Predicting the Dynamics of Ascospore Maturation of *Venturia pirina* Based on Environmental Factors

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ABSTRACT

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Airborne ascospores of *Venturia pirina* were trapped at two sites in northern Italy in 2002 to 2008. The cumulative proportion of ascospores trapped at each discharge was regressed against the physiological time. The best fit ($R^2 = 0.90$, standard error of estimates [SEest] = 0.11) was obtained using a Gompertz equation and the degree-days ($>0^\circ\text{C}$) accumulated after the day on which the first ascospore of the season was trapped (biofix day), but only for the days with ≥ 0.2 mm rain or ≤ 4 hPa vapor pressure deficit (DDwet). This Italian model performed better than the models developed in Oregon, United States ($R^2 = 0.69$, SEest = 0.16) or

Victoria, Australia ($R^2 = 0.74$, SEest = 0.18), which consider only the effect of temperature. When the Italian model was evaluated against data not used in its elaboration, it accurately predicted ascospore maturation ($R^2 = 0.92$, SEest = 0.10). A logistic regression model was also developed to estimate the biofix for initiating the accumulation of degree-days (biofix model). The probability of the first ascospore discharge of the season increased as DDwet (calculated from 1 January) increased. Based on this model, there is low probability of the first ascospore discharge when DDwet ≤ 268.5 ($P = 0.03$) and high probability ($P = 0.83$) of discharge on the first day with >0.2 mm rain after such a DDwet threshold.

Additional keywords: disease warning, modeling, pear scab, primary inoculum.

Venturia pirina Aderh. (the teleomorph of *Fusicladium pyrorum* (Lib.) Fuckel) is the causal agent of scab on pear trees (*Pyrus communis* L.). The disease is common in commercial orchards worldwide where spring rainfall is sufficient and causes heavy crop losses by producing lesions on leaves, young shoots, and fruit; pear fruit with scab lesions are unmarketable (28).

The pear scab pathogen overwinters primarily as pseudothecia in pear leaf litter. In addition, the fungus can overwinter as mycelium in twig lesions of some cultivars (28); however, this phenomenon has never been observed in northern Italy (V. Rossi, unpublished data). During spring, ascospores are released from the pseudothecia and cause infection. Because conidial primary inoculum is usually insignificant (13,30), pear scab is controlled by applying fungicides to prevent infection by ascospores (28).

Fungicide sprays against pear scab are most effective when they coincide with discharge of ascospores and there are optimal temperature and moisture conditions for infection. Ascospore discharge is usually monitored with spore samplers in the field or by crushing the pseudothecia, and favorable temperature and moisture are defined according to the Mill's table (35). Monitoring of ascospore maturation, however, is time consuming and requires adequate laboratory equipment; in addition, the relatively high cost of spore samplers frequently restricts or prohibits their use. In the absence of a network of spore traps, an accurate prediction of ascospore discharge during the primary inoculum season is crucial for the efficient scheduling of fungicide applications.

A model was developed in Oregon, United States, to predict the seasonal dynamics of ascospore maturity as influenced by temperature (31). The proportion of mature ascospores, determined at

weekly intervals by crushing the pseudothecia, was probit-transformed and regressed against cumulative degree-days above 0°C that had accumulated after a "biofix"; in this case, the biofix is the date on which mature ascospores were first observed. A similar model, developed in Victoria, Australia (37), relates the logit of the cumulative percentage of the ascospores trapped by volumetric spore samplers to temperature accumulation ($>0^\circ\text{C}$), calculated daily starting when ascospores were first trapped.

For practical use in disease forecasting, both models need further validation and, if possible, the determination of a biofix that does not require examination of the crushed pseudothecia or the use of spore samplers.

In this work, the seasonal dynamics of the airborne ascospores of *V. pirina* were analyzed at two locations in northern Italy between 2002 and 2008. The objectives were to (i) develop a weather-based tool for determining the first seasonal ascospore discharge, (ii) develop a model for predicting ascospore discharge, and (iii) evaluate the accuracy of the model in comparison with two imported models.

MATERIALS AND METHODS

Ascospore trapping. The study was carried out between 2002 and 2008 at two sites in the western Po Valley, northern Italy. The Migliarino site (district of Ferrara, hereafter referred to as FE) and the Villanova site (Ravenna, hereafter referred to as RA) were commercial orchards of William pear trees, which were 9 and 12 years old, respectively, in 2002. The trees at both sites were trained according to the palmate system with 4-by-3-m spacing. Trees were sprayed with fungicides each year to control scab, following commercial practices. The leaf litter, which is the source of the primary inoculum, was allowed to decompose naturally.

