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BRIEF COMMUNICATION

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***.
*Universidade Federal de Viçosa, Departamento de Engenharia Agrícola, 36571-000 Viçosa, MG, Brazil****.
*Institut fur Pflanzenkrankheiten und Pflanzenschutz, Universität Hannover, 30419 Hannover, Germany*****

Abstract

Isolated and interactive effects of angular leaf spot (caused by *Phaeoisariopsis griseola*) and rust (caused by *Uromyces appendiculatus*) on leaf gas exchange and yield was studied in common bean (*Phaseolus vulgaris* L. cv. Carioca) plants. Gas exchange was measured on 37, 44, 51, and 58 d 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 of net photosynthetic rate (P_N) and yield. The reduction of stomatal conductance (g_s), P_N, and yield was higher under P and combination of P+U than under U treatment. By effect of U, the reduction on yield was higher than the reductions on gas exchange parameters. On the treatment P+U, a reduction of 23 % in P_N and a correspondent reduction of 32 % in yield was observed. The interactive effects of the pathogens on yield could be explained in part by the decreases in g_s and in P_N of diseased bean leaves. The combined effect of both diseases on yield and gas exchange parameters suggests an antagonistic interaction.

Additional key words: healthy leaf area index; *Phaeoisariopsis griseola*; photosynthesis; stomatal conductance; *Uromyces appendiculatus*; water use efficiency.

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 % by effect of angular leaf spot and rust, respectively, were reported (Nasser *et al.* 1977, Brenes *et al.* 1983).

Plants infected with fungi, bacteria, or viruses 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 phosphorylation (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 (Chl) content (Berghaus and Reisener 1985), decrease in mesophyll conductance, reduced activity of ribulose-1,5-bisphosphate carboxylase, RuBPC (Gordon and Duniway 1982), or increase in leaf saccharide content (Livne and Daly 1966). Moore *et al.* (1998, 1999) and Smeekens (2000) showed that excessive saccharide accumulation in leaves might inhibit photosynthesis. It suggests that in plants infected by rust the reduction in photosynthesis may be explained by increase in leaf saccharide content (Livne and Daly 1966).

Because the reduction of photosynthesis is apparently

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

Abbreviations: E = transpiration rate; g_s = stomatal conductance; HLAI = healthy leaf area index; P = *Phaeoisariopsis griseola*; P_N = net photosynthetic rate; P_N/E = photosynthetic water use efficiency; U = *Uromyces appendiculatus*.

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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) should be expected. Because biotrophic pathogens need the vitality of the plant for survival, they cause less effect on the host physiology during colonisation and reproduction than hemibiotrophic pathogens, which are more aggressive and cause more drastic and destructive effect on host physiological processes. Plants affected by these pathogens present considerable differences in leaf growth and photosynthesis (Scholes 1992).

Different modes of action of bean leaf pathogens during the infection and colonisation of the host were observed. Related to angular leaf spot, Bassanezi *et al.* (2000) observed a reduction in Chl content correlated with disease severity. The lowering of carbon assimilation rate was mainly due, probably, to the reduction of RuBPCO activity in the spot regions. The main physiological effects observed during rust infection of bean plants were loss of Chl in the pustule area, reduction of P_N higher than reduction of green leaf area, increase in respiration rate, changes in the patterns of translocation of photosynthates, and increase in invertase activity (Daly *et al.* 1961, Livne 1964, Livne and Daly 1966, Moll *et al.* 1995, Wagner and Boyle 1995, Bassanezi *et al.* 2000, Okwulehie 2000, Lopes and Berger 2001). Lopes and Berger (2001) observed that g_s and photosynthetic capacity of leaves decreased whereas the severity of rust increased. Losses of Chl from leaves affected with rust, in comparison with control leaves, had similar values to the proportion of visually chlorotic tissue.

Thus, our study focuses on the quantification of 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. This study was conducted under field conditions at the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil.

