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Ecology and Epidemiology

Timing of Grape Downy Mildew Onset in Bordeaux Vineyards

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ABSTRACT

Grapevine downy mildew (GDM) is a severe disease of grapevines. Because of the lack of reliable information about the dates of GDM symptom onset, many vine growers begin fungicide treatments early in the season. We evaluate the extent to which such preventive treatments are justified. Observational data for 266 untreated sites for the years between 2010 and 2017 were used to estimate the timing of GDM onset on vines and bunches of grapes in South West France (Bordeaux region) through survival analyses. The onset of GDM was not apparent on vines and bunches before early to mid-May, and the rate of GDM symptom appearance was highly variable across years. Depending on the year, 50% of the plots displayed symptoms between mid-May and late June for vines. For several years, our statistical analysis revealed that the proportion of plots with no symptoms was high in early August on vines (27.5 and 43.7% in 2013 and 2016) and on bunches (between 23 and 79% in 2011, 2013, and 2016). We found a significant effect of the amount of rainfall in spring on the date of symptom appearance. These results indicate that preventive fungicide application is unjustified in many vineyards, and that regional disease surveys should be used to adjust fungicide treatment dates according to local characteristics, in particular according to rainfall conditions in spring.

In France, grapevines (*Vitis vinifera* L.) are susceptible to several diseases, one of the most important being downy mildew. The disease is caused by *Plasmopara viticola*, which has a dimorphic life cycle. In autumn, sexual spores, called oospores (Wong et al. 2001), are produced. They overwinter above the vineyard ground (Dubos 2002) and germinate in spring as macrosporangium, which releases zoospores (Dubos 2002; Gessler et al. 2011). The latter generally spread with rain splashes to leaves, where they germinate and penetrate through stomata, causing primary infection after 7 to 10 days of incubation (Gessler et al. 2011). Sporangia, borne by sporangiophores, then emerge from affected host tissues. They are disseminated through wind and rain splashes to green parts of grapes, where they release asexual zoospores, which can then infect healthy tissues (secondary infection) and lead to yield losses (Dubos 2002). Leaf damage is also responsible for a reduction in the sugar content, which induces a decline in the grape's quality (Jermini et al. 2010).

Given the deleterious effects of grape downy mildew (GDM) on vineyards, fungicides are almost systematically applied to control the disease. Currently, in the Bordeaux region, many vine growers begin applying fungicides early in spring, and spraying is then regularly repeated. This results in a large number of fungicide applications over the course of the growing season, with implications for the public and farmers' health and for the environment, and entails greater production costs (Aubertot et al. 2005; Pimentel 2005). In the Bordeaux vineyards, the mean number of fungicide applications on vines increased from 14.8 to 18.5 between 2010 and 2013, 52% of which were applied to control GDM (Statistical and Prospective Service of the French Ministry of Agriculture 2015).

One widely recommended control strategy involves a first fungicide application after the end of the incubation period for the first primary infection, corresponding to the date on which the first symptoms appear. However, the lack of information on the appearance dates of GDM symptoms may contribute to the overapplication of chemicals in many situations. The dates on which GDM symptoms first appear on vines and bunches of grapes are not well known in France, particularly in the Bordeaux region. Vine growers and extension services need reliable information concerning these dates, which can indicate potential GDM severity. Indeed, the early occurrence of GDM symptoms on vines and on bunches is often associated with high disease severity (Dubos 2002; Galet 1977; Jermini et al. 2010). Accurate information about the date on which GDM symptoms appear is also useful for determining the timing of the first fungicide application.

Typically, the observed dates of symptom onset correspond to time-to-event data. Particular care is required in their analysis, because symptom appearance dates are frequently censored. Plots displaying no observed symptoms during the surveyed period are considered to be right censored, because they may develop disease symptoms at a later stage. Plots displaying symptoms before the start of the surveyed period are considered to be left censored, because the precise date of symptom appearance is known to lie before the first observation date in such situations. Finally, on some plots, GDM symptoms may occur during a follow-up interruption, after the last observation date at which a negative result is obtained and before the first positive observation date. Such data are considered to be interval censored.

Here, we analyzed a unique set of data including weekly monitoring of GDM symptoms on several vine leaves and bunches, collected from untreated plots in Bordeaux vineyards between 2010 and 2017. These data have never before been analyzed in detail with a view to determining the most likely dates of GDM outbreaks. We used survival analysis methods to deal with both censored and uncensored data. Survival analysis is widely used in biomedical sciences, social sciences, engineering, and ecology, but not in plant

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pathology, with a few exceptions (Copes and Thomson 2008; Dallot et al. 2004; Esker et al. 2006; Ojiambo and Kang 2013; Scherm and Ojiambo 2004; Westra et al. 1994).

Based on our statistical analysis, we determined the most likely dates of GDM symptom occurrence on vines and bunches of grapes in Bordeaux vineyards. We analyzed the interannual variability of the date of symptom occurrence and estimated the proportion of sites remaining free from GDM symptoms in the area studied. We compared the results obtained with parametric, semiparametric, and nonparametric methods, and we discuss the practical implications of our results in terms of timing of fungicide applications and for reducing the total number of applications.

MATERIALS AND METHODS

Data. GDM incidence data were collected from 2010 to 2017 by the French Vine and Wine Institute (Institut Français de la Vigne et du Vin) on Bordeaux vineyards. In each vineyard, at least one nontreated row of vine stocks was monitored to detect GDM symptoms. The monitored rows were surrounded by two other nontreated rows, to ensure that the central rows were not unintentionally sprayed with fungicide. The mean number of stocks in the central rows was 53.1. In the monitored central rows, weekly visual inspections of vine stocks were conducted between week 12 (late March) and week 33 (end of August). The observation frequencies are shown in Figure 1. Vine stocks and bunches with GDM symptoms were recorded by visual inspection. Visual observations stopped when the proportions of infected vine stocks and bunches were close to 100%. The vine cultivar, the local name of the vineyard, and geographic coordinates were recorded for each of the monitored plots.

