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Black stem by *Phoma macdonaldii* affected ecophysiological components that determine grain yield in sunflower (*Helianthus annuus* L.)

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ABSTRACT

Black stem (BS) of sunflower (*Helianthus annuus* L.) by *Phoma macdonaldii* has been reported in many production areas of the world associated to losses in grain yield. Their symptoms could be confounded with natural leaf senescence because they appear during the reproductive period. The objective of this work was to evaluate the effect of BS by *P. macdonaldii* on grain yield and the ecophysiological components determining it.

Two field experiments were conducted under natural inoculation of *P. macdonaldii*. Two hybrids were protected or not with a combination of leaf fungicides. Severity of BS, leaf area, leaf senescence, intercepted PAR and dry matter were periodically measured. Nitrogen nutrition index was estimated at flowering. At physiological maturity, BS incidence, grain number and weight, and oil concentration were also measured.

Presence of other diseases was low or null. Incidence of BS ranged from 2.9% to 49% within the protection treatments, hybrids and experiments. The protected treatment diminished BS in both hybrids and experiments in comparison with the unprotected one. Plants from protected treatment showed higher leaf area, leaf area duration, intercepted PAR, radiation use efficiency, dry matter and grain yield and their components, than those from unprotected treatment. Oil concentration was not affected by the disease. Differences in all studied variables were mainly supported for differences in mid and upper leaves.

Black stem was related to premature leaf senescence and thus to reduction of intercepted radiation. An effect not only on the efficiency of radiation interception but also in the efficiency of the radiation conversion was observed.

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1. Introduction

The black stem (BS) of sunflower (*Helianthus annuus* L.) by *Phoma macdonaldii* Boerema (teleomorph *Leptosphaeria lindquistii* Frezzi) has been reported in many sunflower production areas of the world (Acimovic, 1984, 1988) including Argentina where it behaves as an endemic disease (Bertero de Romano, 1978). Losses between 10 and 30% in grain yield were associated to BS (Velásquez and Formento, 2003) and premature ripening (Carson, 1991) both caused by

P. macdonaldii. The use of susceptible hybrids, no-tillage sowing and most intensification practices (high mineral nutrition, high plant density, shortening of row distance) generates predisposing conditions to BS development (Debaeke and Pérès, 2003).

P. macdonaldii is a soil-borne fungus which overwinters as mycelium, pycnidia and pseudothecia in infected stubble residue (Gulya et al., 1997). Black stem symptoms are associated to previous necrosis in veins and/or leaf petiole and to an acropetal progress from bottom to upper leaves. *P. macdonaldii* also attacks roots and collar of the plant producing a stem girdling lesion at the soil level at the beginning of premature ripening (Donald et al., 1987).

As yet, there are no reports of sunflower genotypes resistant to BS. However, several tolerant genotypes with resistance based mostly on additive genes effect were characterized (i.e. Darvishzadeh et al., 2007). The existence of tissue-specific

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resistance genes for *P. macdonaldii* has been suggested (Abou Al Fadil et al., 2007).

The effect of foliar diseases as BS can be evaluated through the study of structural variables (leaf area, chlorophyll, interception of radiation) and/or functional variables (photosynthesis) (Sadras et al., 2000a). A characterization and evaluation of the effects of crop management (irrigation, nitrogen and plant density) via the crop leaf area index or the fraction of PAR on frequency and severity of BS was reported for sunflower (Debaeke and Pérès, 2003). Research in alfalfa (*Medicago sativa*) demonstrated that leaf spot injury caused by *Phoma medicaginis*, *Phoma sclerotioides*, *Leptosphaerulina trifolii* and *Stemphylium botryosum* reduced photosynthetic efficiency (Hwang et al., 2006). In other studies, *Phoma* spp. and *Phyllosticta* spp. decreased leaf area in trees of *Cecropia schreberiana* (Myster, 2002).

Leaf functionality is associated to incident light and therefore to its position on the stem. Bottom leaves receive an intensity and a quality of light (red/far red ratio) poorer than upper ones which make them senesce before (Rousseaux et al., 1996). Leaf age is also a factor which modulates photosynthesis. As was reported in sunflower, photosynthesis capacity in old leaves is lower than in younger ones (English et al., 1979). Consequently, the effect of BS can be more or less significant depending on these factors.

The objective of this work was to evaluate the effect of BS by *P. macdonaldii* on grain yield and ecophysiological related components in sunflower. To accomplish this objective, yield and ecophysiological components were measured in plants with different level of disease manifestation, achieved by (i) applying or not a fungicide protection against BS infection; (ii) selecting two hybrids with different susceptibility to BS and (iii) different environmental conditions as a consequence of experiments performed in two growing seasons.

2. Materials and methods

2.1. Cultural details

Two experiments, hereafter referred to as Exp. 1 and Exp. 2, were conducted at the INTA Balcarce Experimental Station, Argentina (37°45′ S, 58°18′ W). The soil in both experiments was a Typic Argiudol (USDA taxonomy).

Hybrids Payé (KWS, S.A., Balcarce, Buenos Aires, Argentina) and Paraíso 30 (Nidera S.A., Junín, Buenos Aires, Argentina) were sown the 22 Nov. 2002 and the 28 Oct. 2004, in Exp. 1 and Exp. 2, respectively. Hybrid Payé was reported as more susceptible to BS by *P. macdonaldii* and Verticillium wilt than Paraíso 30 in the cultivar evaluation trial network of INTA, year 2001, where severity was evaluated at physiological maturity. Potential number of leaves in experiments with good nutritional and water conditions was 33 in hybrid Payé and 40 in Paraíso 30, while oil potential content was 53.7% in hybrid Payé and 53.1% in Paraíso 30. Emergence occurred 7 and 16 days after sowing, in Exp. 1 and Exp. 2, respectively. Plant density was 7.2 and 5.6 plants m^{-2}, in Exp. 1 and Exp. 2, respectively. The experiments were conducted under conditions of natural inoculation of *P. macdonaldii* in plots infected with the pathogen (verified in previous experiments). Good nutritional conditions were assured by fertilization before sowing with 75 kg ha^{-1} of diammonium phosphate (46% of P_2O_5 and 18% of ammoniacal nitrogen) and at developmental stage V7 (Schneiter and Miller, 1981) with 60 kg ha^{-1} of urea (46% N). Soil water content was monitored every 5–7 days by neutron probe (Troxler 4300, Troxler Electronic Laboratories, Inc., Research Triangle Park, NC). Soil water was maintained by furrow irrigation above 40% of maximum available water in the firsts 0.60 m of soil during the entire growing season. The amount of applied water was 150 mm and 250 mm in

Exp. 1 and Exp. 2, respectively. Weeds and insects were controlled adequately through cultural and chemical techniques.

Flowering date of a plant was considered as the day on which all florets from the outer ring of the head showed their stamens (R5.1, Schneiter and Miller, 1981). Flowering date of a plot was registered as the day on which 95% of the plants had flowered and occurred 55 days after emergence in both hybrids in Exp. 1, and 59 and 63 days after emergence in Exp. 2, in hybrids Payé and Paraíso 30, respectively. Time was expressed on a thermal time basis by daily integration of air temperature with a threshold temperature of 6 °C (Kiniry et al., 1992) and with flowering as thermal time origin.