Seven-day recording volumetric spore samplers (Lanzoni VPPS-2000, Bologna, Italy) were operated continuously within the orchards from March to June. The samplers, which used a

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220-V 50-Hz power source, were adjusted to sample air at 10 liters/min at a 150-cm height above the ground. The tape that served as the trapping surface was 28 mm²/h; it was removed weekly and prepared for microscopic examination. The tape was examined microscopically (×675 magnification) by scanning four equidistant transects across the long axis of the tape at intervals of 2 mm (equivalent to 1 h). The number of ascospores of *V. pirina* observed in each 1-h transect was corrected for the proportion of tape examined and the volume of air sampled: the number was then expressed as trapped ascospores per cubic meter of air per hour. The spore counts were not adjusted for trap efficiency.

The cumulative number of ascospores trapped (that is, the “relative ascospore dose” sensu Hirst and Stedman) (11) was then calculated as the yearly total of hourly ascospore densities; afterward, the cumulative proportion of ascospores trapped (PAT) at each discharge was calculated on a 0-to-1 scale (26).

Environmental data. Air temperature (°C), relative humidity (rh) (%), leaf wetness duration (minutes), and rainfall (millimeters) were recorded hourly by electronic weather stations (model AD2; Silimet S.r.l., Modena, Italy) located within 3 km of the orchards.

To analyze the first discharge of *V. pirina* ascospores of the season and dynamics of PAT over the sampling season, time was expressed in physiological units (14) calculated by four different methods, based on sums of the daily temperatures exceeding 0°C. These variables were calculated as

$$DD_i = \sum_{i=1}^n (T_i); \text{ when } T_i < 0, T_i = 0 \quad (1)$$

where DD = degree-days, *i* is the subscript for days, with *i* = 1 (day of the biofix) to *n*, and *T_i* is the air temperature in each day (daily *T* was calculated as a mean of 24 hourly values);

$$DDlw_i = \sum_{i=1}^n (T_i \cdot LW_i) / 24; \text{ when } T_i < 0, T_i = 0 \quad (2)$$

where *i* is the subscript for hours, with *i* = 1 (hour of the biofix) to *n*, *T_i* is the air temperature in each hour, and LW is a dichotomic variable calculated as follows: when leaf wetness_{*i*} > 30 min, LW_{*i*} = 1; elsewhere, LW_{*i*} = 0;

$$DDvpd_i = \sum_{i=1}^n (T_i \cdot VPD_i); \text{ when } T_i < 0, T_i = 0 \quad (3)$$

where *i* is the subscript for days, with *i* = 1 (day of the biofix) to *n*, *T_i* is the air temperature in each day, and VPD is a dichotomic variable calculated as follows: when vapor pressure deficit (vpd)_{*i*} ≤ 4 hPa, VPD_{*i*} = 1; elsewhere, VPD_{*i*} = 0, with vpd calculated from temperature and rh (%) following Buck (3):

$$vpd = (1 - rh/100) \cdot 6.11 \cdot \exp[(17.47 \cdot T)/(239 + T)] \quad (4)$$

$$DDwet_i = \sum_{i=1}^n (T_i \cdot WET_i); \text{ when } T_i < 0, T_i = 0$$

where *i* is the subscript for days, with *i* = 1 (day of the biofix) to *n*, *T_i* is the air temperature in each day, and WET is a dichotomic variable calculated as follows: when *R_i* ≥ 0.2 mm and vpd_{*i*} ≤ 4 hPa, WET_{*i*} = 1; elsewhere, WET_{*i*} = 0.

Analysis of the first ascospore discharge of the season. The probable timing of the first seasonal ascospore discharge was calculated using the logistic regression procedure of SPSS (ver. 13; SPSS Inc., Chicago). Cases for calculating regression were the days with measurable rainfall (i.e., rainfall [*R*] > 0.2 mm) (22) in the interval 1 January to first seasonal discharge. The dependent variable was the dichotomic response “yes” (value 1, regardless of the daily number of ascospores discharged) or “no” (value 0) ascospore discharge in these days. The independent variables were day of the year (DOY), DD, DDLw, DDvpd, or DDwet, all accumulated starting from 1 January. The best regression model was selected based on the standard error of param-

eters, the significance level calculated by the Wald’s statistic, the dimension of deviance, the number of iterations (1), and the coefficient of determination, adjusted following Nagelkerke (20). Data collected in 2002–2006 were used for model building (167 cases in total), whereas data from 2007 and 2008 were used for model evaluation (43 cases in total).

All the possible combinations of observed versus predicted discharges were organized in a two-by-two contingency table, where the two groups “no (observed), no (predicted)”, and “yes, yes” were the right predictions, whereas the two groups no—yes and yes—no were the wrong ones. These data were evaluated following Madden (17) to calculate sensitivity, specificity, accuracy, and the prior and posterior probabilities of right and wrong predictions.

Modeling PAT. Equations 1 to 4 were used to regress values of PAT registered at FE and RA between 2002 and 2006 against DD, DDLw, DDvpd, or DDwet, all accumulated from the first to the last seasonal ascospore trapping.

