Modeling Cold Curing of Pierce's Disease in *Vitis vinifera* 'Pinot Noir' and 'Cabernet Sauvignon' Grapevines in California

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Accepted for publication 10 July 2011.

ABSTRACT

Lieth, J. H., Meyer, M. M., Yeo, K.-H., and Kirkpatrick, B. C. 2011. Modeling cold curing of Pierce's disease in *Vitis vinifera* 'Pinot Noir' and 'Cabernet Sauvignon' grapevines in California. *Phytopathology* 101: 1492-1500.

Pierce's disease (PD) of *Vitis vinifera* grapevines is caused by the bacterium *Xylella fastidiosa*, a pathogen with a wide plant host range. Exposure of *X. fastidiosa*-infected plant tissue to cold temperatures has been shown to be effective at eliminating the pathogen from some plant hosts such as grapevines. This "cold curing" phenomenon suggests itself as a potential method for disease management and perhaps control. We investigated cold therapy of PD-affected 'Pinot Noir' and 'Cabernet Sauvignon' grapevine. In the fall, inoculated plants and controls of each cultivar were transported to each of four field sites in California (Foresthill, McLaughlin, Hopland, and Davis) that differed in the magnitude of cold winter temperatures. A model for progression of the elimination of plant disease in relation to temperature was conceptualized to be a temperature-duration effect, where temperatures below a particular threshold kill *X. fastidiosa* with increasing efficacy as the temperature

decreases to some value $<6^{\circ}\text{C}$. The temperature effect was modeled as a likelihood of a particular temperature killing the pathogen and is termed the "killing index". We developed a mathematical model for cold curing of grapevines inoculated with *X. fastidiosa* and calibrated the model with cold-curing data collected in a field study. Parameter estimation resulted in lowest sum of squared differences across all 10 trials to be low temperature below which the organism is killed (T_0) = 6°C , number of hours to achieve 100% cure (N_{100}) = 195 h, number of hours to achieve 10% cure (N_{10}) = 20 h, and killing index (K_x) = 0.45 for Pinot Noir and $T_0 = 6^{\circ}\text{C}$, $N_{100} = 302$ h, $N_{10} = 170$ h, and $K_x = 0.41$ for Cabernet Sauvignon. With the parameter estimates optimized by model calibration, the simulation model was effective at predicting cold curing in four locations during the experiment, although there were some differences between Hopland for Pinot Noir and Davis for Cabernet Sauvignon. Using historical temperature data, the model accurately predicted the known severity of PD in other grape-growing regions of California, suggesting that it may have utility in assessing the relative risk of developing PD in proposed new vineyard sites.

Pierce's disease (PD) is a bacterial disease of grapevines (*Vitis vinifera*) caused by the xylem-limited bacterium *Xylella fastidiosa*, which is vectored by xylem-feeding insects. PD was first observed in 1884 in Anaheim, CA by N. B. Pierce. Grapevines infected with *X. fastidiosa* show marginal leaf necrosis, dwarfing of infected vines, irregular lignification of canes, and cordon die back; the entire vine often dies within a few years (10). Other grape species and hybrids such as *V. labruscana* and *Muscadinia rotundifolia* can also be infected by *X. fastidiosa* but, in many of these other species, the symptoms of PD are not as severe as what is observed with all cultivars of *V. vinifera*. The plant host range of *X. fastidiosa* is wide; strains of *X. fastidiosa* cause citrus variegated chlorosis, oleander leaf scorch, phony peach, alfalfa dwarf, almond leaf scorch, and other diseases (10,15).

Various attempts are being made to find ways to manage diseases caused by *X. fastidiosa*. One potential method includes an environmental therapy for grapevines known as "cold curing". In many agronomic crops, *X. fastidiosa* causes disease in geographical areas with mild winter climates. The growth and survival of *X. fastidiosa* in grapevines has been related to temperatures to which the vines have been subjected. PD symptoms appear earlier in the growing season in warmer climates, and the optimum growth conditions for *X. fastidiosa* in vitro is $\approx 28^{\circ}\text{C}$ and pH 6.7 (3,18).

PD symptoms and *X. fastidiosa* are not usually detectable in infected plants early in the spring when temperatures are still low, and PD is not found in North America in areas where average winter temperature drops to $<0^{\circ}\text{C}$ (10,12). Purcell (13,14) demonstrated that relatively brief exposures to subfreezing temperatures eliminated *X. fastidiosa* in cold-treated *V. vinifera* grapevines. Purcell (13) found that exposure of grapevines with severe symptoms of PD to -8 to -12°C for various periods caused a temporary remission of symptoms in some test plants and recovery in other plants. He reported that cold treatments at 0°C for up to 5 days were not therapeutic and multiple treatments (a series of two or three exposures at or below -8°C for two or more hours) were more effective at reducing disease than a single cold exposure. Although there is close correlation between cold curing and temperature, the critical temperature regime and duration that mediates cold therapy under field conditions are not known.