The following treatments were applied from 30 to 104 (743 Cd after first flowering, R5.1, Schneiter and Miller, 1981) and 105 (635 Cd after first flowering, average of the two hybrids) days after emergence in Exp. 1 and Exp. 2, respectively:

(1) A combination of two fungicides in 150 l ha^{-1} spray volume:
   (a) a systemic, preventive, and curative fungicide whose active principle is carbendazim + epoxiconazole (Duett®, Basf Co.) applied every 15 days at 0.750 l ha^{-1} dose, and
   (b) a systemic and contact fungicide with a preventive, curative and antisporulant action, with azoxistrobin as the active principle (Amistar®, Syngenta Co.) applied every 30 days at 0.5 l ha^{-1} dose.

   This treatment will be referred hereafter as F+.
(2) A sole application of water every 15 days, named hereafter as F−.

Hybrids and protection treatments were combined factorially in a randomized split-plot design with three replicates. The hybrids were assigned to the main plots and the protection treatments to the subplots. Each subplot consisted of four rows of 6 m long, spaced at 0.7 m. Physiological maturity in Exp. 1 was taken as the date in which individual grain weight did not increased compared to the previous sample and in Exp. 2 it was estimated visually from the hard yellow color of the head back face and the brown color of the bracts (R9, Schneiter and Miller, 1981) using a correlation between physiological and visual techniques calculated in Exp. 1 (data not shown). Physiological maturity in Exp. 1 occurred 95 (610 Cd after first flowering) and 100 (697 Cd after first flowering) days after emergence in control and protected treatments, respectively, in hybrid Payé, and 105 (752 Cd after first flowering) days after emergence in both treatments in hybrid Paraíso 30 while in Exp. 2, 109 (703 Cd after first flowering) and 111 (731 Cd after first flowering) days after emergence in control and protected treatments, respectively, in hybrid Payé, and 113 (710 Cd after first flowering) days after emergence in both treatments in hybrid Paraíso 30.

2.2. Measurements

2.2.1. Global radiation, temperature and rainfall

Daily global incident radiation, daily mean air temperature and rainfall were measured in a weather station located 400 m from the experiments. Radiation and temperature were averaged weekly after emergence.

2.2.2. Number of leaves and leaf area

The number of total, green and senescent leaves was measured in three plants *per* plot (*n* = 9) at flowering. A leaf was considered as green or senescent when at least 50% of its surface was green or senescent, respectively.

The green and senescent area of each leaf of the plant was periodically measured in three plants *per* plot (*n* = 9) with an area meter (LI 3100 Area meter Li Cor, Lincoln, Nebraska USA). Leaf area *per* plant was calculated as the sum of the area of all leaves. Leaf area index was estimated at first flowering and at physiological

maturity of the control treatment. Leaf area duration was calculated as the integral of the time course of the area of each individual leaf or of the plant leaf area.

2.2.3. Intercepted photosynthetically active radiation

Daily incident photosynthetically active radiation (PAR) was calculated as $0.48 \times$ global daily incident radiation. The proportion of PAR intercepted by the crop at noon was determined according to Gallo and Daughtry (1986) as $(1 - Rb/Ro)$, where Rb is PAR measured below the last green leaf and Ro is PAR measured above the canopy. Rb and Ro were measured weekly at solar noon (± 1 h) with a line quantum sensor (LI-191SB, LI-COR, Lincoln, NE, USA). The daily proportion of PAR intercepted between two measurements was calculated by linear interpolation. The daily intercepted PAR was calculated as the product of the daily incident PAR and the daily proportion of PAR intercepted. The daily intercepted PAR was cumulated from flowering to physiological maturity.

2.2.4. Aboveground dry matter

The aboveground dry matter was measured at flowering and physiological maturity. Three to five plants were cut at the ground level. Each plant was separated in stem, leaves, receptacle and grains. Samples were oven-dried (with air circulating at 60 °C) to constant weight and weighed. The weight of thousand grains was calculated as the ratio between the weight and the number of the grains × 1000. In Exp. 1, head samples were taken in four dates during the filling period in order to follow unit grain weight over time to determine physiological maturity as the time when grain weight does not further increase (Aguirrezábal et al., 2003). The harvest index was calculated as the ratio between the weight of the grains and the weight of the aboveground dry matter. The oil concentration was measured in a 15 g sample of grains by the nuclear magnetic resonance technique (NMR, Analyser Magnet Type 10, Newport Oxford Instruments, Buckinghamshire, England) in duplicate and averaged. Grain yield and oil content were expressed at 6% moisture.

2.2.5. Nitrogen nutrition index

The nitrogen nutrition index was calculated at flowering as the ratio between the actual tissue N concentration obtained from total nitrogen measured in a mixture of all green leaves of three plants per plot ($n = 9$) and weighted to the entire plant through partitioning coefficients calculated from Hocking and Steer (1995), and the tissue N concentration needed to obtain the maximum instantaneous crop growth rate (Debaeke et al., 2012).

2.2.6. Radiation use efficiency

The radiation use efficiency was calculated as the ratio between the aboveground dry matter and the PAR intercepted cumulated from flowering to physiological maturity.

2.2.7. Incidence of BS by P. macdonaldii

Incidence of BS by P. macdonaldii (I %) was evaluated at physiological maturity (stage R9, Schneiter and Miller, 1981) in 10 plants per plot ($n = 30$), as the ratio between the affected to the total number of nodes per plant.

2.2.8. Severity of BS by P. macdonaldii

Severity of BS by P. macdonaldii was evaluated only in Exp. 2 from first flowering (stage R5.1, Schneiter and Miller, 1981) to physiological maturity (stage R9, Schneiter and Miller, 1981) every 10 days. Measurements were performed in leaves 4, 8, 12, 16, 20, 24, 28, 32

and 36 (from the bottom of the plant) selected to obtain a suitable plant profile of BS severity, in three plants per plot ($n = 9$), as Eq. (1):

$$S(\%) = \frac{n_{25} \times 25 + n_{50} \times 50 + n_{75} \times 75 + n_{100} \times 100}{\text{TN}} \qquad (1)$$

where S (%) was the severity of BS by P. macdonaldii; n_{25}, n_{50}, n_{75} and n_{100} were the number of nodes with symptoms of 25, 50, 75 or 100, respectively; 25 means a small spot limited to the region of insertion of the petiole in the stem; 50 means a spot occupying up to a half of the stem perimeter; 75 means a spot occupying more than a half of the stem perimeter without contact between spots of other nodes, and 100 means a spot enclosing the whole stem perimeter; TN was the total number of nodes.

The area under the disease progress curve of BS severity by P. macdonaldii was calculated on the same leaves as was done for leaf area duration. Necrotic to total tissue ratio was measured in the same leaves.

