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Increases in the source to sink ratio related to a higher carbohydrate concentration reduce phoma black stem in sunflower

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Abstract Phoma black stem (BS), caused by *Leptosphaeria lindquistii* (synonymous *Phoma macdonaldii* Boerema) is an endemic disease of sunflower in Argentina and occurs worldwide. The expression of some diseases is related to the translocation of photosynthates during grain fill, which in turn is related to the source to sink ratio (SSr). The aim of this study was to assess the effect of total soluble carbohydrates (TSC) in the stem, and determine the effect of changes in SSr during the grain filling period on the incidence and severity of BS. A range of SSr and TSC was obtained by artificial shading and performing grain excision treatments, in two sunflower hybrids. A positive relationship was determined between SSr and TSC. Both the incidence and severity index of BS decreased with increasing SSr and TSC. This response was affected by the conditions of the experiment and the leaf position, for both incidence and severity of BS, respectively. Rainy days and leaf position (leaf age) were the main factors affecting the relationship between BS and TSC. Results contribute to understanding the physiological basis of the interaction between *L. lindquistii* and sunflower, and suggest how breeding for particular traits might result in improved cultivars. The results should also be useful for guiding management of the disease.

Keywords *Phoma macdonaldii* · *Leptosphaeria lindquistii* · Black stem · Carbohydrates · Source to sink ratio · Sunflower · *Helianthus annuus* L

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Introduction

Black stem (BS) of sunflower (*Helianthus annuus* L.), caused by the necrotrophic fungus *Leptosphaeria lindquistii* (synonymous *Phoma macdonaldii* Boerema), is the most prevalent disease during the grain filling period of sunflower in Buenos Aires province, the main sunflower production area in Argentina (Lazzaro et al. 2013). Production in this province ranges from 1.16 to 2.40 thousand tonnes of grain (SIIA-MAGPyA 2015). Yield losses of 10 to 30% are reported due to BS (Velásquez and Formento 2003; Quiroz et al. 2014). Furthermore, BS occurs in most regions of the world where sunflower is produced (Acimovic 1984, 1988; Miric et al. 1999; Wu et al. 2012), causing considerable damage. The world-wide distribution of BS coupled with the damage it causes makes it desirable to develop methods to minimize the impact of the disease.

Symptoms of BS develop as black oval lesions enlarging from leaf nodes on the stem at flowering, which

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progress from lower to upper leaf nodes, and are usually associated with previous necrosis of the veins, petiole and/or leaf lamina (Bordat et al. 2011). Symptoms of infection caused by *L. lindquistii* on the roots and the collar of the plant seen elsewhere (Donald et al. 1987), are generally less prevalent than those of BS in Argentina. Susceptible hybrids, no-tillage sowing and intensification of production predispose sunflower to BS (Debaeke and Pérès 2003). There are no reports of complete resistance to BS. However, certain morphological traits are associated with reduced disease (Schwanck et al. 2016). Also, fungicides delay senescence triggered by BS in mid-stem and upper leaves (Quiroz et al. 2014).

The expression of some diseases is related to the translocation of photosynthates during grain fill. Davet and Serieys (1987) demonstrated that *Macrophomina phaseolina* colonizes sunflower roots and stems when reducing sugars in the plant fall below a critical level. Reducing sugars include all monosaccharides and some di, oligo and polysaccharides, but do not include saccharose. Genotypes with resistance to *M. phaseolina* had higher levels of reducing sugars in the base of the stem compared to susceptible genotypes. Resistance to root and stalk rot in corn was associated with a high level of reducing sugars, plus saccharose. This fraction of carbohydrates is usually named total soluble carbohydrates (TSC), and is normally available as a reservoir for remobilization toward the developing grains, and contrasts with structural carbohydrates like cellulose, hemicellulose and pectins. Previous research has shown tissues remain healthy provided the sugar level in the plant remains above a critical threshold (Moltimore and Ward 1964).

Source to sink relationships (SSr) are used to estimate the budget between incoming carbon and reproductive sinks (Borrás et al. 2004). This framework has been helpful to interpret plant growth, grain yield and oil production in sunflower (Ruiz and Maddonni 2006). The source is usually quantified through the intercepted photosynthetically active radiation, the dry matter or photosynthesis, while the sink is the number of grain, eventually affected by the potential grain weight (Abbate et al. 2005). SSr has been related to the expression of disease. For example, the removal of fruits decreased susceptibility of banana to crown rot, caused by *Colletotrichum musae* (Lassois et al. 2010).

Infection of sunflower by *L. lindquistii* can occur at any developmental stage of the plant, even on cotyledons (Penaud and Pérès 1994). Susceptibility is related to phenological stage (Larfeil et al. 2010), with the disease incubation period during early reproductive stages being longer compared to later stages, and the flowering stage being the most susceptible (Fayzalla and Maric 1981). In sunflower, grain filling is directly supported by assimilates from photosynthesis (López Pereira et al. 2008; Hall et al. 1990). Photosynthesis is dependent on quantity and quality of incident radiation (red:far-red ratio, Rousseaux et al. 1996), so in this planophile species with a high coefficient of extinction, the age (and position) of the leaf affects photosynthesis and senescence (English et al. 1979; Rousseaux et al. 1996).

The interaction of assimilate source, translocation and subsequent storage in the grain are poorly understood in sunflower in relation to BS. The sunflower/BS pathosystem is a useful model to study plant-pathogen interactions because it is epidemiologically complex (mono and polycyclic components), and the host physiology can affect its susceptibility. The aim of this study was to assess the effect of TSC in the stem, and determine the effect of changes in SSr during the grain filling period on the incidence and severity of BS. Results should contribute to understanding the physiological basis of the interaction between *L. lindquistii* and sunflower, and how breeding for particular traits leading to a high SSr after flowering, might result in improved cultivars with increased disease resistance. The results should be useful for prediction of the impact of the disease on yield through development of models based on the effect of the SSr on BS. The results should also contribute to improved disease management.

Materials and methods

Experiment description

Four experiments, hereafter referred to as Exp. 1, Exp. 2, Exp. 3 and Exp. 4, were conducted at the INTA Balcarce Experimental Station, Argentina (37°45′ S, 58°18′ W) during the 2009/10 (Exp. 1), 2010/11 (Exp. 2 and Exp. 3) and 2011/12 (Exp. 4) growing seasons in a field that had a wheat/sunflower rotation since 1999. The soil in the four experiments was a typic argiudol (USDA–Soil Taxonomy. V. 2014, organic matter 7.4%, P-Bray 25.8 ppm).

In Exp. 1 and Exp. 2, two sunflower hybrids: VDH 487 (Advanta Seeds SAIC, Argentina, 81 days from emergence to flowering) and Baqueano (KWS Argentina SA, 88 days from emergence to flowering) were sown on November 9, 2009 and on November 15, 2010, respectively. Emergence occurred 7 (Exp. 1) and 9 (Exp. 2) days after sowing. Flowering date was considered the day in which 95% of the plants in a plot had flowered (growth stage R5.1, Schneiter and Miller 1981). Hybrid VDH 487 flowered 63 and 61 days after emergence (DAE) and hybrid Baqueano 67 and 65 DAE, in Exp. 1 and Exp. 2, respectively. To manipulate SSr, three treatments were applied after flowering (growth stage R6): (i) grain excision from ½ (G↓) of the head resulting in a semicircle of grain, (ii) grain excision from ¾ (G↓↓) of the head resulting in ¼ segment of grain remaining, and (iii) solar radiation management: a 50% uniform shading with black, synthetic and neutral mesh cloth (S). The control (C) was not treated. SSr treatments and hybrids were factorially combined in a randomized complete block design with three replicates.

In Exp. 3 and Exp. 4, only hybrid Baqueano was sown on November 15, 2010 and on November 16, 2011, respectively. Emergence occurred 9 and 7 days after sowing, and flowering at 65 and 64 DAE, in Exp. 3 and Exp. 4, respectively. In order to evaluate the possible direct effect of BS on SSr (i.e. the extent to which the disease could interfere with applied SSr treatments), the four SSr treatments (G↓, G↓↓, S, C) were factorially combined with a fungicide treatment (Amistar Xtra ®, Syngenta Co. [azoxistrobin 20% + ciproconazol 8% [SC] *w/v*], 0.5 l in 140 l ha^{-1} at growth stages V8, R2, R4 and R6) or a non-treated control in a split plot design with three replicates. The fungicide treatment was assigned as the main plot, and the SSr treatments to the subplots.

