TWO SIMPLE MODELS INCLUDING THE SOURCE/SINK RATIO TO EXPLAIN BLACK STEM BY *PHOMA MACDONALDII* IN SUNFLOWER

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ABSTRACT

Black stem (BS) by *Phoma macdonaldii* Boer of sunflower (*Helianthus annuus* L.) is the most prevalent foliar disease in the Buenos Aires province, main sunflower area of Argentina. The source- sink ratio (SSR) of sunflower crop affects the plant susceptibility to BS, although this effect may be influenced by several factors. The aim of this work was to establish simple models to take into account the SSR of sunflower to estimate BS incidence and severity, in different environmental conditions, hybrid cultivars and leaf stratums. Three field experiments, including two hybrids, were performed at Balcarce, Argentina. The SSR was modified by grain excision or shading, during the grain filling period. BS incidence and severity (in nodes 8, 12 and 20) were evaluated weekly from flowering. SSR took account of a significant fraction of the yearly incidence ($R2 \geq 0.619$) and severity variation ($R2 \geq 0.458$), both hybrids comprised. To include the annual variability, regression analyses were performed between meteorological and physiological variables (PAR, % interception, number of rainy days, mm of rainfall) and BS incidence and severity. In the case of severity, the age of the leaf was also included. Multiple linear and non-linear models were selected by the analysis of stepwise and residual methods. About 80% of the total variation in incidence and severity of BS due to hybrids, experiments and treatments, was explained by simple models including SSR and number of rainy days ($p \leq 0.0001$), or SSR, number of rainy days and leaf age ($p \leq 0.0001$), respectively. Simple models like these are potentially useful in the assistance to crop management, and could also be included to improve simulation models of diseases, growth and development in sunflower.

Key words: source- sink ratio, *Helianthus annuus* L., *Phoma macdonaldii* Boer., rainy days, age of the leaf

INTRODUCTION

The "black stem" (BS) caused by the necrotrophic fungus *Phoma macdonaldii* Boer (teleomorph *Leptosphaeria lindquistii*) is the most prevalent leaf disease in sunflower (*Helianthus annuus* L.) in the Buenos Aires province (Lazzaro *et al*., 2013), the main sunflower production area of Argentina (1.16 to 2.40 thousand ton of grains in the last ten years, SIIA-MAGPyA, 2015). Symptoms appear on the stem near flowering stage, progress from bottom to upper leaves, and are usually associated to previous necrosis in veins, petiole and/or leaf lamina (Bordat *et al*., 2011). *P. macdonaldii* also attacks roots and the 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 with high resistance to BS or premature ripening. Yield losses between 10 and 30 % were reported to be associated to BS (Debaeke and Pérès, 2003; Velásquez and Formento, 2003) or premature ripening (Carson, 1991) via a decrease in intercepted radiation, related to premature leaf senescence and/or in radiation use efficiency (Quiroz *et al*., 2014).

Crop models have many current and potential uses for answering questions in research and crop management. Models can assist in synthesis of research understanding about the interactions of genetics, physiology, and the environment, integration across disciplines, and organization of data □Boote *et al*., 1996□. Often, mathematicians and statisticians models are used to study and describe plant growth, the effect of management practices and development of diseases □Campbell y Madden, 1990□ Hernandez *et al*., 2009□.

BS estimate models have been developed by Debaeke and Peres (2003) and Desanlis (2013). These models consider epidemiological aspects as canopy microclimate (relative humidity and temperature), plant growth (leaf area index) and fungicide treatment, to account for climatic and agronomic limitations to fungus development. Further, in a previous work by our group were determined that BS incidence and severity were inversely related to the source -sink ratio (Nuñez Bordoy *et al*., 2012). These relations suggests the existence of a stem carbohydrates threshold under of this the BS incidence or severity start to increase. The carbohydrate content of the plant could closely relate to the existence of one or more substances that inhibit the growth of fungi, such as phenolics and terpenes (Silva Acuña *et al*., 2000) associated to plant response against pathogens. Because the relation between SSR and BS symptom could be affected by plant intrinsic factors or by meteorological and agronomic conditions we formulate the objective to establish simple models to take into account the SSR of sunflower to estimate BS incidence and severity, in different environmental conditions, hybrid cultivars and leaf stratums.

