

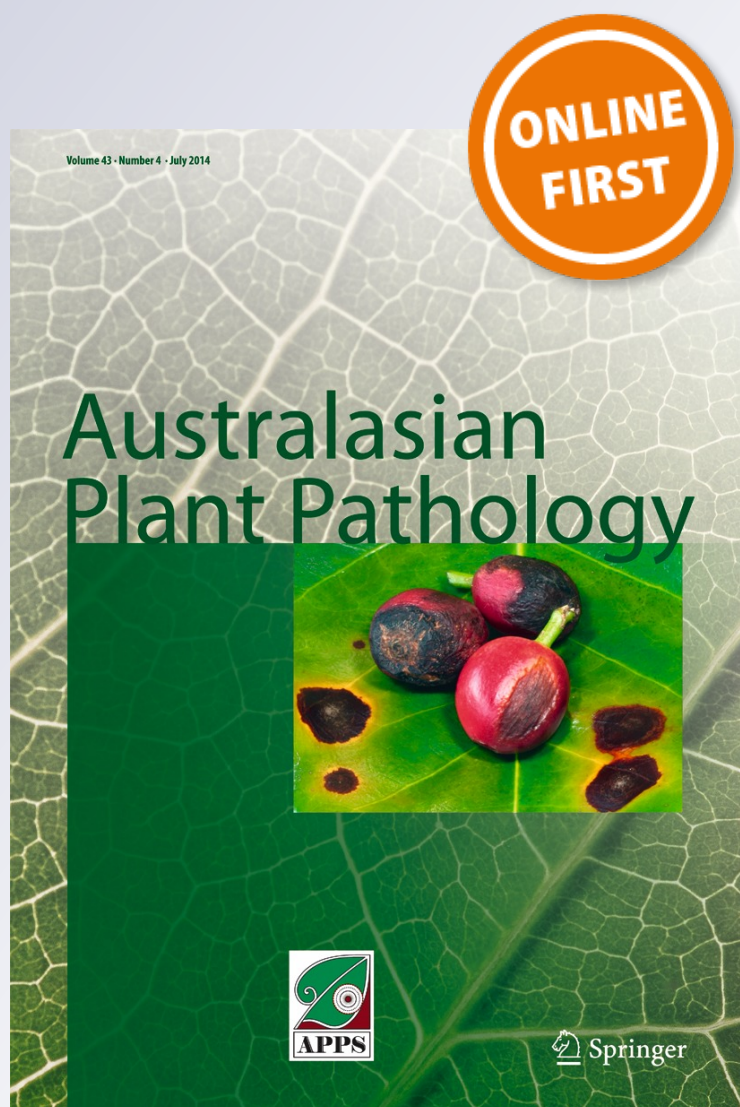
*Quantification of the environmental effect  
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# Quantification of the environmental effect on citrus canker intensity at increasing distances from a natural windbreak in northeastern Argentina

R. C. Moschini · B. I. Canteros · M. I. Martínez · R. De Ruyver

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**Abstract** Citrus canker, caused by the bacterium *Xanthomonas citri* pv. *citri* (Xcc), is an endemic quarantine disease in northeastern Argentina. The objective of this study was to quantify the effect of weather variables on mid-season fruit canker intensity in an experimental grove of the “Red Blush” grapefruit cultivar in Bella Vista, Corrientes Province (Argentina), at two contrasting distances from a natural windbreak (closer, wd=0; farther, wd=1). For the 1991–2010 growing seasons, disease observations were analyzed at both windbreak distances. The variable that best correlated with the disease levels (S: severe, M: moderate and L: light) at both windbreak distances was DPrecW<sub>SBI</sub> (days with precipitation > 12 mm and wind speed > 2.6 km.h<sup>-1</sup>; Kendall Tau-b coefficient (r<sub>k</sub>)=0.71), different from the r<sub>k</sub>=0.60 obtained with DPrec (days with precipitation > 12 mm). Daily wind speed values at both windbreak distances were estimated from wind speeds recorded at Bella Vista meteorological station after fitting linear regression equations. The best ordinal response logistic regression models included DPrecW<sub>SBI</sub> and DT (days with temperatures in the interval 17–27 °C), and DPrec, DDMaxT (sum of the exceeding amounts of daily maximum temperature from 33°C) and windbreak distance (wd) coded as strong (wd=0) and moderate (wd=1) wind protection (prediction accuracy=90 and 88.6 % respectively). Both models classified the nine observations with a severe canker intensity level correctly and their respective precipitation-driven predictors (DPrecW<sub>SBI</sub> and DPrec) achieved a highly satisfactory separation of two observed canker levels (S and M-L). The results may allow us to release

canker risk warnings for scenarios with strong (wd=0) and moderate (wd=1) wind protection. These warnings may assist producers to make bactericide spray applications.

**Keywords** Citrus canker · Windbreak · Logistic models · Weather variables

## Introduction

Citrus canker, caused by the bacterium *Xanthomonas citri* pv. *citri* (Xcc), is an endemic quarantine disease in northeastern Argentina, where weather conditions conducive to disease occurrence prevail. The most aggressive Asian or A strain of Xcc appeared in this region in 1975 (Canteros et al. de Echenique BI et al. 1985; Stall et al. 1993). Different *Citrus* species and cultivars show different susceptibility to this bacterium. Grapefruit is very susceptible, while lemon and some cultivars of tangerine and orange are moderately susceptible (Canteros 2006). Recommended practices for disease management include chemical control with copper-based bactericides, windbreaks planting, leaf miner (*Phyllocnistis citrella*) control and pruning of infected plant tissues (Canteros 1998, 2006, 2009).