In all experiments, plant density was adjusted manually to 5.6 plants m^{-2} on a row spacing of 0.7 m. Although BS was known to occur in the field, 1.6 kg of BS-diseased stalk residues (3–5 cm long) were applied per plot at growth stage V6, to provide additional primary inoculum. Fertilizer was applied before sowing (40 $kg.ha^{-1}$ of diammonium phosphate [46% P_2O_5 and 18% of ammoniacal nitrogen]) and at growth stage V7 (60 $kg.ha^{-1}$ of urea [46% N]). Soil moisture was measured using a time domain reflectometer (TDR, Trase System, Model 6050X1, Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Soil water was maintained above 40% of maximum available water in the first 0.60 m of soil by sprinkler irrigation. The applied water was 107 mm (Exp. 1), 230 mm (Exp. 2 and Exp. 3) and 385 mm (Exp. 4). Weed and insect control was by recommended cultural and chemical methods (Díaz-Zorita and Duarte 2002).

Physiological maturity (PM, maximum grain weight) occurred in the control plants at 102 and 107 DAE, in Exp. 1, and 95 and 103 DAE, in Exp. 2, in hybrids VDH 487 and Baqueano, respectively. Hybrid Baqueano reached PM at 100 and 103 DAE, in Exp. 3, and at 87 and 98 DAE, in Exp. 4, with and without fungicide protection, respectively.

Thermal time was measured by integrating air temperature from flowering using a threshold temperature of 6 °C (Kiniry et al. 1992).

Measurements

Solar radiation, temperature and rainfall

Daily solar incident radiation, rainfall and the number of rainy days were obtained from a weather station located 400 m from the experiments. Daily mean air temperature in control and shaded treatments was measured with copper/constantan thermocouples in both hybrids, at leaf positions 12, 20 and 28 (from the base of the plant). Data was averaged every 3600 s and recorded using a data logger (Cavadevices.com, Buenos Aires, Argentina). Temperature was the average temperature from the three heights. Radiation and temperature were averaged from flowering to PM and on a weekly basis. Rainfall was accumulated from flowering to PM and on a weekly basis.

Intercepted photosynthetically active radiation

Daily incident photosynthetically active radiation (PAR) was calculated as 0.48 × daily solar incident radiation (Monteith and Unsworth 1990). The proportion of PAR intercepted by the crop was determined according to Gallo and Daughtry (1986) as (1 - Rb/Ro), where Rb is the PAR measured below the lowest green leaf and Ro is the PAR measured above the canopy. Rb and Ro were measured weekly at solar noon (± 1 h), using a line quantum sensor (LI-191SB, LI-COR, Lincoln, NE, USA). In accordance with Charles-Edwards and Lawn (1984), the daily proportion of intercepted PAR was estimated as the proportion of PAR intercepted at noon

× 2/(1 + proportion of PAR intercepted at noon). This correction implies a substantial improvement from a single measurement at midday (Trapani et al. 1992). The daily proportion of PAR intercepted between two consecutive measurements was calculated by linear interpolation. Daily intercepted PAR (iPAR) was calculated as the product of the daily incident PAR and the proportion of intercepted PAR. The iPAR accumulated from treatment application to 450 °Cd after flowering was assumed as the "source" value for the calculations of the source to sink ratio.

Number and weight of grains

Three to five heads per plot were cut every 7–10 days (Exp. 1, Exp. 2 and Exp. 3), or every 3–4 days (Exp. 4) from flowering to PM. Grains were separated from the head and manually counted (grain number = GN). Samples were oven-dried (60 °C) to constant weight. Unit grain weight (GW) was calculated by dividing the sample weight by GN. GW, as a function of thermal time after flowering (TT), was adjusted to a two-phase conditional model (linear-plateau). The TT at which GW reached its maximum value was considered to be PM.

Source to sink ratio

The source to sink ratio (SSr) was calculated for each GN and GW sampling date as the ratio between cumulated iPAR (iPARc) and the product of GN and GW (Eq. 1). SSr was estimated at 450 °Cd after flowering by linear interpolation between two successive measurements.

$$SSr_{(d)} = \text{iPARc}/GN_{(d)} * GW_{G\downarrow\downarrow(d)} \quad (1)$$

Where $SSr_{(d)}$ is the source to sink ratio at sampling date (d); iPARc is iPARc from treatment application to the sampling date (d); GN is the grain number at the sampling date (d); $GW_{G\downarrow\downarrow}$ is GW of treatment G↓↓ at the sampling date (d), which is considered the GW closest to the maximum potential weight.

Total soluble carbohydrates in stem

Stems from three plants per plot from Exp. 1, Exp. 2 and Exp. 4 (non-treated plots only) were sampled twice between growth stages R7 and R9, oven-dried and milled. Total soluble carbohydrates (TSC) were quantified by spectrophotometry at 490 nm (Spectronic 20, Baush and Lomb, USA) applying the phenol-sulphuric acid method (Dubois et al. 1956), after extraction (100 °C water bath and centrifugation at 3500 rpm, three times) from a 50 mg milled sample. TSC at 450 °Cd after flowering was estimated by linear interpolation between the sampling dates.

Incidence and severity of BS

Incidence of BS was evaluated every 7–10 days by sampling 3–5 plants per plot (n = 9 to 15), and was calculated as the ratio between the symptomatic and the total number of nodes per plant. A BS severity index was calculated every 7–10 days from 3 plants per plot (n = 9) as (n_{25}× 25 + n_{50} × 50 + n_{75}× 75 + n_{100}× 100) / TN; where: n_{25}, n_{50}, n_{75} and n_{100} were the numbers of evaluated nodes with symptoms of severity rating 25 (a small spot limited to the region of the insertion of the petiole on the stem), 50 (a spot occupying up to a half of the stem circumference), 75 (a spot occupying more than a half of the stem circumference without contact with other spots) and 100 (the spot enclosing the whole stem circumference), respectively; and TN was the total number of evaluated nodes (Quiroz et al. 2014). The BS severity index was measured on leaves 8, 12, 20 and 28, from the base of the plant. Plants under the shading treatment could not be assessed after PM due to stem damage. The BS incidence and severity index were estimated at 450 °Cd after flowering by linear interpolation between two successive measurements.

Statistical analysis

The disease (incidence and severity of BS) and physiological variable (GN, GW, iPARc, SSr, TSC) data were analyzed by a three-way (main factors: SSr, Hybrid and Experiment) analysis of variance (ANOVA procedure, INFOSTAT Professional v.1.1, Córdoba, Argentina), according to the statistical design of the experiments (thus Expts. 1 and 2, and Expts. 3 and 4 were combined in the analysis, respectively). Homoscedasticity and normality of the data were checked by Levene's and Shapiro-Wilk's tests, respectively (p = 0.95). Significance of the factors and their interactions was evaluated based on the F statistics and P values (considered significant if ≤0.05). Means separation of treatments was performed using Fischer's LSD test (α = 0.05). Regression analyses were also conducted (least squares

method; Sigma Plot v. 11.0, Systat Software Inc., Chicago). Incidence and severity of BS were related to SSr and TSC at 450 °Cd after flowering using linear and exponential functions. Similarly, the relationship between TSC and SSr at 450 °Cd after flowering was explored using linear and quadratic functions. In both cases, regression coefficients (R^2), residuals and significance of regression parameters ($p \leq 0.05$) were used to assess the appropriateness of the regressions. A multivariate analysis (principal components, PC) was conducted including SSr treatments, hybrids, experiments, and vectors for physiological (iPARc, GN, GW, SSr and TSC) and BS (incidence and severity) variables, and number of rainy days (INFOSTAT Professional v.1.1, Córdoba, Argentina).