In a preliminary analysis, the following equations were used: asymptotic, monomolecular, logistic, and Gompertz in the forms shown by Campbell and Madden (5). The equation parameters were estimated using the nonlinear regression procedure of SPSS, which minimizes the residual sums of squares using the Marquardt algorithm. Goodness of fit was evaluated based on the magnitude of the standard errors of the model parameters, *R*² adjusted for the number of parameters, the number of iterations taken by the Marquardt algorithm to converge on parameter estimates, and the magnitude and distribution of the residues.

Because the best fits were obtained using the equation of Gompertz, results of the other equations are not shown in this work. The equation of Gompertz has the following form: *y* = exp[−*a* · exp(−*b* · *x*)], where *a* is the equation parameter accounting for the lag phase of the PAT curve and *b* is the rate parameter.

Model evaluation. Predictions of the Gompertz model were compared with the PAT data from RA and FE in 2007 and 2008, which had not been used in model elaboration.

Predicted values were regressed against the observed ones. The null hypotheses that *a* (intercept of regression line) is equal to zero and *b* (slope of regression line) is equal to one were tested using the Student’s *t* test: *t_a* = (*a* − 0)/SE_{*a*}; *t_b* = (*b* − 1)/SE_{*b*}. If the *t* test for *a* and *b* was not significant, then both null hypotheses were accepted and the model was considered to be a statistically accurate estimator of the actual data (33). *R*², the standard error of the estimates (SEest), and the distribution of errors made by the two models in predicting PAT (difference “observed minus predicted”) were also considered.

Comparison with imported models. The Gompertz model (hereafter referred to as the Italian model) was compared with those of Spotts and Cervantes (31) (hereafter referred to as the Oregon model) and Villalta et al. (37) (hereafter referred to as the Victoria model).

The Oregon model fits the proportion of mature ascii within the pseudothecia (*y*, on a 0-to-1 scale) to the degree-days (*x*, base temperature of 0°C) accumulated starting from when the first mature ascospore was observed, as follows: ln[1/(1 − *y*)] = −0.00797 + 0.00415 · *x*.

In this work, DD were accumulated starting from the day when the first seasonal ascospore was trapped, and the model was used to estimate PAT, as follows: PAT = 1 − {1/[exp(−0.00797 + 0.00415 · DD)]}, with a 95% confidence interval for the intercept equal to ±1.00797 (estimated from Figure 1A of Spotts and Cervantes) (31).

The Victoria model fits the PAT (*y*, on a 0-to-1 scale) to the DD accumulated starting from the day when the first ascospore was trapped, as follows: ln[*y*/(1 − *y*)] = −2.952 + 0.0124 · DD, so that PAT = [exp(−2.952 + 0.0124 · DD)]/[1 + exp(−2.952 + 0.0124 · DD)], with a 90% confidence interval for the intercept equal to ±2.248 (estimated from Figure 3C of Villalta et al.) (37).

To compare the three models, the values of PAT predicted for FE and RA in the years 2007 and 2008 were regressed against the observed ones. This evaluation was performed as described for evaluation of the Italian model.

RESULTS

First seasonal ascospore discharge and developing the biofix model. In the data set used for model elaboration, the first *V. pirina* ascospores were trapped between 16 March (FE06) and 9 April (RA05) with a range of 24 days (Table 1). Average temperature (T) of these days was 10°C (minimum 5.2°C, maximum 14.9°C) and discharge was always triggered by at least 1.2 mm of rain (maximum 22.7 mm) (data not shown). DD accumulated between 1 January and the day of first ascospore discharge ranged from 229 (FE06) to 470 (RA05) (Table 1), with significant correlation between DOY and DD ($r = 0.923$, $P = 0.0004$, $n = 9$). In the same period, total rainfall ranged from 36 mm (FE02) to 380 mm (RA04), with 17 (FE02 and FE06) to 46 (RA04) rainy days, and no significant correlation with DOY ($P = 0.5$ and 0.4 for rain and rainy days, respectively).

The best logistic regression model predicting the probability of these ascospore discharges was obtained using DDwet as the independent variable. The model parameters were estimated after eight iterations; low standard errors of parameter estimates, low deviance, and the high R^2 indicated a good fit of the data (Table 2). This logistic regression model (hereafter referred to as the biofix model) is written in the following form: Probability (first discharge) = $1/(1 + e^{-Z})$, where Z is the linear combination: $Z = -11.3114 + 0.0421 \cdot \text{DDwet}$. Based on this model, the probability for the first seasonal ascospore discharge increased as DDwet increased, with probability >0.5 when DDwet > 268.5 (Fig. 1). When DDwet in-

creased by 1, the odds increased by a factor of 1.043, as shown in the $\exp(b)$ column of Table 2; the odds are defined as the ratio of the probabilities that the event will or will not occur. Using a probability >0.5 to predict an actual discharge, the biofix model correctly predicted five of nine actual discharges, giving a true positive proportion (TPP) of 0.56, which indicates