Feil and Purcell (5) found that populations of *X. fastidiosa* in infected grapevines growing in pots decreased 3 days after exposure to 5°C and no culturable *X. fastidiosa* were recovered from plants incubated at 37°C (5). They also reported that temperatures <12 to 17°C and $>34^{\circ}\text{C}$ might affect the survival of *X. fastidiosa* in plants. However, there is little information on the specific temperatures or the duration of exposure to these temperatures that leads to the elimination of *X. fastidiosa* in *V. vinifera* grapevines. To better understand the cold-curing phenomenon, it is necessary to determine the cold-curing temperature threshold that maximizes PD recovery and minimizes vine mortality.

Meyer and Kirkpatrick (12) examined the effects of cold therapy on *X. fastidiosa*-infected *V. vinifera* grapevines. Results of

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doi:10.1094/PHYTO-08-10-0207

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their study showed that there were differences in the incidence of curing (PD-free vines/inoculated grapevines that survived cold treatment), mean disease severity rating, and vine mortality of *V. vinifera* grapevines 'Pinot Noir' and 'Cabernet Sauvignon' placed in field locations in northern California that differed in the severity of winter temperatures. These results were also in agreement with the incidence of cold curing that occurred in *X. fastidiosa*-infected, potted grapevines exposed to varying cold temperatures in cold chambers.

The notion that cold therapy effectively prevents PD from occurring in some geographical areas led us to investigate in this simulation modeling study whether it was possible to provide knowledge or tools that could be used to assess the risk of developing PD in new vineyard locations. Our approach involved the use of mathematical modeling to quantify how low-temperature exposure of grapevines growing in the field might affect the degree of disease curing in plants infected with *X. fastidiosa*. Such a quantitative approach can be expected to shed new light on the dynamics of this plant-bacterium interaction and potentially allow the development of novel strategies for managing *X. fastidiosa* diseases.

The objective of this research was to determine the level of cold curing that occurred at specific levels and durations of cold temperature and to build a mathematical model to predict levels of cold curing of *X. fastidiosa*-infected grapevines grown at particular temperature regimes. We sought to expand this into a simulation model for simulating the incidence of cold curing of grapevines using weather station temperature data collected over four winter months. The model was then calibrated by comparing the simulations with actual cold-curing data collected over several years using experimentally infected grapevines that were located in several Northern California field sites that experience a range of winter climates.

MATERIALS AND METHODS

Plant materials. *V. vinifera* 'Pinot Noir' (susceptible to PD) and 'Cabernet Sauvignon' (less susceptible to PD) were selected for use in this study because the same cultivars were used by Feil and Purcell in their cold-therapy studies (5). Trials were conducted over three winter seasons at several locations, each with a somewhat different winter climate (10 trials total). The experimental design of each trial involved the following time course: inoculating grapevines with *X. fastidiosa* early in the spring, growing these as well as noninoculated control plants during the spring and summer, and then recording the severity of PD symptoms in August. Presence of *X. fastidiosa* in grapevines was assessed using immunocapture polymerase chain reaction (IC-PCR) (2). The test vines were then transported in mid-November to the four Northern California test sites, where they were exposed to ambient winter temperatures. At budbreak the following April, vines were transported back to the University of California (UC) Davis, transplanted into the field, grown over the spring and summer, and again evaluated for the incidence and severity of PD disease symptoms and presence of *X. fastidiosa*.

For each of the trials, 1-year old dormant Pinot Noir and Cabernet Sauvignon scions grafted onto 101-14 rootstock were obtained from a commercial nursery. Plants destined for growth-chamber studies were planted into "one-gallon" (volume = 2.2 liters) plastic pots; plants destined for use in the field were planted into "five-gallon" (volume = 20 liters) plastic pots filled with UC mix (11). All vines were then grown in a greenhouse using drip irrigation and supplemented with 15 g of a slow-release fertilizer (15-15-15 plus micronutrients, Endure with Polyon 43: J. R. Simplot, Boise, ID). Vines were pruned during the growing season to facilitate their transport to field sites or temperature chambers. When inoculated canes were pruned, at least 1 m of cane was retained around the inoculation site.

***X. fastidiosa* strain, inoculation method, and PD symptom evaluation.** Treatments consisted of inoculating grapevines with the *X. fastidiosa* strain 'Stag's Leap' (STL) (American Type Culture Collection [ATCC] 700963) using a pinprick inoculation procedure (8,9,16) while control vines were inoculated with sterile water. Twelve weeks after inoculation, the plants were rated for disease using a symptom severity index of 0 (healthy) to 5 (dead) (7). All *X. fastidiosa* inoculated plants were tested with IC-PCR 14 weeks following inoculation to confirm the presence of *X. fastidiosa* (2). During late summer and fall, the plants were moved into a screen house in order to acclimatize them to decreasing temperatures. While in the screen house, plants were watered by drip irrigation until the first week of November in 2004, 2005, and 2006.