Results

Meteorological conditions

Daily mean air temperature from flowering to PM during the 2011/12 growing season was 0.6 and 1.1 °C higher compared to 2009/10 and 2010/11, respectively (the average of the temperature in plots of hybrids VDH 487 and Baqueano combined, Table 1). Shading treatment did not affect temperature (data not shown). Daily mean solar incident radiation during the same period was 1 and 0.4 MJ m^{-2} day^{-1}greater in 2010/11 (average from plots of both hybrids combined) compared to 2009/10 (average from plots of both hybrids combined) and 2011/12, respectively. Shading reduced radiation by 50, 42 and 56%, in 2009/10, 2010/11 and 2011/12, respectively. Between flowering and PM, rainfall recorded for hybrid VDH 487 in 2009/10 was 66.1 mm higher compared to 2010/11, while for hybrid Baqueano it was 204.6 and 187.3 mm higher in 2009/10 compared to 2010/11 and 2011/12, respectively. There were 15 rainy days during 2009/10, while during 2010/11 there were 7 and 9 rainy days recorded for the hybrids Baqueano and VDH 487, respectively. During the 2011/12 season, the hybrid Baqueano experienced 9 rainy days from flowering to PM.

Results of the analysis of variance

For the combined data from Expts. 1 and 2, there was a consistent effect of the SSr treatment on all measured variables (F = 6 to 291, $p < 0.0001$ to 0.004, Table 2). The factor Hybrid affected GW, iPARc, SSr, and severity of BS on leaves 8 and 12 (F = 6 to 62, $p < 0.0001$ to 0.02). The Experiment had a significant effect on GW, iPARc, SSr treatment and incidence of BS (F = 22 to 152, $p = 0.0002$ to 0.01). In Expts. 1 and 2, there were interactions between the treatment factors for most variables. Those interactions including SSr effects are the most important as they relate directly to the objectives of this research.

Table 1 Daily mean air temperature, solar radiation, and cumulative rainfall at the INTA Balcarce Experimental Station, Argentina

WAF	Temperature (°C)			Solar radiation ($MJ.m^{-2}.d^{-1}$)			Rainfall (mm)		
	2009/10	2010/11	2011/12	2009/10	2010/11	2011/12	2009/10	2010/11	2011/12
1	20.9	21.0	19.9	21.0	25.2	23.6	20.3	9.7	0
2	21.5	19.4	21.4	20.2	22.5	22.6	35.6	55.3	0.4
3	21.4	18.2	19.9	19.0	22.1	22.7	45.4	18.6	0
4	19.4	18.2	21.5	21.8	22.6	16.6	10.0	0	92.5
5	17.5	19.7	16.5	18.5	18.0	16.7	24.9	12.5	12.0
6	15.9	18.0	19.8	18.5	17.8	17.9	27.5	1.5	21.6
7	19.0	20.9	-	13.6	18.4	-	137.3	0	-
$\overline{\chi}/\Sigma$	19.6 [a]	18.9		19.8	20.8		163.7	97.6	
	19.2 [b]	18.9	20.0	19.3	20.3	20.2	292.2	87.6	104.9

Temperature and radiation during the 2009/10, 2010/11 and 2011/12 growing seasons were averaged weekly after flowering (WAF) and from flowering to physiological maturity (PM) ($\overline{\chi}$). Rainfall was cumulated weekly after flowering and from flowering to PM (Σ)

[a] Hybrid VDH 487

[b] Hybrid Baqueano

Table 2 The effects of the Source to Sink ratio (SSr) treatments, Hybrid (Hyb), Experiment (Exp.), and Fungicide treatment (F) and their interactions on grain number per plant (GN) and grain weight (GW) at physiological maturity, intercepted photosynthetically active radiation (PAR) cumulated from treatment application to 450 °Cd after flowering (iPARc), and SSr, total soluble carbohydrates (TSC) and black stem (BS) incidence and severity on leaves 8, 12 and 20 at 450 °Cd after flowering

Expts.	Factor / Interaction	GN	GW	iPARc	SSr	TSC	BS Incidence	BS Severity		
								Leaf 8	Leaf 12	Leaf 20
1 and 2	SSr	85 (<0.0001)	63 (<0.0001)	291 (<0.0001)	218 (<0.0001)	13 (<0.0001)	9 (0.0002)	27 (<0.0001)	12 (<0.0001)	6 (0.004)
	Hybrid	ns [a]	27 (<0.0001)	62 (<0.0001)	45 (<0.0001)	ns	ns	7 (0.01)	6 (0.02)	ns
	Exp	ns	22 (0.01)	104 (0.0005)	50 (0.002)	ns	152 0.0002	ns	ns	ns
	SSr × Hyb	ns	10 (0.0001)	ns	ns	ns	ns	3 (0.03)	ns	ns
	SSr × Exp	ns	3 (0.03)	3 (0.036)	4 (0.02)	ns	ns	3 (0.05)	3 (0.05)	ns
	Hyb × Exp	ns	30 (<0.0001)	59 (<0.0001)	ns	7 (0.01)	ns	ns	ns	ns
	SSr × Hyb × Exp	ns	ns	ns	ns	ns	ns	ns	ns	ns
3 and 4	Fungicide (F)	ns	ns	ns	ns	np [b]	103 (<0.0001)	13 (0.001)	24 (<0.0001)	8 (0.007)
	SSr	93 (<0.0001)	42 (<0.0001)	1453 (<0.0001)	125 (<0.0001)		17 (<0.0001)	11 (0.0001)	14 (<0.0001)	7 (0.001)
	SSr × F	ns	ns	ns	3 (0.05)		3 (0.04)	ns	ns	4 (0.02)

F and *p* values (parentheses) are shown. Analyses are based on combined data from experiments (Expts.) 1 and 2 or Expts. 3 and 4, as indicated

[a] indicates that the factor or interaction is not significant

[b] indicates that the analysis was not performed for this variable

In Expts. 3 and 4, there was no effect of Fungicide treatment on GN, GW, iPARc or SSr, but a consistent effect on incidence and severity of BS (F = 8 to 103, $p < 0.0001$ to 0.007, Table 2). There was an effect of SSr treatment on all variables (F = 7 to 1453, $p < 0.0001$ to 0.001). The interaction of SSr treatment × Fungicide treatment was significant for SSr, BS incidence and BS severity on leaf 20 (F = 3 to 4, $p = 0.02$ to 0.05).

Number and weight of grains

As expected, excision of ½ (G↓) and ¾ (G↓↓) of the grain reduced GN by 35%, and 69%, respectively, compared to the control (Table 3). Shading also reduced GN by 16%. In Expts. 1 and 2, the excision of ½ (G↓) of the grain resulted in a GW 18% higher compared to the control only for hybrid VDH 487 (Table 3). Excision of ¾ (G↓↓) of the grain increased GW by 16 and 34%, in Expts. 1 and 2, respectively, while excision of ½ (G↓) of the grain also increased GW by 19% but only in Exp. 2. Shading decreased GW, but there were slight differences depending on the experiment: shading decreased GW by 23 and 14% compared to the control, in Expts. 1 and 2, respectively. GW in heads with excision of ½ (G↓) and ¾ (G↓↓) of the grain and shading was 16, 14 and 12% higher in Exp. 2 compared to Exp.1, respectively. In addition, Hybrid VDH 487 had grains significantly heavier compared to hybrid Baqueano only in Exp. 2 (66.1 g vs 52.8 g, Hybrid × Experiment interaction: F = 30, $p < 0.0001$).

Cumulative intercepted PAR

As expected, shading reduced iPARc by 46 and 48% compared to the control, in Expt. 1 and 2, respectively (Table 3). iPARc was on average 11% higher in the controls or when either ½ (G↓) or ¾ (G↓↓) of the grain were excised in Exp. 2 compared to Exp. 1. Furthermore, hybrid VDH 487 had significantly higher iPARc in Exp. 2 compared to Exp. 1 (32.3 and 25.2 MJ, respectively, Hybrid × Experiment interaction: F = 59, $p < 0.0001$).