2.2.9. Pathogens identification

Presence of pycnidia and spores of P. macdonaldii was identified in association with BS symptoms at physiological maturity (Boerema et al., 2004). Severity of spots by Alternaria helianthi or Septoria helianthi (Leite and Amorim, 2002), white rust by Albugo tragopogonis (five visual severity levels, Quiroz, unpublished) and black rust by Puccinia helianthi (Gulya et al., 1990) was also evaluated with diagrammatic scales. Observations in Exp. 1 were performed in stages R5 and R8 (Schneiter and Miller, 1981) in leaves 12, 20 and 28, while in Exp. 2, they were done in the same stages and leaves as for BS.

2.3. Data analysis

Data of nitrogen nutrition index, number of total and senescent leaves, leaf area index, leaf area, leaf area duration, aboveground dry matter, intercepted photosynthetically active radiation, radiation use efficiency, grain weight, grain number, grain yield, harvest index, oil content, incidence and area under the disease progress curve of the severity of BS were processed by analysis of variance procedures including the experiment as a factor (Procedure GLM, SAS vs. 12.1, SAS Institute, Cary, NC). Differences among treatments means were evaluated with the LSD test ($P \leq 0.05$). A regression analysis between BS incidence and leaf area index at physiological
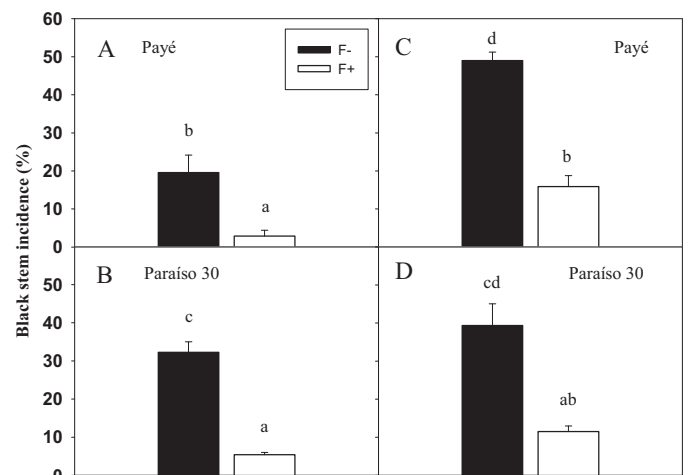

Fig. 1. Incidence of black stem by Phoma macdonaldii. Incidence (%) of black stem by P. macdonaldii. Protection treatments: F+, application of fungicides (white bars); F−, without application of fungicides (black bars). Hybrids: Payé (A and C) and Paraíso 30 (B and D) in Exp. 1 (A and B) and Exp. 2 (C and D). Vertical lines on the bars correspond to the standard error of the mean value ($n = 30$). Different letters mean significant differences ($P \leq 0.05$).
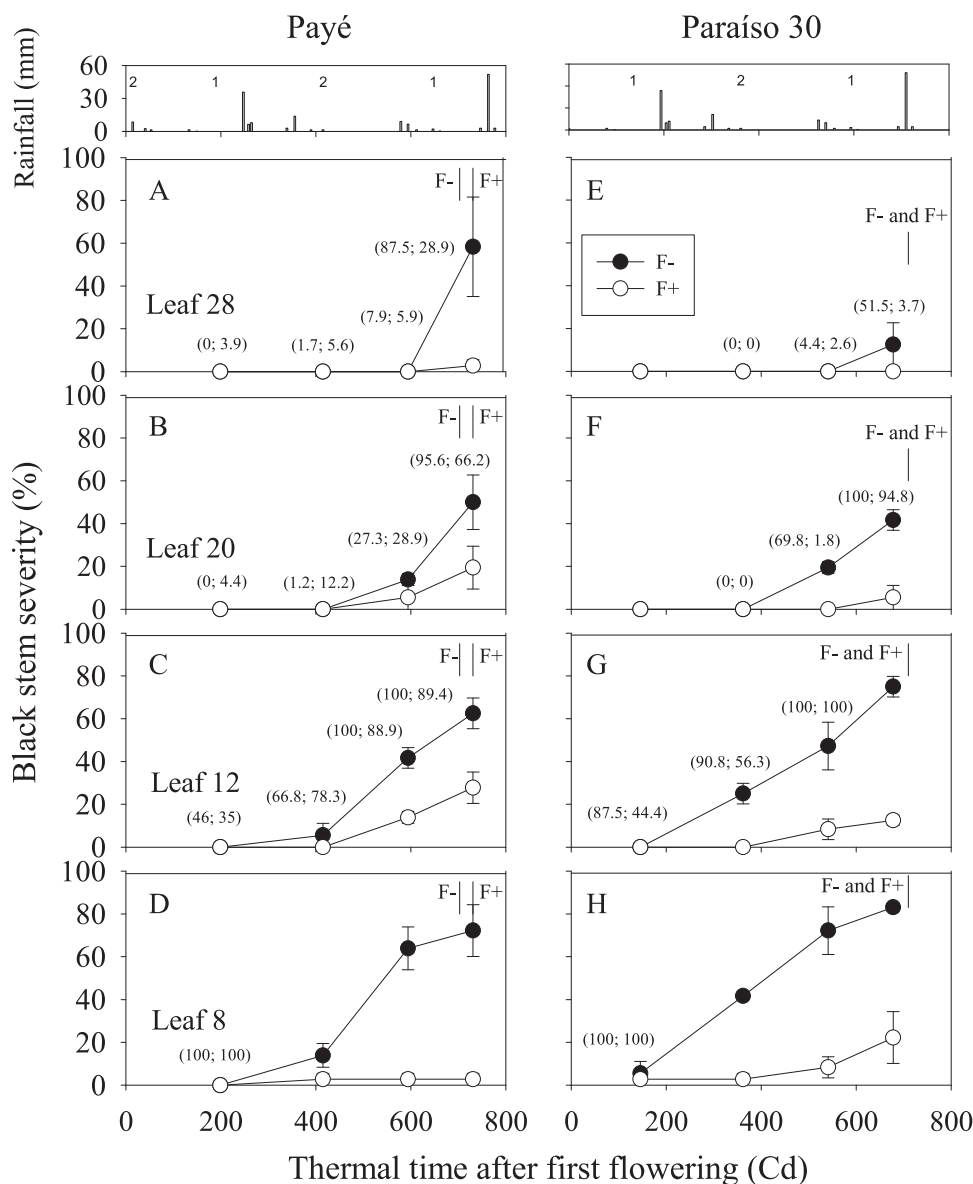
Fig. 2. Severity of black stem by *Phoma macdonaldii* as a function of thermal time. Severity (%) of black stem by *Phoma macdonaldii* as a function of thermal time from first flowering (Cd, Tbase = 6 °C) in leaves 28 (A and E), 20 (B and F), 12 (C and G) and 8 (D and H) from the bottom of the plant in Exp. 2. The proportion of senescent to total leaf tissue (%) is shown in parentheses for F− and F+. Rainfall after flowering are shown above A and E, also with the fungicides application schedule (1: carbendazim + epoxiconazole + azoxistrobin and 2: carbendazim + epoxiconazole). Protection treatments: F+: application of fungicides (open symbols) and F−: without application of fungicides (close symbols). Hybrids: Payé (A–D) and Paraíso 30 (E–H). Physiological maturity in F− and F+ is indicated in each chart with vertical lines. Vertical lines on the symbols correspond to the standard error of the mean value (n = 9).

maturity of the unprotected treatment was performed (Procedure REG, SAS vs. 12.1, SAS Institute, Cary, NC).