Field studies. In three seasons 2004–05, 2005–06, and 2006–07, (26 October to 16 November 2005, 25 October to 1 November 2006, and 28 October to 6 November 2007), 11 plants inoculated with *X. fastidiosa* and 11 uninoculated plants of each cultivar (44 plants total) were transported to each of four field sites in California. In 2004–05, plot sites included Hopland UC Research Station (Mendocino County) and UC Davis (Yolo County). The 2005–06 and 2006–07 plot sites also included locations at Forest-hill (Placer County) and McLaughlin UC Reserve (Lake County). Potted grapevines were planted in the ground to the top of the pot, such that test grapevines were growing in the same UC mix soil at all locations. Pots were buried to prevent roots in the pots from being exposed to abnormally cold air temperatures and to prevent the vines from falling over. When necessary, the vines were manually irrigated on a weekly basis until rain provided adequate moisture for the vines. Vines underwent natural dormancy during the fall and experienced ambient temperatures during the winter.

Cold-chamber studies. The controlled temperature-chamber experiments in this study were undertaken for two reasons. The first was to confirm and expand the results that were previously reported by Feil and Purcell (5). The second reason was to determine whether the incidence of cold curing obtained in the various field sites correlated with curing observed under the strictly controlled temperature conditions of the chambers. In late October or early November (10 November 2005 and 31 October 2006), after the grapevines underwent natural dormancy during the fall, 10 inoculated and 10 controls of each cultivar (40 plants total) were placed into four cold chambers. The cold chamber experiments were replicated over two winters. The chamber temperatures included –5, 0, 2.2, and 5°C. Potted grapevines were placed into each nonilluminated chamber and hand watered as needed. Vines were removed from the cold chambers in early April and planted in the field at UC Davis with vines retrieved from the field sites.

IC-PCR and determination of grapevine curing. At the beginning of bud break in spring (approximately 1 to 15 April, depending on the site location), the plants were collected from the various field locations and cold chambers and planted in the field at UC Davis. The PD symptom ratings were evaluated in mid-August and the presence of *X. fastidiosa* was determined using IC-PCR (2). The mean plant disease severity ratings, PD curing incidence (ratio of PD-free plants to total plants that survived treatment), and incidence of plant mortality were determined for each field and cold-chamber treatment. A vine was considered to be cured of PD if it exhibited no disease symptoms and tested negatively by IC-PCR.

Temperature data. Hourly winter temperatures at the field sites were collected for estimation of model parameters. Temperatures for each field plot were monitored using either California Irrigation Management Information System (CIMIS) weather data or California Data Exchange Center (CDEC) data; these data are available online at the CIMIS and CDEC websites. CIMIS and CDEC temperature data used in developing the model were col-

lected no more than 5 km from the test site. Temperature data used in the final model development and analysis were from the CIMIS weather data source, except for the McLaughlin site where temperature data were obtained from CDEC.

Model development. We sought to develop a model to determine the effect of temperature and its duration on the survival and killing of *X. fastidiosa* in PD-affected grapevines. The model was conceptualized to be a temperature-duration effect, where temperatures below a particular threshold result in killing of the *X. fastidiosa* with increasing efficacy with decreasing temperatures below some low-temperature threshold. The degree of disease elimination was assumed to consist of two elements: (i) the temperature effect on the organism was modeled as a likelihood of a particular temperature killing the disease organism and (ii) the translation of the killing condition into a degree of curing of the whole plant. The former part of the model is termed “killing index” and the later part is termed the “curing effect”.

The killing index consists of a mathematical function, $K(T)$, where the independent variable is temperature (T , in $^{\circ}\text{C}$) and the dependent variable (K , no units) reflect the probability of killing of the bacterium. K takes on values between 0 and 1, where 0 means no killing and 1 means certain death of the bacterium. It had been shown that cold temperatures $<6^{\circ}\text{C}$ affect the survival of *X. fastidiosa* (5), and it is well known that high temperatures also kill bacteria; therefore, K was initially postulated to be of the form

$$K(t) = \begin{cases} 1 & \dots \text{for } T \leq T_0 \\ k_1(T) & \dots \text{for } T_0 < T < T_1 \\ 0 & \dots \text{for } T_1 \leq T \leq T_2 \\ k_2(T) & \dots \text{for } T_2 < T < T_3 \\ 1 & \dots \text{for } T_3 \leq T \end{cases} \quad (1)$$

where the parameter T_0 represents the low temperature point below which the organism is killed, T_1 is the low temperature above which the organism is certain to not be killed, T_2 is the lowest high temperature above which some killing occurs, and T_3 is the high temperature above which the organism is likely to be killed. The functions $k_1(T)$ and $k_2(T)$ represent the mathematical functions that describe the progression of killing probability with temperature. In the absence of knowledge about which models best represent these two functions, we chose the simplest approximation: linear functions (Fig. 1).