Table 3 Grain number per plant (GN) and unit grain weight (GW) at physiological maturity, intercepted photosynthetically active radiation (PAR) cumulated from treatment application to 450 °Cd after flowering (iPARc), and source to sink ratio (SSr) and total soluble carbohydrates (TSC) at 450 °Cd after flowering of the sunflower plants

Exp.	Factor / interaction		GN #		GW (g)		iPARc (MJ)		SSr ($MJ.mg^{-1}$)		TSC ($mg.g^{-1}$)	
1 and 2	SSr	C	1415 a [a]		53.9 c		34.2 a		0.41 c		50.4 b	
		G↓	921 c		59.4 b		34.8 a		0.60 b		66.1 a	
		G↓↓	440 d		67.5 a		35.6 a		1.34 a		70.6 a	
		S	1192 b		43.9 d		18.2 b		0.27 d		38.8 c	
	Hybrid (Hyb)	VDH	1016 a		59.4 a		28.8 b		0.55 b		58.2 a	
		Baq	968 a		52.9 b		32.6 a		0.77 a		54.8 a	
	SSr × Hyb		VDH	Baq	VDH	Baq	VDH	Baq	VDH	Baq	VDH	Baq
		C	1490	1340	54.1 d	53.7 d	31.5	36.9	0.32	0.51	52.0	48.8
		G↓	890	952	64.1 b	54.7 cd	33.4	36.2	0.48	0.71	63.1	69.0
		G↓↓	419	461	75.6 a	59.3 bc	33.9	37.2	1.22	1.47	77.7	63.5
		S	1266	1118	43.9 e	43.9 e	16.3	20.1	0,18	0.37	40.0	37.6
	SSr × Exp		Exp.1	Exp. 2	Exp.1	Exp. 2	Exp.1	Exp. 2	Exp.1	Exp. 2	Exp.1	Exp. 2
		C	1390	1440	53.6 c	54.3 c	32.6 b	35.9 a	0.46 de	0.36 e	51.1	49.7
		G↓	818	1025	54.3 c	64.5 b	32.1 b	37.5 a	0.65 c	0.54 cd	68.5	63.6
		G↓↓	390	489	62.4 b	72.5 a	33.7 b	37.4 a	1.52 a	1.17 b	62.2	79.0
		S	1117	1267	41.2 e	46.6 d	17.7 c	18.8 c	0.33 ef	0.22 f	46.8	30.9

Variables are presented in relation to i) main effects of SSr treatments: control (C), grain excision from ½ (G↓) or ¾ (G↓↓) of the head and shading (S), ii) main effects of Hybrids: VDH 487 and Baqueano, iii) interactions of SSr × Hybrid, and iv) interactions of SSr × Experiment (Exp). Values represent the combined data from Expts. 1 and 2

[a] Means with different letters are significantly different based on the effect of the factors SSr or Hybrid or on the effect of the interactions SSr × Hyb or SSr × Exp (Fisher's LSD, $\alpha = 0.05$). Where means separation is not indicated the interaction was not significant (Table 2)

Source to sink ratio

Excising grain increased the SSr, but there were slight differences depending on the experiment: excising grain from ½ (G↓) or ¾ (G↓↓) of the head increased SSr by 41 and 230%, in Exp. 1 and by 50 and 225%, in Exp. 2, respectively (Table 3). The shading treatment reduced the SSr in Exp. 2 only (by 39%). The SSr in hybrid Baqueano was 40% higher compared to hybrid VDH 487. In Expts. 3 and 4, fungicide treatment applied to a plant with grain removed from ¾ (G↓↓) of the head increased the SSr by17% compared to ¾ (G↓↓) grain excision on the non-treated control (Table 4).

Total soluble carbohydrates in stem

Excising grain from ½ (G↓) or ¾ (G↓↓) of the head increased TSC by 31 and 40%, respectively, compared to the control, while shading reduced it by 23% (Table 3). Hybrid VDH 487 had significantly greater TSC compared to Baqueano, but only in Exp. 2 (62.9 vs 48.7 mg g^{-1}, respectively, Hybrid × Experiment interaction: F = 7, p = 0.01).

Incidence and severity of BS

Incidence of BS increased after flowering in plants receiving the SSr treatments, in both hybrids and in all experiments (Fig. 1). In most cases, incidence was delayed by grain excision (Fig. 1a, c, d) and accelerated by shading in some others (Fig. 1d).

In Expts. 1 and 2, excising grain from ¾ (G↓↓) of the head resulted in a19% decrease in incidence of BS compared to on the control plants (Table 5). Incidence of BS in Exp. 1 was significantly higher compared to Exp. 2 (66.7% vs 51.6%, average of SSr treatments, F = 152, p = 0.0002, Fig. 1). In Expts. 3 and 4, the fungicide treatment decreased incidence of BS in the SSr treatments between 33 and 51% compared to the non-treated control (Table 4). Excising grain from ½ (G↓) or ¾ (G↓↓) of the head decreased incidence of BS compared to the control in non-treated plots only (by 18 and 32%, respectively).

Severity of BS decreased from the lower to the upper nodes. Leaf 8 had the most severe BS whereas leaf 20 was the least affected (Table 5). No symptoms of BS were observed on leaf 28. Both onset and progress of BS severity were delayed by grain excision, but advanced by shading in most cases (Fig. 2).

In leaf 8, excising grain from ½ (G↓) of the head reduced severity of BS on hybrid VDH 487 (by 25%), while shading increased severity on hybrid Baqueano (by 20%, Table 5). Excising grain from ¾ (G↓↓) of the head reduced the severity of BS on leaf 8 by 17 and 32%, in Exp. 1 and Exp. 2, respectively, compared to the control, while excising grain from ½ (G↓) of the head also reduced the severity of BS, but only in Exp. 2 (by 22%). On the other hand, shading increased the severity of BS on leaf 8 by 24% in Exp. 1.

Table 4 Source to sink ratio (SSr), black stem (BS) incidence and severity index on leaves 8, 12 and 20 at 450 °Cd after flowering of the sunflower plants

Experiment	Factor / interaction		SSr ($MJ.mg^{-1}$)		BS Incidence (%)		BS Severity index (%)					
							Leaf 8		Leaf 12		Leaf 20	
3 and 4	Fungicide (F)	T(+)	0.72 a [a]		24.0 b		53.2 b		23.4 b		3.2 b	
		T(−)	0.69 a		41.4 a		65.0 a		39.9 a		11.9 a	
	SSr × F		T (+)	T (−)	T (+)	T (−)	T (+)	T (−)	T (+)	T (−)	T (+)	T (−)
		C	0.39 d	0.43 d	21.9 d	44.9 b	54.4	76.0	27.4	47.9	2.6 b	12.0 b
		G↓	0.67 c	0.74 c	22.4 d	37.0 c	46.4	60.5	19.9	28.8	3.8 b	2.6 b
		G↓↓	1.57 a	1.30 b	20.4 d	30.6 c	43.5	50.3	11.5	24.7	0.0 b	2.1 b
		S	0.25 d	0.28 d	31.2 c	53.0 a	68.3	73.3	34.7	58.0	6.3 b	31.0 a

Variables are presented in relation to i) main effects of Fungicide treatments (F): treated (T+) and non-treated control (T-) and ii) interactions of SSr × Fungicide treatments. SSr treatments: control (C), grain excision from ½ (G↓) or ¾ (G↓↓) of the head and shading (S). Values represent the combined data from Expt. 3 and 4

[a] Means with different letters are significantly different based on the effect of the factor Fungicide treatment or on the effect of the interaction SSr × F (Fisher's LSD, α = 0.05). Where means separation is not indicated the interaction was not significant (Table 2)

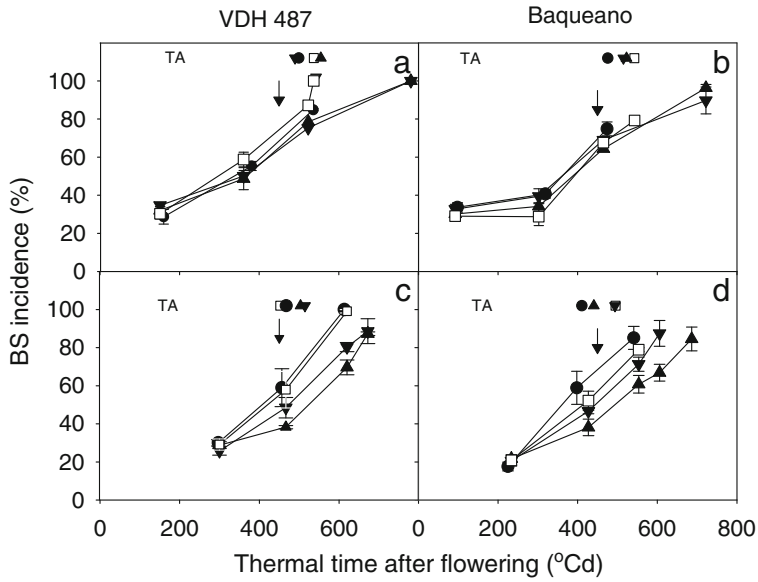

Fig. 1 Incidence of black stem (BS) caused by *Leptosphaeria lindquistii* as a function of thermal time after flowering on hybrids VDH 487 (**a**, **c**) and Baqueano (**b**, **d**), in Exp 1 (**a**, **b**) and Exp 2 (**c**, **d**). Treatments: control (open squares), grain excised from ½ (inverted triangles) or ¾ (triangles) of the head or shading (solid circles). Dates of treatments application (TA) and for physiological maturity (each treatment represented by its corresponding symbol) are indicated at the top of the chart. The arrow indicates 450 °Cd after flowering. Vertical bars on the symbols indicate the standard error of the mean value (n = 9 to n = 15)

