Quantitative Analysis of Two Important Epidemiological Features of the Common Bean-*Phaeoisariopsis griseola* Pathosystem

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ABSTRACT

This work quantifies two important epidemiological features of the bean (*Phaseolus vulgaris*)/*Phaeoisariopsis griseola* pathosystem. The first is the effect of the number of nights of leaf wetness on infection efficiency. Infection efficiency was below 10% when inoculated leaflets were exposed to less than two nights of leaf wetness. Optimum infection efficiencies were obtained after three to four nights of leaf wetness, at about 50%. Further nights of leaf wetness did not increase the infection efficiency. The second feature quantified is the relative rate of leaflet defoliation for varying levels of angular leaf spot severity. It increased with disease severity according to a logarithm-like curve, and a relative rate of 0.23 day^{-1} was estimated for a severity of 18%. The implications of these results on the disease epidemiology are discussed.

Additional keywords: epidemiology, angular leaf spot, *Phaseolus vulgaris*, infection efficiency, defoliation, leaf wetness, monocycle process.

RESUMO

Análise quantitativa de duas importantes características epidemiológica do patossistema feijoeiro comum-*Phaeoisariopsis griseola*

Foram quantificadas duas importantes características do patossistema *Phaeoisariopsis griseola*/feijoeiro comum (*Phaseolus vulgaris*). O efeito do número de noites com umidade foliar na eficiência da infecção foi quantificado. A eficiência de infecção foi menor que 10% quando folíolos inoculados foram expostos a menos de duas noites em condições de umidade. Eficiência de infecção ótima, cerca de 50%, foi obtida após três a quatro noites em que os folíolos permaneceram em condições úmidas. Um maior número de noites nestas condições não aumentou a eficiência de infecção. A taxa relativa de desfolha foi quantificada para diferentes níveis de severidade da mancha angular. Ela aumentou com o aumento da severidade da doença de forma semelhante a uma curva logarítmica e, uma taxa relativa de 0,23 dia^{-1} foi estimada para uma severidade de doença de 18%. A implicação destes resultados na epidemiologia da doença são discutidos.

Angular leaf spot (ALS) of common bean (*Phaseolus vulgaris* L.), caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris, is an important disease in tropical and subtropical areas (Saettler, 1991), and has become a major biotic constraint for bean production in Latin America over the past two decades. Knowledge of the epidemiology of this disease can help to better understand the mechanisms that determine disease dynamics, and may provide the background necessary for managing the disease.

The disease is favored by a warm and humid climate (Saettler, 1991). The optimum temperature for *P. griseola* development is about 24 °C (Cardona-Alvarez & Walker, 1956; Bassanezi *et al*. 1997; 1998). Moisture is required for infection and sporulation of *P. griseola* (Cardona-Alvarez & Walker, 1956; Sindhan & Bose, 1980). The effect of moisture on the efficiency of infection of *P. griseola* has not yet been quantified.

The ALS lesions on leaves can cause severe defoliation (Cardona-Alvarez & Walker, 1956; Ferraz, 1980; Saettler, 1991). On the one hand, this process may have important epidemiological implications, since sporulating lesions on leaves are transferred to the bottom of the canopy, due to defoliation. The importance of this source of inoculum for secondary infections is not known. On the other hand, defoliation caused by ALS has strong implications on bean growth and yield, as it causes a decrease in green leaf area index (LAI) (Bergamin Filho *et al*., 1997; de Jesus *et al*., 2001). Quantification of the relationship between ALS and the bean defoliation rate has not yet been determined either.

The objective of this work was to quantify two important epidemiological features of the ALS-bean pathosystem: the effect of leaf wetness duration on the infection efficiency of the fungus *P. griseola*, and the relationship between ALS severity and the rate of bean

defoliation. This information can help to better understand ALS epidemiology, and can be useful for the parameterization of an epidemiological simulation model for ALS, that may help in assessing management options for the disease.