Because the study reported here addresses only cold temperature conditions, and all our experiments never subjected our plants or bacterial pathogen to temperatures $>T_2$, we simplified the model by constraining the temperature domain to $T < T_2$.

Fig. 1. Conceptualized killing index as a function of temperature for *Xylella fastidiosa*. Scale: T_0 represents the low temperature point below which the organism is killed, T_1 is the low temperature above which the organism is certain to not be killed, T_2 is the lowest high temperature above which some killing occurs, and T_3 is the high temperature above which the organism is likely to be killed.

$$K(T) = \begin{cases} 1 & \dots \text{for } T \leq T_0 \\ m(T_1 - T) + b & \dots \text{for } T_0 < T < T_1 \\ 0 & \dots \text{for } T_1 \leq T \end{cases} \quad (2)$$

Here, m and b are slope and intercept of the linear decline in killing with increasing temperatures between T_0 and T_1 and temperatures are hourly average air temperatures. Because this function is expected to be continuous so that the mathematical description of $K(T)$ is

$$K(T) = \begin{cases} 1 & \dots \text{for } T \leq T_0 \\ (T_1 - T)/(T_1 - T_0) & \dots \text{for } T_0 < T < T_1 \\ 0 & \dots \text{for } T_1 \leq T \end{cases} \quad (3)$$

Thus, observed hourly temperatures represent a vector of temperature values for which a corresponding set of K values were calculated with equation 3.

The curing effect is modeled in relation to K and T vectors for particular locations. Cold curing is assumed to be a matter of the duration of time for which the plants are subjected to temperatures that result in a level of K above some threshold level (K_x). The model calculates for any K vector whether a specific duration of time (in hours) occurred, without interruption, where K is above K_x during the entire period. For those instances, 100% curing is assumed and represented by the variable C . $C = 1$ represents complete curing of all plants, whereas $C = 0$ represents no curing of the plants. Although it was of primary interest to predict absence or presence of curing, we also wanted the model to reflect partial levels of curing in the population of plants. Thus, C was allowed to take on values between 0 and 1 to reflect the degree of curing in a population using the following approach.

We assumed that the curing process would vary in effectiveness with durations at high levels of K but that curing could also be interrupted if the conditions leading to death of the bacterium were interrupted. The model for this was a discrete simulation model running on a one hour time step ($dt = 1$) counting (with N_{count}) the number of hours where the conditions contribute toward a curative effect:

$$N_{\text{count}} = \begin{cases} N_{\text{count}} + 1 & \dots \text{for } K > K_x \\ N_{\text{count}} - N_{\text{reset}} & \dots \text{for } K \leq K_x \end{cases} \quad (4)$$

The reversal of the curing effect for any hour where K is not above the killing index threshold is represented with N_{reset} . The value of N_{count} that is assumed to result in 100% curing is a parameter (N_{100}) in the model. In order to program a linear effect of N_{count} , we used the parameter N_{10} for the level of N_{count} coinciding with 10% curing. Thus, the model for curing efficiency, C , the percentage of previously diseased plants that emerged pathogen free after the cold treatment, is given as

$$C = \begin{cases} 1.0 & \dots \text{for } N_{\text{count}} = 1 \\ 0.1 + 0.9(N_{\text{count}} - N_{10})/(N_{100} - N_{10}) & \dots \text{for } N_{\text{count}} < 1 \end{cases} \quad (5)$$

Further simplification was imposed by setting $T_1 = T_0 + 1$ so that, in summary, the model consists of two main elements, the first represents the killing of the microorganism as a temperature response (equation 3), while the curing of the plant is modeled through an iterative simulation model that simulates and tracks when the killing index is above some threshold (equations 4 and 5). This model included five parameters for which estimates are needed before the model can be used for making calculations: T_0 , N_{10} , N_{100} , N_{reset} , and K_x .

Model calibration. The model for cold curing of PD was calibrated individually for Pinot Noir and Cabernet Sauvignon

cultivars by obtaining temperature data which represent the T vector described above for each of the 10 trials (across four locations over 3 years). Only temperature data recorded between 15 November and 1 April were used in each of the 10 cases. Thus, with specific parameter estimates, the model calculates an incidence of curing that corresponds to observed values across years and locations.