According to several previous studies (Caffi et al. 2009; Hill 2000; Rossi et al. 2008a, b; Rouzet and Jacquin 2003; Tran Manh Sung et al. 1990), GDM is influenced by weather conditions. In order to study the effect of temperature and rainfall on the date of symptom appearance, the daily average amount of rainfall (in millimeters) and the daily average temperature greater than 10°C (temperature – 10°C, in Celsius degrees) were computed for each site-year over two periods: fall (September to November in the year of harvest – 1) and spring (March to June), from the SAFRAN database (Le Moigne 2002) produced by Météo-France (Centre National de Recherches Météorologiques). The 8 years included in our study show contrasted weather characteristics. For example, spring was characterized as warm (5.86°C above 10°C on average) and dry (1.51 mm/day) in 2011, whereas spring was cold (3.91°C above 10°C on average) and wet (5.45 mm/day) in 2013.

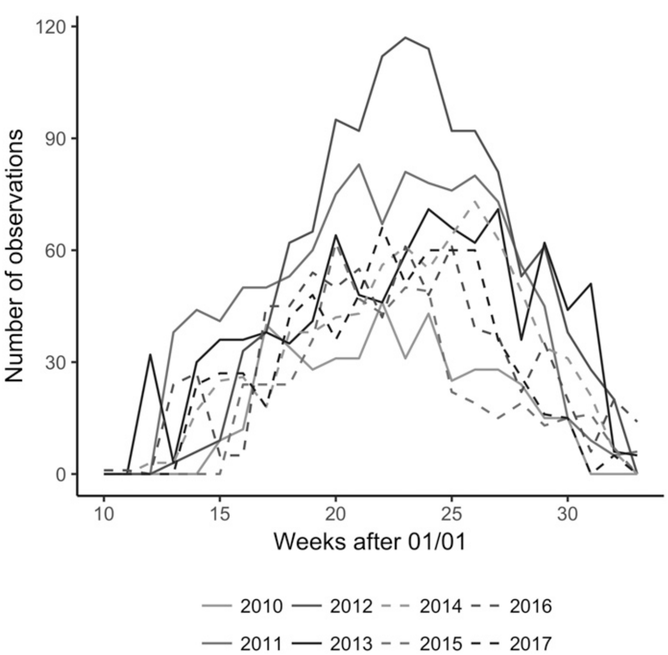

Fig. 1. Number of observations collected per week and per year.

For each plot, survival time was calculated as the number of weeks between the first week of the year and the week in which a certain epidemic threshold was attained. Note that the time origin (here, the first week of the year) has no influence on the results of our analysis. Two types of threshold were considered successively, giving two survival times for each site-year: 1% of vines displaying symptoms and 1% of bunches displaying symptoms. The survival times corresponding to these thresholds have different practical values. The date at which 1% of vines display symptoms corresponds to the time at which GDM symptoms first appeared on vines for each plot. In practice, regional farm advisors use this date to predict subsequent GDM dynamics, as an early disease onset generally leads to more severe disease incidence (Kennelly et al. 2007) and severity (Dubos 2002). In the Bordeaux region, the date at which 1% of bunches display symptoms in a plot is the latest date recommended for the first fungicide application, to prevent the irreversible losses that may occur if climatic conditions are favorable for pathogen development. Dates of bunch infection can also serve as an indicator of subsequent damages. Later infections are usually less damaging because berries acquire an ontogenic resistance after the veraison stage (Kennelly et al. 2005). In total, survival times were analyzed for 266 monitored plots from week 12 to week 33.

Survival times were included in a time-to-event data set. Each survival time was defined as a time interval characterized by a start date and an end date. This time interval was expressed in the following forms: $(-\infty, t]$ for left-censored data, $[t, +\infty)$ for right-censored data, $[t, t)$ for exact survival time, and $(t1, t2]$ for interval-censored data. Interval-censored data occur when the date of symptom appearance is observed 2 weeks or more after the last observation of absence of symptoms. In right-censored time intervals, the infinity symbol was used to indicate that the threshold considered might have been reached after the last observation date. Left-censored data corresponded to site-years for which the threshold had already been reached before the start of the survey. For interval-censored data, the start and end dates of the interval correspond to the last observation at which the threshold was not reached and the first observation date at which incidence exceeded the threshold, respectively. The intervals of interval-censored data were assumed to be open on the left-hand side, and closed on the right.

For vines, the proportions of censored versus uncensored data were 40.6% of right-censored data, 16.9% of left-censored data, 35.0% of uncensored data, and 7.5% of interval-censored data. For bunches, we got 57.5% of right-censored data, 7.5% of left-censored data, 30.5% of uncensored data, and 4.5% of interval-censored data.

Statistical analyses. Survival analysis is a collection of statistical methods for censored time-to-event data (Lee and Wang 2003). One of the main purposes of survival analysis is to estimate survival functions and their dependence on explanatory variables. A survival function is defined as shown in equation 1:

$$S(t) = P(T > t) = 1 - F(t) \qquad (1)$$

where T in this case denotes a random variable representing the time to GDM symptom appearance on 1% of vines or bunches, $P(T > t)$ is the probability that this time exceeds t in a plot, and $F(t)$ is the probability distribution function of T. Survival analysis is also used to calculate a hazard function, $h(t)$, specifying the instantaneous rate of failure at time t (here, the rate of symptom appearance at time t) given that the individual (here, the vineyard plot) has survived to time t. This hazard function is defined as shown in equation 2:

$$h(t) = \frac{f(t)}{S(t)} \quad (2)$$

where $f(t)$ is the probability density function. Here, the survivor function, $S(t)$, is estimated separately for vines and bunches. We used and compared three inference methods: nonparametric, semiparametric, and parametric. All of the estimated survivor functions were used to identify the 10th, 50th (median), and 90th percentiles of time to symptom appearance.