MATERIALS AND METHODS

Three field experiments (Exp. 1, Exp. 2 and Exp. 3) were the INTA Balcarce Experimental Station, Argentina (37º45' S, 58º18' W). 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 the Typic Argiudol soil (USDA taxonomy, organic matter 7.4%, P-Bray 25.8 ppm). Plant density was adjusted manually to 5.6 plants m-2 in the three experiments and rows were 0.7 m apart. The crops were grown under good water and nutrients conditions. Weeds and insects were controlled adequately through cultural and chemical techniques.

The experiments were conducted under conditions of natural inoculation of *P. macdonaldii* in plots infected with the pathogen (verified in previous experiments). Additionally, pieces of infected milled sunflower plants from previous experiments were homogeneously distributed in the plots during V6 stage (Schneiter and Miller, 1981) to assure *P. macdonaldii* presence.

In order to modify the source-sink ratio (SSR) with a different approach (modifying the sink or the source), two sorts of treatments were applied after the end of flowering (R6, Schneiter and Miller, 1981):

1. Grains excision: grains from about two (G↓) or three (G↓↓) quarters of the head were carefully removed (Echarte *et al*., 2012).

2. Reduction of solar radiation: a 38 % uniform shading with black, synthetic and neutral mesh cloth (S) was applied (Dosio *et al*; 2000).

3. An untreated plot was kept as control (C).

Source sink treatments and hybrids were combined in a randomized complete block design with three replicates. Each plot consisted of four rows of 6 m long, spaced at 0.7 m.

Daily global incident radiation and rainfall were measured in a weather station located 400 m from the experiments. Daily mean air temperature in treatments control and shaded (S) was also measured at leaves 8, 12, 20 and 28 level (from the bottom of the plant) with copper/constantan thermocouples. The average of the temperature at these three levels was used for thermal time estimates. Data were averaged every 3600 s, and recorded by a data logger (Cavadevices.com, Buenos Aires, Argentina). Thermal time was calculated by daily integration of air temperature and a base of 6 °C (Kiniry *et al.* 1992), and cumulated from flowering.

Daily incident photosynthetically active radiation (PAR) was calculated as 0.48 × 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 (iPAR) was calculated as the product of the daily incident PAR and the daily proportion of PAR intercepted. The iPAR was cumulated from flowering to physiological maturity (PM).

Incidence of BS by *P. macdonaldii* (I %) was evaluated every 7-10 d in 3 plants per plot (n=9), as the ratio between the affected to the total number of nodes per plant. Severity of BS by *P. macdonaldii* (S %) was evaluated every 7-10 days in leaves 8, 12, 20 and 28 (from the bottom of the plant), selected to obtain a suitable plant profile of BS severity, in 3 plants per plot (n=9), following the methodology proposed in Quiroz *et al*. (2014). BS incidence and BS severity were estimated at 300, 350, 400 °Cd and PM by interpolation between two successive measurements.

The source–sink ratio (SSR) was periodically calculated during the grain filling period as the quotient of accumulated iPAR and the grain number per plant affected by the grain weight of treatment G↓↓, considered the closest to the potential weight. SSR was estimated at 300, 350, 400 °Cd and PM by interpolation between two successive measurements.

Data of SSR, incidence and severity of BS were processed by analysis of variance procedures (INFOSTAT Professional v.1.1, Di Rienzo *et al*., 2010). Differences among treatments means were evaluated with the LSD test ($P \leq 0.05$). Data of incidence and severity of BS as a function of SSR were adjusted to exponential models (Nuñez Bordoy *et al*., 2012) at 300, 350 and 400 °Cd from flowering and at PM (Sigma Plot v. 11.0 Systat Software Inc., 2010). For each variable the model with the greatest signification (α) and coefficient of determination (R2) was selected. Models did not include outliers.