Bean plants (*P. vulgaris* L., the susceptible cv. Carioca) were inoculated with *Phaeoisariopsis griseola* (P), *Uromyces appendiculatus* (U), and the combination of P+U. Treatments were set in a randomised complete block design with four treatments and three replications. Plot size was 16 m^2. Ten plants were allowed to grow per linear meter of row. The plots were maintained with the conventional cultural practices used in commercial fields. The plots were inoculated, always at nightfall, with P and/or U at 10^4 spores per cm^3 for each pathogen. The first inoculation was made when the second trifoliolate leaf had expanded (*ca.* 30 d after planting), and the second inoculation followed 10 d later. The control plots were sprayed with tebuconazole at 0.75 kg per ha [18.75 mg(active substance) m^{-2}] the day before each inoculation. In the four central rows of each plot, five plants of similar height and vigour were selected 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 [%] 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] of selected plants was weekly determined using the empirical relationship of Bergamin Filho *et al.* (1997).

Gas exchange was measured 37, 44, 51, and 58 d after planting using a *LI-6400* Portable Photosynthesis System (*LI-COR*, Lincoln, NE, USA). P_N [μmol m^{-2} s^{-1}], g_s, [mol m^{-2} s^{-1}], and E [mmol m^{-2} s^{-1}] were recorded at an irradiance of 650 μmol m^{-2} s^{-1}. Measurements were taken between 08:00 and 11:00. Readings were done at the central leaflet of each leaf. For each plot, five plants were marked and five leaves of the same physiological age were used per plant. Water use efficiency was calculated as P_N/E. Yield was determined at the end of the crop cycle in terms of seed mass (12 % moisture).

Healthy leaf area index (HLAI) was estimated according to Waggoner and Berger (1987) and Bergamin Filho *et al.* (1997).

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

The visual severity of ALS was higher than the severity of R (Fig. 1*A,B*). There was no significant difference among all treatments in terms of plant growth. Yield reductions of 21, 20, and 32 % were observed in plants infected by P, U, and P+U, respectively (Table 1). For all treatments, similar progress of healthy leaf area index (HLAI) was observed, but in the control treatment the leaf area remained on a high level for a longer time compared to inoculated treatments. The maximum HLAI in the control was less than 3.5 (Fig. 1*C*).

The inoculation of bean plants with P, U, and P+U caused a significant ($p = 0.05$) reduction of P_N (Fig. 1*D*, Table 1). P and P+U induced also a significantly reduction of g_s, E, and P_N/E. However, the intercellular (C_i) to ambient (C_a) CO_2 concentration ratio increased by effects of U and P+U (Table 1).

The 21 % P-induced reduction in yield was associated with 17 % reduction in P_N, 21 % reduction in g_s, and 14 % reduction in P_N/E. The P+U inoculation ended in 32 % reduction of yield which was associated to 23 % reduction in P_N and g_s and 14 % reduction in P_N/E. However, the 20 % reduction in yield induced by U-inoculation was associated only with 11 % reduction in P_N, 14 % reduction in g_s, and 10 % reduction in P_N/E.

There was a high relationship between g_s and E in all treatments. However, there were low correlations between g_s and P_N in all treatments ($r^2 = 0.58$, 0.56, 0.75, and 0.64 for P, U, P+U, and control, respectively).

Table 1. Net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), photosynthetic water use efficiency (P_N/E), ratio of intercellular to ambient CO_2 concentration (C_i/C_a), and yield of bean plants inoculated with *P. griseola* (P), *U. appendiculatus* (U). and *P. griseola* + *U. appendiculatus* (P+U). Means from 4 evaluations (yields were calculated from 15 plants per treatment). Values in a column followed by the same letters are not significantly ($p = 0.05$) different according to ANOVA, by Tukey's test.

Treatment	P_N [μmol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	P_N/E	C_i/C_a	Yield [g m^{-2}]
P	14.77 c	0,32 b	3.01 c	0.0049 b	0.69 b	123.7 a
U	16.99 b	0.36 a	3.28 b	0.0051 b	0.69 b	126.1 a
P+U	15.84 b	0.33 b	3.23 b	0.0049 b	0.67 a	107.1 a
Control	19.22 a	0.42 a	3.36 a	0.0057 a	0.66 a	158.1 b