The nonparametric approach is distribution free but is less efficient than the parametric approach when survival times follow a theoretical distribution. Here, we used the nonparametric maximum likelihood estimator (NPMLE), also known as Turnbull's estimator, for the nonparametric approach. This method is a generalization of the Kaplan-Meier method that allows for interval censoring (Anderson-Bergman 2017b). We first applied this method to the whole data set to obtain a global survival function for all years, and then we estimated yearly survival functions for vines and bunches, separately. Calculations were performed with the ic_np function of the icenReg package (version 2.0.4; Anderson-Bergman 2017a) of R software (version 3.3; R Core Team 2017).

The semiparametric approach involved the fitting of a Cox proportional hazards model to the data set. This model assumes that the hazard function is defined as shown in equation 3:

$$h(t, X, \beta) = h_0(t) e^{X^T \beta} \quad (3)$$

Where $h_0(t)$ is a nonparametric baseline function, X is a set (vector) of covariates, and β is a set of parameters. We fitted model 3 with year effects considered as covariates for vines and bunches, separately. We then introduced four climate input variables in model 3 to explain the between-year variability of dates of symptom appearance on vines and bunches: mean temperature above 10°C in fall (September to November in year of harvest – 1), mean temperature above 10°C in spring (March to June), mean rainfall (in millimeters per day) in fall, and mean rainfall in spring. For bunches, we also then fitted a variant of model 3 in which the date of symptom appearance on the vine was included as a covariate (the covariate values were imputed from the vine survival model using the function imputeCens from icenReg). The model including year effects was used to assess whether survival functions differed significantly between years. Significant climatic variables ($P < 0.05$) were used to quantify the effects of weather conditions on dates of symptom appearance. The model including the date of symptom appearance on the vine as a covariate was used to determine whether the dates of GDM symptom appearance on bunches were related to the dates of GDM symptom appearance on vines. The significance of the effects of X was estimated by bootstrap resampling. Calculations were performed with the ic_sp function of the R icenReg package.

Finally, we fitted several parametric models based on exponential, Weibull, gamma, log-normal, and log-logistic hazard functions. First, these models were fitted to the whole data set without covariates, and the model with the lowest Akaike information criterion (AIC) was selected, for vines and bunches separately. Based on the AIC, the log-logistic distribution was selected for both vines and bunches. The year effects (X) were then incorporated into a parametric model defined as follows (equation 4):

$$Y = \ln(T) = \beta_0 + X^T \beta + \sigma Z \quad (4)$$

where β_0, β, and σ (scale) are parameters and Z is a random variable defining the baseline hazard function. Several distributions were compared for Z, and the distribution resulting in the lowest AIC was selected (the log-normal model presented the lowest AIC for both vines and bunches). The four weather input variables mentioned above were introduced in model 4 to estimate the effect of weather conditions on dates of symptom appearance (the log-normal model presented the lowest AIC for vines and the log-logistic model presented the lowest AIC for bunches). Finally, for bunches, we fitted a variant of the parametric model 4 in which date of symptom appearance on the vine was included as a covariate. In this case, the log-normal model resulted in the lowest AIC. Calculations were performed with the ic_par function of the R icenReg package.

RESULTS

Global survival analysis for the 2010 to 2017 period. In nonparametric survival analysis (NPMLE model), GDM onset on vines did not become apparent until week 19 (early to mid-May) (Fig. 2A). Thereafter, the proportion of plots with symptomless vines decreased steadily, to 90% in week 21 (mid- to late May) and 50% in week 24 (mid-June). By week 32, at the end of follow-up period, the proportion of plots with symptomless vines had fallen to 29.3% (Fig. 2A).

According to the fitted parametric model (log-logistic) (Fig. 2A), the proportion of plots with no GDM on vines was close to 100% before week 15.1 (95% confidence interval [CI] = 14.1, 16.2) (mid- to late April) but decreased to 90% in week 19.1 (95% CI = 18.2, 19.9) (early to mid-May), 50% in week 25.3 (95% CI = 24.5, 26.1) (mid-June), and 10% in week 33.6 (95% CI = 31.8, 35.5) (mid-August).

According to the NPMLE model, GDM onset on bunches was not apparent before week 21 (late May). Bunches were symptomless in 90% of plots in week 23 (early June) and 50% of plots in week 27 (early July) (Fig. 2B). This proportion had fallen to 42.1% by week 29 (mid-July) and did not further decrease. According to the log-logistic model estimates, the proportion of plots with symptomless bunches was close to 100% before week 17.9 (95% CI = 16.7, 19.1) (late April, early May) and reached 90% in week 22.6 (95% CI = 21.8, 23.3) (late May, early June), 50% in week 27.8 (95% CI = 27.2, 28.6) (early to mid-July), and 10% in week 34.4 (95% CI = 32.8, 36.3) (mid- to late August) (Fig. 2B).

Thus, the proportion of symptomless plots estimated by both log-logistic and NPMLE survival curves was close to 100% in mid-May, except for log-logistic model for vines, where it was close to 100% until late April to early May. The survival curves subsequently

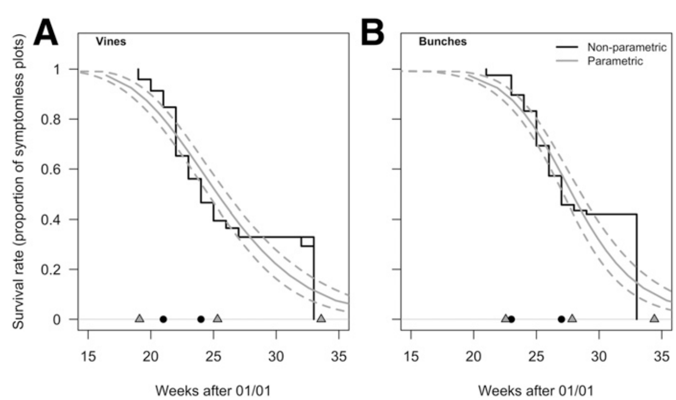

Fig. 2. Proportions of plots with symptomless **A,** vines and **B,** bunches over the period from 2010 to 2017 estimated by nonparametric and parametric (log-normal model) survival analysis methods. Black and gray circles at the base of the graph indicate the estimated dates at which the proportion of symptomless plots decreased to 90, 50, and 10%, for the nonparametric maximum likelihood estimator (NPMLE) and log logistic models, respectively. With nonparametric methods (i.e., NPMLE method), the 10% level was never reached. Dashed lines indicate 95% confidence intervals.