To include the annual variability, regression analyses were performed between meteorological variables (PAR, % interception, number of rainy days, mm of rainfall) and BS incidence and severity. In the case of severity, the age of the leaf was also included. Multiple linear and non-linear models were done by the analysis of stepwise and residual methods (INFOSTAT Professional v.1.1, Di Rienzo *et al*., 2010).

RESULTS AND DISCUSSION

Source-sink ratio after flowering slightly increased or remained steady in plants from control and shading treatments, while grain excision (G↓ and G↓↓) mainly increased it in all tested situations (Fig. 1). SSR increase by grain removal is attributable to a higher retention of green leaves which still intercept solar radiation (data not shown), inclusive in some cases, SSR still increases yet after physiological maturity. Although productive sense of SSR after the end of grain filling is questionable, if photosynthesis is yet detectable, assimilates could be stocked in alternative sinks like stem.

An interaction between the effect of the experiment and that of the treatment ($p<0.0044$) was observed for SSR at 400ºCd after flowering (Fig. 1.A, B, C and D). No differences between S and control treatments were observed in Exp.1 and Exp.2 ($p>0.05$), probably because of a mild drop in the grain number (data not shown). Since grain number in sunflower is finally set near 20 days after flowering (Connor and Hall, 1997), in some cases we probably applied shading treatment just before grain number was set. Grain excision treatments showed a higher SSR in Exp.1 (216%, G↓↓) and Exp.2 (47% and 200%, G↓ and G↓↓, respectively) than that of the control (Fig. 1.A, B, C and D). Furthermore, hybrid Baqueano had SSR more than 30% higher than hybrid VDH 487 at this thermal time after flowering ($p<0.0001$, Fig. 1.A, B, C and D), due to a lesser demand from grains with a small potential weight than hybrid VDH 487.

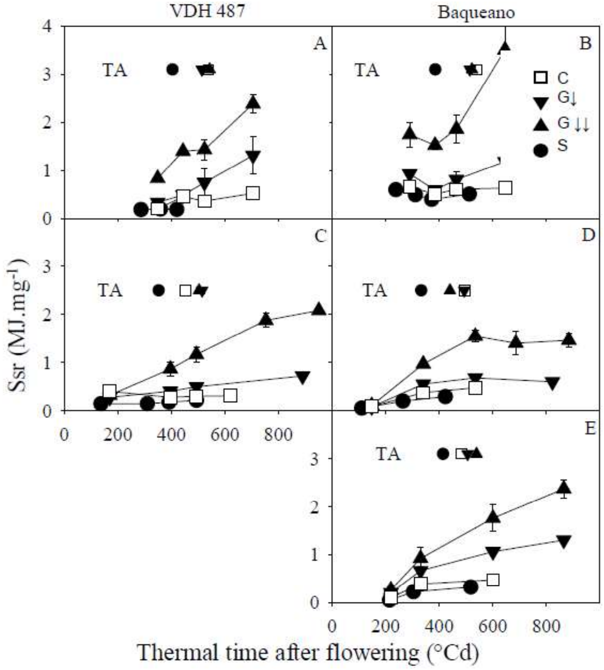

Fig. 1. Source-sink ratio (SSR) as a function of the thermal time after flowering for hybrids VDH 487 (A and C) and Baqueano (B, D and E), in Exp.1 (A and B), Exp. 2 (C and D) and Exp.3 (E). Treatments: grain excision from about two (G↓, inverted triangles), or three (G↓↓, triangles) quarters of the head, shading during the filling period (S, solid circles) and control (C, open squares). Treatment application (TA) and physiological maturity for each treatment are indicated at the top of the chart. Vertical bars on the symbols indicate the standard error of the mean value (n=9).