The bacterium Xcc enters the young tissue of leaves, fruits, and twigs via wounds or, more importantly, via natural openings such as stomata, especially on the leaf abaxial surface, where stomatal density is higher. Young leaves are more susceptible than mature leaves (Stall et al. 1982; Gottwald and Graham 1992). Low temperatures affect the infection process. In controlled environmental conditions, Dalla Pria et al. (2006) observed the disease in oranges in a thermal range of 12–40 °C. These authors found that canker severity was greater with 24 h of leaf wetness than with 4 h, the minimum wet duration enough to cause 100 % of incidence at optimum temperatures (25–35 °C). After the infection,

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bacteria multiply, causing a typical type of corky lesion known as canker. Bacteria exude from cankers after wet by dew, rain or irrigation. The bacterial inoculum is easily dispersed in rain splash and spread is especially favored by wind (Bock et al. 2005, 2012). It has been reported that precipitations combined with wind speeds greater than  $8 \text{ m s}^{-1}$  could produce numerous new Xcc infections (Serizawa and Inoue 1974). In simulated wind-driven rain splash, Bock et al. (2006) concluded that at great wind speeds (up to  $19 \text{ m s}^{-1}$ ) Xcc bacteria disperse up to 5 m from an inoculum source and that bacteria disperse in large quantities immediately when a wind-driven rain splash occurs (Bock et al. 2005). These authors consistently detected the lowest bacteria flux density at the greatest sampling height (from 30 to 180 cm above ground), resulting in studies of short-distance splash dispersal of Xcc from cankered grapefruit canopies affected by a turbulent flux (Bock et al. 2012). Besides direct effects on the dispersal process, stronger winds occurring before an infection event predispose citrus trees to more infection (Bock et al. 2006). Because inocula come from canker lesions in trees, elimination of infected tissues will help to reduce the inoculum potential at the time of recording precipitations (Graham et al. 1987). Gottwald et al. (2009) have recently studied the epidemiological effect of harvested fruits as inoculum source. These authors showed that it is highly unlikely that citrus fruits harvested and disinfected at packing sites be the source of bacteria that reach and infect susceptible citrus species and colonize a disease-free area.

In order to reduce citrus canker intensity, frequent chemical applications (which help mitigate negative effects on the productivity and external appearance of harvested fruits) and windbreak planting are needed. This leads to high production costs. Bock et al. (Bock et al. 2010a) highlighted the importance of the interaction of precipitation and high speed winds in the dispersal of large quantities of bacteria from infected citrus trees. These authors concluded that reducing inoculum sources and wind speed could help to minimize disease spread. In northeastern Argentina, natural windbreaks are frequently planted to protect citrus groves from southern winds. This southern windbreak location can be explained analyzing the synoptic-scale atmospheric circulation pattern associated with precipitation events. The normal atmospheric circulation cycle in large scale starts with wind blows from the South Atlantic Anticyclone ( $30^{\circ}\text{S}$  latitude in average) over South America, accompanied by anticyclonic conditions in the midtroposphere. Normally, after a few days, a wave front moves from the Pacific Ocean across the Andes and forms an extra-tropical cyclone somewhere in eastern or northeastern Argentina. A cold front moving to northeastern Argentina is associated to the cyclone. The presence of the cold front causes the main precipitation events. In this cyclone stage over northeastern Argentina, the wind blows from the south or southwest.

In Concordia (North East of Entre Ríos Province, northeastern Argentina), Gottwald and Timmer (1995) found that the use of windbreaks alone or in combination with copper-based bactericides led to significant reductions in citrus canker progress. In Bella Vista, Canteros (1998; 2006) studied the windbreak effect over canker intensity observed in *Citrus* species planted in three blocks, at increasing distances to the north from a natural windbreak (Fig. 1). By using regression analysis, this author found high positive correlations between windbreak distance and disease intensity weekly observed ( $R^2$ : 0.62–0.96). Citrus canker intensity observed at 117 m from the windbreak (last row of the grove) was 2- to 10-fold greater than that observed at 19 m (first row), on different dates and for all the cultivars. In the same experimental grove, Moschini et al. (2005; 2010) used mid-season grapefruit canker observations (average of observations of three blocks) for 14 and 18 growing seasons respectively, in order to identify the weather variables more significantly associated with the disease, without considering the effect of distance to the windbreak. In both studies, among the weather variables calculated in spring time windows, total days with precipitation  $>12 \text{ mm}$  and total days with simultaneous occurrence of precipitation  $>12 \text{ mm}$  and mean daily wind speed (measured at Bella Vista meteorological station)  $>2.6 \text{ km h}^{-1}$  were the most significantly correlated. For the present study, we hypothesized that similar weather variables can be identified to explain the variability of disease levels observed at contrasting windbreak distances, including daily wind speed values estimated for both scenarios.

The main objective of this study was to quantify the effect of environmental factors on mid-season grapefruit canker intensity (Bella Vista, Corrientes Province), under two wind protection scenarios (strong and moderate), according to their distances to a natural windbreak.