decreased rapidly to 50% in mid-June for vines and in early to mid-July for bunches. However, there were marked differences between survival curves estimated with the two methods. The proportion of plots with no symptoms on vines and on bunches decreased more rapidly between weeks 20 and 22 for vines and between weeks 21 and 25 for bunches for the log-logistic than for the NPMLE model. In addition, during the period in which survival decreased rapidly (from weeks 22 to 25 to week 27), the proportion of plots without symptoms estimated with the log-logistic model was higher than that estimated with the NPMLE model, especially for vines. During this period, the maximum difference between the two methods reached 15%. Finally, the log-logistic curves tended to zero, whereas the NPMLE curves reached a plateau after week 29, at 29.3% for plots with no symptoms on vines and 42.1% for plots with no symptoms on bunches (Fig. 2).

Variability of the proportion of symptomless plots between years. The observed dates on which symptoms were first observed varied both within and between years (Figs. 3 and 4). For example, in 2015, 10% of the plots that reached a threshold of 1% of vines with GDM were recorded at week 19 (i.e., early May). Fifty percent of the surveyed plots still had no symptoms at week 21 (i.e., late May). At week 27 (i.e., early July), 90% of the plots showed symptoms on vines. In 2011, the first plot reaching a threshold of 1% of vines with GDM symptoms was recorded at week 19 (i.e., mid-May). At week 21 (i.e., late May), the threshold of 1% infected vines was reached in 10% of the plots in 2011 (i.e., 2 weeks later than in 2015). GDM symptoms were recorded on 50% of the plots at week 33 (i.e., mid-August) in 2011. The observed dates of disease onset were also highly variable for bunches of grapes (Fig. 3).

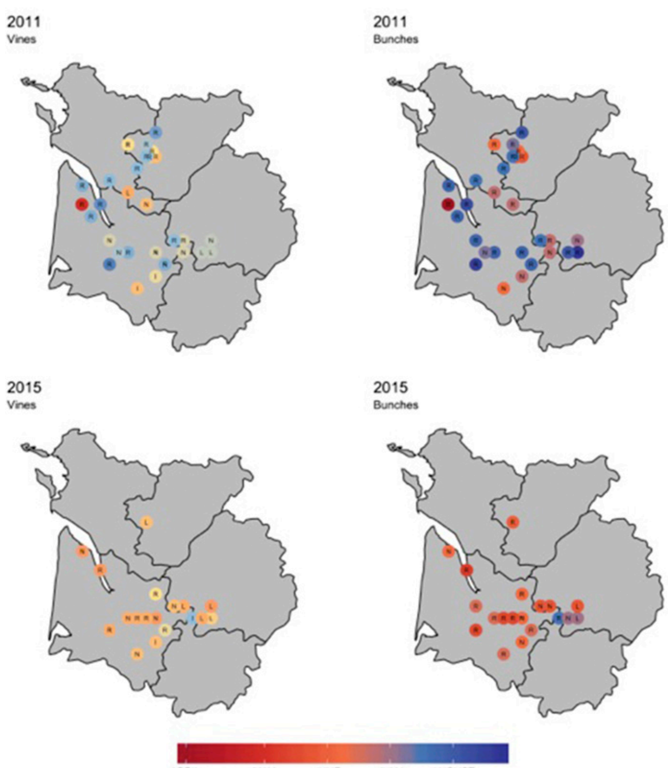

Fig. 3. Examples of observed dates of GDM onset for two different years (2011 and 2015). Each point corresponds to a single untreated site in a Bordeaux vineyard. Recorded dates of symptom onset are represented by a palette of colors. Letters indicate censoring status as follows: N = noncensored data, R = right-censored data (the last recorded date is presented), L = left-censored data (the first recorded date is presented), and I = interval-censored data (the first recorded date with an incidence of at least 1% of vines or bunches with symptoms is presented).

According to the semiparametric estimations (Cox model), 2010 and 2011 were the first and second years with the latest dates of GDM onset on vines, and 2015 was the year showing the earliest symptom appearance dates (Table 1). In 2011, the estimated proportion of plots with symptomless vines was 100% until week 19 (early May), whereas the proportion of symptomless plots had already started to decrease 2 weeks earlier in 2015. On vines, the proportion of symptomless plots reached 50% in week 21 (late May) in 2015, whereas 90% of the surveyed plots still had no symptoms in week 21 in 2011. The 50% level was reached 3 weeks later in 2011, according to the Cox survival curve (Fig. 4C). Parametric estimates (log-normal model) (Fig. 4E; Table 2) confirmed that symptoms appeared on vines earlier in 2015 than in 2011. After week 25, the differences observed for log-normal estimates between 2011 and 2015 were smaller than the differences observed for Cox estimates.

