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ORIGINAL PAPER

Qualitative and quantitative factors affecting the relationship between Canola leaf spot epidemic and stem base canker (*Leptosphaeria maculans*) in Argentina

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Abstract Stem base canker caused by *Leptosphaeria maculans* (SBC) has been reported to affect canola production worldwide. Ascospores infections produce leaf spots (LS) in early vegetative stages. From these initial spots, the fungus grows towards the stem crown and may cause SBC, lodging and premature ripening at maturity. Growth stage at LS presence, cultivar resistance and environment conditions are factors associated with SBC severity. Since there are no studies that associate the LS epidemic with SBC for the Argentine cropping conditions we performed two experiments to address this essential knowledge for SBC management. In 2010, plants of four cultivars (varying in their level of resistance to LS and SBC) ranging from cotyledon stage up to 9 developed leaves were transplanted to a field where artificial inoculation was carried out by spreading infected canola stubble from the previous year. The probability of SBC occurrence depended on the interaction between cultivar resistance level and the number of developed leaves at the moment of LS detection. Based on these results, in 2011 plots of the most susceptible cultivar (Nexera 8450) received different fungicide protection treatments (azoxystrobin or propiconazole and timing of spraying) and were exposed to natural *L. maculans* infections. We observed that the maximum incidence of LS up to 8th developed leaf best correlated with SBC severity at maturity and a linear regression was fitted to model the relationship between these two variables.

Keywords Phoma lingam · Oilseed rape · Chemical control

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Introduction

Stem base canker by *Leptosphaeria maculans* (*syn. Phoma lingam*) is the most important disease of canola (*Brassica napus* L. var. *oleifera*) worldwide (West et al. 2001). Airborne ascospores released from pseudothecia developing on infected stubbles of previous crops are the main source of primary inoculum, however, infections can also be initiated by conidia from alternative cruciferous hosts or infected seed (Salisbury et al. 1995). Liberation of ascospores generally occurs simultaneously with the emergence and establishment of new canola crops in autumn (Travadon et al. 2007). Compatible infections in leaves and cotyledons by ascospores produce leaf spot (phoma leaf spot): light-brown spots, from 3 to 7 mm in diameter, with irregular edges. Black globose structures (pycnidia) emerge from lesions containing asexual spores (conidia) which, splashed by rain drops can infect adjacent plants. Following the initial leaf infection, mycelium colonizes intercellular spaces between mesophyll cells, and grows down along the petiole without visible symptoms, protected from fungicide effects (Gladders et al. 1999; Sexton and Howlett 2001). At the end of winter/early spring, after a period of dormancy inside the stem crown, the pathogen restarts its activity and can kill the cells of the stem cortex causing a dark-colored canker, which can reduces yield by restricting water and nutrient transport through the stem or predisposing the plant to lodging or premature senescence

and ripening (Sansford et al. 1996). On the other hand aerial cankers, in middle-upper portion of stems or even in pods can be observed, mainly due to later infections of the fungus, however they are not reported to produce yield losses as the stem base canker does (hereafter referred simply as canker for stem base canker). Despite leaf spot epidemic has a polycyclic dynamic, canker develop is considered a monocyclic process (since new cankers are not originated from present cankers) in Australia, Canada and Europe (Salam et al. 2007). Canker epidemic is a consequence of the initial leaf spot epidemic, and the longer the period between pathogen infection and leaf abscission the higher the risk of canker occurrence (Hammond and Lewis 1987). For an effective management of canker it is required the integration of: genetic resistance (qualitative - seedling stage resistance or quantitative - adult stage resistance); agronomic factors like location of the paddock or crop rotations; and use of fungicides (Gladders et al. 2006). Fungicide sprays of difenoconazole and prothioconazole, alone or in mixtures with tebuconazole, on plots with 10 – 20% of plants with visible leaf spots, produced significant reductions of canker severity (Gladders et al. 2006). In England, when leaf spots appear in early autumn, fungicide sprays up to the tenth developed leaf stage are essential to prevent canker development (West et al. 2002). On the other hand, in Australia, greenhouse inoculations on spring varieties at the stages of 3 to 5 developed leaves produced the most severe stem base canker (Marcroft et al. 2005). A similar pattern was observed in Canada where vegetative growth stages (up to five developed leaves) had higher risk of canker development (Chigogora and Hall 1995). Steed et al. (2007) suggested that the phenological stage with presence of leaf spots has greater influence in the canker epidemic than the severity or leaf spot epidemic duration. Identifying the stage of crop development (or crop growth stages) when leaf spots have higher risk of canker developing would be a key knowledge to determine the best fungicide timing sprays, however testing the phenological stage effect under a unique environment and in field growing conditions may not be an easy task.