All experiments reported here involved artificial inoculations, performed with the *P. griseola* isolate PTURRI that was isolated from a sporulating lesion sampled from an infected leaflet, in an experimental bean field at CATIE (Turrialba, Costa-Rica) in 2001. Three to four week-old bean plants (cv Negro Huasteco) grown in a greenhouse were inoculated with a spore suspension prepared by cutting sporulating lesions from infected leaves. The cuttings were placed in a beaker, water (with Tween 80 at 0.01%) was added, and the spore suspension was agitated for 5 min. The spore concentration was measured with a hemacytometer observed under the microscope (x100, six countings), and the volume of suspension was adjusted to obtain the desired spore concentration. The final suspension was then placed into a vaporizer and sprayed (0.3 ml) at 10 cm from the leaf, onto the abaxial face of each leaflet to be inoculated. The inoculated plants were then placed in the greenhouse, and incubated for three consecutive nights in polyethylene cages to insure a high level of humidity. At the end of the incubation period (ten-14 days), the infected leaflets were cut from the plants, placed in a Petri dish onto a humidified filter paper, and incubated for three days in a growth chamber (24 °C, 12-h photoperiod). After this period, the lesions on the leaflets had formed spores that could be used as inoculum.

The measurement of the effect of leaf wetness duration on infection efficiency required to estimate the spore density of inoculated leaflets and the number of lesions generated from these spores. Prior to inoculation, the length and width of the central leaflet of each trifoliate leaf to be inoculated were measured, so as to estimate the area of the trifoliate leaf, using a regression parameterized from previous measurements ($A = 1.52\ l.w + 0.69$, $R^2 = 0.95$, where A = area of trifoliate leaf, l =length of central leaflet, w = width of central leaflet). The first expanded leaf of three week-old bean plants (cv Negro Huasteco) was inoculated as described above, with a spore concentration of 5 spores/µl. A 20x20 mm cover slip was placed onto the central leaflet before inoculation, and was observed under a microscope (x100) after inoculation to determine the density of spore deposition. Inoculated plants were then caged for zero to five consecutive nights, thus allowing the quantification of the effect of the number of nights of leaf wetness on infection efficiency. Cages were 50-cm high and 30-cm wide and consisted of a transparent plastic held together with a wooden frame. Four potted plants were placed within each cage between 4 p.m. and 8 a.m. After the incubation period (ten-14 days), the number of lesions per leaf was counted from visual observation every other day for two weeks, and the maximum number of lesions over the period of observation (n) was retained for the computations. Infection efficiency (IE) was computed as the ratio of the number of lesions over the number of spores deposited per surface unit:

$$IE = n / [(A - 4) \times d],$$

where n is the number of lesions per leaf, A is the area of the leaf (cm^2), and d is spore density (nb spores.cm^{-2}). The area of leaf was decreased by 4 cm^2 to account for the spores trapped by the cover slip that was placed onto the leaf to estimate the density of spores deposited.

The experiment involved five replications (leaves) in each of the five leaf wetness duration treatments, and was conducted twice. The effect of leaf wetness duration on infection efficiency was tested with the procedure GLM of the statistical software SAS (SAS, 1989).

During the incubation period, relative humidity ranged between 50 and 80% during the daytime (8 a.m. to 4 p.m.), and between 70 and 100% during the caging period (4 p.m. to 8 a.m.). Daily temperatures ranged from 18 to 31 °C. Small droplets could be observed on both sides of bean leaves when cages were removed in the morning, whereas leaves of uncaged plants did not have any droplets. In the first experiment, infection efficiency was below 0.05 for plants that had not been caged, and increased up to 0.5 for plants caged for three nights (Figure 1). There was no significant ($P<0.05$) difference in infection efficiency between plants caged for three, four, and five nights. In the second