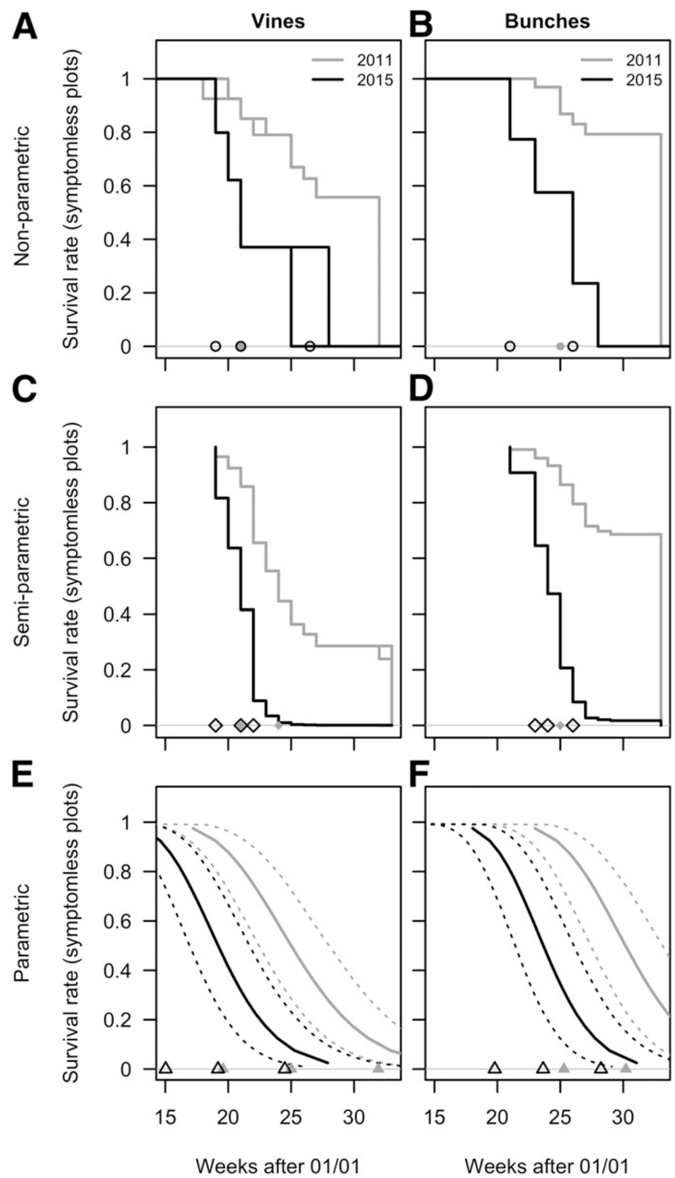

Fig. 4. Proportions of plots with symptomless vines and bunches estimated by **A and B,** nonparametric, **C and D,** semiparametric (Cox model), and **E and F,** parametric (log-normal model) survival analyses for 2011 (gray) and 2015 (black). Gray dots indicate the estimated dates by which the proportion of symptomless plots would decrease to 90, 50, and 10% in 2011, by method. Black dots indicate the estimated dates by which the proportion of symptomless plots would decrease to 90, 50, and 10% in 2015, by method. Dotted lines correspond to the 95% confidence interval estimated by the log-normal model for each year.

According to the Cox model, the years with the latest and earliest dates of GDM onset on bunches were 2011 and 2015, respectively (Table 1). According to this model, 90% of plots had no symptoms on bunches in week 23 (early June) and 50% had no symptoms in week 24 (early to mid-June) in 2015 (Fig. 4D). The corresponding date for the 90% level in 2011 was week 25 (late June), and the 50% level was never reached in 2011, even in August. The results obtained with the NPMLE and log-normal models (Fig. 4B and F) confirmed that the proportion of symptomless plots in summer was higher in 2011 than in 2015. As for disease on vines, the differences between years were smaller with the log-normal model than with the Cox model after week 25. For example, at week 27 in 2011, the proportion of symptomless plots was 34.4% with the log-normal model but 55.7% with the NPMLE method (Fig. 4B and F).

The contrasts between the different years surveyed are reflected in the estimated parameter values of the fitted Cox and log-normal models (Tables 1 and 2). These parameter values reveal the differences in rates of GDM symptom appearance between years, relative to 2010. The Cox model suggested that the rate of GDM symptom appearance on vines was higher in 2012 ($P = 0.050$), 2013 ($P = 0.029$), 2014 ($P < 0.001$), 2015 ($P = 0.011$), and 2017 ($P < 0.001$) than in 2010 (Table 1), and the rate of GDM symptom appearance on bunches was higher in 2015 ($P = 0.025$) than in 2010. No statistically significant difference was found for the other years.

The values estimated with the log-normal model confirmed that the rate of GDM symptom appearance on vines was higher in 2012 ($P = 0.026$), 2013 ($P = 0.016$), 2014 ($P < 0.001$), 2015 ($P < 0.001$), and 2017 ($P = 0.002$). The rate of GDM symptom appearance on bunches was higher in 2014 ($P = 0.020$) and in 2015 ($P = 0.004$) than in 2010 (Table 2).

Weather conditions (temperature and rainfall) in fall and temperature in spring had no significant effect on symptom appearance on vines ($P > 0.05$), but results obtained with the Cox and the log-normal models showed that rainfall between March and June had a significant effect on the date of first symptom appearance on vines ($P < 0.01$). High amounts of rainfall in spring led to early dates of symptom appearance on vines. A significant effect of rainfall in spring was also found for bunches with both types of models ($P < 0.001$).

The effect of rainfall in spring is illustrated on Figure 5 for two contrasted years with a dry (1.51 mm/day in 2011) and a wet (5.45 mm/day in 2013) spring, respectively. According to the semiparametric (Cox) and parametric models, symptoms appeared

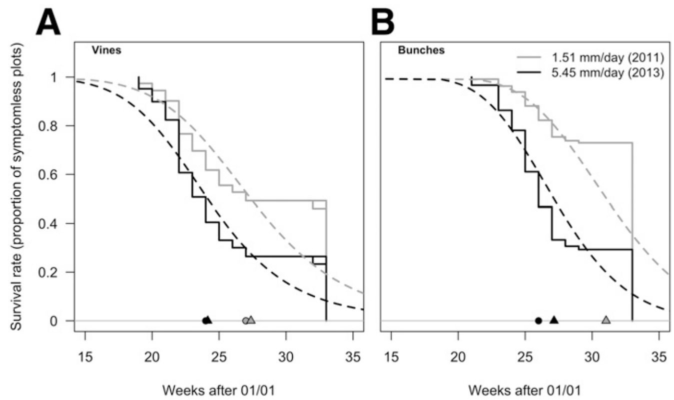

Fig. 5. Proportion of plots with symptomless **A,** vines and **B,** bunches estimated by semiparametric (Cox model) and parametric (log-normal or log-logistic model) survival analyses as a function of the average spring rainfall measured in 2011 (1.51 mm/day, gray lines) and 2013 (5.45 mm/day, black lines). Plain lines represent estimation for Cox models. Dotted lines represent the estimation of the log-normal model for vines and the log-logistic model for bunches, respectively. Dots indicate the dates where the proportion of plots with symptomless vines or bunches reached 50% for each model. Gray dots represent the estimations for 2011 and black dots represent the estimations for 2013. Circles represent the estimations of Cox models and triangles represent the estimations of log-normal models for vines and log-logistic models for bunches, respectively.