Incidence of BS by *P. macdonaldii* increased during the grain filling period in all treatments, hybrids and experiments (Fig. 2). Shading treatment almost always accelerated the incidence increase (Fig. 2.A, B, C and D), while grain excision usually reduced it (Fig. 2.A, C, D and E). BS incidence at 400ºCd after flowering was highly affected by applied treatments ($p<0.0001$, Fig. 2 A, B, C and D). Plants from shading treatment increased 42% BS incidence ($p<0.05$), while those from the higher grain excision treatment (G↓↓) decreased it 16%, in comparison with control plants ($p<0.05$, Fig. 2.A, B, C and D). No hybrid effect was observed for this variable ($p=0.4732$). The lower values of BS incidence were observed in Exp. 3, while the highest in Exp. 1 (14% and 59%, respectively, mean of all applied treatments, Fig. 2.B, D and E). An interaction between the treatment and the experiment was observed for this variable ($p<0.0001$).

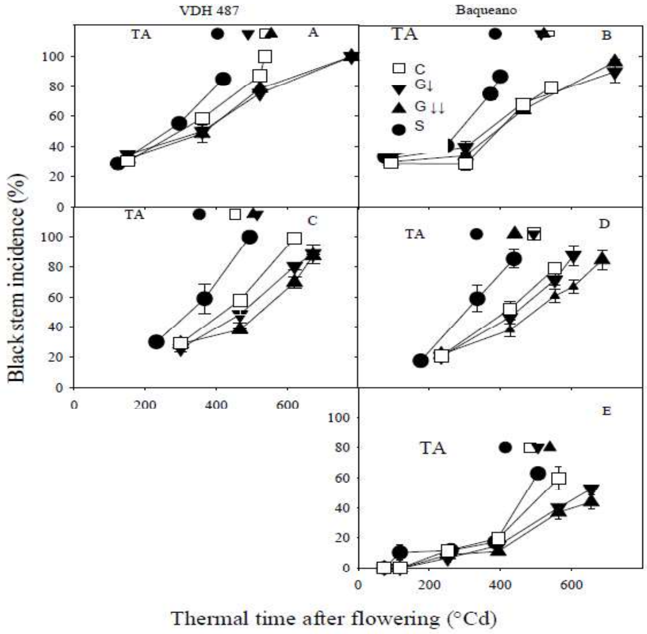

Fig. 2. Black stem incidence (%) as a function of the thermal time after flowering for hybrids VDH 487 (A and C), Baqueano (B, D and E) in Exp.1 (A and B), Exp. 2 (C and D) and Exp.3 (E). Treatments: grain excision from about two (G↓, inverted triangles), or three (G↓↓, triangles) quarters of the head, shading during the filling period (S, solid circles) and control (C, open squares). Treatment application (TA) and physiological maturity for each treatment are indicated at the top of the chart. Vertical bars on the symbols indicate the standard error of the mean value (n=9).

Symptoms of BS by *P. macdonaldii* appeared first in leaves from the bottom of the plant and progressed upwards to upper leaves in all hybrids, treatments and experiments ($p<0.0001$). This result corroborates the rise acropetal nature of this disease (Quiroz, *et al*. 2014). In turn, leaf functionality is associated to incident light and therefore to its position on the stem. Bottom leaves receive an intensity and aquality of light (red/far red ratio) poorer than upper ones which make them senesce before (Rousseaux *et al*., 1996), may be more susceptible to *P. macdonaldii* infections for photosynthetic stress-translocation balance.

The onset and the progress on the node of BS severity symptoms were advanced in shading treatment (S) and delayed in grain excision treatments (G↓, G↓↓) in most hybrids and experiments (Fig. 3, leaf 20 as example).

A significant effect of the treatments ($p<0.0001$), the hybrid ($p=0.0074$) and the leaf ($p<0.0001$) on BS severity was observed at 400ºCd after flowering. Shading increased 50% BS severity while grain excision reduced it 18% and 33% (treatments G↓ and G↓↓, respectively, $p<0.05$) in comparison with the control (Fig. 3.A, B, C and D, leaf 20 as example). Hybrid VDH 487 showed severity symptoms more than 10% higher than hybrid Baqueano ($p<0.05$). Leaf 8 was the most affected by BS severity, followed by leaf 12 and leaf 20 which was the less affected (difference of 80% between leaves 8 and 20, $p<0.05$).