Excising grain from ¾ (G↓↓) of the head reduced the severity of BS on leaf 12 only in Exp. 2 (by 59%, Table 5). The severity of BS on leaf 12 was 16% higher on hybrid VDH 487 compared to Baqueano. On leaf 20, excising grain from ¾ (G↓↓) of the head reduced the severity of BS by 57% compared to the control.

In Expts. 3 and 4, severity of BS was decreased by 18 and 41% on leaves 8 and 12, respectively, in plots treated with fungicide compared to non-treated plots (Table 4). On leaf 20, the fungicide treatment applied to a plant under shading decreased severity of BS by 80% compared to the same treatment in the non-treated control.

Relationships between BS and SSr, TSC and SSr, and BS and TSC

Both incidence and severity of BS decreased with increasing SSr (Fig. 3a–d). Incidence and severity of BS in Expts. 1 and 2 and in the non-treated control from Exp. 4, were in most cases exponentially related to SSr (for incidence, R^2 = 0.73 and 0.96 for Expts. 2 and 4, respectively, Fig. 3a, and for severity, R^2 = 0.53, 0.54 and 0.50, for leaves 8, 12 and 20, respectively, Fig. 3b–d), the exception being incidence of BS in Exp. 1 where the regression was not significant (p = 0.1, Fig. 3a, dotted line). An increase in SSr from 0.1 to 1 MJ.mg^{-1} was associated with a decreased incidence of BS between 10 (Exp. 1) and 49% (Exp. 4), and a decrease in severity of BS between 35 (leaf 8) and 79% (leaf 20). An SSr greater than 1 MJ.mg^{-1} had little effect on incidence or severity of BS.

The relationship between TSC and SSr was described by a positive quadratic function (Fig. 4). An increase in SSr from 0.1 to 1 MJ.mg^{-1} resulted in a higher TSC content, which according to the model reached a maximum of 80 mg.g^{-1}. In some cases a slight decrease in TSC was observed when grain was excised from ¾ (G↓↓) of the head compared to when grain was excised from ½ (G↓) of the head (hybrid Baqueano in Expts. 1 and 4).

Incidence and severity of BS decreased with increasing TSC in the stem (Fig. 3e–h). The relationship between incidence or severity of BS and TSC were described in most cases by negative linear models (for incidence of BS R^2 = 0.90 and 0.87, for Expts. 2 and 4, respectively, Fig. 3e, and for severity of BS R^2 = 0.49, 0.72 and 0.54, for leaves 8, 12 and 20, respectively, Fig. 3f–h), the exception being incidence of BS in Exp. 1 where the regression was not significant (p = 0.2, Fig. 3e, dotted line).

Table 5 Black stem (BS) incidence and severity index on leaves 8, 12 and 20 at 450 °Cd after flowering of sunflower plants. Variables are presented in relation to i) main effects of SSr treatments: control (C), grain excision from ½ (G↓) or ¾ (G↓↓) of the head and shading (S), ii) main effects of Hybrids: VDH 487 and Baqueano, and iii) interactions of SSr × Hybrid, and iv) interactions of SSr × Experiment (Exp)

Exp	Factor / interaction		BS Incidence (%)		BS Severity index (%)					
					Leaf 8		Leaf 12		Leaf 20	
1 and 2	SSr	C	62.8 ab [a]		82.6 b		59.4 ab		19.6 ab	
		G↓	57.2 b		68.9 c		52.5 b		10.0 bc	
		G↓↓	50.9 c		62.0 c		37.9 c		8.5 c	
		S	65.6 a		90.9 a		68.4 a		25.7 a	
	Hybrid (Hyb)	VDH	58.5 a		79.4 a		59.2 a		17.4 a	
		Baq	59.8 a		72.7 b		49.8 b		14.5 a	
	SSr × Hyb		VDH	Baq	VDH	Baq	VDH	Baq	VDH	Baq
		C	64.8	60.8	89.5 a	75.6 b	66.3	52.4	24.8	14.5
		G↓	55.8	58.6	67.4 b	70.5 b	59.8	45.2	12.8	7.2
		G↓↓	50.1	51.7	69.6 b	54.4 c	38.1	37.6	7.8	9.3
		S	63.2	68.0	91.3 a	90.4 a	72.8	64.1	24.3	27.1
	SSr × Exp		Exp.1	Exp. 2	Exp.1	Exp. 2	Exp.1	Exp. 2	Exp.1	Exp. 2
		C	69.5	56.1	76.6 b	88.6 a	60 ab	58.7 ab	24.0	15.3
		G↓	65.9	48.6	68.5 bc	69.3 bc	55.2 ab	49.7 b	10.5	9.4
		G↓↓	62.3	39.5	63.7 c	60.4 c	51.6 b	24.2 c	16.1	1.0
		S	69.1	62.1	94.7 a	87.0 a	68.3 a	68.7 a	26.8	24.6

Values represent the combined data from Expts. 1 and 2

[a] Means with different letters are significantly different based on the effect of the factors SSr and Hybrid or on the effect of the interactions SSr × Hyb and SSr × Exp (Fisher's LSD, $\alpha = 0.05$). Where means separation is not indicated the interaction was not significant (Table 2)

Multivariate analysis

The biplot accounted for 77% of total variability (Fig. 5). Disease- and epidemiological-related variables (incidence and severity of BS, the number of rainy days RD) had positive scores, whereas physiological variables (SSr, iPARc, GW, TSC) had negative scores for PC 1, with the exception of GN. PC 1 accounted for 58.4% of total variability. The variables positively related to SSr (iPARc, TSC, GW) were situated together and opposite to sink size (GN) and variables related to BS (INC, SEV, RD). SSr treatments ranged from lower to higher SSr (grain excision from about ¾ or ½ of the head, control and shading) for PC 1 regardless of the hybrid or the experiment.

The relative weight of the experiments and to a lesser extent the hybrid could be detected in PC 2, which accounted for 19.1% of total variability. The main differences observed in this axis were explained by the positively associated variables RD and INC.

Discussion

The increase in SSr observed in these experiments was due mainly to grain excision, while decrease in SSr was due to a reduction in incident radiation by the artificial shading, and the subsequent decrease in iPARc. A decrease in SSr due to BS was observed only when grain was excised from ¾ (G↓↓) of the head. The response could be in part the consequence of decreased interception of radiation due to advanced leaf senescence induced by *L. lindquistii* (Quiroz et al. 2014).