Yield losses up to 50% have been registered as a consequence of canker development in canola crops in Western Australia from 1993 to 1998 (Barbetti and Khangura 1999). In France, crop grain losses of 5% - 20% were documented in winter oilseed rape (Aubertot et al. 2004), while losses of 8% - 29% were reported in the UK (Fitt et al. 2006).

Canola represents 15% - 20% of world oil crop production and it is the third most grown vegetable oil across the globe (FAOstat 2016). Argentina has excellent agronomic conditions to produce canola, and including a winter oil crop, represents an interesting diversification alternative for the predominant rotation wheat/soybean for the main agricultural region. Canker is commonly observed in Argentine canola's crops (Iriarte and Valetti 2008), probably due to the wide presence of brassica weeds representing a widespread alternative host (Hall 1992); the massive adoption of no-till practices; or humid early autumns. All major canola growing countries have developed management programs that target the most susceptible growth stages when leaf spot infections has the higher risk to produce canker, however this information is totally unknown in Argentina (also Brazil, Paraguay and Uruguay).

The objectives of this study were: (i) to identify the canola's growth stages period in which phoma leaf spots present the highest risk to produce canker, and (ii) to establish quantitative relationships between the leaf spot epidemic and canker severity.

Material and methods

Two field experiments were carried out at Balcarce Experimental Station, National Institute of Agricultural Technology, Buenos Aires, Argentina (37°45′S, 58°15′W, 130 mamsl): Experiment 1 (2010) and Experiment 2 (2011), aiming to explore the relationships between early leaf spot epidemics and stem base canker. Both experiments were established on typical argiudol soil (loamy texture in top horizon; 5.6% of organic matter; 32 mg kg^{-1} of Bray and Kurtz P; pH 6.1). Growth stages (GS) were registered using the crop development stage coding of Sylvester-Bradley and Makepeace (1985). Daily temperature and precipitations were recorded with a conventional meteorological station located less than 1 km away from the experimental site.

Qualitative analysis of the relationship phoma leaf spot – stem base canker

The objective of the first experiment was to analyze the relationship between leaf spot epidemic and the stem base canker occurrence. We had particular interest in determine at which phenological stages leaf spot symptoms would consequently present the highest risk to develop canker. One week spaced sowings (a total of seven cohorts) were done for generating plants with a wide range of vegetative stages (represented by the number of developed leaves) for a same date. Four canola cultivars varying in cycle length (first case, W: winter or S: spring varieties) and in resistance to *L. maculans* (second case, S: susceptible or R: resistant): Gospel (WR), Hydromel (WS), Hyola 61 (SR) and Nexera 8450 (SS) were sown in 2 L pots. Plants were kept healthy and safety from *L. maculans* ascospores infections in a greenhouse located more than 500 m away from arable areas until the inoculum exposition at the field, distance enough to avoid the ascospores deposition from infected stubbles (Salam et al. 2003). Leaves were considered developed when they reached 3 cm in its longer section. All at once, plants from the stage of cotyledon up to ninth developed

leaves were transplanted to the field (28/5/2010) and exposed to the pathogen: infected stubble with *L. maculans* pseudothecia containing ascospores from previous crops (observed under a light microscope, 40×) was homogeneously spread in the area (~200 g.m^{-2}) and high relative humidity was maintained by daily manual water aspersion for the first two weeks. Individual plants (experimental unit, 50 per cultivar) were distributed in the experimental area in a completely randomized design, evenly distanced in 10 cm each other to minimize plant growth interference due to the differences in size. Leaf spot was daily scouted and the number of developed leaves at the first leaf spot detection was registered. The canker presence was assessed all at once, when 100% of all the plants had at least presence of discolored grains inside the pods (≥ GS6).

Logistic regression analysis**.** This analysis technique is widely used in epidemiological research (medical or phytopathological) where the binary response usually is the presence or absence of a disease and the predictor variables can be considered as risk factors (De Wolf et al. 2003). For the purposes of our work we modeled whether the plants exposed to natural infections (at a same time) presented (Y = 1) or not (Y = 0) stem base canker. We fitted individual linear logistic models for each cultivar including the number of developed leaves at first spot detection (X_1= LS_1) as a predictor variable, giving the equation:

$$Y = \beta_0 + \beta_1 X_1 = Ln\left(\frac{p}{1-p}\right) \quad (1)$$

for the "log odds" of Y. Therefore, in case of β_1 result significant ($\beta_1 \neq 1$) we can stay that the effect of a unit increment in LS_1 is to increase the "log odds" of Y by an amount of β_1. Equivalently, the model may be written in terms of the "odds" of canker occurrence, giving:

$$Odds\ of\ canker\ occurrence = e^{\beta_0+\beta_1 X_1} = \frac{p}{1-p} \quad (2)$$