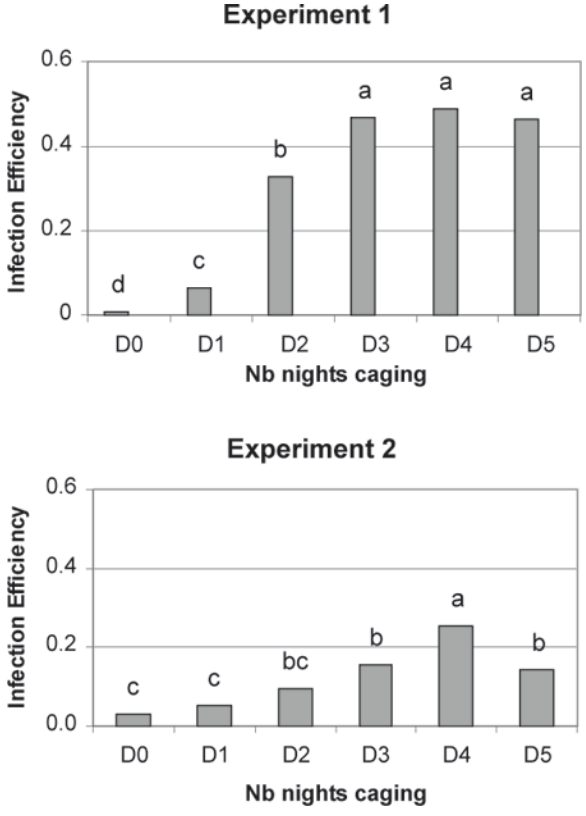

FIG. 1 - Infection efficiency of *Phaeoisariopsis griseola* in bean (*Phaseolus vulgaris*) submitted to increasing number of nights with leaf wetness after inoculation. Treatments for which bars have the same letters are not significantly different (P<5%) according to the Student-Newmann-Keuls test.

experiment, infection efficiency increased with the number of nights with leaf wetness until the fourth night, and then declined (Figure 1). The maximum infection efficiency was 0.25.

Maximum infection efficiency was 0.5 and 0.25 in the first and second experiments, respectively. This difference cannot be attributed to differences in temperature or relative humidity, which were similar during both experiments. Differences between the two experiments in other non-monitored factors, such as radiation or soil moisture, may be the cause of the difference in maximum infection efficiency observed between the two experiments. In both experiments, infection efficiency increased with the number of nights of leaf wetness until three to four nights after inoculation, and then remained stable or decreased. These results are in agreement with the general view that moisture favors infection of *P. griseola* (Allen *et al*., 1998), and with results from Cardona-Alvarez & Walker (1956), which have shown that the number of ALS lesions increased when the period of moisture after inoculation varied between 0 and 25 h. Infection processes take place mainly during the three first days after spore deposition (Cardona-Alvarez & Walker, 1956). Our results thus suggest that once the infection process is achieved, fungal progression between leaf cells does not depend on leaf wetness anymore. Reported values of infection efficiency of pathogenic fungi range between below 0.01 to around 0.5 (Gregory, 1961; Sache & de Valavieille-Pope, 1995). In our case, the maximum level of infection efficiency estimated for *P. griseola*, 0.5, is among the highest reported. The large difference in infection efficiencies between the treatments (from 0.05 to 0.5) indicate that leaf wetness duration has to be considered to better understand, and to model, the effect of climate on angular leaf spot epidemics. The results presented here provide a quantitative background for both purposes.

The determination of the relationship between ALS severity and bean defoliation required monitoring defoliation from leaflets with varying levels of disease. In a first set of two experiments, varying levels of ALS severity were obtained by inoculating plants with suspensions having increasing levels of spore concentration (0, 1.25, 2.5, and 5 spores/µl). The two experiments carried out to measure the effect of leaf wetness on infection efficiency (described above) also allowed for a determination of different levels of ALS severity. There were five replications (plants) per treatment for each of the four experiments.