GW was indirectly affected by shading and grain excision and followed source availability. GW was reduced by shading but increased by excision of grain, reduction in weight being due primarily to reduction in photosynthesis (López Pereira et al. 2008). Moreover, plants with a low SSr (shading) remobilize reserves from the stem (an 18% decrease in stem dry matter weight between flowering and PM) (Hall et al. 1990), while those with a high SSr (grain excised from the

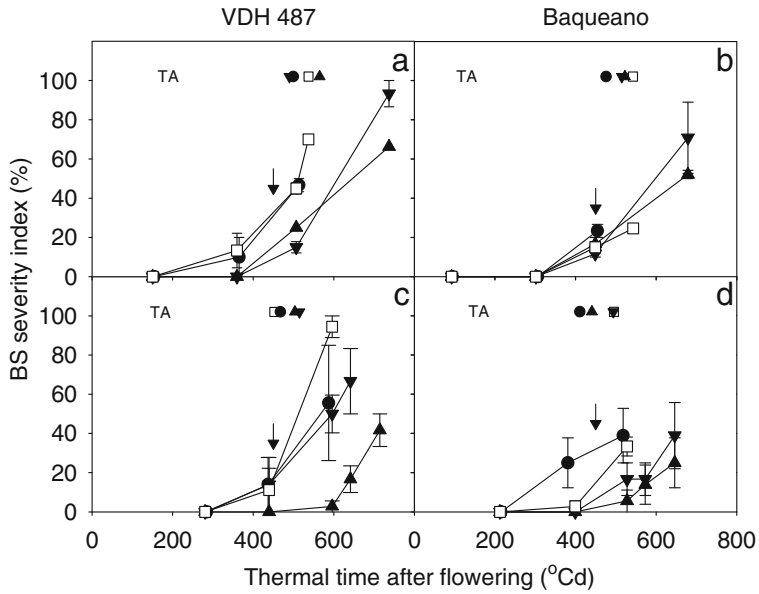

Fig. 2 Severity of black stem (BS) caused by *Leptosphaeria lindquistii* on leaf 20 as a function of thermal time after flowering on hybrids VDH 487 (**a**, **c**) and Baqueano (**b**, **d**), in Exp 1 (**a**, **b**) and Exp 2 (**c**, **d**). Treatments: control (open squares), grain excised from ½ (inverted triangles) or ¾ (triangles) of the head or shading (solid circles). Dates of treatments application (TA) and for physiological maturity (each treatment represented by its corresponding symbol) are indicated at the top of the chart. The arrow indicates 450 °Cd after flowering. Vertical bars on the symbols indicate the standard error of the mean value ($n = 9$)

head) accumulate reserves in the stem (Ho and Below 1989). In this work and previous research (Ruiz and Maddonni 2006), the stem reserves were not considered in the calculation of the SSr, neither as a source nor as a sink. Carbohydrate availability depended upon actual photosynthesis, accumulation or remobilization of reserves from the stem, and GN and GW. Similarly in our studies TSC in the stem increased with a high SSr (excising grain from the head) and decreased with a low SSr (shading). Physiological maturity, and therefore crop development was not affected by any SSr treatments, so we dismiss the hypothesis that degree-days to different developmental stages depends on SSr treatments.

However, our work demonstrated that BS was related to SSr. A high SSr delayed disease progress, while a decrease in SSr accelerated disease in most cases. Dodd (1980) observed an increase in stalk rot in corn plants (*Zea mays*) when the plant had a large number of kernels, and Lassois et al. (2010) determined that banana fruit from plants with a high SSr were less susceptible to crown rot; although a decrease in SSr was not associated with more disease. Our results and these other studies suggest photosynthetic translocation can affect the resistance response of a plant to a disease. In our work, the delay in disease progress observed in plants with a high SSr could be a consequence of the greater carbohydrate content increasing available energy to trigger defense metabolic processes, as suggested by Dangl and Jones (2001). In this respect, Alignan et al. (2006) suggested a model in which the negative regulation of the Mitogen-activated protein kinase (MAPK) cascade could subsequently trigger sunflower defense responses to infection by *L. lindquistii*.

In our studies, incidence and severity of BS was affected by SSr, experiment and hybrid. Rainfall, along with the number of rainy days are environmental variables that affect development of BS (Schwanck et al. 2016). The hybrid VDH 487 was more susceptible to developing BS compared to the hybrid Baqueano, and VDH 487 had lower SSr, probably due to the higher potential weight of the grains. Supporting this contention, remobilization of reserves from the stem was also greater in hybrid VDH 487 compared to hybrid Baqueano (a 20% vs a 5% decrease in stem dry matter weight, respectively, data not shown).

The observation that TSC reached saturation at high values of SSr suggests that an adjustment in photosynthesis by the plant occurs due to lack of a sink, as previously shown by Sadras et al. (2000), also in

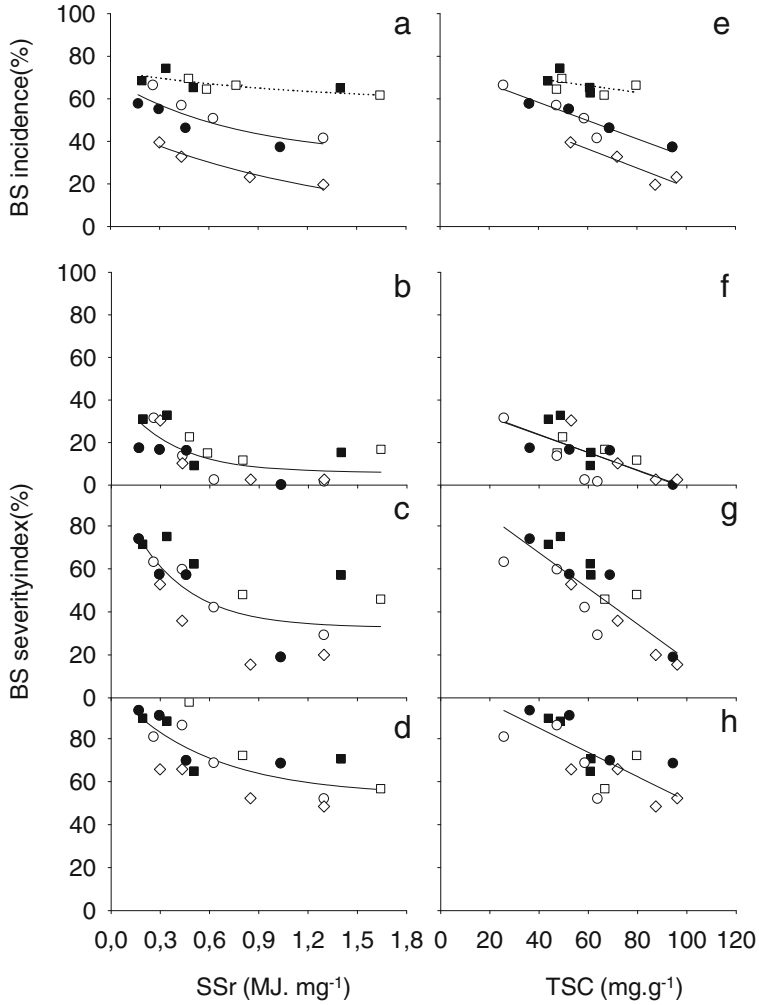

Fig. 3 Incidence of black stem (BS) caused by *Leptosphaeria lindquistii* (**a**, **e**) and severity of BS on leaf 20 (**b**, **f**), 12 (**c**, **g**) and 8 (**d**, **h**) as a function of the source to sink ratio (SSr, **a**, **b**, **c** and **d**) or the total soluble carbohydrates in the stem (TSC, **e**, **f**, **g** and **h**) at 450 °Cd after flowering for sunflower hybrid VDH 487 (black symbols) and Baqueano (white symbols), in Exp 1 (squares), Exp 2 (circles) and the non-treated control plots from Exp 4 (diamonds). Regression solutions are as follows: a: for Exp. 1, BS_{inc} (%) = 58.3 + 14.9 * exp.$^{(-0.880 * SSr)}$, $p = 0.10$, $R^2 = 0.60$, $n = 8$; for Exp.2, BS_{inc} (%) = 32.0 + 37.3 * exp.$^{(-1.307 * SSr)}$, $p = 0.04$, $R^2 = 0.73$, $n = 8$; for Exp. 4, BS_{inc} (%) = 47.4 * exp.$^{(-0.753 * SSr)}$, $p = 0.02$, $R^2 = 0.96$, $n = 4$. b: BS_{SEV20} = 5.81 + 40.58 * exp.$^{(-3.10*SSr)}$, $p = 0.0028$, $R^2 = 0.50$, $n = 20$. c: BS_{SEV12} = 32.65 + 70.33 * exp.$^{(-3.01*SSr)}$, $p = 0.0029$, $R^2 = 0.54$, $n = 18$. d: BS_{SEV8} = 53.43 + 49.80 * exp.$^{(-1.74*SSr)}$, $p = 0.0022$, $R^2 = 0.53$, $n = 19$. e: for Exp.1, BS_{inc} (%) = 75.8–0.16 * TSC, $p = 0.23$, $R^2 = 0.23$, $n = 8$; for Exp.2, BS_{inc} (%) = 75.45–0.43 TSC, $p = 0.0004$, $R^2 = 0.90$, $n = 8$; for Exp. 4 BS_{inc} (%) = 63.2–0.45 TSC, $p = 0.07$, $R^2 = 0.87$, $n = 4$. f: BS_{SEV20} = 40.01–0.41 * TSC, $p = 0.0002$, $R^2 = 0.54$, $n = 20$. g: BS_{SEV12} = 100.77–0.83 * TSC, $p < 0.0001$, $R^2 = 0.72$, $n = 18$. h: BS_{SEV8} = 107.58–0.57 * TSC, $p = 0.0008$, $R^2 = 0.49$, $n = 19$

sunflower. Similar to our observations, *Fusarium* spp. caused more severe symptoms in maize plants with low SSr (Eslava et al. 2007). Also in sunflower, severe infections by *M. phaseolina* were related to a decreased TSC (Davet and Serieys 1987). Carbohydrates could be associated with the plant response to pathogens including production of phenolics and terpenes, which inhibit fungal growth (Molot 1969).