## Materials and methods

### Disease data

An experimental grove with Red Blush grapefruit (*Citrus paradisi* Macf.) and other *Citrus* species was planted at the National Institute of Agricultural Technology (INTA) Bella Vista Experimental Station ( $28^{\circ}26'\text{S}$ ,  $58^{\circ}55'\text{W}$ ) in 1991. Trees were planted at a distance of  $7 \times 7 \text{ m}$  in a randomized complete block design with three replications or blocks (5 plants per plot). The three blocks were deployed at increasing distances to the north from a natural windbreak (*Casuarina* sp. and *Grevillea robusta*; 25 m high) (Fig. 1). Disease severity in fruit was assessed in each block at different times (from near mid-season to harvest), using a three-grade scale: 0=no symptoms, 1=one large or three small citrus canker lesions per fruit, 2=more than one large or three small

**Fig. 1** Experiment citrus grove (bottom: with Robinson anemometers) planted with grapefruit and six other citrus species in a randomized complete block design (right) with three blocks (5 plants per plot). The blocks were deployed at increasing distances to the north from the natural windbreak



		Effect of windbreak on citrus canker intensity							
		0	Windbreak						
<b>Block 1</b>	19 m	+	*	+	+	+	+	+	+
		+	*	+	+	+	+	+	+
		+	*	+	+	+	+	+	+
		+	*	+	+	+	+	+	+
<b>Block 2</b>	47 m	+	*	+	+	+	+	+	+
		+	+	+	+	+	*	+	+
		+	+	+	+	+	*	+	+
		+	+	+	+	+	*	+	+
<b>Block 3</b>	82 m	+	+	+	+	+	*	+	+
		+	+	+	+	+	*	+	+
		+	+	+	+	+	*	+	+
		+	+	+	+	+	*	+	+
<b>Block 3</b>	89 m	+	+	+	+	+	+	*	+
		+	+	+	+	+	+	*	+
		+	+	+	+	+	+	*	+
		+	+	+	+	+	+	*	+
	117 m	+	+	+	+	+	+	*	+

S      W \* Grapefruit plants  
E      N + Other citrus species plants

lesions per fruit. The observed fruit disease intensity (OI%) was obtained using the following formula:  $OI\% = (\% \text{ of grade 0 fruits} + \% \text{ grade 1 fruits} + \% \text{ grade 2 fruits}) / 3$ . The maximum fruit canker intensity value observed between December 27 and February 1 was selected for each growing season. The set of maximum values of OI% (per block) defined the level of mid-season fruit disease (Table 1). For the 2002–2003 growing season, a single canker intensity observation was available on February 19. Categorized observations of OI% from blocks 1 and 3 (more contrasting) for 20 growing seasons (1991–1992 to 2010–2011) were used to develop predictive logistic models ( $n=40$ ). Citrus canker intensity was ordinally coded as 0 (OI<10.6 %; L: light epidemic), 1 (OI>=10.6 % and <=45 %; M: moderate) or 2 (OI>45 %; S: severe). Such thresholds were defined following a statistical criterion and corresponded to 75 % (OI=45 %) and 20 % (OI=10.6 %) percentiles regarding the 40 canker intensity values observed (blocks 1 and 3; Table 1).

**Weather data**

The influence area of Bella Vista has a humid subtropical climate, with heavy precipitations (average annual values of 1179.4 mm) and high temperatures, with little daily and seasonal variation, and no dry season (Canteros 2006).

*Wind speed estimation within each block (at increasing distances from the windbreak)*

To estimate the wind speed within each block, three Robinson mechanical anemometers were located in each of the three blocks of the experimental citrus grove in 2008 (Fig. 1).

Subsequently, the mean daily wind speed values ( $W_{SBI}$ ;  $km.h^{-1}$ ) recorded by the anemometers from 2008 to 2010 ( $n=277$ ) in each block were related with those measured at Bella Vista INTA meteorological station ( $W_{Sst}$ ; 300 m from the grove) and linear regression equations were adjusted. Daily wind speed values estimated by the fitted linear regression equations for the closest and farthest blocks regarding the windbreak (blocks 1 and 3) were available for both sectors.

*Explanatory weather variables*

Readings of daily maximum (MaxT; °C) and minimum (MinT; °C) temperatures and precipitation (Prec; mm) were collected from Bella Vista INTA meteorological station (70 m over sea level). Daily average temperature (Td) was calculated as half the sum of MaxT and MinT. Based on these meteorological data (including daily estimations of  $W_{SBI}$  from daily  $W_{Sst}$  values by the linear regression equations adjusted per block), weather variables were calculated in a time period (CPL: critical period length) beginning after accumulating 372°-days as from July 10, and finishing when the sum reached 985°-days (base Td=12.5 °C). The base temperature was appropriate to simulate the development of citrus species, according to Davies and Albrigo (1994). CPL and weather variables were mostly adapted from Moschini et al. (2005, 2010). The following weather variables were calculated: total days with Prec>12 mm (**DPrec**), total days with simultaneous occurrence of Prec>12 mm and mean daily  $W_{SBI}>2.6 km h^{-1}$  (**DPrec $W_{SBI}$** ), total accumulated millimeters of daily Pr>12 mm (**TPrec**), mean maximum temperature (**MMaxT**), mean minimum temperature (**MMinT**), sum of the exceeding amounts of daily MaxT

**Table 1** Mid-season fruit grapefruit canker intensity values (OI%) observed in each block at increasing distance from a natural windbreak (Bella Vista citrus experiment grove)