3. Results

3.1. Meteorological conditions during crop development

Solar radiation during the vegetative period (weeks after emergence 1–8 and 1–9, in Exp. 1 and Exp. 2, respectively) was around 10% higher in Exp. 1 than in Exp. 2 (Table 1). Rainfall was also higher in Exp. 1 than in Exp. 2 during this period (34 mm) but the number of weeks with more than 5 mm of rainfall was greater in Exp. 2 than in Exp. 1 (8 vs. 3 weeks, Table 1). During the reproductive period (weeks after emergence 9–14 and 10–15 in Exp. 1 and Exp. 2, respectively) only a slight increase in temperature and radiation was observed in Exp. 1 in comparison with Exp. 2. Rainfall in this

period was rather similar between experiments. In Exp. 1, rainfall observed in week 15 after emergence occurred after physiological maturity of the crop (Table 1).

3.2. Disease symptoms

Incidence of BS by *P. macdonaldii* at physiological maturity ranged from 2.9% to 49% among all studied situations and was 83% and 69% lower in protected treatment, in Exp. 1 and Exp. 2, respectively (protection × experiment effect: P = 0.063, Fig. 1). In Exp. 1 incidence of BS was higher in hybrid Paraíso 30 than in hybrid Payé while in Exp. 2 the opposite was observed (hybrid × experiment effect: P = 0.0058, Fig. 1).

Leaves below position 8 were senescent at 100 Cd after first flowering without symptoms of BS in the node. Leaf 8 was already senescent at the first severity measurement in both protection

Table 1

Daily mean air temperature, daily mean solar radiation and cumulated rainfall during crop development. Data were weekly averaged (temperature, radiation) or cumulated (rainfall) from emergence. Flowering occurred within the 8th and 9th week after emergence (WAE) in experiment 1 (Exp. 1) and experiment 2 (Exp. 2), respectively. Physiological maturity occurred within the 14th and 15th WAE, in Exp. 1, and within the 16th and 17th WAE in Exp. 2.

WAE	Temperature (°C)		Solar radiation (MJ)		Rainfall (mm)	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
1	18.3	14.1	24.9	16.9	0.8	28.5
2	18.9	17.8	21.9	18.7	5.0	2.5
3	19.6	21.8	22.4	19.6	32.5	18.0
4	20.1	17.7	24.0	21.5	1.0	9.1
5	19.4	20.1	23.4	23.8	0.0	15.0
6	20.2	19.4	22.5	21.6	43.8	20.6
7	23.5	21.4	23.0	23.3	69.3	5.8
8	19.5	24.1	21.6	21.7	2.6	10.6
9	23.2	20.8	23.4	21.7	8.0	11.1
10	23.4	19.2	21.2	26.4	1.4	2.9
11	20.7	20.1	19.1	24.7	25.8	0.3
12	16.6	16.8	16.9	18.1	24.6	50.2
13	21.3	21.4	20.1	13.2	39.0	19.9
14	22.9	22.1	19.8	17.9	0.0	0.0
15	19.9	23.0	13.5	17.0	88.4	19.3

treatments and hybrids (severity was only measured in Exp. 2). Severity of BS increased after end of flowering in both hybrids and the symptoms progressed from bottom to upper leaves (i.e. between 200 and 400 Cd after first flowering in leaves eight and 12, between 400 and 600 Cd in leaf 20 and after 600 Cd in leaf 28, Fig. 2). The senescent to total leaf tissue ratio (%) in the last two measurements of BS was mostly higher in plants from unprotected than protected treatment (Fig. 2, in brackets) and sometimes differences were observed even with low or null symptoms of the disease (Fig. 2C and G, in brackets). At physiological maturity BS severity in protected treatment decreased 85, 71, 73 and 96% (average of both hybrids) in leaves 8, 12, 20 and 28, respectively (Fig. 2). The area under the disease progress curve from leaf eight to 28 decreased in the protected treatment in both hybrids (higher P value: $P = 0.0581$) showing bottom leaves a greater and more significant effect than upper leaves (Fig. 3A and B).

The symptomatic manifestation of the other measured diseases was low (i.e. the highest value of severity of leaf spot by *A. helianthi* was 4% in bottom leaves) or null (i.e. black rust by *P. helianthi*, spot by *S. helianthi* and BS in the base or premature ripening by *P. macdonaldii*).

3.3. Number of leaves and leaf area

The number of total and senescent leaves at flowering was 16% (hybrid effect: $P = 0.0022$) and 11% (hybrid effect: $P = 0.0198$) higher in hybrid Paraíso 30 than in Payé, respectively (Table 2).

Unprotected plants showed a lower leaf area than protected ones with the exception of hybrid Payé in Exp. 1 where differences were not significant (Fig. 4A). Physiological maturity in this hybrid was delayed 84 and 28 Cd in the protected treatment in Exp. 1 and Exp. 2, respectively (Fig. 4A and C). Differences on leaf area in hybrid Paraíso 30 were mainly observed from 400 Cd after first flowering in Exp. 1 (Fig. 4B).

Leaf area duration from first flowering to physiological maturity was 21% higher in protected than unprotected treatment ($256.7 \pm 15.4 \, \text{m}^2$ Cd vs. $216.5 \pm 16.3 \, \text{m}^2$ Cd, respectively, protection effect: $P = 0.0032$). In the same period, hybrid Paraíso 30 showed a leaf area duration 32% higher than Payé ($281.7 \pm 11.7 \, \text{m}^2$ Cd vs. $191.4 \pm 8.5 \, \text{m}^2$ Cd, respectively, hybrid effect: $P = 0.005$). The protection treatment had a higher leaf area duration in comparison with the unprotected treatment in leaves 20 (protection effect: $P = 0.0124$), 24 (protection effect: $P = 0.0159$) and 28 (protection
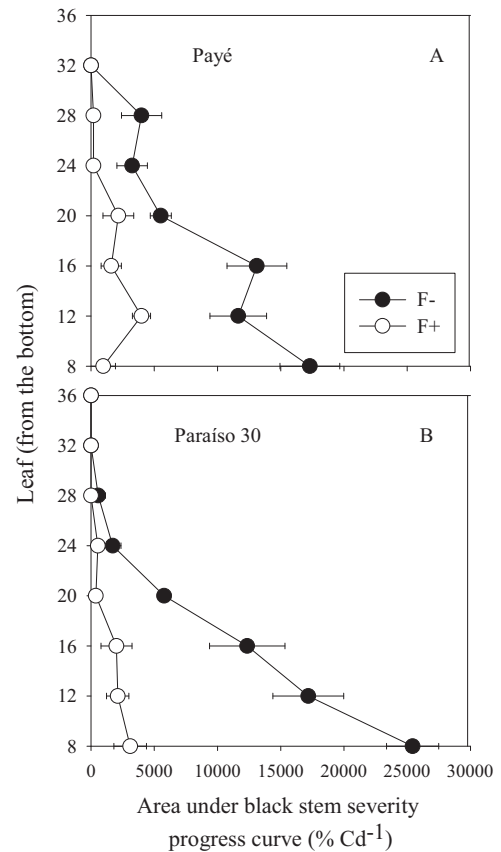