Now, this "odds" can be defined as the ratio of the probability (p) of canker occurrence over the probability of absence (1-p). For example, considering an specific situation of our data (Table 1), for the cultivar WR we observed that when first leaf spot was detected at the 3th developed leaf (LS_1 = 3) canker was developed in 3 out of 4 plants and was absent in 1 out of 4 plants. So the odds of canker occurrence for this situation were: ¾ / ¼ = 0.75/0.25 = 3 (then it is three times more likely that canker will occur than will not occur). An opposite situation could be observed for cultivar SR when leaf spot was detected at the 4th developed leaf stage: for the 5 plants exposed 1 of them developed canker and 4 did not, so the odds can be calculated as 1/5 / 4/5 = 0.2/0.8 = 0.25. These values of odds (in natural log scale) are modeled and quantified by the β_1 coefficient in eq. 1. Finally, the probability of canker occurrence can be described as:

$$p = probability\ of\ canker\ occurrence = \frac{e^{\beta_0+\beta_1 X_1}}{1+e^{\beta_0+\beta_1 X_1}} \quad (3)$$

With the estimated probabilities for each cultivar for the range of LS1: 3 – 13 we generate the Fig. 1. Model fitting and parameter estimation were implemented in R by the GLM function using the maximum likelihood method, and the plots were done with the R base package.

Quantitative study of the relationship phoma leaf spot - stem base canker

A second and complementary experiment was aimed to analyze the relationship between leaf spot epidemic and canker severity. Field plots of the most susceptible genotype, which presented the widest high risk period in the first experiment, Nexera 8450, were sown (12/05/2011) under conventional tillage. The seeding density was 6 kg.ha^{-1}, resulting in an average of 30 plants per linear meter (140 plants.m^{-2}) after 15 days from emergency. The plots were 8 m long and 1.47 m wide (seven rows 21 cm apart). Infected stubble (with presence of pseudothecia containing ascospores of *L. maculans* observed under light microscope, 40×) from the previous year was chopped and evenly spread around the plots (~200 g.m^{-2}) after sowing to provide inoculum enough to initiate leaf spot epidemic. To favor natural infection, high relative humidity was maintained by moistening the foliage twice a day during the first 2 weeks with micro sprinkler irrigation (5 mm.cm^{-2}) arranged homogeneously between the blocks. With the aim of generate a wide range of leaf spots epidemic intensity, fungicides were sprayed with CO_2 backup sprayer (130 l.ha^{-1}, 3 bars) at crop growth stages in which leaf spot presence leads to higher probability of canker develop (results from experiment 1). Two fungicides: azoxystrobin (Az) and propiconazole (Pr) (FRAC 11/Qol group - Amistar® and FRAC 3/DMI group - Tilt® respectively, Syngenta) were singly sprayed (75 g i.a.ha^{-1}, 300 cm^3. ha^{-1}) at the second, fourth, sixth and eighth developed leaf stages (Az_2, Az_4, Az_6, Az_8, and Pr_2, Pr_4, Pr_6, Pr_8) or sprayed at all these phenological stages (full protected treatments: Az_Full or Pr_Full). The selected fungicides vary in their fungitoxic effect, since propiconazole acts in the fungus ergosterol biosynthesis (mycelium growth) representing a curative effect and azoxystrobin acts in the spore germination, considered as a preventive fungicide. Both fungicides are systemic via xylem with limited acropetal movement and have no protective effect on tissues developed after its application. Non protected treatment (check) was included in each block by spraying water. A sampling area was delimited by the three central grooves and the remaining four external ones were considered as intra-plot buffer area. Treatments (11) were randomized inside three homogenous blocks, obtaining a total of 33

plots, observations enough (> 30 plants) were done to establish relationships between leaf spot epidemic and canker severity. Leaf spot incidence (infected plants / total plants, in proportion) was assessed at 42, 47, 54, 62, 69 and 75 days after emergence, considering infected plants those with at least one typical lesion. Each plot incidence value was estimated by the mean value of three 0.5 m^2 subsamples areas. Thermal time was calculated by subtracting 4 °C, the base temperature for canola developing (Gabrielle et al. 1998) to the daily mean temperature and the accumulated value was used as independent variable as the epidemic time for the following calculations and model fittings (Lovell et al. 2004). The area under disease incidence progress curve (AUDIPC) was calculated by the trapezoidal method. Nonlinear models were fitted individually to each plot (experimental unit) aiming to fit a unique global growth model with the use of the nonlinear function (NLS) from the STATS package in R software. Gompertz, logistic and monomolecular models were fitted to the incidence of leaf spot progress through thermal time, giving the equations:

$$Gompertz: y_t = A \exp\left(-\exp\ \left(B-(r \cdot t)\right)\right)$$

$$Logistic: y_t = A/(1 + exp\ (B-r \cdot t))$$

$$Monomolecular: y_t = A \cdot\ (1-B \cdot (exp-r \cdot t))$$

Where, y_t is the disease incidence for the t-th thermal time; A is the curve asymptote parameter; B is a parameter indicating the initial value of y at the beginning of the epidemic (for this analysis fixed to B = 0.01, due to the small sample of disease incidence assessments and the assisted inoculum spread in the experimental area); r is the rate of curve progress. The lowest sum of the model residual was the criteria for model selection. Diagnostic plots of the model residuals were checked: standardized residuals vs fitted values (for homoscedasticity assumption) and the quantile-quantile plot (for the normality distribution of the dependent variable assumption). With the selected model function the leaf spot incidence was estimated for each combination of aplication moment and fungicide, and the control treatments.

Canker severity was estimated at crop maturity (174 days after emergence) with the aid of a scale widely used in France (Pierre and Regnault 1982) and modified by Aubertot et al. (2004). For this purpose, 30 plants were cut from the sampling area in each plot and rated in one of the 6 severity classes as a function of the percentage of the discolored cross-section of the stem crown: 1, healthy plant, no visible lesion; 2, [0 - 25%] of discolored cross-section; 3, [25 – 50%] of discolored cross-section; 4, [50 – 75%] of discolored cross-section; 5, [75 – 100%] of discolored section; 6, section without any living tissue. Then, the canker severity index of each plot was calculated as follows:

$$Canker\ severity = \frac{\sum_{i=0}^{5} i * n_i}{\sum_{i=0}^{5} n_i} \cdot \frac{100}{6}$$

Where n_i is the number of plants in class of canker severity of i. To confirm the variation between fungicide treatments in leaf spot incidence and canker severity, variance analysis (ANOVA) were performed to the leaf spot AUDIPC and the logit transformation canker severity (to improve normality, and stabilize variance), following the next model:

$$\gamma_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \quad e_{ij} \sim \mathrm{N}\left(0, \sigma^2\right)$$

Where, γ is the AUDIPC or canker severity at crop maturity; μ is the overall mean; α_i is the effect of the i-th fungicide treatment (i = 1,2,…,11); β_j is the effect of the j-th block (j = 1,2,3); and e_{ij} is the random error term associated with the ijth plot.

Correlation and regression analyses were performed to examine the relationship leaf spot - canker and to establish whether a single critical point model (maximum incidence observed up to eighth developed leaf; estimated incidence for the fourth, fifth, sixth, seventh or eighth developed leaf stages) or AUDIPC (multipoint model) fit better for our data. The Akaike information criterion (AIC) and residuals distribution were used to select the best model that would allow predicting stem canker severity given the leaf spot epidemic metrics.

Results

Qualitative analysis of the relationship phoma leaf spot – stem base canker

At the first experiment, a period of 39 days after plants exposition to *L. maculans* inoculum was enough to infect and show leaf spots in 100% of the plants of the SS cultivar. Mean daily temperature was 8.1 °C during this period. From this moment to canker assessment (113 days, ~463 degree-days) mean temperature was 11.5 °C. An overall average of three leaves developed between the moment of plants exposition to inoculum and the first leaf spot detection. From 3 to 13 developed leaves ranged the vegetative stage in which was detected the first leaf spot. The canker incidence presented a wide range of levels between cultivars (Table 1). For the two spring cultivars canker incidence presented the most extreme values, with overall means of SR = 6% and SS = 96%. Intermediate incidence was observed for the two winter cultivars: WR = 38% and WS = 46%. We observed a differential effect of the cultivar for modeling the probability of canker occurrence (Fig. 1). For the two spring cultivars the stage of development at the detection of phoma leaf spot was not significant for modeling the probability of canker (Table 2). In cultivar SR stem canker

Table 1 Canker occurrence counts relative to the number of developed leaves at first leaf spot detection, in four cultivars of canola exposed to natural infection of *L. maculans*

		Cultivar							
		WR		WS		SR		SS	
SBC occurrence →		No	Yes	No	Yes	No	Yes	No	Yes
Developed leaves at detection of leaf spot	3	1	3	0	1	3	0	0	7
	4	0	4	2	8	4	1	0	7
	5	3	2	0	2	3	2	0	7
	6	2	2	3	2	3	0	-	-
	7	1	2	2	4	5	0	0	5
	8	5	1	1	2	6	0	-	-
	9	5	2	1	2	3	0	0	2
	10	-	-	1	2	4	0	0	7
	11	-	-	2	2	7	0	0	7
	12	4	1	2	1	5	0	2	3
	13	3	1	2	0	1	0	0	2
	Total[y]	29	18	17	29	44	3	2	47
	Canker Incidence[z]	38%		46%		6%		96%	