The effect of the experiment interacted with those of the treatment and the leaf ($p=0.0033$ and $p=0.0025$, respectively) Treatment S during Exp. 1 and Exp. 2 presented higher BS severity values than the rest of the treatments at 400ºCd after flowering ($p<0.05$). In Exp. 3, we observed the lesser BS severity in all treatments (Fig. 3. B, D and E, leaf 20 as example). Leaves 8, 12 and 20 presented lower BS severity values in Exp. 3 in comparison with Exp. 1 and Exp. 2 ($p<0.05$, Fig. 3. B, D and E, leaf 20 as example). There were not observed symptoms of BS severity in leaf 28 at 400ºCd after flowering (data not shown).

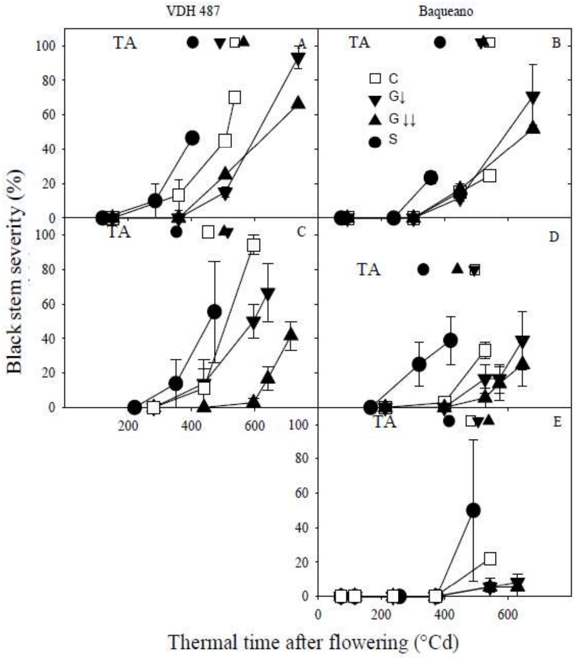

Fig. 3. Black stem severity (%) in leaf 20 as a function of the thermal time after flowering for hybrids VDH 487 (A and C), Baqueano (B, D and E), in Exp.1 (A and B), Exp. 2 (C and D) and Exp.3 (E). Treatments: grain excision from about two (G↓, inverted triangles), or three (G↓↓, triangles) quarters of the head, shading during the filling period (S, solid circles) and control (C, open squares). Treatment application (TA) and physiological maturity for each treatment are indicated at the top of the chart. Vertical bars on the symbols indicate the standard error of the mean value (n=9).

As stated in MM, results of incidence and severity of BS as a function of SSR were adjusted to exponential models at 300°Cd, 350°Cd and 400°Cd from flowering and at physiological maturity, however we decided to carry out modeling with results from 400°Cd from flowering for three reasons: (i) before this date, disease symptoms were not important yet (Fig. 2 and Fig. 3), (ii) this was the very last date in which we kept all the treatments for the analysis, since in shading treatment, the stem of the plants became black, impeding measurements, and (iii) adjustments at 300°Cd, 350°Cd and 400°Cd from flowering and at physiological maturity presented lower significance and/or determination coefficient (data not shown). Both incidence and severity of BS by *P. macdonaldii* decreased with increasing SSR. As a consequence of the highly significant effect of the experiment and the leaf presented above, results from treatments at 400°Cd from flowering adjusted to negative exponential models for each experiment in the case of BS incidence (R2≥0.619, Fig. 4.A), and for each measured leaf in the case of BS severity (R2≥0.458, Fig. 4.B).

Similar results were reported by Eslava *et al.* (2007) observed root and presence of mycelia from *Fusarium spp*. on the stem base. This function suggests the existence of a stem carbohydrates threshold under of this the BS severity start to increase, as Davet and Serieys (1987) had shown for *Macrophomina phaseolina* infection at base stem in sunflower.

The higher BS incidence was observed in Exp. 1 (Fig. 4.A), while the higher BS severity was almost always observed on leaf 8 and the lower on leaf 20 (Fig. 4.B). Increasing SSR from 0.1 to 0.6 MJ.mg-1 was associated to a decrease in BS incidence up to near 40% of the maximum incidence observed (Exp. 2, Fig. 4.A), and 70% of the maximum severity observed (leaf 20, Fig. 4.B). Values of SSR higher than 0.6 MJ.mg-1 did not affect significantly, neither incidence, nor severity of BS (Fig. 4.A and B).