Estimation of model parameters (model calibration) was done using a grid-search approach because the model is too complex to use nonlinear regression techniques. This technique involves setting all parameter estimates to some initial estimate and then systematically varying each parameter, one at a time, by small increments. At each iteration, the model calculates percent curing at each location and for each year for which we had observations of degree of curing. The sum of squared differences (SSQ) between predicted and observed across all these simulations was then used as the measure of goodness of fit. Thousands of combinations of parameter estimates were tested in this way, using the following ranges: T_0 at 5 to 10°C, N_{100} at 100 to 400 h, $N_{10} = 5$ h to N_{100} , and K_x of 0.10 to 0.95. This grid search was iteratively refined as ranges of parameter estimates were found that minimized the SSQ. For parameters where no minimum SSQ value was found, we identified which of the data sets showed disagreement with the model. Our search focused on finding the greatest number of data sets for which a specific set of parameter estimates would yield reasonable results. The model calibration procedure also assumed that all parameter estimates would be dependent on grapevine cultivar. Thus, we carried this out separately for Pinot Noir and Cabernet Sauvignon grapevines.

Model application to other production areas and time frames. Simulations were also run with the resulting (unvalidated) model for selected wine-growing regions in California to allow discussion related to potential cold curing of PD-affected grapevines. In those instances, climate data were obtained from CIMIS or the CDEC to provide T vectors for use in the simulations.

RESULTS

Vine curing, disease ratings, and vine mortality. The results for the field and cold-chamber trials showed lower mean disease ratings and higher curing efficiencies in the colder temperature

treatments. The Davis field location had less cold curing (infected plants exposed to cold temperatures during the winter that emerged disease free in the spring), with 9 to 36% curing compared with the coldest field location, Foresthill, which had curing incidences of 91 to 100% for Cabernet Sauvignon or Pinot Noir, respectively (Table 1).

In 2005–06, 56% of the Cabernet Sauvignon vines placed in the coldest chamber (–5°C) and 100% of the Pinot Noir vines were cured of PD symptoms and *X. fastidiosa* was not detected in these vines by IC-PCR. In contrast, in the warmest chamber (+5°C), only 10% of the Cabernet Sauvignon vines and 17% of the Pinot Noir vines were cured and free of the pathogen. In 2006–07, the –5°C chamber had 100% curing of both Cabernet Sauvignon vines and Pinot Noir vines, whereas vines incubated in the warmest chamber (+5°C) had 0% curing of Cabernet Sauvignon vines and 33% curing of Pinot Noir vines.

CIMIS and CDEC weather station data from the field studies provided the basis for model development (Fig. 2). Low and high average daily temperatures were –1.5 to 21.5°C for Foresthill, 0.16 to 22.5°C for McLaughlin, –0.58 to 20.0°C for Hopland, and 1.71 to 18.8°C for Davis during the field studies. The average minimum temperatures for 3 years were –0.43°C for Foresthill, 0.87°C for McLaughlin, 1.49°C for Hopland, and 2.43°C for Davis.

Model development and calibration. Simulated K(T) patterns (Fig. 2) in relation to temperatures during 2004 to 2007 field seasons at the four locations suggest that there are few periods of time at any of the locations and years where the K levels were continuously near 1.0 for extended periods. The results showed that there were differences between K levels of PD-affected grapevines Pinot Noir and Cabernet Sauvignon from each field location and field season (Table 1). However, at all of the locations, even those with mild winters, there were periods of time where K was above some intermediate threshold value. This suggests that the model does have the capacity to represent the level of harshness of winter cold conditions that might lead to cold curing.

The model includes a reasonable representation for instances where killing conditions are interrupted with milder temperatures. One result from early testing, where we had assumed that an episode of mild conditions would completely reverse any potential curing, showed that complete resetting of N_{count} never resulted in reasonable predictions with any parameter estimates. Thus, we

TABLE 1. Model parameter estimates and simulation performance for data collected from four northern California field locations in 2005 to 2007

Parameter name, data sets ^a	Pinot Noir				Cabernet Sauvignon			
	EV ^b	Fraction of plants cold-cured ^c			EV ^b	Fraction of plants cold-cured ^c		
		Observed	Simulated	Discrepancy		Observed	Simulated	Discrepancy
Parameter								
N_{100} (h)	195	...	...	...	302.5	...	...	...
N_{10} (h)	20	...	...	...	170	...	...	...
K_x	0.45	...	...	...	0.41	...	...	...
T_0 (°C)	6	...	...	...	6	...	...	...
N_{reset} (h)	3	...	...	...	2	...	...	...
Data sets								
2004–05 Davis	...	0.36	0.48	–0.12	...	0.00	0.00	0.00
2005–06 Davis	...	0.27	0.38	–0.11	...	0.44	0.00	0.44
2006–07 Davis	...	0.27	0.32	–0.05	...	0.09	0.00	0.09
2004–05 Hopland	...	0.80	0.75	0.04	...	0.64	0.64	–0.00
2005–06 Hopland	...	0.44	0.44	0.00	...	0.44	0.61	–0.17
2006–07 Hopland	...	0.82	0.47	0.34	...	0.73	0.61	0.12
2005–06 McLaughlin	...	0.56	0.72	–0.16	...	0.64	0.68	–0.04
2006–07 McLaughlin	...	0.73	0.65	0.07	...	0.64	0.64	0.00
2005–06 Foresthill	...	1.00	1.00	0.00	...	1.00	1.00	0.00
2006–07 Foresthill	...	1.00	1.00	0.00	...	0.91	1.00	–0.09

^a N_{100} and N_{10} are values of N_{count} that result in 100 and 10% curing, respectively, where N_{count} is the number of hours where the conditions contribute toward a curative effect; K_x is the K(t) threshold above which curing is assumed to occur; T_0 is low temperature (°C) below which organism is killed; and N_{reset} is the subtracted hours (reversal of the curing effect) for any hour where K is not above the killing index threshold K_x .

