Manifestation of "black stem" *by Phoma macdonaldii* and its relationship with reserves in stem and the source/sink ratio in sunflower

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ABSTRACT

• Black stem (BS) by *Phoma macdonaldii* Boer of sunflower (*Helianthus annuus* L.) is an endemic disease in Argentina. The manifestation of this disease is affected by assimilate availability and requirements during the grain filling period (Nuñez Bordoy et al, 2011). High demand of assimilates during grain filling and leaf senescence cause a decrease in the source-sink ratio (SSR) which could favor the manifestation of this disease. This response, in turn, may be associated with remobilization of stem reserves, as was reported for *Fusarium spp*. in corn (Eslava et al, 2007). The aim of this work was to study the manifestation of BS by *Phoma macdonaldii* to changes in the SSR, and its association with the remobilization of stem reserves during grain filling in sunflower.

• Two field experiments were performed at Balcarce INTA Station (37° 45´S, 58° 18´O). Hybrids VDH 487 (Advanta) and Baqueano (KWS) with different health behavior were grown under good water and nutrients conditions. Treatments imposed to modify the SSR were the extraction of (i) 0% (T), (ii) 25 % (R1) or (iii) 75 % (R2) of total grains, or (iv) 50% shading (S) during the filling period. Stubble of sunflower crop with symptoms of BS was distributed previously in the experimental field. BS incidence (nodes with symptoms / total nodes) and severity (on leaves 8, 12 and 20) were evaluated during grain filling, as well as dry matter per plant. SSR (at physiological maturity) was calculated as the ratio of total dry matter per plant and number of grains per head corrected by the weight of the grains of treatment R2, considered close to the potential weight. Remobilization of stem reserves was calculated as the difference in dry weight of this organ between end of flowering and physiological maturity.

• The severity of BS varied between treatments for VDH 487 (range 2.8 – 94.4%, average= 49%) and Baqueano (range 5.6- 69.4%, average=23.8%). The incidence of BS was associated with severity. The range of SSR explored was 1.9 to 10.8 (g.g-1). The remobilization of reserves accumulated in the stems was associated with the SSR (P = 0.0008), and was higher for hybrid VDH 487 (average= 20%) than for Baqueano (average= 5%). At physiological maturity, BS severity of leaves 8, 12 and 20 and incidence were inversely related to SSR, adjusting both hybrids and years of experimentation to an unique relationship (P=0.0008, P=0.0022, P=0.0023 and P = 0.0145, respectively). Both severity and incidence of BS were explained by the variation in the remobilization of stem reserves, adjusting the results to an exponential model (P= 0.0001).

• These results strongly suggest that a simple model based on SSR can predict the BS of sunflower in the range of hybrid and environments used. The stem reserves could be an important factor in modulating this relationship.

• The knowledge achieved in this work could help in better understanding how crop structure and reserve management contribute to tolerance of sunflower genotypes to BS.

Keywords*:* assimilate supply and demand- *Helianthus annuus L. - Phoma macdonaldii Boer.*- reserves management.

INTRODUCTION

Black stem (BS), caused by necrotrophic fungus *Phoma macdonaldii* Boer (teleomorph *Leptosphaeria lindquistii),* is an important disease of sunflower (*Helianthus annuus* L.) in the word. It is an endemic disease in Argentina and it can occur in all developmental periods, although usually appears after flowering. Initially, the pathogen causes leaf necrosis (blade and petiole), and subsequently produces the symptom known as black shield (spot dark brown to black with well defined margins) at the insertion of the petiole in the stem (Velazquez y Formento, 2000). Yield losses due to *Phoma* stem lesions are generally moderate in natural conditions (Debaeke and Pérès, 2003).

The manifestation of BS is affected by assimilate availability and requirements during the grain filling period (Nuñez Bordoy et al., 2011). High demand of assimilates during grain filling causes a decrease in the source-sink ratio (SSR) which could favor the manifestation of this disease. This response, in turn, may be associated with remobilization of stem reserves, as was reported for *Fusarium* spp. in corn (Eslava et al., 2007) and for *Macrophomina phaseolina* in sunflower (Davet and Serieys, 1986). The utilization of sunflower cultivars with partial resistance to BS in combination with appropriate crop management practices is an effective way to control the disease (Darvishzadeh et al., 2007).