Differences in incidence of BS among experiments were most likely due to epidemiological (amount and type of inoculum, etc.) and/or meteorological (moisture, vapor pressure deficit, etc.) variables. Under conditions of high disease pressure, neither the SSr nor TSC were related to incidence of BS (Exp.1, Fig. 3a, e). Since incidence mostly relies on the infection process while severity mostly relies on the extent of tissue colonization

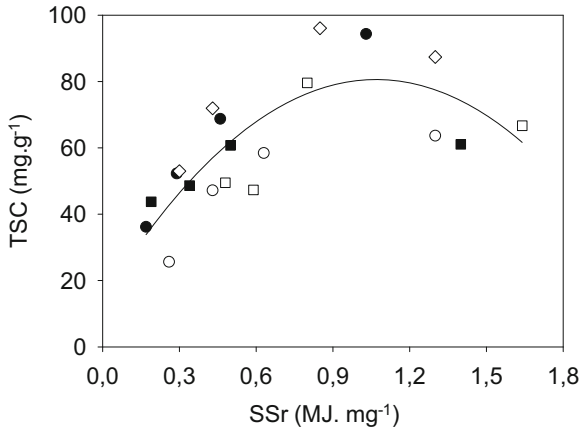

Fig. 4 Total soluble carbohydrates in stems (TSC) as a function of the source to sink ratio (SSr) at 450 °Cd after flowering, for hybrids VDH 487 (black symbols) and Baqueano (white symbols), in Exp. 1 (squares), Exp. 2 (circles) and non-treated control plots from Exp.4 (diamonds). The relationship was described by a positive quadratic regression solution: TSC = 14.46 + 123.77 * SSr – 57.82 * SSr^2, $p = 0.0003$, $R^2 = 0.62$

by the pathogen, the results can be used to infer that under these conditions (high disease pressure) tissue colonization by *L. lindquistii,* more than the infection process, would be affected by levels of SSr or TSC. The association between the number of rainy days and incidence of BS indicates that potential infection with *L. lindquistii* in sunflower could be affected by the production of primary inoculum (Délos and Moinard 1997) from nearby sources or by more favorable conditions for the penetration of the pathogen. On the other hand, differences in severity of BS could be associated with the age of the leaf. The incubation period for *L. lindquistii* is known to be shorter in older leaves (Larfeil et al. 2010), which have decreased photosynthetic efficiency (English et al. 1979) and reduced TSC, and based on our results, probably a greater susceptibility to BS. We observed a severe BS on lower leaves and when the SSr was low. This is consistent with previous studies showing that early initiated fruit were more susceptible to banana crown rot compared to later initiated fruit (Lassois et al. 2010). Furthermore, a high iPAR at flowering is related to a favorable microclimate for the disease (more BS according to Debaeke and Pérès 2003), but also to greater production of grains (Cantagallo et al. 2004) that will subsequently induce a decrease in SSr during the grain filling period (corroborated by the severe BS we observed).

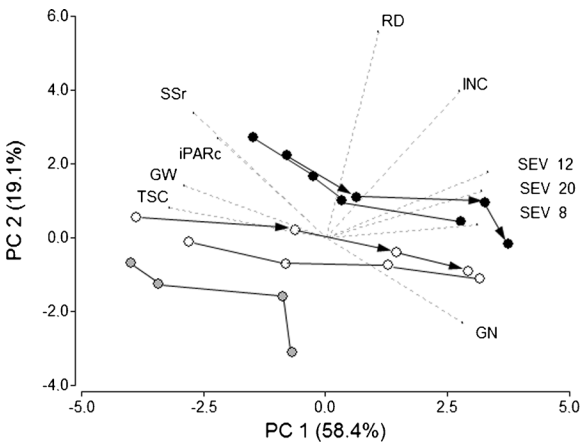

Fig. 5 Biplot derived from the analysis of principal components of hybrids VDH 487 (symbols connected by arrows) and Baqueano (symbols connected by lines), in Exp. 1 (black symbols), Exp. 2 (white symbols) and Exp. 4 (grey symbols), and four SSr treatments (grain excision from ¾ or ½ of the head, a control and a shading treatment), associated with physiological (grain number per plant, GN; unit grain weight, GW; intercepted PAR cumulated from treatment application to 450 °Cd after flowering, iPARc; source to sink ratio, SSr; and total soluble carbohydrates in stem at 450 °Cd after flowering, TSC) and epidemiological variables (incidence of BS, INC and severity of BS for leaf node 8, 12 and 20, SEV-8, SEV-12 and SEV-20, respectively, and number of rainy days, RD), represented by dashed vectors

The sunflower/BS pathosystem used in this work is a useful model system to study plant-pathogen interactions as it is epidemiologically complex (mono and polycyclic components), and the host physiology affects susceptibility to the disease. The results should contribute to understanding the physiological basis of interactions between *L. lindquistii* and sunflower, and how breeding for particular traits might result in improved cultivars (for example, leaf number and plant height, Schwanck et al. 2016). Agronomic factors including plant density, row spacing, etc., are management techniques associated with SSrs, and further crop management could be adjusted to reduce infection by BS. For example, it has been reported that BS and premature ripening in sunflower decreased at lower plant densities (Debaeke and Pérès 2003; Seassau et al. 2010). These results should also be useful in helping to predict BS and its impact on yield, by developing models based on the effects of SSr on BS, the capture of resources and the conversion efficiency of remaining healthy tissue. A similar model based on healthy leaf area and pre-anthesis reserves predicts wheat growth and yield under late epidemics of foliar diseases (Bancal et al. 2007).

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Compliance with ethical standards This article complies with the EJPP Ethical Standards.

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights and informed consent The research did not include humans or animals.

References

Abbate, P. E., Lázaro, L., Montenegro, A. A., Bariffi, J. H., & Gutheim, F. (2005). Potential yield of argentine vs. foreign wheat cultivars. In: Proceedings of the 7th International Wheat Conference, Mar del Plata (Argentina).

Acimovic, M. (1984). Sunflower diseases in Europe, the United State and Australia, 1981-1983. *Helia, 7*, 45–54.

Acimovic, M. (1988). Sunflower disease in Europe and some countries outside Europein the period 1984–1986. *Helia, 14*, 129–144.

Alignan, M., Hewezi, T., Petitprez, M., Dechamp-Guillaume, G., & Gentzbittel, L. (2006). A cDNA microarray approach to decipher sunflower (*Helianthus annuus*) responses to the necrotrophic fungus *Phoma macdonaldii*. *New Phytologist, 170*, 523–536.

Bancal, M.-O., Robert, C., & Ney, B. (2007). Modelling wheat growth and yield losses from late epidemics of foliar diseases using loss of green leaf area per layer and pre-anthesis reserves. *Annals of Botany, 100*, 777–789.

Bordat, A., Debaeke, P., Dechamp-Guilaume, G., Mestries, E., Seassau, C., & Vincourt, P. (2011). *Phoma et dessèchement précoce du tournesol*. Toulouse: CETIOM.

Borrás, L., Slafer, G. A., & Otegui, M. E. (2004). Seed dry weight response to source-sink manipulations in wheat, maize and soybean: a quantitative reappraisal. *Field Crops Research, 86*, 131–146.