Fig. 1. Predicted probability (based on a logistic regression) of the first discharge of *Venturia pirina* ascospores of the season as a function of degree-days (above 0°C) accumulated after 1 January only for days with ≥0.2 mm of rainfall or ≤4 hPa vapor pressure deficit (DDwet). Points show the probability for the observed DDwet values, while the solid line is the estimated probability for the remaining cases: ○ and ● indicate DDwet data leading to or not leading to an observed ascospore discharge, respectively. Statistics for the regression are shown in Table 2.

TABLE 1. Dates of first and last trapping of the airborne ascospores of *Venturia pirina* at two locations in northern Italy between 2002 and 2008, and summary data of rain (R) and temperature (Tm)

Location ^b	Trapping ^a		1 January to first trapping				First to last trapping			
	First	Last	R (mm) ^c	R (days) ^d	Tm (°C) ^e	DD ^f	R (mm) ^c	R (days) ^d	Tm (°C) ^e	DD ^f
FE02	22 March	30 May	36	17	4.8	431	149	26	14.0	968
FE04	24 March	6 June	326	42	3.8	318	241	37	14.0	1,061
FE05	24 March	24 May	39	26	2.8	242	132	29	13.8	858
FE06	16 March	11 May	42	17	2.9	229	71	16	12.4	710
FE07	8 March	29 May	36	11	6.3	417	151	21	14.7	1,223
FE08	4 March	21 May	50	12	5.1	323	176	32	12.4	978
RA02	23 March	25 May	75	18	5.1	452	173	33	13.6	869
RA03	1 April	4 June	114	25	4.2	393	161	23	16.1	1,045
RA04	24 March	21 May	380	46	4.4	366	142	41	12.7	752
RA05	9 April	11 May	104	21	4.7	470	97	10	14.2	470
RA06	22 March	5 June	70	17	3.2	271	201	20	14.7	1,120
RA07	7 March	27 May	30	14	6.5	423	140	19	14.7	1,208
RA08	5 March	6 June	43	9	5.6	357	146	28	13.7	1,289

^a Seasonal ascospore trapping. Airborne ascospores were trapped with a volumetric spore sampler placed within the pear orchard.
^b Location (FE = Ferrara, RA = Ravenna) and year (02 = 2002, 08 = 2008); data from 2002–2006 were used for model building while data from 2007 and 2008 were used for model evaluation.
^c Total rainfall in the period.
^d Number of rainy days with ≥0.2 mm rainfall in the period.
^e Average air temperature of the period.
^f Degree-days (DD) (above 0°C) accumulated for the period.

TABLE 2. Parameters and statistics of the logistic regression accounting for the effect of physiological time on the probability of first discharge of *Venturia pirina* ascospores of the season

Variables included ^a	b	se ^b	Wald's statistic	df	P	exp(b)	Deviance	R ^{2c}
Constant	-11.311	2.621	18.63	1	<0.0001	1.22 × 10 ⁵	35.0	0.55
DDwet ^d	0.042	0.011	13.82	1	0.0002	1.043		

^a Logistic regression is: probability (first discharge) = $1/(1 + e^{-(-11.311+0.042 \cdot \text{DDwet})})$.
^b Standard error of the parameter estimates.
^c Coefficient of determination adjusted following Nagelkerke (20).
^d Degree-days (above 0°C) accumulated from 1 January only for days with ≥0.2 mm rainfall or ≤4 hPa vapor pressure deficit.

an intermediate sensitivity of the model (Table 3). Also, 157 of 161 “no discharges” were correctly predicted, giving a true negative proportion (TNP) of 0.99, which indicates a high specificity of the model. In four cases (FE05, FE06, RA02, and RA06), the discharges were wrongly predicted, giving a false negative proportion (FNP) of 0.44. In one case (RA04), the model predicted a discharge that did not result in airborne ascospores, giving a false positive proportion (FPP) of 0.01; the wrong prediction occurred on 20 March, 4 days before the right one. Overall accuracy was 0.97 [(TPP + TNP)/total cases] considering accurate versus total predictions, whereas it was 0.55 using the Youden’s index ($J = \text{TPP} - \text{FPP}$). The likelihood ratio of discharge ($LR(O+) = \text{TPP}/\text{FPP}$) was 87.8 and the likelihood ratio for no discharge ($LR(O-) = \text{TNP}/\text{FNP}$) was 0.45. These results also had good accuracy, because an accurate model has, in general, a large $LR(O+)$ and a small $LR(O-)$. The model had posterior probabilities of 0.833 ($P(O+,P+)$) that there is discharge when discharge was predicted, and 0.975 ($P(O-,P-)$) that there is no discharge when discharge was not predicted (Table 3).