Some sunflower crop practices like density, N fertilization, irrigation, use of fungicides, has been reported to have incidence on BS (Debaeke and Pérès, 2003; Velazquez and Formento, 2000, 2003), but how crop structure and reserve management could contribute to tolerance of sunflower genotypes to BS, has not yet been properly tested.

The aim of this work was to study the manifestation of BS by *Phoma macdonaldii* in response to changes in the SSR, and its association with the remobilization of stem reserves during grain filling.

MATERIALS AND METHODS

Two field experiments (Exp. 1 and 2) were performed at Balcarce INTA Station (37° 45´S, 58° 18´O) in two growing seasons. Hybrids VDH 487 (Advanta Seeds SAIC, Murphy, Argentina) and Baqueano (KWS Argentina SA, Balcarce, Argentina) were selected for their different cycle, 81 and 88 days to flowering. The crops were grown under good water and nutrients conditions. Stubble of sunflower crop with symptoms of BS was distributed previously in the experimental field to ensure the presence of *Phoma macdonaldii*´s inoculums. Treatments imposed to modify the SSR were the extraction of 25 % (R1) or 75 % (R2) of total grains, 50% shading (S) during the filling period and the control (T).

BS incidence was determined as the ratio between nodes with symptoms and total nodes, expressed as a percentage. BS severity was determined on leaves 8, 12 and 20 (numbered from the bottom) depending of the size of lesion, according to the scale proposed by ASAGIR (2002).

Dry matter per plant was recorded from flowering to physiological maturity. SSB was calculated as the ratio of total dry matter per plant at physiological maturity and the number of grains per head corrected by the weight of the grains of treatment R2, considered close to the potential weight. Remobilization of stem reserves was calculated as the difference in dry weight of this organ between end of flowering and physiological maturity.

Statistical differences between treatments were tested through standard analyses of variance, the mean treatment values were compared using Tukey test with significance level of 0.05. The Software used for statistical analysis was INFOSTAT Professional v 1.1. (Di Rienzo and Robledo, 2002). Simple regressions between the severity of BS and the SSR and remobilization of stem reserves were performed using the Sigma Plot 11.0 Software (Systat Software Inc., 2010).

RESULTS AND DISCUSSION

The evolution of BS severity was different according to the leaf considered. BS severity of leaf 8 was higher than leaves 12 and 20, in both hybrids and experiments (Fig.1 only exp. 2 is showed). This is because BS initially affects lower leaves and then goes upward to upper ones, therefore oldest leaves are exposed to the pathogen for a longer period than the upper leaves.

The BS severity and incidence of VDH 487 were significantly higher than Baqueano, comparing the treatment T in both experiments. These differences between hybrids may be the consequence of the difference in cycle. Velazquez and Formento (2000) observed that at a similar phenological stage, an intermediate-long cycle hybrid had lower incidence and number of spots on stem than a short cycle cultivar. The greater leaf area and leaf area duration of the long-cycle cultivars probably retard the penetration of necrotrophic pathogens, giving a better performance to BS compared to short-cycle genotypes.

The greatest differences in BS severity between treatments were observed since 500°Cd from anthesis, approximately in all studied leaves. In hybrid VDH 487, both severity and incidence of BS at physiological maturity, were significantly reduced in grain extraction treatments (R1 and R2), while in shading treatment did not differ significantly from the control.

In hybrid Baqueano, severity of BS at physiological maturity of treatment R2 was lower than T, in leaves 8 (exp. 1 and 2) and 12 (exp. 2). The incidence of BS at physiological maturity of treatment S was significantly higher than T only in experiment 2.