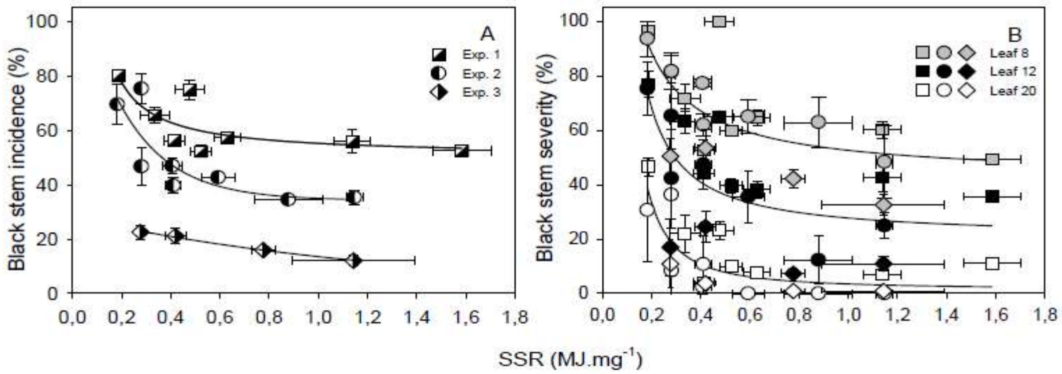

Fig. 4. Incidence (A) and severity (B) of black stem by *P. macdonaldii* as a function of the source-sink ratio (SSR, MJ.mg-1) at 400°Cd after flowering in Exp. 1 (squares), Exp. 2 (circles) and Exp. 3 (diamonds). Symbols filled in combined black/white indicate BS incidence (A). BS severity is indicate with grey (leaf 8), black (leaf 12) or white (leaf 20) symbols (B). Shading value from Exp. 1 in hybrid Baqueano corresponded to 372Cd after flowering. Curvilinear lines illustrate the adjustment of the results to the model: *BSincidence(%)=50.524*exp(0.089/(SSR+0.007)), p=0.0895, R2=0.619, n=8, for Exp.1; BSincidence(%)=34.216+89.563*exp(-4.774*SSR), p=0.05, R2=0.698, n=8, for Exp.2, and BSincidence(%)=27.991*exp(-0.730*SSR), p=0.0025, R2=0.995, n=4, for Exp.3. BSseverity(%)=48.051+70.593*exp(-3.066*SSR), p=0.0053, R2=0.46, for leaf 8; BSseverity(%)=24.721+97.108*exp(-4.186*SSR), p=0.0055, R2=0.458, for leaf 12, and BSseverity(%)=3.962+146.129*exp(-7.9*SSR), p=0.0002, R2=0.63,* for leaf 20.Vertical and horizontal bars on the symbols indicate the standard error of the mean value of BS incidence or severity and SSR, respectively (n=9).

After a multiple regression analysis including, SSR, the number of rainy days, the photosynthetically active radiation (PAR), the % of interception of radiation, the mm of rainfall and the age of the leaf, the models: BS incidence = -26.78+7.56*number of rainy days+56.52*exp(-4.138*SSR), s.e.=11.97%, and BS severity = -374+0.26*age of the leaf+10.96*number of rainy days+95.067*exp(-5.033*SSR), s.e.=12.15%, explained about 80% of the variability in BS incidence and BS severity, in an estimated/observed plot (Fig. 5.A and B).

While in the case of BS incidence a linear model setting between estimated and observed values did not differ from the 1:1 bisector (p=0.17), in BS severity, both severity intercept (p=0.0006) and the slope (p=0.0004) differed from "0" and "1", respectively. The observed values of BS severity were slightly underestimated or overestimated by the model in low and high ranges of the scale (0-20% and 80-100%, respectively, Fig. 5.A and B). Nevertheless, the magnitude of these differences was lower than the s.e. of the model.