In the data set used for model evaluation, the first seasonal ascospores were discharged between 4 March (FE08) and 8 March (FE07), generally earlier than in the previous period (2002 to 2006) because of the higher temperatures in 2007 and 2008 (Table 1). The biofix model showed very high sensitivity ($\text{TPP} = 1$) and specificity ($\text{TNP} = 0.95$) in predicting these discharges (Table 3). Overall accuracy was also high (0.95), with a Youden’s index showing almost perfect prediction. Only the posterior probability $P(O+,P+)$ was lower than in the original model (Table 3) because of two discharges predicted at FE07 and RA07 (4.9 and 0.3 mm of rain on 25 February, respectively) that did not result in ascospore trapping. In both cases, this wrong prediction occurred 10 days before the first seasonal discharge.

Dynamics of ascospore discharge and developing the Italian model. Both the cumulative number of ascospores trapped and its seasonal dynamic differed among years and study sites (Fig. 2).

At both study sites, highest numbers of ascospores were trapped in 2002 (average of 3,055 ascospores/ m^3 of air), and the lowest ones in 2005 and 2006 (178 and 139 ascospores/ m^3 of air, respectively) (Fig. 2). The ascospore season was 68 days long on average, with a minimum of 32 days at RA05 and maximum of 93 days at RA08; in the last 2 years, the ascospore season was longer than in the previous years (average of 84 days for 2007 and 2008, and 61 days for 2002–2006) (Table 1). Seven (RA05) to 29 (FE08) ascospore discharges occurred during the primary inoculum season (Fig. 2).

In 2007, ascospores matured earlier than in the other years. At both study sites, >60% of the season’s ascospores were already discharged at the end of March; most of these ascospores were

trapped between 20 and 31 March (Fig. 2C), a period with frequent and heavy rain (88 and 108 mm of rain at FE and RA, respectively; data not shown). In this year, there was a long period (26 days of April) with no rainfall (data not shown) and no rele-

Fig. 2. Cumulative proportion of the *Venturia pirina* ascospores trapped (PAT) from the air of **A** and **B**, two pear orchards in northern Italy between 2002 and 2006 (used for model building) and **C**, in 2007 and 2008 (used for model evaluation). Numbers between parentheses are total ascospores trapped per cubic meter of air for each combination of location (FE = Ferrara, RA = Ravenna) and year (02 = 2002, 08 = 2008).

TABLE 3. Comparison between the first discharge of *Venturia pirina* ascospores of the season predicted by the logistic regression (Table 2) and the first discharge observed in two pear orchards in two periods, 2002 to 2006 used for model building and 2007 and 2008 used for model evaluation

Period, obs. ^a	Predicted discharge ^b		Total	Youden's index (J) ^c	Likelihood ratio (<i>LR</i>)	Prior probability (<i>P</i>)	Posterior probability (<i>P</i>)	
	Yes (P+)	No (P−)						
2002–2006								
Yes (O+)	5 (0.56) ^d	4 (0.44) ^e	9	0.55	<i>LR</i> (O+) 87.8	<i>P</i> (O+) 0.05	<i>P</i> (O+,P+) 0.83	<i>P</i> (O+,P−) 0.03
No (O−)	1 (0.01) ^f	157 (0.99) ^g	158		<i>LR</i> (O−) 0.45	<i>P</i> (O−) 0.95	<i>P</i> (O−,P+) 0.17	<i>P</i> (O−,P−) 0.97
Total	6	161	167					
2007 and 2008								
Yes	4 (1.00) ^d	0 (0.00) ^e	4	0.95	19.5	0.01	0.67	<0.01
No	2 (0.05) ^f	37 (0.95) ^g	39		0	0.99	0.33	0.99
Total	6	37	43					

^a Years and observed discharge.

^b Predicted discharges are discharges with a probability >0.5, that correspond to DDwet > 268.5.

^c $J = \text{true positive proportion (TPP)} - \text{false positive proportion (FPP)}$.

^d TPP (sensitivity).

^e False negative proportion (set to 0.001 for calculation of LR and P for 2006 and 2007).

^f FPP (accuracy).

^g True negative proportion (specificity).

vant ascospore discharge (Fig. 2C) that did not occur in the other cases (Fig. 2A and B).

Also at FE06, ascospores matured early in the season: on 6 April, after a 12-day hot and moist period (average 11.9°C and 78% rh; data not shown) with no discharge, 7 mm of rain triggered the discharge of ≈70% of the total ascospores (Fig. 2A).

The Gompertz model accurately described the PAT for the nine combinations of site and year (period 2002–2006) over the physiological time, DDwet (Fig. 3): the values of R^2 were 0.92 to 0.99, the standard errors of model parameter estimates were low, and the differences between observed and predicted values were small, except for the trappings on 3 May at FE02 (−0.20, Fig. 3A), 7 April at RA02 (−0.17, Fig. 3E), and 13 April at RA06 (0.25, Fig. 3I).