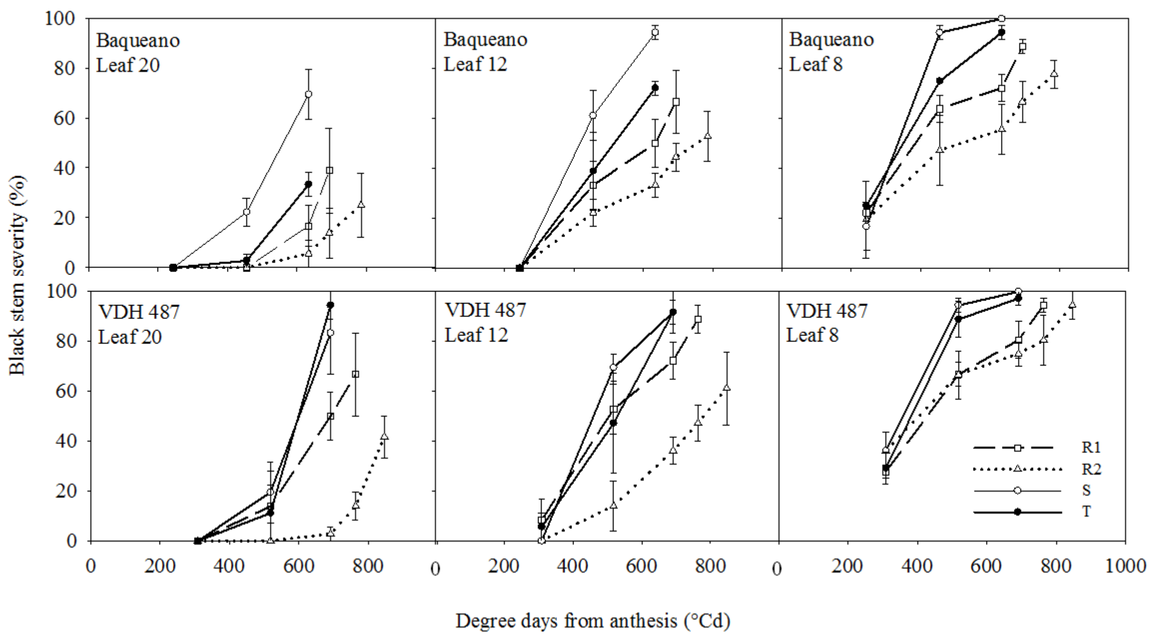

Fig.1. Evolution of the black stem severity (%) during the grain filling period, for leaves 20, 12 and 8, in experiment 2, for Baqueano and VDH 487 hybrids. Treatments: extraction of 25 % (R1, squares with medium dash line), or 75 % (R2, triangles with dotted line) of the total grain, 50% shading during the filling period (S, open circles with continue line) and control (T, solid circles with continue line). Vertical bars indicate standard error.

Table 1. Mean values of sink- source relationship (SSR) and remobilization of stem reserves for all treatments evaluated during experiment 1 and 2. Standards errors are between parentheses.

		Source/sink ratio ($g.g^{-1}$)		Remobilization stem reserves (%)	
Hybrid	Treatment	Exp. 1	Exp. 2	Exp. 1	Exp. 2
VDH 487	R1	3.7 (0.9)	3.5 (0.4)	19.6	15.3
	R2	15.3 (3.2)	7.8 (1.2)	7.9	-9.8
	S	2.0 (0.1)	2.0 (0.0)	34.7	34.9
	T	2.3 (0.1)	2.1 (0.1)	17.1	35.0
Baqueano	R1	5.6 (0.9)	4.5 (0.3)	-15.4	11.1
	R2	10.9 (1.2)	10.7 (1.5)	-9.0	3.0

S	3.0 (0.3)	3.7 (0.1)	15.8	29.4
T	3.5 (0.2)	3.6 (0.2)	-3.8	9.5

The range of SSR explored was from 2 to 15 ($g.g^{-1}$) approximately. As expected, R1 and R2 treatment showed the higher SSR, but shading treatment presented values close to those of T treatment (Table 1). This was probably the consequence of the slight diminish of grain number observed in treatment S (data not shown).

Remobilization of stem reserves was higher in hybrid VDH 487 (average= 19.33 %) than Baqueano (average= 5.07%). The remobilization of reserves accumulated in the stems was associated with the SSR (*P*= 0.0008, data not shown).