Growing season	Observed canker intensity			
	Block 1 19–47 m	Block 2 54–82 m	Block 3 89–117 m	Average (three blocks)
1991–92	0.6	17.0	28.4	15.3
1992–93	65.0	83.0	81.0	76.3
1993–94	36.0	50.0	41.5	42.5
1994–95	10.8	42.0	40.0	30.9
1995–96	2.8	2.0	10.0	4.9
1996–97	11.8	53.0	61.2	42.0
1997–98	46.0	71.0	85.0	67.3
1998–99	29.0	45.0	43.0	39.0
1999–00	27.0	38.6	44.4	36.7
2000–01	40.0	55.0	63.0	53.8
2001–02	45.0	44.0	40.0	43.0
2002–03	20.0	43.0	63.0	42.0
2003–04	45.0	72.0	74.0	63.7
2004–05	16.0	30.0	50.0	32.0
2005–06	11.0	10.5	15.5	12.3
2006–07	10.4	8.0	18.0	12.1
2007–08	6.0	11.4	30.0	15.8
2008–09	1.6	13.0	20.0	11.5
2009–10	1.2	9.0	12.0	7.4
2010–11	8.0	26.0	31.0	21.7
2011–12	6.7	2.5	12.0	7.1
2012–13	20.7	14.2	30.0	21.6

from 33 °C when  $\text{MaxT} > 33$  °C (**DDMaxT**), accumulated differences between 12 °C and  $\text{MinT}$  when daily  $\text{MinT} < 12$  °C (**DDMinT**), total days with  $\text{MaxT} \leq 27$  °C and  $\text{MinT} \geq 17$  °C (**DT**).

#### Explanatory discrete variable

To consider the effect of windbreak distance (wd) of citrus plants over canker levels, a discrete binary variable (wd) was also included as explanatory variable. This variable was coded as 0 when disease data of block 1 were analyzed (strong wind protection scenario) or as 1 for farther disease observations (block 3) regarding the windbreak (moderate wind protection scenario).

#### Statistical analysis

First, the Freq procedure in SAS (Statistical Analysis Systems, version 8.0; SAS Institute, Inc., Cary, NC, USA) was used to calculate Kendall Tau-b nonparametric correlation coefficients ( $\tau_k$ ) between the three canker intensity categories (S, M and L) and weather variables. Second, by logistic regression techniques (Hosmer and Lemeshow 2000), weather-based models were developed to estimate the probabilities of occurrence of the ordinal disease categories. The

SAS Logistic procedure fits a parallel lines regression model to ordinal response data by the method of maximum likelihood. One of the assumptions underlying ordinal logistic regression is that the relationship between each pair of outcome categories is the same. A chi-squared score statistic was calculated to test the parallel regression assumption. The logit function  $[\ln(p/(1-p))]$ , where  $\ln$  is the natural logarithm and  $p$  is the cumulative probability of disease categories, establishes the connection between the stochastic component and explanatory variables. In the present study, the canker levels were treated in a descending order (severe to light). Each logistic model fits two equations, one estimating  $\ln[pS/(1-pS)] = \alpha_S + \beta'x$  and the other estimating  $\ln[pMc/(1-pMc)] = \alpha_M + \beta'x$ , being  $pS$  the probability of observing a severe (S) epidemic,  $pMc$  the cumulative probability of an epidemic  $\Rightarrow$  to moderate (M),  $\alpha_S$  and  $\alpha_M$  the intercept parameters,  $\beta'$  the common vector of slope parameters and  $x$  the vector of explanatory variables. Each parameter was evaluated using the Wald test. The Wald chi-square statistic was computed by squaring the ratio of the parameter estimate divided by its standard error estimate values. Solving the expressions  $\text{Exp}\{\ln[pS/(1-pS)]\} / \{1 + \text{Exp}\{\ln[pS/(1-pS)]\}\}$  and  $\text{Exp}\{\ln[pMc/(1-pMc)]\} / \{1 + \text{Exp}\{\ln[pMc/(1-pMc)]\}\}$ ,  $pS$  and  $pMc$  are obtained. The probabilities of observing a moderate (M) and light (L) epidemic result from the next two differences:  $pM = pMc - pS$  and

$pL=1-(pS+pM)$ . The probability function that gave the highest probability value was considered to be the forecasted canker level (S, M or L) for that growing season. Stepwise logistic regression was used to select the most appropriate weather-based model. Several models were built using significance levels ranging from 0.25 to 0.05 as entry (SLE) and retention (SLS) criteria for the variables. The predictive ability of the models was calculated based on the number of pairs of observations (t) with different outcome category (9S\*23M+23 M\*8 L+9S\*8 L=463 pairs, being S, M and L the epidemic categories observed). A pair of input observations with different responses is said to be concordant (or discordant) if the larger ordered value of the response has a higher (or lower) predicted event probability than the smaller response. If the pair is neither concordant nor discordant, it is a tie. Somers'D and Gama correlation indices were calculated from the number of concordant (nc) and discordant (nd) pairs of observations. The values of both indices range from -1.0 (all pairs disagree, no association) to 1.0 (all pairs agree, perfect association). The prediction accuracy of the models selected was also calculated as the percentage of cases analyzed ( $n=40$ ) in which there was agreement between the observed canker intensity category and that predicted by the logistic equations with the highest probability.

**Validation** The disease levels predicted from the selected weather-based logistic models were validated against the canker intensity levels observed in both blocks 1 and 3 (moderate to light) for the 2011–2012 and 2012–2013 growing seasons (Table 1).