^b Estimated value.

^c Sum of squared differences = 0.18 and 0.25 for Pinot Noir and Cabernet Sauvignon, respectively.

concluded that reversal of potential curing due to mild conditions could be modeled through subtraction of N_{reset} from N_{count} where the value of N_{reset} would be >1 (i.e., each hour of interruption of curing resulted in more than an hour of reversal). We found that N_{reset} estimates of 3.0 and 2.0 for Pinot Noir and Cabernet Sauvignon, respectively, resulted in excellent model simulation performance.

The grid search resulted in lowest SSQ across all 10 trials to be for the following parameter values: for Pinot Noir, $T_0 = 6^\circ\text{C}$, $N_{100} = 195$ h, $N_{10} = 20$ h, and $K_x = 0.45$; and, for Cabernet Sauvignon, $T_0 = 6^\circ\text{C}$, $N_{100} = 302$ h, $N_{10} = 170$ h, and $K_x = 0.41$. Parameter estimates along with how the simulation performed for each data set are shown in Table 1.

With the parameter estimates optimized by model calibration, the simulation model was effective at predicting cold curing in four locations (Fig. 3). The predicted cold-curing efficiencies matched the actual data closely, although there were some differences between Hopland for Pinot Noir and Davis for Cabernet

Sauvignon. The cold-curing efficiency for Pinot Noir during 2006–07 was not well predicted in Hopland compared with other locations. These simulation results also show that there is a difference between actual data and simulated level of cold curing for Cabernet Sauvignon during 2005–06 in Davis.

The simulation model was applied to winter temperatures that occurred during 2007 to 2009 using estimated parameters to determine how the model might perform for other time frames. The levels of cold curing of Pinot Noir and Cabernet Sauvignon grapevines at four locations were predicted by using temperature data from 15 October 2007 to 15 April 2008 and from 15 October 2008 to 15 April 2009. Areas with a high level of cold curing and low risk for PD were Foresthill and McLaughlin, which had the lowest average winter temperatures among the four locations. The susceptible Pinot Noir had a higher cold-curing efficiency in field conditions compared with the moderately susceptible Cabernet Sauvignon (Table 2; Fig. 4). Of the four locations, only the Davis location was predicted to have a low cold-curing efficiency during

Fig. 2. Daily averages of the calculated killing index of *Xylella fastidiosa* as influenced by temperatures during 2004 to 2007 field seasons at the Davis and Hopland locations using a value of 6 for T_0 , the low temperature ($^\circ\text{C}$) below which the organism is killed.

2007 to 2009 while Foresthill, McLaughlin, and Hopland all showed high efficiencies of cold curing.

DISCUSSION

The results of these studies suggest that the proposed model for cold curing using temperature and duration can be used with reasonably good predictive accuracy to predict cold curing of Pinot Noir or Cabernet Sauvignon grapevines infected with *X. fastidiosa*. It should be noted that, as described above, the model is not as yet validated; therefore, forecasts or diagnoses made with this model should be considered speculative. It would also be useful to calibrate the model for other grape cultivars. In addition, it may also be feasible to test this approach for other host plants, such as almonds, that can be infected with *X. fastidiosa*. It was interesting to note that the killing index parameters ended up with similar values for Pinot Noir (0.45) and Cabernet Sauvignon (0.41) and that the main cultivar differences in the model were in

the cold curing. This is certainly reasonable because the killing index portion of the model is a reflection of what is happening with the bacterium while the cold-curing part of the model reflects the plant's response to cold temperatures.

Equation 1 is a model for predicting the level of cold curing at specific levels of cold temperature and duration. Results also suggest that there is a threshold of killing index for curing duration at specific cold temperatures T_0 and T_1 for cold curing of PD-infected grapevine. Feil (4) showed that whole potted vines infected with *X. fastidiosa* exposed to low temperatures had a higher efficiency of recovery than PD-affected detached bud sticks exposed to the same cold temperatures. This implies that some factor or factors expressed in the intact plant but not in detached bud sticks helped eliminate *X. fastidiosa* from the plants.