The severity of ALS was monitored every other day for each leaflet from the appearance of lesions until the defoliation of the leaflets. The ALS severity was assessed using the severity scale developed by Godoy *et al*. (1997). Defoliation was monitored for each leaflet every day. The mean severity over the period of assessment was computed for each leaflet. Leaflets were then grouped according to severity classes:0; 0 to 1%; 1 to 5%; 5 to 10%; 10 to 15%; and above 15%. For each group of leaflets, the number of non-defoliated leaflets was computed over the period of assessment. It was hypothesized that the number of live (un-defoliated) leaflets declined exponentially over time. The relative rate of defoliation (*RRDEF*) is defined as the number of defoliated leaflet per leaflet and per day, and its dimension is day^{-1}. When plant growth is halted (no new leaves emitted), the dynamics of the number of (live) leaflets on a plant can thus be written as follows:

$dNFV/dt = - RRDEF \times NFV$, which can be integrated as:

$\ln(NFV_t) = \ln(NFV_{t0}) - RRDEF \times t$,

where NFV_t is the number of live leaflets at time t (in days), and t_0 is the time of appearance of symptoms.

In each experiment, a linear regression of the naperian logarithm of the number of live leaflets over time was performed for each group of leaflets defined according to the severity range, using the procedure REG of SAS (SAS, 1989). The slope parameter of the regression, that is, the relative rate of defoliation, was then estimated for a range of ALS severity.

Estimated values of *RRDEF* for the different severity ranges of ALS were obtained from the four experiments (Figure 2). The relative rate of defoliation increased with ALS severity, and averaged 0.23 day^{-1} for an ALS severity of 18%. A linear regression of *RRDEF* over the logarithm of ALS severity was performed. It corresponds to the regression equation:

$RRDEF = a \times \ln(SEV + 1) + b$,

where *SEV* is ALS severity in percentage. The model fitted reasonably well the observed data (n = 18, R^2 = 0.88; Figure 2), with regression coefficients a = 0.077 and b = -0.0063.

Our results indicate that relatively low levels of ALS severity can generate a large defoliation. This agrees with several studies (Cardona-Alvarez & Walker, 1956; Bergamin Filho *et al*., 1997; de Jesus *et al*., 2001) which showed the importance of defoliation in the bean-*P. griseola* pathosystem. Compared to other diseases, ALS causes a strong defoliation. For example, for a disease severity of 10%, estimated *RRDEF*

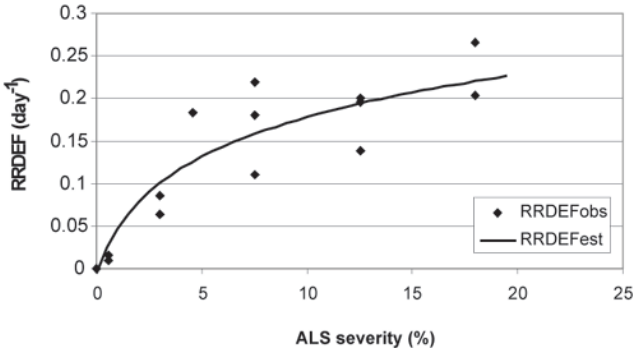

FIG. 2 - Relationships between angular leaf spot (ALS) severity (%) and the relative rate of bean (*Phaseolus vulgaris*) leaflet defoliation (*RRDEF*). RRDEFobs: observed value; RRDEFest:values estimated according to the regression equation $RRDEF = a \times \ln(SEV + 1) + b$, where *SEV* is ALS severity in percentage. R^2 = 0.88, a = 0.077 and b = -0.0063.

is 0.17 in ALS (this article), 0.035 in rice (*Oryza sativa* L.) blast (Bastiaans, 1993), 0.08 in rice sheath blight (Willocquet *et al*., 2000), and 0.024 in groundnut leafspot (Savary *et al*., 1990).

Much is known on the severity-loss relationships in the ALS-bean pathosystem (Bergamin Filho *et al*., 1997; de Jesus *et al*., 2001). Comparatively less is understood about the epidemiology of the disease. Epidemiological work might focus on the characteristics of the disease, such as defoliation, and the role of infected, defoliated leaflets, as well as features such as the wide variation in infection efficiency. This could enable the development of both a better understanding, and a better management of the disease.

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