BS incidence and severity of leaves 8, 12 and 20 were inversely related to SSR (Fig 2), adjusting both hybrids and experiments to a unique relationship (*P*=0.0145, *P*= 0.0008, *P*=0.0022 and *P* = 0.0145 respectively).

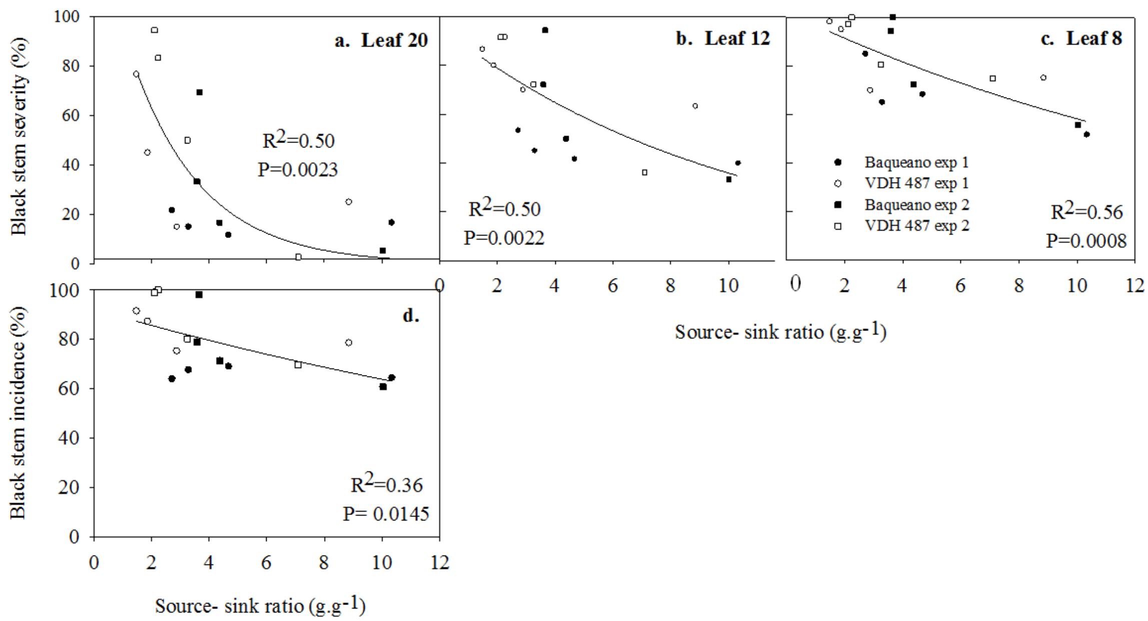

Fig.2. Black stem severity and incidence (%) related to the source sink ratio ($g.g^{-1}$) at physiological maturity, for exp. 1 and 2 (2009/2010 and 2010/2011) and two hybrids (VDH 487 and Baqueano). a. Severity of leaf 20 y=142.5*exp (-0.46*x). b. Severity of leaf 12 y= 102.3*exp (-0.056*x). c. Severity of leaf 8 y= 102.3*exp (-0,056*x). d. Incidence y= 92.21*exp(-0.04*x).

Severity of leaves 20, 12 and 8, and incidence of BS were well explained by the variation in the remobilization of stem reserves, adjusting both hybrids and experiments to a unique relationship (Fig. 3).

From the flowering period in sunflower, there is a migration of photoassimilates to the capitulum, and a decrease in the content of them in leaves and stem in response to the demand exerted by the fruits (Aguirrezábal et al, 1996). SSR variation affects the accumulation and partitioning of dry matter produced. Thus, in sunflower crops with low SSR, during the grain filling, we expect a reduction in total photosynthesis and therefore a rapid decline of the carbohydrate content in stems, to meet the demand of the fruits. By contrast, a higher SSR at this stage would lead to an accumulation of photoassimilates in the stems (Molot, 1969).