## Results

The linear regression equations presented in Table 2 were fitted to estimate mean daily wind speed ( $\text{km}\cdot\text{h}^{-1}$ ) in each block ( $W_{S_{BI}}$ ) from wind speed values measured at Bella Vista meteorological station ( $W_{S_{st}}$ ) (300 m from the grove). According to their slopes, daily wind speed is approximately one-third and one-half of the wind speed measured at the meteorological station for block 1 ( $wd=0$ ) and block 3

**Table 2** Coefficient estimates of linear regression equations fitted for estimating daily wind speed at each block ( $W_{S_{BI}}$ ) from wind speed values registered at the Bella Vista meteorological station ( $W_{S_{st}}$ )

Block	Distance from windbreak	Intercept	Slope	R <sup>2</sup> <sup>a</sup>	RMSE <sup>b</sup>
1	19–47 m	0.56	0.27	0.74	0.71
2	54–82 m	0.25	0.29	0.53	0.67
3	89–117 m	-0.07	0.49	0.45	0.74

<sup>a</sup> Coefficient of determination

<sup>b</sup> Root mean square error

( $wd=1$ ) respectively. Using the linear regression equations fitted to estimate the wind speed at both contrasting windbreak distances ( $wd=0$  and  $wd=1$ ),  $DPrecW_{S_{BI}}$ , which counts the total days with  $Prec>12$  mm and  $W_{S_{BI}}>2.6$   $\text{km}\cdot\text{h}^{-1}$ , was calculated.

The correlations ( $r_k$ : Kendall Tau-b coefficient) between weather variables and canker intensity levels are presented in Table 3. The variable  $DPrecW_{S_{BI}}$  reached the maximum  $r_k$  value (0.709). Along the CPL (beginning and finishing mean dates of the CPL: September 24–November 26), for the 1991–2010 growing seasons, 268 days recorded  $Prec>12$  mm and  $W_{S_{BI}}>2.6$   $\text{km}\cdot\text{h}^{-1}$  simultaneously, corresponding this last wind speed threshold to the 43 % percentile.  $DPrecW_{S_{BI}}$  calculated with other wind speed thresholds, such as those corresponding to the quartiles 75 % (3.69  $\text{km}\cdot\text{h}^{-1}$ ), 50 % (2.75  $\text{km}\cdot\text{h}^{-1}$ ) and 25 % (2.08  $\text{km}\cdot\text{h}^{-1}$ ), showed lower correlations ( $r_k=0.48$ ,  $r_k=0.707$ ,  $r_k=0.667$ , respectively).  $DPrec$ , which only considers the effect of daily precipitations exceeding 12 mm on bacterial dispersion, showed a lower Kendall Tau-b correlation ( $r_k=0.60$ ) than  $DPrecW_{S_{BI}}$ . By setting daily precipitation thresholds other than 12 mm for the variables  $DPrec$  and  $DPrecW_{S_{BI}}$ , we obtained lower correlations with canker levels. By setting thresholds of 1 mm, 5 mm, 10 mm, 15 mm, and 20 mm, respectively, we obtained values of  $r_k$  of 0.43, 0.48, 0.60, 0.58, and 0.58 for  $DPrec$  and of 0.49, 0.54, 0.63, 0.69, and 0.68 for  $DPrecW_{S_{BI}}$ . According to Table 3, among the temperature-related variables,  $DT$  and  $DDMaxT$  were the most highly correlated with disease levels.

Including all the explanatory weather variables analyzed, the stepwise logistic regression (0.10 for SLE and SLS) selected model I (Table 4) as the most adequate model. The chi-

**Table 3** Kendall Tau-b correlation coefficients ( $r_k$ ) for weather variables and canker epidemic levels (severe, moderate and light)

Weather variables <sup>a</sup>	Coefficient values
Days with $Prec>12$ mm and $W_{S_{BI}}>2.6$ $\text{km}\cdot\text{h}^{-1}$ ( <b><math>DPrecW_{S_{BI}}</math></b> )	0.709
Days with $Prec>12$ mm ( <b><math>DPrec</math></b> )	0.602
Total accumulated millimeters of daily $Prec>12$ mm ( <b><math>TPrec</math></b> )	0.462
Total days with $MaxT\leq 27$ °C and $MinT\geq 17$ °C ( <b><math>DT</math></b> )	0.426
Sum of exceeding amounts of daily $MaxT$ from 33°C ( <b><math>DDMaxT</math></b> )	-0.401
Mean maximum temperature ( <b><math>MMaxT</math></b> )	-0.232
Mean minimum temperature ( <b><math>MMinT</math></b> ),	0.061
Accumulated differences between 12 and $MinT$ ( <b><math>DDMinT</math></b> )	-0.020

<sup>a</sup>  $Prec$ : precipitation,  $W_{S_{BI}}$ : wind speed at each block,  $MaxT$ : maximum temperature,  $MinT$ : minimum temperature. Weather variables were calculated in a time period (CPL) beginning after accumulating 372°-days since 10 July, and finishing when the sum reached 985°-days (base  $T_d=12.5$  °C)

**Table 4** Parameter estimates of the stepwise logistic regression models for estimating the probability of occurrence of each epidemic category: severe (S), moderate (M) and light (L), based on weather and discrete (windbreak distance: wd) variables

Model <sup>a</sup>	Variable <sup>b</sup>	Parameter estimate	Standard error	Wald Chi-Square <sup>c</sup>	Pr>ChiSq
I	Intercept S	-13.2049	3.5588	13.7674	0.0002
	Intercept M	-5.4635	1.7213	10.0748	0.0015
	DPrecW <sub>SBI</sub>	1.7122	0.4817	12.6367	0.0004
	DT	0.3504	0.1827	3.679	0.0551
II	Intercept S	-15.4604	4.796	10.3916	0.0013
	Intercept M	-6.6910	2.5244	7.0251	0.008
	DPrec	1.4393	0.445	10.4605	0.0012
	Wd	4.9754	1.7254	8.315	0.0039
	DDMaxT	-0.0657	0.0491	1.7945	0.1804