Fig. 3. Area under the black stem by *Phoma macdonaldii* severity curve. Area under the black stem by *Phoma macdonaldii* severity curve (% Cd^{-1}) in leaves 8, 12, 16, 20, 28, 32 (hybrids Payé and Paraíso 30) and 36 (hybrid Paraíso 30) from the bottom of the plant in Exp. 2. Protection treatments: F+, application of fungicides (open symbols) and F−, without application of fungicides (close symbols). Hybrids: Payé (A) and Paraíso 30 (B). Horizontal lines on the symbols correspond to the standard error of the mean value ($n = 9$).

effect: $P = 0.0063$) in both hybrids and in hybrid Paraíso 30 also in leaf 32 (protection × hybrid effect: $P = 0.0091$, Fig. 4 insets). In the same leaves, hybrid Paraíso 30 had always a higher leaf area duration than hybrid Payé (hybrid effect; $P = 0.0466$, $P = 0.0171$ and $P = 0.0096$, for leaves 20, 24 and 28, respectively, Fig. 4 insets).

3.4. Leaf area index (LAI) ant its relationship with BS incidence

Leaf area index at flowering was 24% higher in Exp. 1 than in Exp. 2 (experiment effect: $P = 0.0083$). Neither a hybrid ($P = 0.1330$) nor a protection ($P = 0.2537$) effect was observed for this variable at this stage (Table 2). When assessed at physiological maturity (of non-protected treatment) LAI in protected treatment was 49% higher than in unprotected one ($P = 0.0002$, Fig. 5). Black stem incidence explained 79% ($P = 0.003$) of the differences observed in LAI at physiological maturity through a negative linear model (Fig. 5). No relationship between BS and LAI was observed at flowering ($R^2 = 0.30$; $P = 0.1614$).

3.5. Interception of radiation and radiation use efficiency (RUE)

The interception of solar radiation decreased faster in unprotected than in protected treatment (Fig. 6). Differences in Exp. 1 were observed later than in Exp. 2, especially in hybrid Payé (Fig. 6A). According to these differences and those arising as a consequence of a delayed physiological maturity (hybrid Payé) intercepted PAR cumulated from flowering to physiological

Table 2
Nitrogen nutrition index (NNI), number of total (TL) and senescent (SL) leaves, leaf area index (LAI) and aboveground dry matter (DM) at flowering, in hybrids Payé and Paraíso 30 during experiments 1 (Exp. 1) and 2 (Exp. 2). Protection treatments (Treat.) were the application (F+) or not (F−) of fungicides.

Exp.	Hybrid	Treat.	NNI	SL	TL	LAI (m² m⁻²)	DM (g m⁻²)
1	Payé	F−	1.15 ± 0.05	4.4 ± 0.4	29.7 ± 0.4	2.60 ± 0.27	442 ± 58
		F+	1.16 ± 0.06	4.2 ± 0.6	30.8 ± 0.7	2.76 ± 0.09	450 ± 9
	Paraíso 30	F−	1.39 ± 0.10	4.6 ± 0.4	36.1 ± 0.3	3.17 ± 0.06	444 ± 21
		F+	1.30 ± 0.14	5.2 ± 0.3	36.7 ± 0.5	3.13 ± 0.58	499 ± 68
2	Payé	F−	0.75 ± 0.03	4.5 ± 0.4	30.1 ± 0.6	1.59 ± 0.13	494 ± 30
		F+	0.80 ± 0.02	4.3 ± 0.5	31.6 ± 0.6	2.01 ± 0.15	551 ± 42
	Paraíso 30	F−	1.06 ± 0.01	4.6 ± 0.4	36.8 ± 0.6	2.43 ± 0.26	700 ± 38
		F+	1.05 ± 0.03	5.2 ± 0.2	36.3 ± 0.4	2.76 ± 0.13	807 ± 39

Mean value ± standard error ($n = 9$ in Exp. 1 and $n = 15$ in Exp. 2)
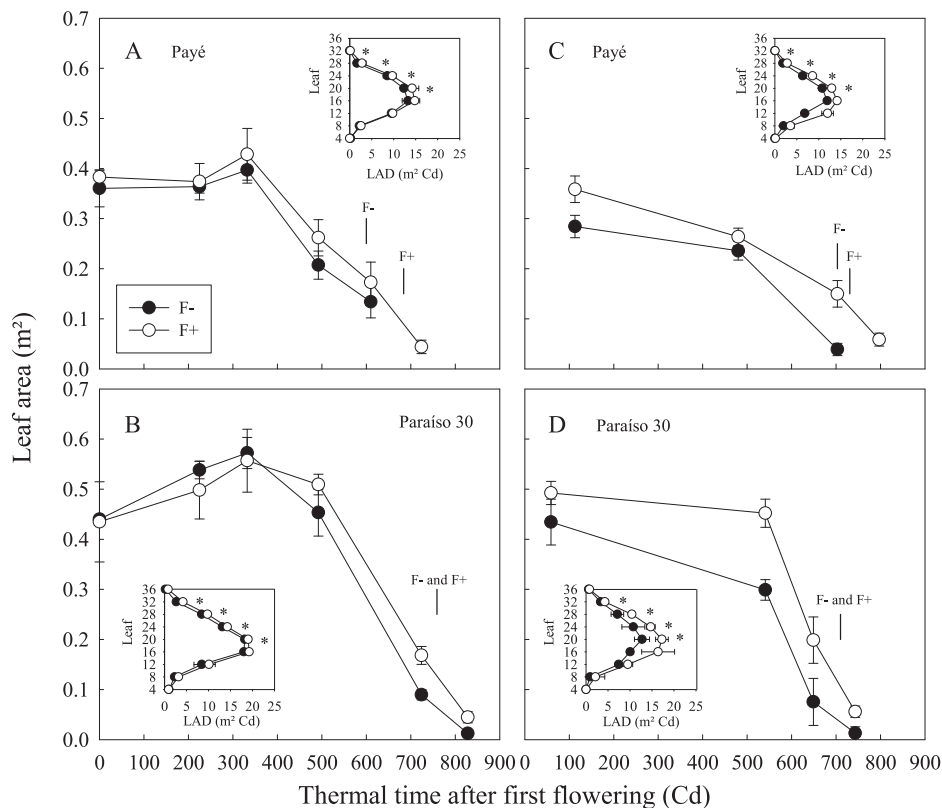

Fig. 4. Leaf area as a function of thermal time. Leaf area per plant (m²) as a function of thermal time after first flowering (Cd, Tbase = 6 °C). Protection treatments: F+, application of fungicides (open symbols) and F−, without application of fungicides (close symbols). Hybrids: Payé (A and C) and Paraíso 30 (B and D). Exp. 1 (A and B) and Exp. 2 (C and D). Physiological maturity in F− and F+ is indicated in each chart with vertical lines. Vertical lines on the symbols correspond to the standard error ($n = 9$). Insets: leaf area duration from flowering to physiological maturity in leaves 4, 8, 12, 16, 20, 24, 28, 32 (hybrids Payé and Paraíso 30) and 36 (hybrid Paraíso 30) (LAD, m² Cd). Leaves in which protection effect was significant are indicated with * ($P \leq 0.05$). Horizontal lines on the symbols correspond to the standard error of the mean value ($n = 9$).