The aggregate values of PAT were described by the following model with $R^2 = 0.90$: $\text{PAT} = \exp[-5.31 \cdot \exp(-0.015 \cdot \text{DDwet})]$.

Standard errors of the model parameters (± 0.758 and ± 0.001 for 5.31 and 0.015, respectively) were used to calculate the 99% confidence band of the estimated PAT (Fig. 4A). In all, ≈75% of the observed data fell inside this band: 7% of the outsider cases were below the lower limit of the band while 18% were above the upper one. The residuals, calculated as observed minus predicted, had an average of 0.0006 and a range of −0.276 to 0.327. Most data were within the interval ± 0.15 (Fig. 5A). The linear regression of predicted versus observed data accounted for 90% of the total variance ($R^2 = 0.90$) with low SEest (SEest = 0.109) (Fig. 4B). The model overestimated the lowest values of PAT and underestimated the highest ones, because the intercept and slope were slightly but significantly different from zero ($a = 0.06$, $P = 0.005$) and one ($b = 0.91$, $P = 0.001$), respectively.

Model evaluation. The Italian model was evaluated against the independent data set collected in 2007 and 2008. Only 26% of the

Fig. 3. Gompertz equations (dashed line) describing the relationship between the degree-days (above 0°C) accumulated after the first seasonal ascospore discharge only for days with ≥ 0.2 mm of rainfall or ≤ 4 hPa vapor pressure deficit (DDwet) and the cumulative proportion of the *Venturia pirina* ascospores trapped (PAT, ●) from the air of two pear orchards in northern Italy between 2002 and 2006: A, FE02; B, FE04; C, FE05; D, FE06; E, RA02; F, RA03; G, RA04; H, RA05; and I, RA06 ((FE = Ferrara, RA = Ravenna). The letters a and b are the parameter estimates of the equation $y = \exp[-a \cdot \exp(-b \cdot x)]$, with their standard error, and adjusted R^2 .

observed data were outside the confidence band of the model: 8% of these outsider cases were below the lower limit of the band while 16% were above the upper one (Fig. 6A). The distribution of observed minus predicted data was similar to that of the original data set, with an average of -0.003 and a range of -0.276 to 0.237 (Fig. 5B). The linear regression of predicted versus observed data accounted for more than 90% of the total variance ($R^2 = 0.92$), with $SE_{est} = 0.101$ (Fig. 6B). The parameter estimates of this line ($a = 0.04$, $P = 0.03$; $b = 0.93$, $P = 0.02$) were nearly equal to those of the line calculated for the original data.

Comparison of models. The Oregon model predicted the PAT with lower accuracy than the Italian one (Fig. 6C). Even though 80% of the PAT values fell inside the 95% confidence band of this model, there was a great difference between observed and predicted data: the average of the residuals of observed minus predicted was -0.097 , with a minimum of -0.404 and a maximum of 0.207 , with most of the data showing negative residuals (i.e., overestimation) (Fig. 5C). The linear regression between these data accounted for less than 85% of the total variance ($R^2 = 0.84$), with $SE_{est} = 0.114$ (Fig. 6D). The intercept was 0.27 (different from zero at $P < 0.0001$) and the slope was 0.71 (different from one at $P < 0.0001$); therefore, the Oregon model substantially overestimated low values of PAT.

The Victoria model also estimated PAT with lower accuracy than the Italian model (Fig. 6E). Frequency of cases falling within the 90% confidence band was 63% and the average difference of observed minus predicted was -0.063 ; distribution of these residuals was similar to that of the Oregon model but the maximum difference was lower ($= -0.387$); also for this model, most of the residuals were negative (Fig. 5D). The linear regression between observed and predicted data accounted for $\approx 90\%$ of the total variance ($R^2 = 0.899$), with $SE_{est} = 0.111$ (Fig. 6F). The intercept was 0.12 (different from zero at $P < 0.0001$) and the slope was 0.90 (different from one at $P = 0.0015$); therefore, the Victoria model also overestimated low values of PAT.

DISCUSSION

The main objective in scab management is the prevention of primary infections in spring, because extensive primary infections result in poor fruit set and make scab control during the season

more difficult (7). If the orchard is free of scab up to the end of the primary inoculum season, additional fungicide applications are usually unnecessary (22). In this respect, pear scab is similar to apple scab. Dynamic models have been developed as decision aids for controlling primary apple scab infections (25,34,38). A decision support system for controlling primary pear scab infections needs the following information: (i) the beginning and end of the primary inoculum season, (ii) the density of mature ascospores discharged on each event, and (iii) the infection risk after ascospore discharge.