The simulation model might be improved with parameters estimated by separate fittings for seven to nine data sets by excluding the Hopland or Davis data from the calibration (Table 3). We noted that the simulation model showed lower predict-

Fig. 3. Daily averages of the calculated killing index of *Xylella fastidiosa* as influenced by temperatures during 2004 to 2007 field seasons at the McLaughlin and Foresthill locations using a value of 6 for T_0 , the low temperature ($^{\circ}\text{C}$) below which the organism is killed.

TABLE 2. Predicted levels of cold curing (C) of grapevine 'Pinot Noir' and 'Cabernet Sauvignon' in four field study locations based on temperature data from 15 October 2007 to 15 April 2008 and from 15 October 2008 to 15 April 2009

CIMIS ID ^a	Station name	Location (latitude, longitude)	Levels of cold curing (C)			
			Pinot Noir		Cabernet Sauvignon	
			2007–08	2008–09	2007–08	2008–09
6	Davis	N38°32'09", W121°46'32"	0.44	0.50	0.00	0.00
85	Hopland	N39°00'25", W123°04'45"	1.00	1.00	0.63	0.92
KNO ^b	McLaughlin (Knoxville)	N38°49'40", W122°20'26"	1.00	1.00	0.89	1.00
13	Foresthill (Camino)	N38°45'13", W120°43'57"	1.00	1.00	1.00	1.00

^a California Irrigation Management Information System (CIMIS) identification number.

^b California Data Exchange Center (CDEC) letter code.

Fig. 4. Simulated levels and actual observed data of cold curing of *Vitis vinifera* A, 'Pinot Noir' and B, 'Cabernet Sauvignon' in each location using N_{reset} values of 3 and 2, respectively. N_{reset} is the subtracted hours (reversal of the curing effect) for any hour where the killing index (K) is not above the killing index threshold (K_x).

ability for Davis with mild winter climates in the simulation for Cabernet Sauvignon (Fig. 3). Restricting the analysis for Pinot Noir to exclude the Hopland data also resulted in improved performance. The optimized parameters of the cold-curing model that result in lowest SSQ resulted when excluding Hopland data for Pinot Noir and by excluding Davis data for Cabernet Sauvignon. These regions are the ones with the mildest winter climates (Table 3). Further model refinement should focus on improving predictive performance in regions with milder winter climates. This also suggests that, although our results represent a powerful approach to identifying regions where cold conditions are adequate for curing, the model is not suitable for predicting which milder temperature conditions might result in an increase in the incidence of curing or no curing.

Purcell (13) reported that a higher percentage of plants that were given multiple cold exposures recovered from PD than plants that received a single treatment. He suggested that every cold exposure contributed to pathogen mortality that approached or reached 100% as the number and severity of treatments increase.

Based on in vitro and in planta studies, it appears that temperatures of 25 to 30°C are suitable for rapid growth rate of *X. fastidiosa* (5,18). Feil and Purcell (5) investigated populations of *X. fastidiosa* in grape petioles after grape seedlings were incubated at 5, 10, 17, or 25°C for 18 days. In order to measure the effect of temperatures on established *X. fastidiosa* populations, they incubated the inoculated seedlings for 2 weeks in a greenhouse heated to 25°C prior to incubation at the various regulated temperatures. After incubation, grape seedlings had 230-fold lower populations of *X. fastidiosa* when kept at 5°C but populations declined slightly at 10°C, remained constant in plants at 17°C, and increased 5-fold at 25°C over 6 days. They determined the minimum threshold temperature for growth of *X. fastidiosa* in plants was between 17 and 25°C. As previously discussed, there was a direct correlation between the curing efficiency observed in the coldest field sites and the coldest temperature chambers. The chamber data were not used in the construction of the model because it obviously did not have the same fluctuating low and high temperatures that vines planted in the field sites or actual vineyards would experience.

The levels of cold curing of grapevines in other production areas, as well as the four field locations, were predicted by using the optimized parameters and CIMIS or CDEC temperature data sets for 2008–09. Predicted levels of cold curing of grapevine Pinot Noir in other California wine-growing regions using temperature data from 15 October 2008 to 15 April 2009 are shown in Table 4. Temecula, located in Southern California where PD recently caused extensive losses of *V. vinifera* grapevines, had some of the lowest levels for cold curing and average winter temperatures of 14.5°C. Our results showed that cold-curing levels of PD-affected grapevines are low in California and that PD is a threat throughout many wine-grape-growing regions in Southern California; however, there are areas suitable for wine grape production, such as Sierra Nevada foothill vineyard sites above 600 m, where cold curing can be an important factor in preventing *X. fastidiosa*'s survival over the winter.