Table 3
Intercepted PAR (iPARc) and aboveground dry matter (DMc) cumulated during the grain filling period, radiation use efficiency (RUE) during the same period and grain yield (GY), grain number (GN), thousand grain weight (1000GW) and oil content (OC) at physiological maturity, in hybrids Payé and Paraíso 30 during experiments 1 (Exp. 1) and 2 (Exp. 2). Protection treatments (Treat.) were the application (F+) or not (F−) of fungicides.

Exp.	Hybrid	Treat.	Ecophysiological variables			Grain yield components			
			iPARc (MJ m⁻²)	RUE (g MJ⁻¹)	DMc (g m⁻²)	GY (g m⁻²)	GN	1000GW (g)	OC (%)
1	Payé	F−	343 ± 11	1.50 ± 0.11	516 ± 46	332 ± 23	8179 ± 379	40.5 ± 1.1	47.3 ± 0.1
		F+	368 ± 3	1.68 ± 0.11	618 ± 45	354 ± 19	8461 ± 299	41.7 ± 0.8	47.6 ± 0.4
	Paraíso 30	F−	413 ± 24	1.84 ± 0.07	762 ± 55	306 ± 15	7150 ± 203	42.7 ± 1.0	48.3 ± 0.3
		F+	429 ± 13	2.49 ± 0.11	1070 ± 74	383 ± 3	8191 ± 220	46.8 ± 0.9	47.9 ± 0.3
2	Payé	F−	390 ± 4	1.27 ± 0.08	494 ± 26	408 ± 14	8303 ± 394	49.3 ± 0.7	48.2 ± 0.6
		F+	406 ± 3	1.41 ± 0.12	574 ± 49	458 ± 11	8440 ± 151	54.3 ± 1.4	48.7 ± 0.2
	Paraíso 30	F−	369 ± 30	1.39 ± 0.15	516 ± 77	351 ± 21	6353 ± 312	55.3 ± 2.3	49.8 ± 0.5
		F+	435 ± 10	1.53 ± 0.02	667 ± 21	440 ± 9	6886 ± 279	64.1 ± 1.5	50.0 ± 0.9

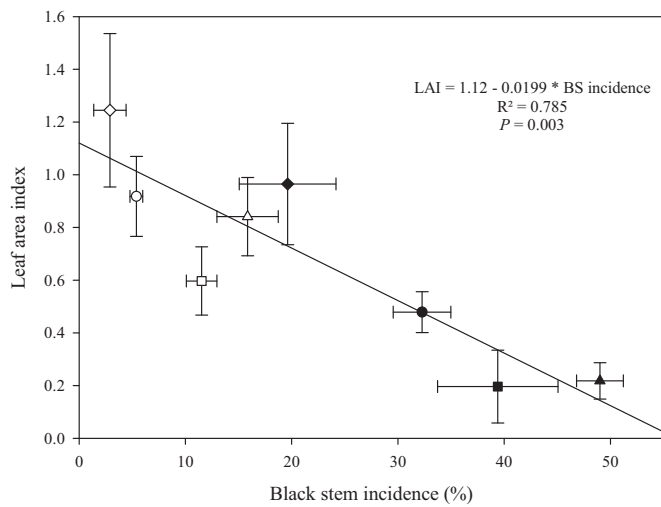Mean value ± standard error ($n = 9$ in Exp. 1 and $n = 15$ in Exp. 2).

Fig. 5. Relationship between black stem incidence and leaf area index. Leaf area index as a function of black stem incidence by *Phoma macdonaldii* measured at physiological maturity of each control treatment. Diamonds and circles correspond to hybrids Payé and Paraíso 30, respectively, in Exp. 1. Triangles and squares correspond to hybrids Payé and Paraíso 30, respectively, in Exp. 2. White symbols: application of fungicides (F+) and black symbols: without application of fungicides (F−). Horizontal and vertical lines on the symbols correspond to the standard error of the mean value of incidence black stem ($n = 30$) and leaf area index, respectively ($n = 9$).

maturity in protected treatment was 7% higher than in unprotected one (protection effect: $P = 0.0079$, Table 3). Intercepted PAR cumulated in hybrid Payé was 11% higher in Exp. 2 than in Exp. 1 (hybrid × experiment effect: $P = 0.007$, Table 3).

Radiation use efficiency during grain filling increased 16% in protected treatment (protection effect: $P = 0.0071$, Table 3). Hybrid Paraíso 30 converted PAR in dry matter 32% more efficiently in Exp. 1 than in Exp. 2 (hybrid × experiment effect: $P = 0.0207$).

3.6. Nitrogen nutrition index, aboveground dry matter and harvest index

The nitrogen nutrition index at flowering was 37% higher in Exp. 1 than in Exp. 2 (experiment effect $P = 0.0024$) and 19% higher in hybrid Paraíso 30 than Payé (hybrid effect; $P = 0.0610$). No protection effect was observed in this variable ($P = 0.7886$, Table 2). An interaction between the experiment and the hybrid was detected in the aboveground dry matter at flowering ($P = 0.0077$). In Exp. 2, dry matter in hybrid Paraíso 30 was 31% higher than in hybrid Payé (Table 2). No protection effect was observed in dry matter at flowering (Table 2), but plants from protected treatment cumulated 28% more dry matter from flowering to physiological maturity than those from the unprotected treatment (protection effect: $P = 0.0017$, Table 3). In Exp. 1, hybrid Paraíso 30 cumulated 62% (average of protected and unprotected treatments) more dry matter than hybrid Payé (hybrid × experiment effect, $P = 0.0032$, Table 3).

The protection treatments did not affect the harvest index (mean value = 0.33, protection effect: $P = 0.4151$). This variable was 29% higher in hybrid Payé than in Paraíso 30 (0.38 vs. 0.27, hybrid effect: $P = 0.0004$) and 21% higher in Exp. 2 in comparison with Exp. 1 (0.35 vs. 0.29, experiment effect: $P = 0.0007$).