Not all growth stages (developed leaves stage) at leaf spot detection were present (we inputted "- " both for Yes or No canker occurrence in those cases)

[y] Sum of counts of a cultivar < 50 since dead plants (mainly due to mechanical damage) were removed from the experiment

[z] Calculated for each cultivar dividing the total counts of positive (Yes) values by the total of plants (Yes + No) * 100

was observed only when leaf spot was detected in the fourth and fifth developed leaves stages. For this cultivar the mean probability of occurrence of canker in all phenological stages for the detection of leaf spot was 0.023 [ranging from 0.001 to 0.28]. For cultivar SS it did not matter in which developed foliar stage leaf spot was detected, canker occurred for all phenological stages with presence of LS, with an average probability of developing canker of 0.95 [0.64 to 1]. For the winter cultivars the inclusion of the number of developed leaves at first leaf spot detection as predictor contributed significantly to predict the probability of canker occurrence: WR ($P = 0.017$) and WS ($P = 0.037$). The increase of one developed leaf at first leaf spot detection decreases the odds of canker occurrence in 25% for WR and 22% for WS (odds ratios: 0.75 and 0.78 respectively). This differential effect in the canola's cultivars of the developed leaf at leaf spot

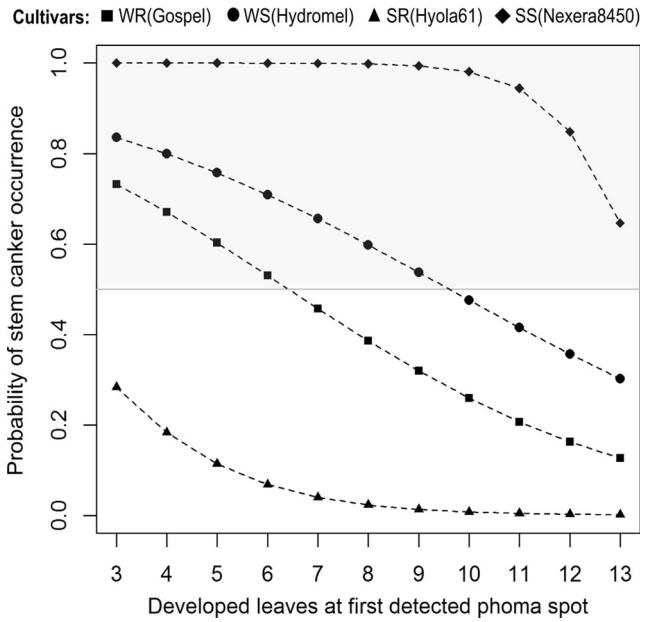

Fig. 1 Probability of stem base canker occurrence in plants of canola in relation to the number of developed leaves stage at the first phoma leaf spot for cultivars WR, WS, SR and SS. The grey shaded probability area represents a higher risk situation (probability > 0.5)

Table 2 Logistic regression coefficients (intercepts β_0 and slopes β_1) of canker occurrence on the number of developed leaves at first leaf spot detection in four cultivars of canola exposed to natural infection of *L. maculans*

Cultivar	Parameter	Coefficient	SE	*P*-value	OR [CI]
SR	β_0	0.76	1.776	-	
	β_1	−0.562	0.343	0.1	
SS	β_0	15.147	0.397	-	
	β_1	−1.119	0.072	0.17	
WS	β_0	2.373	0.984	-	
	β_1	−0.247	0.118	0.037	0.78 (0.62, 0.98)
WR	β_0	1.884	0.94	-	
	β_1	−0.293	0.123	0.017	0.75 (0.59, 0.95)

SE Standard error of the estimated coefficient. *OR* Odd ratio with it CI: 95% (confidence interval)

detection for modeling the stem canker occurrence means an interaction between both factors (Fig.1). The fitted models allowed us to estimate the probability of canker occurrence at crop maturity: it was higher than 0.5 when leaf spot were detected up to the sixth developed leaf for WR and the ninth developed leaf for WS. For cultivar SR the probability of canker occurrence was lower than 0.5 for all the growth stages when phoma leaf spot was detected and for Nexera 8450 leaf spots all the phenological stages had a probability higher than 0.5 to develop canker at crop maturity.

Quantitative analysis of the relationship phoma leaf spot – stem base canker

At the second experiment, the crop duration (from emergence to maturity) was 196 days with an accumulated precipitation of 469 mm. The stem elongation started at day 100 (427 degree-days) after crop emergence and beginning of flowering after 132 days (609 degree days) from emergence. During the vegetative stages the mean temperature was 9.4 °C, with 46 days with minimum temperature ≤ 0 °C (at 5 cm height from ground). Flowering to maturity period lapsed from September to December with a mean monthly temperature of 11.5, 13, 18.8 and 19 °C, with a total accumulated precipitation of 262 mm.