Cantagallo, J. E., Medan, D., & Hall, A. J. (2004). Grain number in sunflower as affected by shading during floret growth, anthesis and grain setting. *Field Crops Research, 85*, 191–202.

Charles-Edwards, D. A., & Lawn, R. J. (1984). Light interception by grain legume row crops. *Plant, Cell & Environment, 7*, 247–251.

Dangl, J. L., & Jones, J. D. G. (2001). Plant pathogens and integrated defense responses to infection. *Nature, 41*, 826–833.

Davet, P., & Serieys, H. (1987). Relation between the amount of reducing sugars in sunflower tissues and their invasion by *Macrophomina phaseolina* (Tassi) Goid. *Journal of Phytopathology, 118*, 212–219.

Debaeke, P., & Pérès, A. (2003). Influence of sunflower (*Helianthus annuus* L.) crop management on Phoma black stem (*Phoma macdonaldii* Boerema). *Crop Protection, 22*, 741–752.

Délos, M., & Moinard, J. (1997). *Asphodel: modèle de simulation des épidémies de Phomopsis du tournesol (Diaporthe helianthi)*. Tours: Conférence Internationale sur les Maladies des Plantes.

Díaz-Zorita, M., & Duarte, G. A. (2002). *Manual Práctico para el Cultivo de Girasol*. Buenos Aires: Editorial Hemisferio Sur.

Dodd, J. L. (1980). Grain sink size and predisposition of *Zea mays* to stalk rot. *Phytopathology, 70*, 534–535.

Donald, P. A., Vanette, J. R., & Gulya, T. J. (1987). Relationship between *Phoma macdonaldii* and premature death of sunflower in North Dakota. *Plant Disease, 71*, 366–368.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356.

English, S. D., McWilliam, J. R., Smith, R. C. G., & Davidson, J. L. (1979). Photosynthesis and partitioning of dry matter in sunflower. *Australian Journal of Plant Physiology, 6*, 149–164.

Eslava, F., Vega, C., & Vargas Gil, S. (2007). Relación fuente-destino durante el llenado de granos y su asociación con la susceptibilidad al quebrado de tallos y el vuelco en maíz (pp.10–11). Actas del Workshop Internacional "Eco Fisiología Vegetal Aplicada al Estudio de la Determinación del Rendimiento y la Calidad de Cultivos de Granos", Primer encuentro de Red Raíces de Ecofisiología SECyT, Mar del Plata (Argentina).

Fayzalla, S., & Maric, A. (1981). Contribution à l'étude de la biologie et de l'épidémiologie de *Phoma macdonaldii* Boerema provoquant la maladie des taches noires dutournesol. *Zastita Bilja, 32*, 13–27.

Gallo, K. P., & Daughtry, C. S. T. (1986). Techniques for measuring intercepted and absorbed photosynthetically active radiation in corn canopies. *Agronomy Journal, 78*, 752–756.

Hall, A., Whitfield, D., & Connor, D. (1990). Contribution of pre-anthesis assimilates to grain filling in irrigated and water-stressed sunflower crops II. Estimates from a carbon budget. *Field Crops Research, 24*, 273–294.

Ho, I., & Below, F. (1989). Whole plant senescence of sunflower following seedhead removal. *Plant Physiology, 91*, 85–90.

Kiniry, J. R., Blanchet, R., Williams, J. R., Texier, V., Jones, C. A., & Cabelguenne, M. (1992). Simulating sunflower with the EPIC and ALMANAC 4 models. *Field Crops Research, 30*, 403–423.

Larfeil, C., Barrault, G., & Dechamp-Guillaume, G. (2010). Assessment of sunflower genotype tolerance to *Phoma macdonaldii*. *OCL Oléagineux Corps Gras Lipides, 17*(3), 161–166.

Lassois, L., Bastiaanse, H., Chillet, M., Jullien, A., Jijakli, M. H., & De Lapeyre de Bellaire, L. (2010). Hand position on the bunch and source-sink ratio influence the banana fruit susceptibility to crown rot disease. *Annals of Applied Biology, 156*(2), 221–229.

Lazzaro, N., Velázquez, L., Aguirrezabal, L.A.N., Escande, A., & Quiroz, F.J. (2013). Impacto de las enfermedades de fin de ciclo en girasol sobre rendimiento y porcentaje de aceite en ambientes de Balcarce, Argentina. Libro de Actas XXII Congreso Peruano and XVII Congreso Latinoamericano de Fitopatología, Lambayerque (Perú).

López Pereira, M., Berney, A., Hall, A. J., & Trápani, N. (2008). Contribution of pre-anthesis photoassimilates to grain yield: its relationship with yield in Argentine sunflower cultivars released between 1930 and 1995. *Field Crops Research, 105*, 88–96.

Miric, E., Aitken, E. A. B., & Goulter, K. C. (1999). Identification in Australia of the quarantine pathogen of sunflower *Phoma macdonaldii* (Teleomorph: *Leptosphaeria linquistii*). *Australian Journal of Agricultural Research, 50*, 325–332.

Molot, P. M. (1969). Recherches sur la resistance du maïs al´ Helmithosporiose et aux Fusarioses. *Annales de Phytopathologie, 1*, 55–74.

Moltimore, C. G., & Ward, G. M. (1964). Root and stalk of corn in southwester Ontario. III. Sugar levels as a measure of plant vigor resistance. *Canadian Journal of Plant Science, 44*, 451–457.

Monteith, J. L., & Unsworth, M. H. (1990). *Principles of environmental physics*. Oxford: Elsevier Academic Press.

Penaud, A., & Pérès, A. (1994). The Phoma of sunflower. *Oléoscope, 15*, 37.

Quiroz, F. J., Edwards Molina, J. P., & Dosio, G. A. A. (2014). Black stem by *Phoma macdonaldii* affected ecophysiological components that determine grain yield in sunflower (*Helianthus annuus* L.) *Field Crops Research, 160*, 31–40.

Rousseaux, M. C., Hall, A. J., & Sanchez, R. A. (1996). Far-red enrichment and photo-synthetically active radiation level influence leaf senescence in fieldgrown sunflower. *Journal of Plant Physiology, 96*, 217–224.

Ruiz, R. A., & Maddonni, G. A. (2006). Sunflower seed weight and oil concentration under different postflowering source-sink ratios. *Crop Science, 46*, 671–680.

Sadras, V. O., Echarte, L., & Andrade, F. H. (2000). Profiles of leaf senescence during reproductive growth of sunflower and maize. *Annals of Botany, 85*, 187–195.

Schneiter, A. A., & Miller, J. F. (1981). Description of sunflower growth stages. *Crop Science, 21*, 901–903.

Schwanck, A. A., Savary, S., Lepennetier, A., Debaeke, P., Vincourt, P., & Willocquet, L. (2016). Predicting quantitative host plant resistance against phoma black stem in sunflower. *Plant Pathology, 65*, 1366–1379.

Seassau, C., Dechamp-Guillaume, G., Mestries, E., & Debaeke, P. (2010). Nitrogen and water management can limit premature ripening of sunflower induced by *Phoma macdonaldii*. *Field Crops Research, 115*, 99–106.

SIIA-MAGPyA (2015). Sistema integrado de información agropecuaria. Ministerio de Agricultura, Ganadería, Pesca y Alimentación de la República Argentina. http://www.siia.gov.ar. Accesed 26 June 2015.

Trapani, N., Hall, A. J., Sadras, V. O., & Vilella, F. (1992). Ontogenetic changes in radiation use efficiency of sunflower (*Helianthus annuus* L.) crops. *Field Crops Research, 29*, 301–316.

Velásquez, P. D., & Formento, N. (2003). Efecto de la infección natural de *Phoma oleracea* var *Helianthi-tuberosi* Sacc. sobre algunos caracteres agronómicos y el rendimiento de aceite de cuatro genotipos de girasol (*Helianthus annuus* L.) con dos niveles de fertilización nitrogenada. *Agrisciencia, 20*, 29–34.

Wu, P. S., Du, H. Z., Zhang, X. L., Luo, J. F., & Fang, L. (2012). Occurrence of *Phoma macdonaldii*, the causal agent of sunflower black stem disease, in sunflower fields in China. *Plant Disease*. doi:10.1094/PDIS-05-12-0485-PDN.