3.7. Grain yield and its components

Grain yield in protected treatment was 17% higher than in unprotected one (protection effect: $P = 0.0008$, Table 3). Hybrid
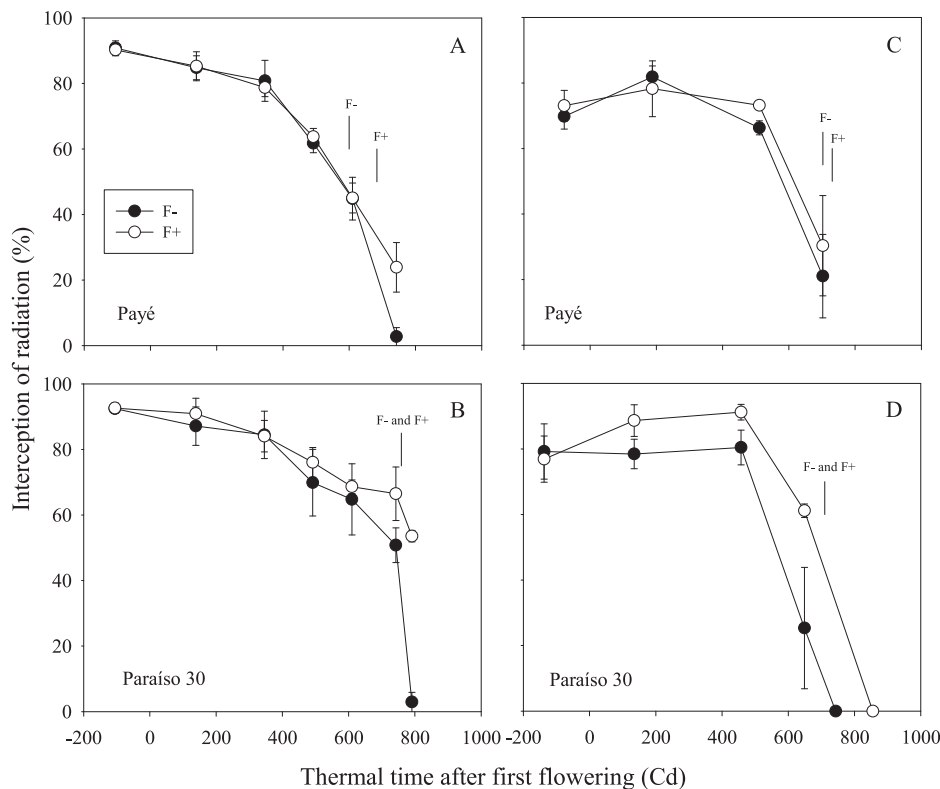

Fig. 6. Interception of radiation as a function of the thermal time. Interception of radiation (%) as a function of thermal time after first flowering (Cd, Tbase = 6 °C). Protection treatments: F+, application of fungicides (open symbols) and F−, without application of fungicides (close symbols). Hybrids: Payé (A and C) and Paraíso 30 (B and D). Exp. 1 (A and B) and Exp. 2 (C and D). Physiological maturity in F− and F+ is indicated in each chart with vertical lines. Vertical lines on the symbols correspond to the standard error of the mean value ($n = 9$).

Payé yielded 5% more than Paraíso 30 (hybrid effect: $P = 0.0058$, Table 3) and Exp. 2, 21% more than in Exp. 1 (experiment effect: $P = 0.0019$, Table 3). Grain number increased 7% in protected treatment in comparison with unprotected one (protection effect: $P = 0.0419$, Table 3). An interaction between the experiment and the hybrid was detected for this variable ($P = 0.0275$). Grain number in hybrid Payé was 26% higher than Paraíso 30 in Exp. 2 (LSD, $P < 0.05$, Table 3) and hybrid Paraíso 30 had a grain number 16% greater in Exp. 1 than in Exp. 2 (LSD, $P < 0.05$, Table 3). The application of fungicides increased 10% the grain weight in comparison with the control (protection effect $P = 0.0008$, Table 3). Grains in Exp. 2 were 30% heavier than in Exp. 1 (experiment effect: $P \leq 0.0001$, Table 3) and in hybrid Paraíso 30, 12% heavier than in hybrid Payé (Table 3, hybrid effect: $P = 0.0324$). The relative oil content was not affected by the protection treatments (protection effect: $P = 0.6229$, Table 3). Oil content in Paraíso 30 was 2% higher than in Payé (hybrid effect: $P = 0.0348$) and in Exp. 2 was 3% higher than in Exp. 1 (experiment effect: $P = 0.0423$).

4. Discussion

Black stem by *P. macdonaldii* was the only conspicuous disease found in our experiments. The absence of other diseases even those caused by the same pathogen, black stem at the stem base and premature ripening indicates that differences between protected and unprotected treatments in measured variables were certainly the consequence of the effect of BS by *P. macdonaldii*. The protection treatments, the hybrids and the experimental environments generated a wide range of BS incidence (i.e. from 2.9 to 49%; Fig. 1).

As expected, leaf area index and dry matter at flowering were not affected by protected treatment since BS principally occurs during the grain filling period. Leaf area at flowering seems to be underestimated in hybrid Paraíso 30 during Exp. 1 (Fig. 4B), and that was probably the consequence of the limited number of measured plants. In Exp. 2, dry matter of the same hybrid was higher than hybrid Payé at flowering (Table 2). These results and those of nitrogen nutrition suggest that hybrid Paraíso 30 takes advantage of lower plant density in vegetative stages. The higher number of leaves at flowering (Table 2) which favor leaf area index was probably one of the causes of this response. Canopy status at flowering (leaf area index, fraction of intercepted radiation) was positively associated to BS in sunflower (Debaeke and Pérès, 2003). In our work, a higher BS incidence was observed in Exp. 2 even with lower leaf area index and nitrogen nutrition index than in Exp. 1. During Exp. 2 occurred more rainfall events than in Exp. 1 (>5 mm week^{-1}, Table 1) and the number rather than the amount of rainfall events are positively associated to the maturation and release of *L. lindquistii* (anamorph *P. macdonaldii*) spores (Gulya et al., 1997; Descorps et al., 2012). Moreover, in not nitrogen limiting conditions like ours a lower plant density was reported to be associated to higher BS levels (Debaeke and Pérès, 2003).

The relationship between disease symptoms and yield in many foliar diseases arise because production is a function of the healthiness of the leaf area (Waggoner and Berger, 1987). In this work we analyzed biomass production in terms of the amount of intercepted light and the efficiency with which it is converted to biomass (Monteith and Moss, 1977). Both ecophysiological variables could be affected by foliar diseases (Waggoner and Berger, 1987; Johnson, 1987). Dry matter production from flowering to physiological maturity increased by fungicide protection in all studied conditions (Table 3). According to our results the effect of BS during this period was associated with: (1) a decrease in leaf area (Fig. 4) and LAI (Fig. 5) by premature leaf senescence (Fig. 2), (2) a reduction in interception of radiation (%) (Fig. 6) under a critical value of LAI (Carretero et al., 2010) and (3) a decrease in RUE

(Table 3). Depressing effect of diseases on light interception and photosynthesis were reported in sunflower for black rust by *P. helianthi* (Siddiqui, 1980), *Verticillium* wilt (Sadras et al., 2000a; Creus et al., 2007) and Alternaria leaf spot (Leite et al., 2006). The main physiological effect of BS was observed on leaf area duration (21%) as a consequence of premature leaf senescence since no effect was observed on leaf expansion (data not shown). In Exp. 2 this effect was detected at early stages of the disease even with no observed symptoms in stem (around 100 Cd, Fig. 4C and D). In hybrid Payé a delay in physiological maturity in protected plants was also involved in increasing differences especially in Exp. 1.