Phoma leaf spot epidemic

The first leaf spot were observed 42 days (150 degree-days) after the emergence and the maximum incidence in the untreated plots was 45% at 64 days after the emergence. Gompertz model yielded the lowest residual standard error to describe the relationship between leaf spot incidence and thermal time: 0.0219 (vs. 0.048 monomolecular model) and the best distribution of residuals. Maximum levels of incidence ranging from 7 to 50% (Fig. 2) confirmed that the fungicide treatments were effective to generate a wide variability of leaf spots epidemics. There was a significant effect of the fungicide treatments on the AUDIPC ($P < 0.01$). Post hoc comparisons using the Tukey HSD (Honest Significant Difference) test indicated that the mean AUDIPC for the treatments with azoxystrobin sprayed at 6 or 8 developed leaves or sprayed with propiconazole at 4, 6 or 8 developed leaves, were not significantly different from the untreated control (88.6). On the other hand full protected treatments with both fungicides (azoxystrobin = 32.7; propiconazole = 22.3) or single sprays with azoxystrobin at 2 or 4 developed leaves had significantly lower AUDIPC than the non-sprayed check (mean = 42.45 and 60.6, respectively) (Table 3).

Stem base canker

Fungicide treatments had also a significant effect on the canker severity ($P < 0.001$). Multiple comparisons among means with Tukey HSD test indicated that full protected treatments with either azoxystrobin or propiconazole (7.8% and 18.8%, respectively), or single sprayed with azoxystrobin at 2 (15.9%) or 4 developed leaves (20.6%) had significantly lower canker severity at crop maturity than the check treatment (Table 3).

The Pearson's *r* correlation coefficient between canker severity and leaf spot incidence were significant for: maximum incidence up to the eighth developed leaf ($r = 0.795$), AUC disease incidence progress ($r = 0.782$), incidence at 8th developed leaf ($r = 0.781$) or 7th ($r = 0.780$) (Table 4). Maximum incidence up to the eighth developed leaf was the best correlated variable with canker severity (Single critical model). The linear regression model had the best goodness of fit among the tested models (AIC: linear model = 239.5, power function model = 240; exponential model = 240.4) to describe canker

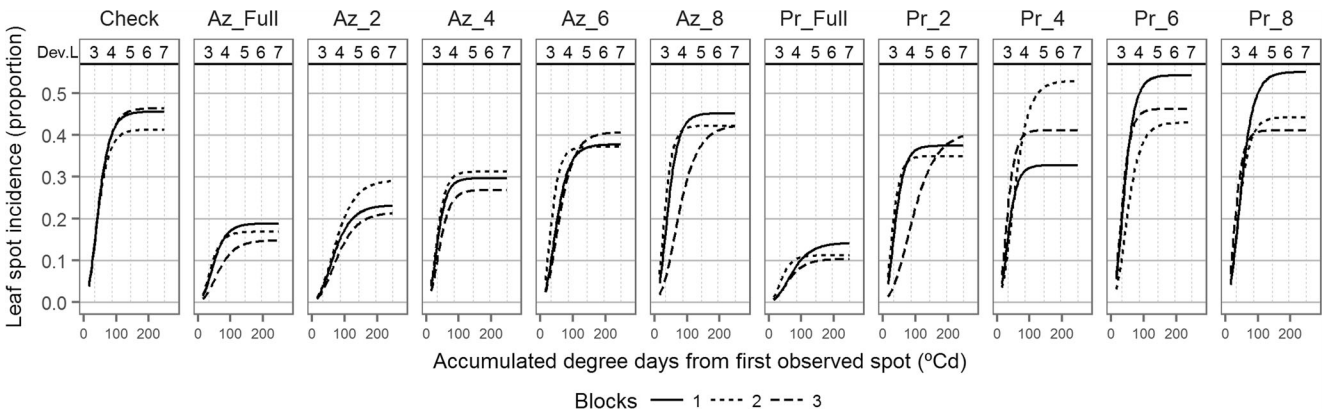

Fig. 2 Leaf spot incidence through thermal time at plot level: fitted curves by the Gompertz model. Vertical dashed lines are the moment of leaf development (detailed in numbers at the top of each plot, "Dev.L" in y-axis). Fungicide treatments: Check (non-sprayed treatment); sprayed with azoxystrobin at *i*-th developed leaf (Az_2, Az_4, Az_6, Az_8) or sprayed at all these phenological stages (Az_Full); sprayed with propiconazole at *i*-th developed leaf (Pr_2, Pr_4, Pr_6, Pr_8) or sprayed at all these phenological stages (Pr_Full)