Results obtained in this study showed that treatments which increased RSS (extraction of grains), promoted the accumulation of reserves in the stem and reduced expression of BS in comparison with the control. In this study, shading did not decrease markedly RSS respect to the control, probably because it reduced total dry matter, but also slightly the number of filled achenes per plant, especially those located in the capitulum centre, as reported by Aguirrezábal et al, (1996) and Alkio, et al (2003). Although no differences were found in RSS between S and T, the remobilization of stem reserves in the S treatment was higher than in T (with the exception of VDH 487, exp. 2). In turn, only S and T were differenced in BS incidence in hybrid Baqueano, in exp 2. This suggests the existence of a stem carbohydrates threshold over which BS severity start to decrease. . Similar results were mentioned by Davet and Serieys (1987) for *Macrophomina phaseolina* in sunflower. However, carbohydrates are involved in the carbon nutrition of fungi, therefore we cannot grant a fungistatic role, at least in the concentrations found in the tissues. From these observations, we assume the existence of one or more substances that inhibit the growth of fungi, closely related to the carbohydrate content of the plant, such as phenolics and terpenes (Silva Acuña et al., 2000) associated to plant response against pathogens.

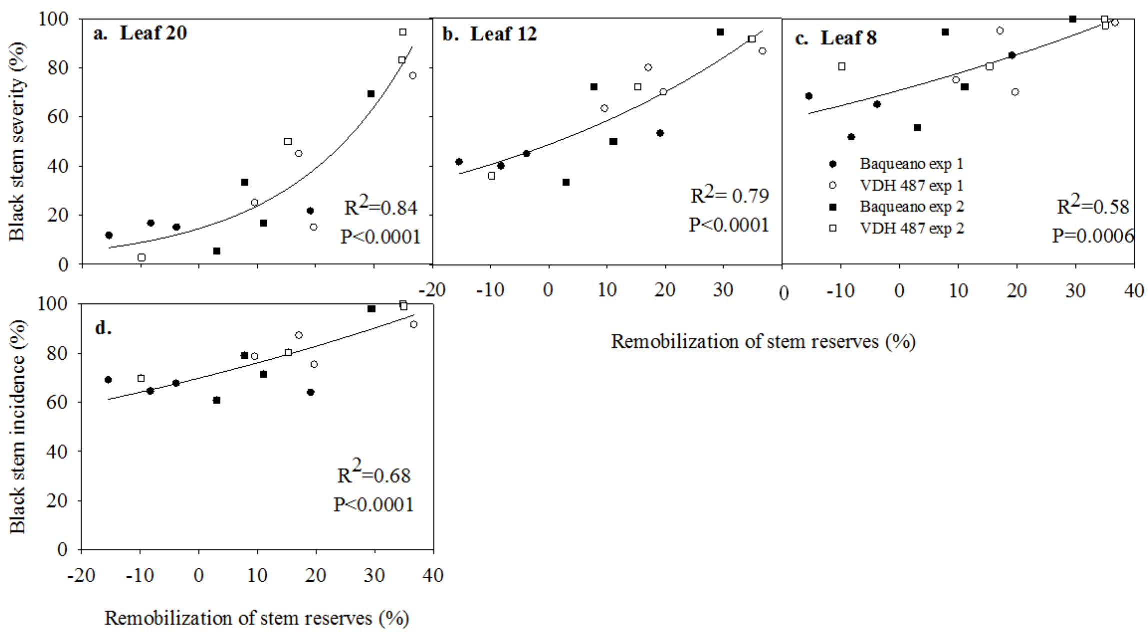

Fig.3. Black stem severity and incidence (%) related to the remobilization of stem reserves (%) at physiological maturity, for exp. 1 and 2 (2009/2010, circles, and 2010/2011, squares) and two hybrids (VDH 487, open symbols, and Baqueano, solid symbols). a. Severity of leaf 20 y=14.51*exp (0.049*x). b. Severity of leaf 12 y= 48.76*exp (0.018*x). c. Severity of leaf 8 y= 70.86*exp (0.009*x). d. Incidence y=69.81*exp (0.009*x).

These results strongly suggest that in our experimental conditions a model based on SSR may be adequate to predict the BS of sunflower. The stem reserves could be an important factor in modulating this relationship. Not because of the intrinsic effect of carbonated reserves, but probably by the relationship between carbohydrates metabolism with phenol compounds.

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