$ in two of the three trials in this study, even when data from each trial were analyzed individually, was probably due to three main factors: (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.

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 and 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 use of *HAD*, *HAA*, *ELAD* or *ELAA* models avoid these problems, because yield is estimated with the true determinant of yield, the duration or absorption of the healthy or effective leaf tissue (Johnson et al., 1987; Lopes et al., 1994).

The results of this study were consistent with previous data (Bergamin Filho et al., 1997; Carneiro et al., 1997; Silva et al., 1998; Jesus Junior et al., 2001a). However, together with the work of Bassanezi et al. (2001), it is the first time that virtual disease has been considered in disease assessment. Yield cannot be accurately predicted with disease parameters, such as disease severity, alone. Rather, parameters of host growth, such as the healthy and effective leaf area duration (*HAD* and *ELAD*) and healthy and effective leaf area absorption (*HAA* and *ELAA*), have to be considered.

The use of parameter β to calculate the area under virtual disease progress curve ($AUDPC^*_{virtual}$) still was not enough to obtain a significant relationship between this variable and 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.

Models that predict yield based on integral variables like *HAD*, *HAA*, *ELAD* or *ELAA* 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 are more useful. Based on this fact, empirical single-point-type models using *HLAI*, *ELAI*, *HRI* and *EHRI* to estimate yield for the main bean growth stages were determined for the three trials. The slopes of these relationships were shown to be stable, independent of planting date and bean growth stage (especially from R6 to R8, Figure 2). The stable level considering yield-*HLAI* or yield-*HRI* is in agreement with Bergamin Filho et al. (1997), however is in contrast with Stern et al. (1959) and Zadoks (1985). Thus, these variables could be used as key explanatory variables for a transportable system of disease management. These variables may be useful to produce precise recommendations at the farm level, as suggested by Lopes et al. (1994) and Bergamin Filho et al. (1997). The sharp increase of the slopes of the regression lines in growth stage R9 probably was not caused by the disease. In this growth stage most of the leaves had already fallen due to natural senescence. This caused a decrease in the values of *HLAI*, *ELAI*, *HRI* or *EHRI* (x-axis), but the yield (y-axis) was the same, and then the slope increased.

Bergamin Filho et al. (1997) discussed some requirements for decision-making based on the approach studied in this paper. They commented on the difficulties of the methods used to determine leaf area (to estimate *LAI*). To minimize some of these problems, Jesus Junior et al. (2001b) demonstrated that a LAI-2000 Plant Canopy Analyzer (LI-COR, 1990) can be used to estimate the *LAI* in common bean. The use of this equipment to calculate *LAI* saves time and may aid in calculation of variables related to healthy and effective leaf area to quantify yield loss due to bean diseases. To be able to understand and explain the effect of angular leaf spot on the yield of common bean, further studies need to be conducted to address the effects of defoliation and the effect of the pathogen on the radiation use efficiency of the remaining healthy leaves on diseased plants and the redistribution of photosynthates.

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