Table 3 Estimated mean values (and standard error) for area under the disease incidence progress curve (AUDIPC) of phoma leaf spot epidemics and canker severity (%) at canola maturity stage for each fungicide treatments

Treatment	*AUDIPC*		Stem canker severity (%)	
1. Check	88.6 ± 2.4	ab	48.3 ± 7.7	a
2. Az_2,4,6,8	32.7 ± 3.4	e	7.8 ± 1.1	c
3. Az_2	42.4 ± 4.4	de	15.9 ± 2.9	bc
4. Az_4	60.6 ± 2.6	cd	20.6 ± 4.2	bc
5. Az_6	76.7 ± 0.7	abc	25.6 ± 4.1	abc
6. Az_8	86.1 ± 6.8	abc	35.1 ± 3.8	ab
7. Pr_2,4,6,8	22.3 ± 1.7	e	18.8 ± 1.7	bc
8. Pr_2	73.0 ± 7.2	bc	36.3 ± 2.9	ab
9. Pr_4	88.0 ± 8.7	abc	30.4 ± 6.1	abc
10. Pr_6	101.6 ± 8.7	a	37.1 ± 6.9	ab
11. Pr_8	97.7 ± 7.7	ab	47.6 ± 3.9	a
Tukey HSD	27.8		24.1	

Values followed by different letter are significantly different in a Tukey's HSD test ($P < 0.05$). Treatments codes are: Az(i) or Pr(i) represent azoxystrobin or propiconazole applied at *i*-th developed leaf stage(s)

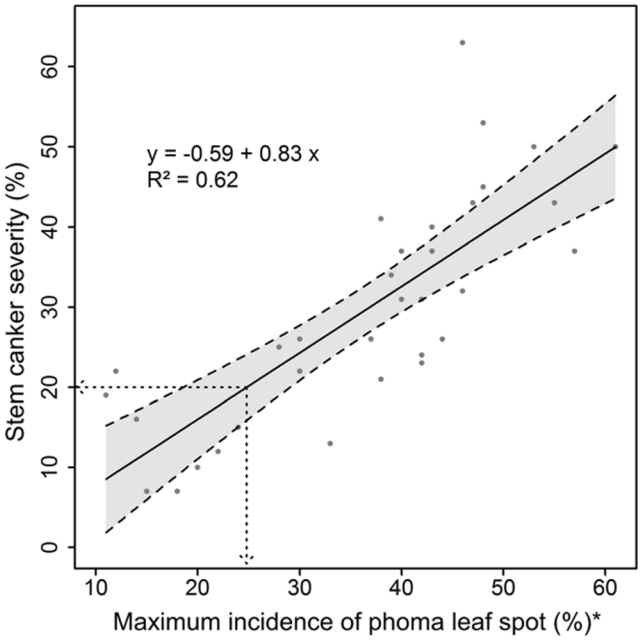

Fig. 3 Linear regression between stem base canker severity at crop maturity in function of maximum incidence of phoma leaf spot. Model fitted line (*black filled line*) and its 95% confidence interval (gray area). *Maximum leaf spot incidence up to 8th developed leaf stage

severity (y, %) related to maximum incidence of leaf spot up to the 8th developed leaf (x), with the estimated equation: $y = -0.59 + 0.83\ x$ ($R^2 = 0.62$) (Fig. 3).

Discussion

This is the first study in canola to examine the relationship between leaf spot epidemic and the consequent stem base canker occurrence and severity in one of the main Argentine growing regions. We modeled the canker occurrence combining the cultivar resistance and developed leaf stage at first leaf spot detection, and then canker severity relative to the leaf spot epidemic incidence.

We observed a wide variability in the cultivar resistance to *L. maculans* in the used genotypes, what is well known in the major canola's producer countries like Canada or Australia, however it is due to a major gene resistance which can be broken down (Li and Cowling 2003; Sprague et al. 2006). The presence of resistance in the commercial cultivars available in Argentina allows the growers to consider it as a main management mean to adopt, however this information is still scarce. Growers do not take into account the level of resistance to *L. maculans* when selecting cultivars, despite fungicide sprayings are mainly targeted to stem base canker (Iriarte L, personal communication). Cultivar Hyola 61(SR) was highly resistant to *L. maculans* infections, and even with presence of leaf spots at initial growth stages canker only developed 6% of plants. On the other hand Nexera8450 (SS) was highly susceptible, totally dependent of fungicide sprayings to avoid the canker damage. For the cultivars with intermediate resistance we observed a trend: the lower the numbers of developed

Table 4 Correlation coefficients (and *P*-values) between leaf spot incidence epidemic metrics and canker severity at crop maturity