<sup>a</sup> Criteria for assessing model predictive ability: model I: concordant(%)=91.4, discordant (%)1.1, tied (%)=7.6; Somers'D=0.903; Gamma=0.977; prediction accuracy (%)=90. Model II: concordant (%)=88.6, discordant (%) 2.4, tied (%)=9.1; Somers'D=0.862; Gamma=0.948; prediction accuracy (%)=87.5

<sup>b</sup> *Prec*: precipitation, *W<sub>SBI</sub>*: wind speed at each block, *MaxT*: maximum temperature, *MinT*: minimum temperature. *DPrec*: days with *Prec*>12 mm, *DPrecW<sub>SBI</sub>*: days with simultaneous occurrence of *Prec*>12 mm and *W<sub>SBI</sub>*>2.6 km h<sup>-1</sup>, *DDMaxT*: sum of the exceeding amounts of daily *MaxT* from 33°C, *DT*: days with *MaxT*≤27°C and *MinT*≥17°C. *wd*: windbreak distance: wd=0 (closer); wd=1 (farther). *CPL*: beginning after accumulating 372°-days since 10 July, and finishing when the sum reached 985°-days (base Td=12.5°C)

<sup>c</sup> Wald Chi-Square test statistic is the squared ratio of the estimate to the standard error of the respective predictor and Pr>ChiSq the corresponding probability value

squared score test for the parallel line assumption indicated that the model was appropriate for the data (chi-square=3.7296 with two degrees of freedom, Pr>chi-square=0.1549). Model I included the effect of DT and DPrecW<sub>SBI</sub>. This model classified correctly 36 out of 40 observed cases (prediction accuracy=90%). Two of the four misclassified cases were overestimated (observed as a light epidemic and predicted as moderate), whereas the other two were observed as moderate and predicted as a light epidemic. The number of concordant pairs was very high (91.4%). Accordingly, values of Somer'D (0.903) and Gamma (0.977) correlation indices were also high.

To calculate the values of DPrecW<sub>SBI</sub>, daily wind speed records at two contrasting windbreak distances in the citrus grove must be available. In the present study, these records were estimated by the linear equations adjusted for blocks 1 and 3 from the daily weather station wind speed data. Due to this site-specific wind speed data demand, we run a stepwise logistic regression including not only all the weather variables, except DPrecW<sub>SBI</sub>, but also a discrete binary variable (wd: coded as 0 or 1) that took into account the two wind protection scenarios (strong: block 1 and moderate: block 3), according to their distances to the natural windbreak. Model II (Table 4), which included the weather variables DPrec and DDMaxT and the discrete variable wd, resulted the most adequate model (0.25 as SLE and SLS). The model was also appropriate for the data according to the chi-squared test (chi-square=2.431 with three degrees of freedom, Pr>chi-square=0.4879). Model II misclassified five out of the 40 mid-season canker intensity observations ordinally categorized (prediction

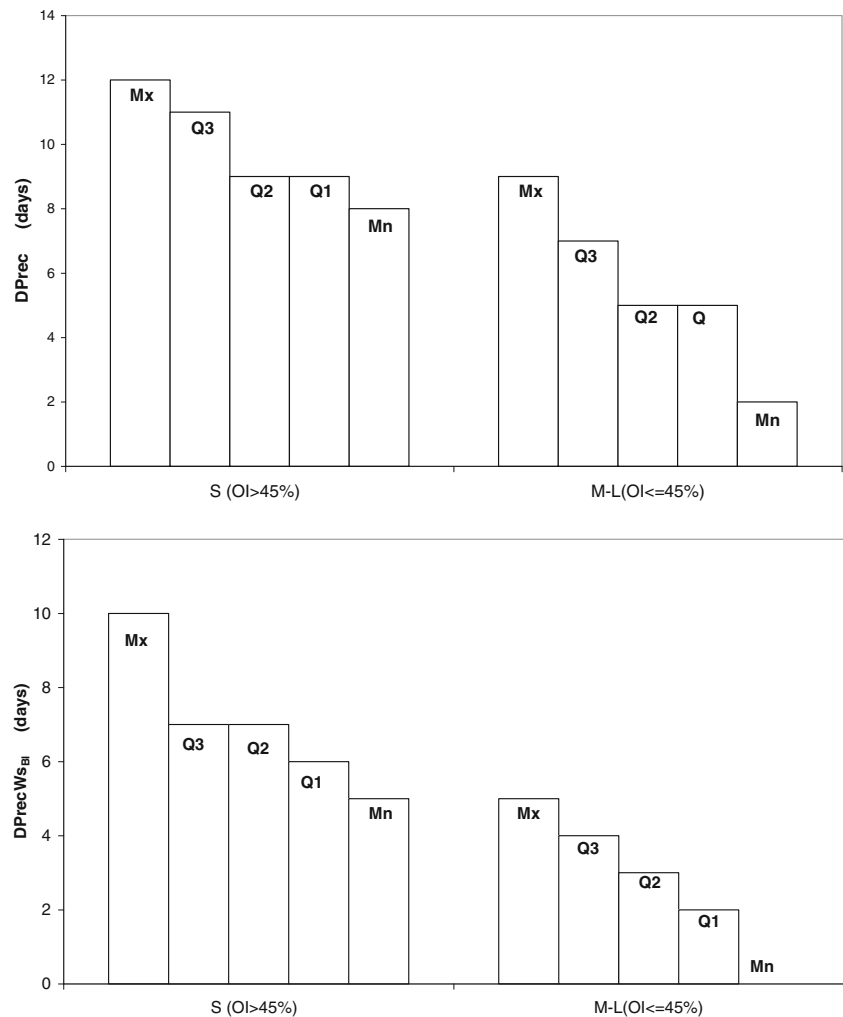
accuracy=87.5%). Three cases were overestimated and two underestimated. In relation to its predictive ability, the number of concordant pairs (88.6%) and both Somer'D (0.862) and Gamma (0.948) correlation indices resulted slightly lower than those found by model I, but highly satisfactory. Dropping wd from model II, the resulting logistic model integrating only the effect of weather-driven variables (DPrec and DDMaxT) misclassified 11 cases out of 40 (prediction accuracy: 72.5%).