TABLE 1. Estimated parameters, exponential values for estimates [Exp(Est)], standard errors (SEs), and P values for each of the years surveyed (2010 to 2017) for the Cox model[a]

	Vines				Bunches			
Year	Estimate	Exp(Est)	SE	P	Estimate	Exp(Est)	SE	P
2010								
2011	0.048	1.049	0.383	0.901	–1.010	0.364	0.574	0.078
2012	0.763	2.144	0.389	0.050[b]	0.508	1.662	0.434	0.242
2013	0.850	2.340	0.391	0.029[b]	0.692	1.998	0.403	0.086
2014	1.685	5.395	0.448	<0.001[b]	0.757	2.132	0.482	0.116
2015	1.795	6.019	0.703	0.011[b]	1.370	3.935	0.609	0.025[b]
2016	0.348	1.416	0.394	0.378	–0.320	0.726	0.484	0.508
2017	1.279	3.591	0.326	<0.001[b]	0.382	1.465	0.375	0.309

[a] Each estimate corresponds to the difference in the rate of grapevine downy mildew appearance on vines or bunches of grapes between years, relative to 2010.
[b] Statistically significant at $P < 0.05$.

TABLE 2. Estimated parameters, exponential values for estimates [Exp(Est)], standard errors (SEs), and P values for each of the years surveyed (2010 to 2017) for the parametric models (log-normal models)[a]

	Vines				Bunches			
Year	Estimate	Exp(Est)	SE	P	Estimate	Exp(Est)	SE	P
2010	3.228	25.230	0.014	0	3.329	27.900	0.012	0
2011	–0.010	0.990	0.063	0.870	0.092	1.096	0.052	0.078
2012	–0.127	0.881	0.057	0.026[b]	–0.059	0.942	0.044	0.180
2013	–0.147	0.864	0.061	0.016[b]	–0.081	0.922	0.047	0.086
2014	–0.277	0.758	0.066	<0.001[b]	–0.116	0.890	0.050	0.020[b]
2015	–0.275	0.759	0.066	<0.001[b]	–0.154	0.858	0.053	0.004[b]
2016	–0.037	0.964	0.062	0.547	0.051	1.052	0.049	0.303
2017	–0.203	0.816	0.066	0.002[b]	–0.053	0.949	0.051	0.306
Scale	–1.658	0.191	0.073	0	–1.976	0.139	0.078	0

[a] Each estimate corresponds to the difference in the rate of grapevine downy mildew appearance on vines or bunches between years relative to 2010.
[b] Statistically significant at $P < 0.05$.

earlier in wet conditions. According to the parametric log-normal model, the proportion of plots with symptomless vines reached 50% at week 24 (95% CI = 23.3, 25.1) in 2013 (mid-June), but this level was only reached at week 27 (95% CI = 25.5, 29.4) in 2011 (early July) (i.e., about 3 weeks later). According to the parametric log-logistic model, the proportion of plots with symptomless bunches reached 50% at week 27 (95% CI = 26.4, 28.0) in 2013 (early July), and this level was reached at week 31 (95% CI = 29.1, 33.1) in 2011 (early August) (i.e., about 4 weeks later). Large differences between 2011 and 2013 were also obtained with the Cox model (Fig. 5).

Figure 6 compares the annual estimates of the proportion of symptomless plots obtained with the log-normal, Cox, and NPMLE models. Comparisons were performed at three different dates: weeks 22, 26, and 31 (early June, early July, and early August).