Pear scab infection periods can be determined by measuring temperature and leaf wetness (22). The Mills table for apple scab (19) is currently used to evaluate temperature and wetting periods for pear scab (30,35). Tools for predicting the dynamics of ascospore maturation represent an economical and effective alternative

Fig. 5. Box plots representing the distribution of the errors made by three different models in predicting the proportion of the *Venturia pirina* ascospores trapped from the air in two pear orchards in northern Italy. Data from A, Figure 4B; B, Figure 6A; C, Figure 6C; D, Figure 6E; errors are calculated as observed minus predicted. The box contains 50% of the data, the line within the box is the median, whiskers extend to lower and upper quartiles, while points are outliers.

Fig. 4. Observed cumulative proportion of the *Venturia pirina* ascospores trapped from air (PAT) versus PAT predicted by the Italian model. A, Observed PAT data (●) were from two pear orchards in northern Italy, between 2002 and 2006. Predicted PAT (dashed line) (with 99% confidence band indicated by solid line) was based on the degree-days (above 0°C) accumulated after the first seasonal ascospore discharge only for days with ≥ 0.2 mm of rainfall or ≤ 4 hPa vapor pressure deficit (DDwet). B, Model predictions were plotted against the observed data, with the correspondent regression line (dashed line); a and b are the parameter estimates of the regression line, with their standard error and adjusted R^2 .

to labor-intensive and time-consuming methods, such as trapping of the airborne ascospores and microscopic observation and counting of mature pseudothecia. The mathematical models developed in Oregon (31) and Victoria (37) are valuable but they have not been validated outside the areas where they were developed and they require a biofix, which has to be determined directly in the orchard by crushing pseudothecia or using spore samplers, respectively.

The pattern of ascospore discharge observed in northern Italy was similar to that reported in other locations (2,6,13,39), including Oregon (31) and Victoria (37). In spite of that, the models developed in Oregon and Victoria did not accurately describe the ascospore trapping data in northern Italy. Differences between observed and estimated data were large for both models, and both overestimated the proportion of mature ascospores ready to be discharged in the early season. The Oregon and Victoria models

Fig. 6. Observed cumulative proportion of the *Venturia pirina* ascospores trapped from air (PAT) versus PAT predicted by the **A and B**, Italian; **C and D**, Oregon; and **E and F**, Victoria models. The observed PAT data (●) were from two pear orchards in northern Italy in 2007 and 2008. The predicted PAT (dashed line) (with 99, 95, and 90% confidence band, solid lines, for the three models, respectively) was based on the degree-days (above 0°C) accumulated after the first seasonal ascospore discharge **A**, only for days with ≥ 0.2 mm of rainfall or ≤ 4 hPa vapor pressure deficit (DDwet) or **C and E**, for all days (degree-days [DD]). **B, D, and F**, Model predictions were plotted against the observed data, with the correspondent regression line (dashed line); a and b are the parameter estimates of the regression line, with their standard error and adjusted R^2 .

predict ascospore maturation based only on DD accumulation. The low accuracy of the Oregon model (31) when used outside of Oregon was also demonstrated by Zoller et al. (39). A part of these inaccuracies could rise from some differences in the procedures used in Oregon and Victoria. In Oregon, the model was initiated based on actual observation of mature ascospores in crushed pseudothecia (31); in Victoria, the model was developed using a Burkard spore trap (37).

Compared with the Oregon and Victoria models, the Italian model developed in this work was a better predictor of *V. pirina* ascospore discharge. This model differs from the other two models in two main ways. First, the Gompertz equation of the Italian model has an initial lag phase that is completely lacking in the Oregon model. Because the model of Spott and Cervantes (31) was developed by determining the proportion of asci that contained mature ascospores, we infer that maturation and discharge of *V. pirina* ascospores do not coincide. For the apple scab pathogen, *V. inaequalis*, ascospore maturation precedes ascus maturation (9) so that, early in the season, ascospores are not discharged until at least 10 to 15% of the asci contain morphologically mature ascospores (15). The Italian model agrees with the Victoria model, which also has a lag phase for early season ascospore discharge. Unlike the model of Villalta et al. (37), who linearized the logistic equation through the logit transformation (5), the Gompertz equation is not symmetrical around the inflection point: the rate of ascospore discharge approaches the inflection point more rapidly and declines more slowly than in the logistic model. This lack of symmetry is biologically reasonable because humidity is higher in early spring than later in the season. Therefore, the DDwet of the Italian model accumulates faster than the DD of the Victoria model in the early stage of ascospore maturation.