Both models I and II classified correctly the nine observations with a severe canker intensity level (S: OI>45%). Accordingly, Fig. 2 shows how well both moisture predictors (DPrecW<sub>SBI</sub> and DPrec) achieved the separation of two canker levels (S and M-L). The minimum value of DPrecW<sub>SBI</sub> for the group of observations (9) with a severe canker intensity level was equal to the maximum observed for the observations (31) with a moderate-light disease intensity level (M-L: OI≤45%). Although slightly less evident, separation between the two groups was also achieved with DPrec.

As a next step, the logistic models I and II were run for the 42-year daily weather series (1971–2012) of Bella Vista, calculating the number of growing seasons with severe (S), moderate (M), and light (L) canker intensity levels, for both scenarios of wind protection (strong and moderate). The results showed the low probability of occurrence of a severe epidemic (21.4 and 11.9% of the years, respectively) in scenarios with strong wind protection (block 1), contrasting with 35.7 and 40.5% at greater distance to the windbreak (block 3). For the 22-year mid-season canker intensity observations (Table 1), the percentages of years with severe disease



**Fig. 2** Distribution of the weather-based variables D<sub>Prec</sub> (upper) and D<sub>PrecW<sub>SBI</sub></sub> (bottom) within two observed canker intensity levels (Severe and Moderate-Light) defined by a 45 % intensity threshold. Mx and Mn: maximum and minimum values. Q3, Q2 and Q1: 75th, 50th (median) and 25th percentiles of the data



levels (OI > 45 %) were 9.1 and 31.8 % for the strong (block 1) and moderate (block 3) wind protection scenarios respectively, showing a similar trend to that estimated by both models I and II in 42 years.

In two out of four cases, the observed mid-season citrus canker intensity category agreed with those predicted by both models I and II, with the maximum probability of occurrence for the 2011–2012 and 2012–2013 growing seasons and for both block 1 and block 3 (Table 1). Model I correctly classified the moderate canker levels observed in block 3 for both growing seasons, whereas model II overestimated the disease level observed for the 2011–2012 growing season in both blocks. Nevertheless, for block 3, the probability of occurrence of a severe epidemic (0.56) was slightly greater than that predicted for a moderate epidemic by model II (0.43).

## Discussion

Ordinal logistic regression models have been developed to predict levels of crop diseases such as gray leaf spot on maize

(Paul and Munkvold 2004), black point on wheat (Moschini et al. 2006) and late blight on potato (Henderson et al. 2007). Similarly, in the current study we used logistic regression techniques to quantify the effect of weather variables on canker intensity ordinal levels. The aim of this study was to identify weather variables calculated during spring in Bella Vista in order to try to explain the observed variability in mid-season fruit canker intensity in grapefruit at two contrasting distances from a natural windbreak located at the south of the experimental grove. From previous studies carried out in Bella Vista (Moschini et al. 2005, 2010) without considering the effect of windbreak distance, the correlation of D<sub>PrecW<sub>Sst</sub></sub> with average disease levels failed to differ significantly from that achieved by D<sub>Prec</sub>. In contrast, in the current study, D<sub>PrecW<sub>SBI</sub></sub> showed a higher correlation with canker levels at two distances from the natural windbreak ( $r_k=0.71$ ) in comparison with D<sub>Prec</sub> ( $r_k=0.60$ ). When precipitation thresholds lower than 12 mm were used to calculate both D<sub>PrecW<sub>SBI</sub></sub> and D<sub>Prec</sub>, their Kendall correlations with disease levels decreased. This might be related to the fact that light daily precipitations would not have enough energy for bacterial

dispersion from cankers. Coincidentally, Wischmeier and Smith (1958) selected only storms with rains greater than 12.7 mm to estimate their energy and their relationship to soil loss. Both precipitation-driven variables would explain the variability in the bacterial dispersion process and, to a lesser extent, in infection initiation. Studies in a controlled environment (Dalla Pria et al. 2006; Christiano et al. 2009) found that bacterial infection occurred when wet events lasted only 4 h, which is frequent in Bella Vista during spring from precipitation and dew. Pruvost et al. (2002) confirmed that short precipitations are more efficient for spread of Xcc, in contrast with long-duration precipitations, which may cause washing of the inoculum. These authors also noted that an efficient bacterial entry would be associated with simultaneous occurrence of precipitation and wind, which ensures a water level in excess from the mesophyll, through stomata, to the leaf surface. This requirement confirms the biological meaning of the weather variable  $DPrecW_{S_{BI}}$ .