In the present work grain yield decreased 15% without a frequent fungicide protection (every 15 days). Losses around 10% were associated to BS lesions by Velásquez and Formento (2003). The effect of BS on grain yield was mainly explained for differences in grain weight ($P = 0.0008$) however the number of grains was also affected ($P = 0.0419$). Velásquez and Formento (2003) also reported a decrease in grain weight associated to BS for four sunflower genotypes and in agreement with our results for both hybrids they did not observe a disease effect on oil content (Table 3).

A decrease in photosynthesis from flowering could explain the effect of BS on weight (Goffner et al., 1998) and number (Cantagallo et al., 2004) of grains. However, except for hybrid Paraíso 30 in Exp. 2, the largest differences in iPAR were detected in advanced stages of grain filling (more than 400 Cd after first flowering). Grain number is usually affected by stresses around flowering (Cantagallo et al., 2004) and remained steady after the end of flowering (R6, Schneiter and Miller, 1981). Under this work conditions the fall on grain number could be explained by an early disease effect on the last flowering stages (R5.8–R5.10) without detectable symptoms on the stem. This would be possible since at early stages of the infection *P. macdonaldii* produces necrosis in ribs and/or petiole which generate chlorophyll loss and leaf tissue necrosis (Gulya et al., 1997; Bordat et al., 2011). Water and nutrient transport was probably affected in this case. A greater senescent to green tissue proportion in leaf lamina of unprotected plants (Fig. 2C and G) was usually observed in correspondence with necrosis on ribs and petiole (data not shown). The appearance of BS symptoms delayed between 2 and 3 weeks after pycnidiospores inoculation on the insertion point of the petiole to the stem at early flowering stages (Larfeil et al., 2010) so in conditions of natural infection as ours it was expected that the appearance of symptoms on stem could be even more delayed after infections of ribs or mid petiole. This makes possible that BS could affect grain number prior to the appearance of symptoms on the stem. On the other hand, in our experiments we measured intercepted and not absorbed radiation. Differences between them have been reported for foliar diseases on sunflower and other crops (Leite et al., 2006; Carretero et al., 2010). Diseased leaves do not absorb all intercepted radiation leading to an overestimation of intercepted PAR at flowering, which probably explains part of the differences observed in grain number, and during grain filling provoking an underestimation of radiation use efficiency in unprotected treatment (Carretero et al., 2010). Finally, the limited number of measured plants could also lead to an underestimation of grain number.

Knowledge about the side-effects of fungicides from the strobilurins group is contradictory. Some authors reported benefit on physiological variables affecting yield grain in wheat and soybean (Beck et al., 2002; Fagan et al., 2010) however, other studies found no such effects in maize and soybean (Blandino et al., 2012; Swoboda and Pedersen, 2009). Preliminary works of our group in sunflower demonstrated no benefit of the application of azoxystrobin (Quiroz and Dosio, 2010) or pyraclostrobin (unpublished results) on leaf area and chlorophyll under a low presence of foliar diseases restricted to bottom leaves.

Several authors reported differential behavior among sunflower genotypes to assisted inoculation of *P. macdonaldii*, and genetic resistance was mostly characterized by genes of additive effects (i.e. Darvishzadeh et al., 2007). Working with natural infections we also detected genotype differences in BS incidence. In Exp. 1, hybrid Payé showed lesser symptoms than Paraíso 30, nevertheless in an environment with a higher disease manifestation as Exp. 2 the opposite was observed. This could be explained by the quantitative nature of genetic resistance to BS that limits the level of resistance when the expression of the disease was more favored.

The effect of BS on sunflower canopy and ecophysiological variables related to yield could be influenced by the environment and the genotype. In our work, interactions between the protection treatment and hybrid or experiment in leaf variables measured during the grain filling period as leaf area duration and cumulated intercepted PAR were not detected. Nevertheless, comparing within the same measurement date we observed sometimes a different response between experiments (i.e. leaf area or intercepted PAR around 450 Cd after flowering in Exp. 1 showed a lower response to the fungicide protection than in Exp. 2). In the first experiment, differences in BS incidence between protection treatments were highly significant (Fig. 1A and B). However, the effect of the disease on leaf area and intercepted PAR was less evident and concentrated in the final stage of the grain filling period. The low BS incidence in Exp. 1 implies presence of the disease mainly on bottom leaves. These leaves are smaller than middle ones and consequently have low impact on leaf area or PAR interception. In agreement with this conjecture the effect of leaf diseases on ecophysiological variables in wheat was related to the presence of symptoms in the upper leaves of the canopy (Carretero et al., 2010).

In our work, BS was first detected in leaves 8 and 12 between 200 and 400 Cd after first flowering depending on the hybrid and later progressed to upper leaves (Fig. 2). In concordance with this, the protection effect against BS decreased in higher leaves because they were less affected by the disease (Fig. 3). Disease severity on leaf 28 of hybrid Payé seemed to be excessive in comparison with leaf 20 (Fig. 2) probably because the perimeter became thinner toward the top of the plant allowing disease to advance faster around the stem than in lower leaves. Leaves below the eight were already senescent without symptoms of BS the day of the first measurement (data not shown). These leaves probably died by the low intensity of radiation received (Rousseaux et al., 1996) when BS was just commencing symptomatic development.

The highest BS effect on leaf area duration was mostly observed in middle and some top leaves (between leaves 20 and 28). The main decrease of leaf area as a consequence of *Verticillium dahliae* in sunflower was also reported in intermediate leaves (Sadras et al., 2000a). Analyzing senescence profiles in sunflower under two contrasting source/sink ratios the same authors determined greater differences between treatments in upper or lower strata than in mid strata (Sadras et al., 2000b). These results suggest a differential response between an internal physiological condition of the plant and a biotic stress like a disease.

As discussed by Johnson (1987) the vertical position of the leaves also need to be taken into account in the disease study. The quantification of BS and its physiological effect at different leaves layers would be useful to develop prediction models of the disease and its impact on yield. A simple model based on green leaf area *per* layer and pre-anthesis reserves could predict growth and yield of wheat under epidemics of foliar diseases over a range of crop practices (Bancal et al., 2007). On the other hand, the identification of strata of leaves more susceptible to the effect of BS can contribute to improve techniques of fungicides application. The application of fungicides based on the developmental stage of these susceptible leaves and the residuality of the active ingredient can be considered as a promising strategy to increase the effectiveness of fungicides,

and then to reduce the use of chemical products with the consequent benefit to the environment.

5. Conclusions

In this work we observed that the effect of BS on sunflower was related to the decrease in leaf area by premature senescence and thus to the reduction of intercepted radiation. Although the effect on senescence was observed from early stages of the grain filling period, that on intercepted radiation was delayed to advanced stages of this period. Black stem incidence and leaf area index was negative related at physiological maturity. Radiation use efficiency was also diminished by BS indicating an effect not only in the efficiency of radiation interception but also in the conversion efficiency of the radiation. As a consequence, dry matter production and also grain yield were reduced. The physiological effects of BS were especially important in mid and upper leaves.

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