Applying simulation for other geographic areas allows modeling as to where cold curing is likely to occur and where it is not. This simulation may enable vineyard managers or prospective vineyard owners to make improved management decisions concerning the potential impact of PD at these sites. It should be cautioned that this model has not been field tested or validated in all grapevine-growing regions where PD occurs. This model should be tested for one or more seasons under local conditions to verify that it will work in the desired location. In the future, it would be of particular interest to create a risk map for PD to develop in existing wine-grape-growing regions based on cold temperatures. Various measures of temperatures have been used by others to define the highest or lowest risk areas for PD. Cur-

TABLE 3. Optimized parameters of cold-curing model for *Vitis vinifera* ‘Pinot Noir’ and ‘Cabernet Sauvignon’ affected with Pierce’s disease

Cultivar, parameter ^a	Optimized parameters				
	10 data sets	9 data sets	8 data sets	7 data sets	
	All locations	Without 2005–06 Hopland	Without 2004–05 & 2006–07 Hopland	Without Hopland	Without Davis
Pinot Noir					
N ₁₀₀	195	195	225	230	210
N ₁₀	5–20	5–20	5–20	5–20	120–125
K _x	0.45	0.45	0.45	0.45–0.55	0.45
T ₀	6	6	6	6	6
SSQ	0.184	0.184	0.042	0.040	0.098
Cabernet Sauvignon					
N ₁₀₀	302	302	180	213	302
N ₁₀	170	170	5	120–125	170
K _x	0.45	0.45	0.95	0.90–0.95	0.45
T ₀	6	6	6	6	6
SSQ	0.255	0.062	0.088	0.210	0.054

^a N₁₀₀ and N₁₀ are values of N_{count} that result in 100 and 10% curing, respectively, where N_{count} is the number of hours where the conditions contribute toward a curative effect; K_x is the K(t) threshold above which curing is assumed to occur; T₀ = low temperature (°C) below which organism is killed; and SSQ = sum of squared differences.

TABLE 4. Predicted levels of cold curing (C) of ‘Pinot Noir’ grapevines in several California viticultural areas based on temperature data from 15 October 2008 to 15 April 2009

Region	ID ^a	Station	County	Location (latitude, longitude)	Levels of cold curing (C)	
					Pinot Noir	Cabernet Sauvignon
South Coast Valleys	62	Temecula	Riverside	N33°29'25", W117°13'20"	0.24	0
North Coast Valleys	77	Oakville	Napa	N38°26'02", W122°24'35"	0.33	0
North Coast Valleys	83	Santa Rosa	Sonoma	N38°24'04", W122°47'56"	0.30	0
San Joaquin Valley	92	Kesterson	Merced	N37°13'55", W120°52'51"	0.28	0
North Coast Valleys	103	Windsor	Sonoma	N38°31'35", W122°49'42"	0.45	0.62
San Francisco Bay	109	Carneros	Napa	N38°13'08", W122°21'14"	0.26	0
Sacramento Valley	121	Dixon	Solano	N38°24'55", W121°47'12"	0.53	0
Sacramento Valley	139	Winters	Yolo	N38°30'05", W121°58'39"	0.39	0.61
North Coast Valleys	144	Petaluma East	Sonoma	N38°16'02", W122°36'58"	0.31	0
Sierra Foothill	195	Auburn	Placer	N38°53'15", W121°06'11"	0.49	0
Monterey Bay	129	Pajaro	Monterey	N36°54'12", W121°44'31"	0.17	0
Mountain	LOS	Lake Lois	El Dorado	N38°55'30", W120°11'49"	1	1

^a California Irrigation Management Information System identification number or California Data Exchange Center (CDEC) letter code.

rently available PD-risk maps (1,17) show areas in North and South Carolina, Georgia, and Alabama, where the disease is rare, were based on an average minimum January temperature <1.7°C. Anas et al. (1) collected weather data for November to March from 84 weather stations in 1972 to 2005 and PD incidence data from viticultural areas in the southeastern United States. They reported that areas at low risk for developing PD corresponded most closely with minimum winter temperatures of ≤–12.2°C for 2 to 3 days or ≤–9.4°C for 4 to 5 days. Our findings suggest that these very low thresholds are perhaps too conservative. Using our model would allow development of PD risk-assessment maps with greater resolution, perhaps even allowing cold-curing potential to be calculated for individual growing operations by collecting temperature from individual vineyards rather than assessing risk using regional temperature data.

Recently, several papers on the potential impact of global warming on plant diseases and their management have been reported (1,6). As a result of warmer winter temperature over the recent years, the number of PD-affected grapevines could increase. Because our data were collected in northern California, this model could be used as a guide for those considering establishing new vineyards in California and, potentially, other areas on the west coast of the United States.

ACKNOWLEDGMENTS

Support for M. Meyer was provided by a grant from the California Department of Food and Agriculture Pierce’s disease/Glassywing Sharpshooter Program. We thank Sunridge Nursery (Bakersfield, CA) for providing the grapevines used in this study, G. Kasun for lab and field

assistance, and the staff at all of our study locations (UC Davis Pomology, UC Davis Plant Pathology, UC Hopland, McLaughlin UC Reserve, and US Forest Service Foresthill).

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