		Pearson'r coefficient with Stem canker severity	*P*-value
Leaf spot incidence	AUDIPC [1]	0.782	0.001
	Maximum value (up to eighth DL[2])	0.795	0.001
	at eighth DL	0.781	0.001
	at seventh DL	0.780	0.003
	at sixth DL	0.762	0.060
	at fifth DL	0.621	0.094

[1] Area under disease incidence progress curve of leaf spot epidemic

[2] *DL* Developed leaf stage

leaves at first leaf spot detection (at the early vegetative stages) the higher the probability of canker symptoms occurrence. One of the probable reasons of this decrease in canker development in large mature plants (with more developed leaves than small young plants) may be the longer distance between leaf spots and the stem crown which increases the difficulties of the pathogen to reach the stem base (Sun et al. 2000). Despite all leaves at rosette stage (up to 8–10 developed leaves) have a quite similar position in terms of height, it will be interesting to study (in further works) the influence of leaf position both for the likelihood of being infected by ascospores and also to develop canker in case of leaf spot presence. The differential effect of the temperature over the pathogen and the plant growth could also determine whether the fungus can approach the stem crown or not: it seems that temperatures lower than 6 °C can slow down the mycelium systemic growth or stop it while the plants continue growing and developing at temperatures around 4 °C (West J, personal communication, June, 2010).

Confirming the existence of a most susceptible crop phase to model the canker occurrence we explored which epidemic metric in this phenological window could better explain the severity level of canker at the crop maturity stage. We observed that the maximum leaf spot incidence previous to the eighth developed leaf had the highest correlation with canker severity at crop maturity. Gompertz growth function was the most suitable to model the incidence of leaf spots in function of the crop thermal time. This model allowed us to estimate the incidence at all the other developed leaf stages. We found that seventh developed leaf was the earliest growth stage with presence of leaf spot that correlated significantly with canker severity. This piece of information may lead growers to achieve more efficient fungicide sprayings, confirming the need of early scouting of the leaf spots incidence and highlighting the relevance of the so-called fungicide spray timing (Gladders et al. 1998). Timing of fungicide application studies for the Argentine growing conditions requires further investigation efforts in order to maximize economic response and to avoid unnecessary fungicide sprayings. We showed in this first approach that canker can be effectively controlled by a single fungicide spray at the very beginning of the leaf spot epidemic, when the pathogen has not yet spread from leaves to the stems. Steed et al. (2007) observed that the most effective timings for flusilazole + carbendazim application were when leaves 7 to 11 were present on most plants and at least 10% of plants were affected by phoma leaf spots. It has been established that, in Europe, autumn fungicide sprays control canker more effectively than spring sprays. On the other hand, Kaczmarek et al. (2014) demonstrated that knowledge about the concentration of pathogen inoculum in the air allows more efficient protection of oilseed rape against stem canker: fungicide spray was most efficient, when applied 4 to 14 days after the highest concentration of *L. maculans* ascospores was found in air samples.

Even though our results can be considered as a very first approach to understand the stem canker causes, some basis for a disease management program could be established integrating these both experiment results for the argentine cropping conditions: using genetic resistance may be a main technique to consider; late planting dates could result in lower temperatures, making more difficult to the fungus to approach the stem canker before the plant elongation; spraying fungicides to avoid the leaf spot arise high levels of incidence in the initial crop growth stages, what is linearly related to canker severity. We also agree with Sun et al. (2000) about the high correlation between leaf spot incidence and canker previous to stem elongation: even we didn't explore leaf spot epidemic post stem elongation our results showed that the incidence at the seventh developed leaf was the earliest predictor of canker severity at crop maturity. The obtained simple linear function (Stem canker severity = −0.59 + 0.83 *MaxI%*) should be calibrated and validated under several field conditions, experimental seasons as well as tested in other canola growing areas in Argentina for forecasting canker severity purposes. Replacing the value of *MaxI%* in the equation one can predict the stem canker severity at crop maturity with a 95% of confidence (Fig. 3) In the inverse direction, thresholds of leaf spot incidence can be estimated in future studies of crop losses, for example: if 20% of canker severity would trigger economical losses at harvesting, hence some fungicide should be sprayed before 25% of leaf spot incidence. As Zhou et al. (1999) explained, leaf spot – canker relationships models could be coupled with canker - yield loss relationship to guide decisions in the autumn about application of fungicides. This latter (relationship canker - yield losses) study is of high priority to be executed in Argentina for guiding canola's growers decision –making and achieve an efficient disease management. Other factors that should be considered in further experiments are: cultivar resistance screenings; fungicides efficiency; *L. maculans* host range; canola's planting date and plant density; genetic population structure of the *L. maculans*; and patterns of ascospores release from canola stubbles.

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