In Concordia (North East of Entre Ríos Province, north-eastern Argentina), Gottwald and Timmer (1995) provided valuable information about the important role of windbreaks to reduce citrus canker progress. In Bella Vista, Canteros (1998) also demonstrated the importance of windbreaks by showing the very high correlation between the distance from a natural windbreak and citrus canker intensity (experimental grove). Bock et al. (2010b) concluded that the reduction of wind speed with the aid of windbreaks could decrease the bacterial dispersion and infection events, and thus reduce the disease severity. In the present study, the windbreak effect on disease variability was addressed fitting logistic models (models I and II, Table 4). Although model II showed values of prediction accuracy (87.5 %) and other correlation indices slightly lower than those derived from model I, the former has the advantage that its weather variables only need daily temperature and precipitation records, more easily available than wind speed. Nonetheless, it should be emphasized that both models I and II classified correctly the nine observations with a severe canker intensity level (S:  $OI > 45\%$ ) and their respective precipitation-driven predictors ( $DPrecW_{S_{BI}}$  and  $DPrec$ ) achieved a highly satisfactory separation of two observed canker levels (S and M-L). Also, the evaluation of model I and II outputs over a 42-year period (1971–2012) and 22-year mid-season canker intensity observations in Bella Vista showed a similar low probability of occurrence of severe epidemics in scenarios with strong wind protection (range: from 21.4 to 9.1 % of the years), contrasting with higher probabilities in moderate wind protection scenarios (range: from 31.8 % to 40.5 %). These results support the findings of many studies about the overriding importance of windbreaks in relation to citrus canker epidemic progress. When validated with independent disease data (only light to moderate canker intensity levels were observed in blocks 1 and 3 in the 2011–2012 and 2012–2013 growing seasons), both

models correctly predicted disease levels in 50 % of the cases. Further efforts are needed to make a greater and complete (all disease levels) data set available for model validation purposes.

In the present study, the thermal window defined by daily temperatures  $\geq 17\text{ }^{\circ}\text{C}$  and  $\leq 27\text{ }^{\circ}\text{C}$  (variable DT of model I) was found to be favorable for the disease intensity. This result is quite similar to the 14–28  $^{\circ}\text{C}$  interval established by Vernière et al. (2003) in the Isle of Reunion. The 17–27  $^{\circ}\text{C}$  temperature range was lower than the 25–35  $^{\circ}\text{C}$  reported by Dalla Pria et al. (2006) and Christiano et al. (2009) in orange and Tahiti lime respectively, in controlled environment chambers. In natural spring season conditions, it would be unlikely to reach this last temperature interval when wetting events caused by rain or dew occur simultaneously. Daily maximum temperatures above 33  $^{\circ}\text{C}$  and disease levels were found to be inversely related, according to the negative Kendall correlation value of the  $DDMaxT$  (included in model II).

Gottwald and Irej (2007) pointed out that meteorological events like gentle rain, rain with wind, rain storms, tropical storms, and hurricanes are progressively more effective in dispersing the Xcc inoculum to greater distances. In north-eastern Argentina, the first three events occur frequently. For the current study, only daily weather data were available to calculate the explanatory variables analyzed. The standard meteorological station of Bella Vista records wind speed at 2 m height, and does not have the ability to capture the intensity of gusts in short time intervals. In addition, the wind speed cannot be combined with the total precipitation recorded in those intervals due to the lack of pluviograph. Only records of daily accumulated precipitation in millimeters were available. The maximum mean daily wind speed estimated at increasing distances from the natural windbreak, combined with the occurrence of daily precipitation  $> 12\text{ mm}$ , was only  $7.66\text{ km h}^{-1}$  ( $2.13\text{ m s}^{-1}$ ), significantly lower than those analyzed in other works under natural or semi-controlled environmental conditions. By working with artificial inoculation and artificially-generated wind, Bock et al. (Bock et al. 2010b) concluded that the higher the wind speeds ( $> 10\text{ m s}^{-1}$ ), the higher the canker incidence and the severity levels. This increased level of disease was not strictly associated with the occurrence of visible injuries caused by the greater wind energy.

In previous studies, Canteros (1999) found a direct relationship between the mid-season disease intensity and the canker intensity at harvest, adjusting the following equation:  $OIH\% = 7.915 + 0.98\ OIm\%$ , where  $OIH\%$  and  $OIm\%$  are the canker intensities observed (three grade scale formula) at harvest and mid-season, respectively (correlation coefficient = 0.88). Extending these findings to the present study, the canker intensity levels predicted at mid-season by the spring weather-based logistic models will also likely occur at harvest.

Factors other than weather and windbreak distance-driven variables, such as canopy height growth after planting the experimental citrus grove, were not included in the analysis. Trend lines, adjusted by simple linear regression techniques, showed a slight decrease in canker intensity over time (over 22 citrus growing seasons), being  $-1.07$  ( $R^2=0.15$ ,  $Pr>F: 0.08$ ) and  $-1.58$  ( $R^2=0.20$ ,  $Pr>F: 0.03$ ) the negative slopes corresponding to the analyses of blocks 1 and 3, respectively. The current study sustains the hypothesis that the increase in canopy height (citrus plants protect one another from the wind) along the growing seasons is not a significant factor to explain the variability of citrus canker intensity over time.

Both logistic models I and II quantified and contrasted the environmental effect on disease intensity in strong wind protection (close to the windbreak;  $wd=0$ ) and moderate wind protection (farther;  $wd=1$ ) scenarios. From both models, in the springtime, we were able to daily calculate the values of the probability of occurrence of severe and moderate-light disease levels and the respective main precipitation-based variables ( $DPrecWs_{BI}$  or  $DPrec$ ) and display their progress curves graphically. Thus, canker risk warnings for scenarios with strong and moderate wind protection could be released and thus assist producers to make bactericide spray decisions and likely reduce the number of applications. Other epidemic pyramid components such as the behavior of the planted species and variety regarding the disease, grove age, last chemical spray, and pruning of affected plant tissues, should be considered in the decision-making process of canker chemical control.

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