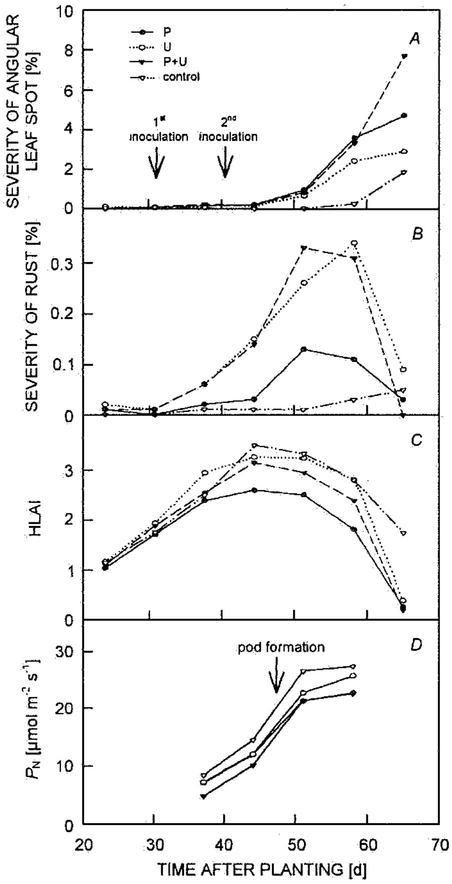

Fig. 1. Visual severity of angular leaf spot (*A*) and rust (*B*), healthy leaf area index, HLAI (*C*), and net photosynthetic rate (*D*) of bean plants inoculated with *Phaeoisariopsis griseola* (P), *Uromyces appendiculatus* (U), and *P. griseola* + *U. appendiculatus* (P+U) during the experiment. Pathogens were inoculated on 30 and 40 d after planting. Means of 15 plants per treatment.

The direct assessment of photosynthetic competence of a diseased leaf, defined here as the ability of that leaf to photosynthesise when compared with a similar healthy leaf, allows to quantify the impact of one or more diseases on the remaining green area of diseased leaves. We observed a significant difference of the severity of angular leaf spot and rust in bean plants. The reduction of g_s, P_N, and yield was larger under P and P+U treatments than under U treatment. By effect of U, the reduction of yield was larger than the reductions of gas exchange. However, on the treatment P+U, transpiration of the plant continued, but photosynthetic activity decreased. The higher correlation observed between g_s and E than between g_s and P_N suggests that P_N would have been reduced by a non-stomatal effect of the diseases.

Because biotrophics pathogens such as *U. appendiculatus* have little influence on the stomata physiology, the resistance to carboxylation, caused by metabolic alterations on chloroplasts, could explain the reduction on P_N of leaves infected with rust as observed on barley (Owera *et al.* 1981). We demonstrated that rust and angular leaf spot reduced P_N, however, we did not specifically examine the relative contributions of biochemical limitations on photosynthesis by effect of the pathogens. Lower reductions in P_N induced by rust were associated with reductions of the green leaf area (Shtienberg 1992). Bergamin Filho *et al.* (1997) and Silva *et al.* (1998) determined the effects of angular leaf spot on leaf area reduction. When conditions were favourable to *U. appendiculatus*, no significant defoliation was observed. In that case, the yield reduction probably occurred because the rusted leaves are sinks of saccharides (Livne and Daly 1966) and the remaining leaves could not compensate the carbon requirement for growth.

In addition to effects reducing leaf area and thus the amount of intercepted radiation, some pathogens can also affect the efficiency of radiation used by the plant that might explain the reduction of yield. Under rust infection, probably other metabolic processes as the increase of the resistance to carboxylation and reduction of Chl content could be affected (Owera *et al.* 1981, Bassanezi *et al.* 2000, Lopes and Berger 2001).

The slight increase in C_i/C_a in the U-treatments is an indication that the flow of CO_2 from the stomata cavities to the carboxylation sites was not affected (Table 1). Consequently, we suggest that mesophyll resistance to carboxylation could increase in leaves infected with rust, which is in agreement with Bassanezi *et al.* (2000) and Lopes and Berger (2001).

The comparison of the effect of P+U inoculation on the reductions in yield and P_N with the effects of the separate treatments suggests a less-than-additive interaction of both diseases on bean plants. This interaction may result from competition for plant resources between pathogen populations or from stimulation of active defence mechanisms in the plant (Waller and Bridge 1984).

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