The second main difference between the Italian and the other two models is that the Italian model accumulates degree days (DD) above 0°C daily only on days with ≥ 0.2 mm of rain or ≤ 4 hPa vapor pressure deficit. These criteria have been used to estimate the effect of moisture on fungal development and sporulation on leaf residue on the soil surface (23,24). In apple scab models, inclusion of variables accounting for moisture improved the effect of temperature as a predictor of ascospore maturation (27,32). The same is likely true for pear scab because accumulation of DD only on rainy days sometimes improved predictions of ascospore maturation (37). The effect of moisture on ascospore production by *V. pirina* has been demonstrated by Borecki (2), who found that mass production of ascospores within pseudothecia occurred at temperature sums (above 0°C, from 1 February) of 147 to 155 under controlled environmental conditions at 90 to 100% rh but increased to 176 to 240 under orchard conditions with fluctuating humidity. Sobreiro and Mexia (29) found that the scab epidemics started earlier in the season when the winter was cold and rainy than when it was warm and less rainy. Villalta et al. (37) found that total rain accumulation was strongly associated with ascospore maturation but, when both temperature and rain accumulations were considered, rain accumulation did not appreciably contribute any additional variation in ascospore maturation.

The Italian model, as well as the imported models, is based on DD. It assumes a linear response of ascospore maturation to temperature, or at least a piecewise linear relationship in the range of temperature commonly observed under natural conditions (5 to 21°C in the present work) (14). Data on temperature response across a range of constant temperatures are not available for *V. pirina* but, in *V. inaequalis*, temperature is directly proportional to the rate of ascospore maturation at 6 to 20°C (8).

Following MacHardy (15), we consider three phases of particular value when the Italian model is used in pear scab management programs. The lag phase lasts from 1 to 81 DDwet, when 20% of the seasonal ascospores are ready to be discharged. In this work, this phase began from 10 March to late March, lasted 10 days in

humid years and 3 weeks in dry years, and ended in late March to early April. The accelerated phase lasts from 82 to 267 DDwet, when $\approx 70\%$ of the season ascospores are ready for discharge. This phase lasted 1 month and ended between late April and early May. The final phase lasts from 268 to 428 DDwet, when the final 10% of the ascospores become ready for discharge. These ascospores matured over a period of 3 to 5 weeks, so that the primary season ended between mid-May and early June.

The first *V. pirina* ascospores of the season are usually discharged when pear trees are between the swollen-bud and green-tip stage (13,31,36), so that Villalta et al. (37) proposed using the green-tip stage of the fruit bud as a biofix for ascospore maturity models; the silver-tip stage of McIntosh is used in a similar manner for predicting apple scab (16). Nevertheless, observations in Poland showed that the use of the tree development for inferring perithecial development is a great simplification (2). The Oregon State University Extension considers that the pear scab infection season starts when 320 DD (base 0°C) have followed at least 0.25 mm of rain or dew (22).

Models have been developed to predict the first ascospore discharge of *V. inaequalis* based on weather data (12,21). Giosuè et al. (10) accurately predicted this event by accumulating 80 DD (only on hours with $>0^\circ\text{C}$ and presence of wetness) starting from the date when the model of James and Sutton (12), modified by Mancini et al. (18), estimated that the first pseudothecia contained morphologically mature ascospores.

In the present work, a model was developed to estimate the biofix for the maturation of *V. pirina* ascospores based on the D above 0°C, accumulated daily from 1 January only on days with ≥ 0.2 mm of rain or ≤ 4 hPa vapor pressure deficit (DDwet). The date 1 January was chosen because, at this date, all the pseudothecia were in an undifferentiated stage (V. Rossi, unpublished data). This biofix model had high overall accuracy (0.97) and satisfactory properties, especially in predicting no discharge, as indicated by high specificity (TNP = 0.99) and high likelihood ratios of discharge ($LR(O+) = 87.8$) (16). Based on this model, there is low probability of ascospore discharge when DDwet ≤ 268.5 ($P(O+,P-) = 0.03$), and high probability ($P(O+,P+) = 0.83$) of the first seasonal discharge on the first day with >0.2 mm of rain after such a DDwet threshold. The model was validated in two independent years (2007 and 2008) at two locations. Winter temperatures in these years were higher than in the previous years and the ascospore season began earlier. Nevertheless, the time of first seasonal ascospore discharge was correctly estimated by the model in both years. A small FPP (0.01 in the original data set and 0.05 in 2006 and 2007) was observed, that led to a 4- to 10-day-early biofix. This error should have little impact for the following prediction of ascospore maturation because DDwet accumulates slowly in this period of low air temperature. Still, further studies are necessary to improve these model predictions.

We consider these two models (the biofix model and the Italian model) suitable for use in our regional "forecasting and warning system" for plant protection (4). The required environmental data (temperature, rh, and rainfall) are provided by a widespread network of weather stations in the Po Valley, northern Italy. With these data, the two models correctly predict the beginning and end of the primary season of *V. pirina* inoculum and the proportion of the season's ascospores that are mature and ready for discharge on each discharge event. Although immediately useful in northern Italy, these models require validation in other locations.

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