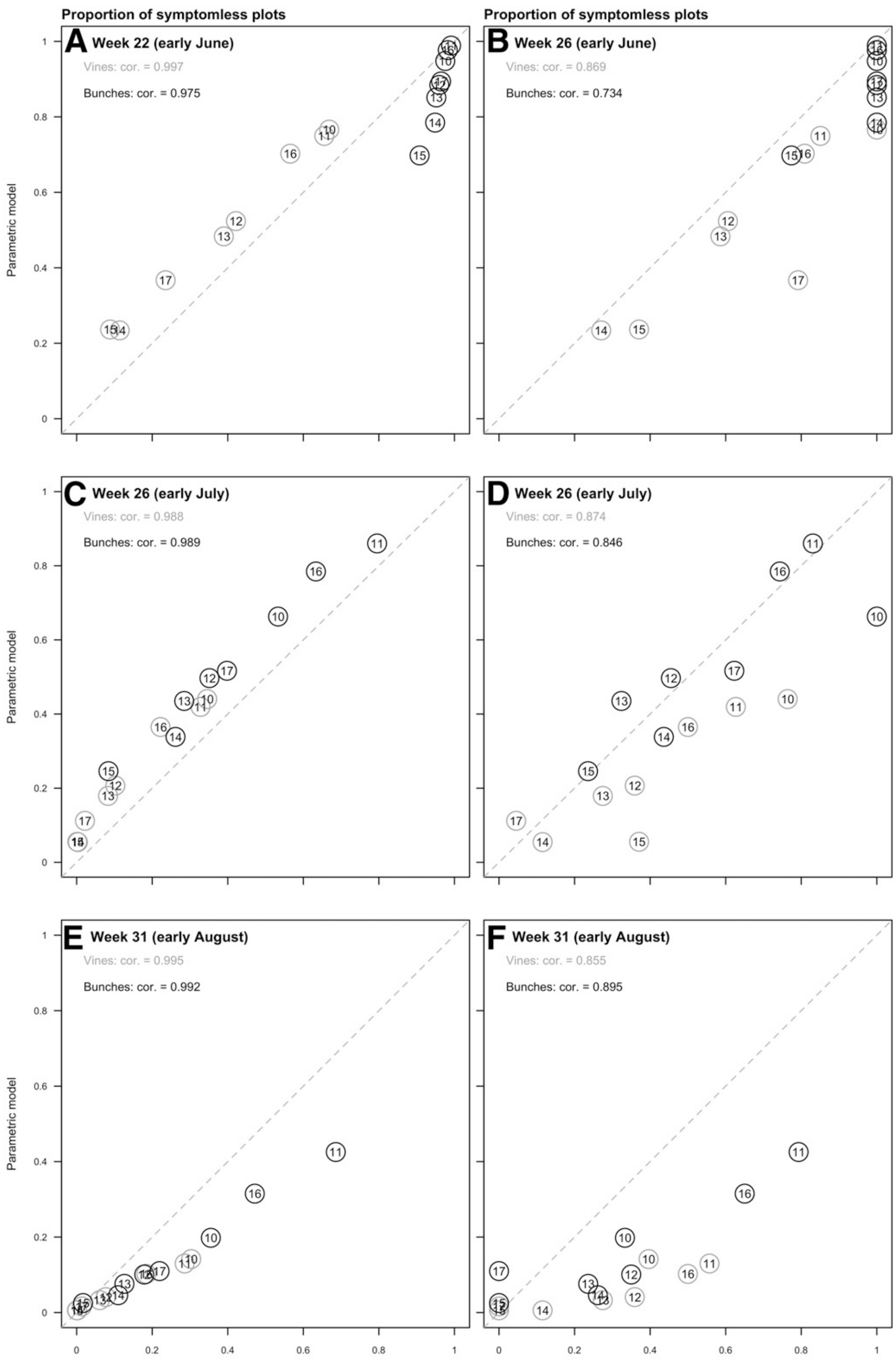

Fig. 6. Proportions of symptomless plots estimated each year with the **A, C, and E,** parametric (log-normal model) and semiparametric (Cox model) or **B, D, and F,** nonparametric (nonparametric maximum likelihood estimator [NPMLE]) models on three different dates (weeks 22, 26, and 31; i.e., early June, early July, and early August). Gray and black circles correspond to vines and bunches, respectively. The numbers in the circles indicate the years.

Relative to the Cox model, the log-normal model tended to estimate higher proportions of symptomless vines and bunches at week 26 and lower proportions at week 31 (Fig. 6C and E). For bunches, higher proportions of symptomless plots were obtained with the log-normal model than with the Cox model at week 22, but lower proportions of symptomless plots were obtained with the log-normal model for vines (Fig. 6A).

The correlations between NPMLE and log-normal estimates were weaker (Fig. 6B, D, and E). The proportions of symptomless plots estimated with the log-normal method were frequently lower than those estimated with the NPMLE method, especially at weeks 22 and 31 (Fig. 6B and D).

Relationship between the date of GDM appearance on vines and the proportion of plots with symptomless bunches. We found a significant relationship between the date of GDM appearance on vines and the proportion of plots with symptomless bunches ($P < 0.0001$ for the Cox and log-normal models). The early occurrence of GDM on vines resulted in a low proportion of plots with symptomless bunches, whereas a late occurrence of GDM on vines was associated with a high proportion of plots with symptomless bunches (Fig. 6). With both the Cox and log-normal models, the proportion of plots with symptomless bunches was higher if the first GDM symptoms were observed on vines at week 22 than if such symptoms were not observed until week 25 (Fig. 7). For example, with the log-normal model, the proportion of plots with symptomless bunches reached 10% at week 23 (95% CI = 22.6, 23.5) (early June) when the first GDM symptoms occurred on vines at week 22 (late May, early June). By contrast, this proportion was not reached until week 25.1 (95% CI = 24.6, 25.5) (mid-June) if the first GDM symptoms were observed on vines at week 25 (mid-June) (Fig. 7).

DISCUSSION

We were able to estimate the dynamics of the appearance of GDM symptoms on vines and bunches of grapes in Bordeaux vineyards. The onset of GDM was not apparent on vines and bunches before early to late May. Thereafter, we found that the thresholds of 90 and 50% of plots with symptoms were attained at very different dates over the 2010 to 2017 period in this region and sometimes were never even reached, even in August. For example, in 2015, 50% of the plots showed symptoms on vines and bunches in mid-May and in late June, respectively, but this threshold was never reached for vines and for bunches in 2011. Thus, according to the NPMLE estimates, the proportion of plots with symptomless vines in August exceeded 25% in 2013 and 2016 (27.5 and 43.7%). The proportion of plots with symptomless bunches in August exceeded 25% in 2011, 2013, and 2016 (79.3, 23.6, and 65%). Parametric models tended to give lower estimated proportions of symptomless plots in summer than Cox models, but the proportion of symptomless plots estimated in early July with parametric models nevertheless exceeded 30% for vines in 2010, 2011, and 2016 (44.0, 41.9, and 36.5%) and 45% for bunches in all years except for 2013, 2014, and 2015 (43.5, 33.8, and 24.6%).

Our data set included a substantial proportion of censored data. These data were subjected to several classic procedures for survival analysis, and we assessed the robustness of our results to the chosen survival model. We found some discrepancies between the results obtained with different techniques, particularly for estimations of the proportions of symptomless plots in summer with the nonparametric and parametric methods. However, agreement was found between the tested methods for many aspects. For example, both the semiparametric and parametric methods showed that the rates of GDM symptom occurrence were higher in 2012, 2013, 2014, 2015, and 2017 than in 2010 for vines in the Bordeaux vineyards. Both these methods also showed that the rate of GDM symptom appearance on bunches was higher in 2015 than in the other years. These results were consistent with the reports of the French Agricultural Warnings published from 2011 to 2017. In the Bordeaux region, these reports indicated that GDM incidence and severity were "low" in 2011 and 2017 (Gironde Regional Agricultural Chamber 2011, 2017), "medium" in 2012, 2013, and 2014, "medium to low" in 2015, and "high" in 2016, but damages and yield losses were "low" (Gironde Regional Agricultural Chamber 2012, 2013, 2014, 2015, 2016).