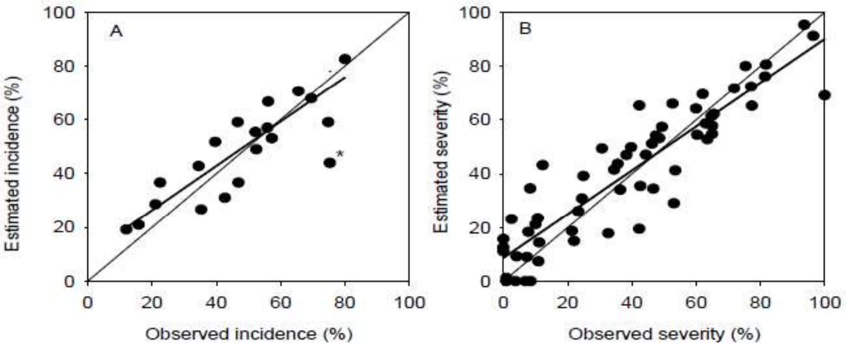

Fig. 5. Observed and estimated values of BS incidence (A) and BS severity (B) obtained from the field experiments and from the models BS incidence=-26.78+7.56*number of rainy days+56.52*exp(-4.138*SSR), s.e.=11.97%, and BS severity=-374+0.26*age of the leaf+10.96*number of rainy days+95.067*exp(-5.033*SSR), s.e.=12.15%, respectively. The thick lines result from the adjustment of the results to linear models (estimated incidence=9.909+0.822*observed incidence; R2=0.798; $p<0.0001$; n=19, and estimated severity=8.625+0.815*observed severity; R2=0.825; $p < 0.0001$; n=60). The asterisk near a symbol indicates that this result was an outlier (estimated - observed > 2.5 standard deviation), and was not consider in the adjustment. The thin line represents the 1:1 values.

The function between SSR and BS symptom was strongly affected by disease variable (incidence or severity), the experimental years and by number of nude. In concordance with Quiroz (2015), the number of rainy days from flowering to 400ºCd after flowering was the meteorological variable which better explained the effect of the experiment on BS incidence.

Rainfall events were the principal epidemiological indicator affecting diseases expression in the relationship SSR vs BS. In agreement, Délos *et al.* (1997) demonstrated that rainfall correlated with ascospores release from the primary source of inoculum (stubble, seeds, etc.), and not with inoculum amount (Descops *et al.*, 2012).

The age of the leaf (in ºCd from its appearance on the apex) was the variable which better explained differences observed among leaves. Time passed between *P. macdonaldii* inoculation in petiole and symptons appearance in stem, depend on phenological stage (related to leaf age) and the cultivar (Larfeil *et al.*, 2010), exhibiting later stages the shortest period. Leaf age affects photosynthesis, being older leaves less efficient than younger ones (English *et al.*, 1979) resulting in early senescence and probably with a higher susceptible to *P. macdonaldii* infections.

Our models were constructed without water and nutrients limitation during three years and with two hybrids. We considered ecophysiological and epimediological interactions from the crop and disease during the grain filling period. Other empiric predictive models, developed including a great range of crop management and environments (Debaeke and Péres, 2003), established a positive relationship between LAI or iPAR at flowering and BS and postulate that the more favourable microclimate of dense stands canopies could explain the higher level of infection of *P. macdonaldii.* Later, Desanlis (2013) deepened this epimediological approach proposing a conceptual model where the potential infection rate could be reduced by several reduction factors (RF): microclimate (HR, T), plant growth (LAI) and fungicide treatment, to account for climatic and agronomic limitations to fungus development.

CONCLUSION

The estimate BS models proposed in this paper include a new concept relating SSR, as a crop ecophysiological condition, to the plant susceptibility to *P. macdonaldii* infection. In turn, these models can contemplate different environmental conditions, hybrid cultivars and leaf stratums. This approach could be combined with the other mentioned models. For this it would be necessary to broaden the crop managements (according to Debaeke y Peres 2013) and stressed environments (e.g., crop damage, water or nutrient deficits, etc.).

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