High variability of GDM incidence across years was reported by Kennelly et al. (2007) and Carisse (2016). According to previous experimental and modeling studies, the variability of the GDM appearance rate and GDM incidence is at least partly attributable to climatic factors (Gessler et al. 2011). The occurrence of GDM is determined principally by rainfall and temperature, which affect the various steps in the lifecycle of the pathogen, such as oospore germination (Rossi and Caffi 2007; Rossi et al. 2007; Vercesi et al. 2010) and sporulation (Kennelly et al. 2007). In our study, we found a significant effect of rainfall in spring on the date of symptom appearance on vines and on bunches. A dry (wet) spring led to a late (early) date of symptom appearance on vines and on bunches. These results are consistent with those from Rossi et al. (2002) (cited by Rossi and Caffi 2007), who found that dry periods in spring delay the date of first symptom appearance. This is at least partly attributable to the fact that litter and leaf moisture stimulates the development of oospores (Rossi and Caffi 2007). Furthermore, the survival of zoospores is strictly dependent on the presence of a film of water (Gessler et al. 2011).

We did find a significant relationship between the proportion of plots with symptomless bunches and the date of appearance of GDM on vines. The proportion of plots with bunches displaying symptoms was higher if GDM symptoms appeared on vines early in the season. The variability of the timing of GDM symptom onset on bunches is thus partly explained by the variability in the timing of GDM symptom appearance on vines in the Bordeaux region. Our approach based on survival analysis could be applied to determine dates of symptom appearance for other vine diseases like powdery mildew or black rot and also for diseases of other crops. Censored data are quite common in regional disease survey data sets such as those described for wheat and rapeseed in Northern France (Sine et al. 2010) or for Yellow Sigatoka on banana and weevils on sweet potato in tropical regions (Michel et al. 2017). Our approach is generic and can be applied in many situations.

Our results have several practical implications for the Bordeaux region. The date of first application has a strong influence on the total number of fungicide treatments during the growing season because after the first seasonal treatment, fungicides are applied at an interval of about 2 weeks, on average, in the Bordeaux region. Our results indicate that fungicide treatments against GDM should

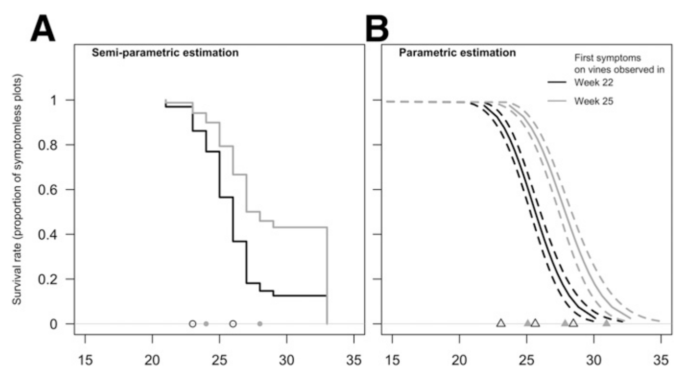

Fig. 7. Estimated proportions of plots with symptomless bunches for two different dates for the first observation of GDM symptoms on vines (week 22 and week 25). The dotted lines correspond to the 95% confidence intervals. Empty black and filled gray circles at the base of the graph indicate the estimated dates at which the proportion of symptomless decreases to 90, 50, and 10%, for the **A,** Cox and the **B,** log-normal models, respectively.

not be applied before early to mid-May in Bordeaux vineyards. Fungicide applications before this date would not be effective and would unnecessarily increase the number of fungicide applications in the vineyards of the Bordeaux region, potentially increasing the environmental impact of vine production and the risk of fungicide resistance (Chen et al. 2007).

Our results also showed that no GDM symptoms were ever recorded in some vineyards in certain years, indicating that systematic preventive fungicide treatments against the disease may not be justified in every vineyard in the Bordeaux region. Considering the large variability of the date of first symptom appearance (i.e., from early May to early July) both within and between years, we suggest delaying the application of the first fungicide treatment in the case of low rainfall in spring. Our survival models could be used to estimate the date of symptom appearance as a function of the amount of rainfall in spring. This strategy could reduce the number of pesticide treatments compared with systematic preventive treatments in the Bordeaux region. This is consistent with a study by Mailly et al. (2017), who indicated that fungicide use could be reduced by postponing the date of first fungicide spray in the French vineyards. From an economic point of view, the systematic use of fungicide treatments remains the most effective solution to control GDM compared with biocontrol agent use (Dagostin et al. 2011) and resistant vine varieties (Pertot et al. 2017). However, regulations on pesticide use may become more restrictive in the future. For example, in France, the "Plan Ecophyto 2" was established to reduce pesticide use by 50% before 2025 (French Ministry of Agriculture and Food 2018), and its implementation may encourage farmers to reduce the number of pesticide treatments during the next decade.

The practicality of this control strategy could be assessed in close collaboration with vine growers. The approach presented here could benefit from various tools, such as alert bulletins based on yearly field surveys (Michel et al. 2017), climate and/or phenological indicators (Caffi et al. 2010; Kennelly et al. 2007), on-farm measurements collected by sensors on drones (Rieder et al. 2014), and by systems of in-vineyard inoculum detection (Thiessen et al. 2016). The latter could make first symptom detection easier in the near future. Several epidemiological models (Raynal et al. 2010; Rossi et al. 2008b; Tran Manh Sung et al. 1990) and warning systems (Caffi et al. 2010; Delière et al. 2015; Madden et al. 2000; Raynal et al. 2012) were developed for simulating GDM epidemic dynamics or optimizing the timing of fungicide sprays. In the future, the output of some of these models could be used as inputs of survival models in order to predict dates of GDM symptom appearance as a function of local characteristics.

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