

Release of *Erysiphe necator* Ascospores and Impact of Early Season Disease Pressure on *Vitis vinifera* Fruit Infection

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Abstract: Populations of *Erysiphe necator* cleistothecia can dehisce and release ascospores over an extended period ranging from fall through late spring and are an important source of primary inoculum for grapevine powdery mildew epidemics. The temporal distribution of ascospore maturity was monitored and measured as ascospore release from field-stored samples in a controlled environment assay. Assays were conducted over multiple seasons, in multiple locations, using multiple source populations. The influence of primary inoculum quantity and how that influences subsequent fruit disease severity was assessed. Cumulative ascospore release was positively correlated with accumulated wetting events and heat units; however, in most situations, >50% of the season-total ascospores were released before the date of local budbreak of *Vitis vinifera*. Both abbreviated and season-long fungicide programs suppressed mildew on fruit across a 100-fold gradient of inoculum dose when seasonal weather was relatively unfavorable for epidemic development. Suppression was degraded with progressive 10-fold increases of ascosporic inoculum dose when seasonal weather was more conducive to epidemic development. Combined, these findings suggest that differences between severe and mild years in grape powdery mildew can relate to the amount of primary inoculum present in the vineyard, that the levels of primary inoculum can be influenced by pre-budbreak weather conditions, and that effectiveness of spray programs at controlling primary infection events is related to the favorability of in-season weather conditions.

Key words: disease foci, chasmothecia, powdery mildew management, ascospores, primary inoculum, cleistothecia

Grapevine powdery mildew, caused by the fungal obligate biotroph *Erysiphe necator* (Schwn.) Burr (syn. *Uncinula necator*), threatens the sustainable production of grapes in vineyards irrespective of climate or locale (Pearson 1988). Suppression of primary infection is a critical early step in disease management within commercial vineyards. Given the relatively high susceptibility of all *Vitis vinifera* cultivars to *E. necator*, this suppression requires carefully timed

applications of fungicides during the period when vines are at the greatest risk for infection. In regions where winter temperatures favor the survival of *E. necator* mycelia in dormant buds, the pathogen can overwinter in a vegetative state (Pearson and Gartel 1985). In the spring, these dormant buds can give rise to shoots heavily coated with colonies of the sporulating pathogen (termed “flag shoots”). Flag shoots were once presumed to be the principal form of overwinter survival of the pathogen (Pearson and Gadoury 1987). Irrespective of winter temperatures, the pathogen also survives the dormant season as cleistothecia (syn. chasmothecia), a stage confirmed as the principal overwintering form in many locations worldwide (Cortesi et al. 1997, Gee et al. 2000, Grove 2004, Hal-leen and Holz 2000, Hoffmann and Virányi 2007, Pearson and Gadoury 1987, Steinkellner 1998). Clarification of the role of ascosporic inoculum (Pearson and Gadoury 1987) and quantification of ontogenic resistance in berries (Ficke et al. 2003, Gadoury et al. 2003, Gee et al. 2008, Stark-Urnau and Kast 1999) have shifted the focus of management programs toward the suppression of primary infection from ascosporic inoculum, particularly during the period of maximal ontogenic susceptibility of berries (Falacy et al. 2007, Gadoury et al. 1994, 2001). Studies in which ascosporic inoculum was reduced by chemical eradication of cleistothecia (Gadoury et al. 1994, Legler et al. 2012b) or by the use of biological control agents (Falk et al. 1995) have indicated a substantial effect of primary inoculum dose on epidemic development and performance of disease management programs.

The processes of ascocarp maturation, dehiscence, and ascospore discharge in *E. necator* have been studied extensively (Diehl and Heintz 1987, Gadoury and Pearson 1990, Grove

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2004, Jailloux et al. 1998, Legler et al. 2012a, Rossi et al. 2010). Ascospore release is generally associated with rainfall amounts of 2.0 to 2.5 mm (Gadoury and Pearson 1990), although ascospores have been infrequently detected following lesser amounts of precipitation or during wetting periods not initiated by rain (Rossi et al. 2010). Ascospores are typically released under field conditions between budbreak until shortly after bloom in New York State (Gadoury and Pearson 1990, Pearson and Gadoury 1987), but fall and midwinter release has been reported for comparatively warmer climates in South Australia (Gee et al. 2000) and northern Italy (Rossi et al. 2010). A widely used threshold for ascospore release and subsequent infection of grape tissue was proposed as liquid precipitation amounts of ≥ 2.5 mm coincident with temperatures $\geq 10^{\circ}\text{C}$ (Gadoury and Pearson 1990).

Many previous studies of ascospore release have been restricted to only a few years, such as ≤ 3 (Cortesi et al. 1997, Rossi et al. 2010), and to a few years and a limited number of sites (Gadoury and Pearson 1990, Gee et al. 2000). Some focused primarily on ascocarp maturation and the mechanism of ascospore release (Gadoury and Pearson 1990), release of ascospores under laboratory conditions (Gadoury and Pearson 1990, Jailloux et al. 1998), or timing of ascospore release immediately before or during active vine growth (Cortesi et

al. 1997, Gadoury and Pearson 1990, Grove 2004, Jailloux et al. 1999). Collectively, these studies have added breadth to our knowledge of ascospore release. However, the degree to which inoculum potential might be reduced in certain climates by pre-budbreak dehiscence of cleistothecia and premature release of ascospores remains poorly understood. The occurrence of pre-budbreak release of ascospores in the more temperate viticulture regions of North America has not been thoroughly investigated. The objectives of the two studies presented herein were to evaluate the influence of variable overwintering environments on the seasonal distribution of ascospore release and to determine the impacts of overwintering inoculum levels on following year fruit disease severity and relationship to timing of fungicide programs.

Materials and Methods

Cleistothecia collection, overwintering, and discharge assessments. *Distribution of ascospore release at diverse sites.* Cleistothecia that developed and matured in New York (NY), New Jersey (NJ), North Carolina (NC), and Washington (WA), were collected and overwintered at both their location of origin and in NY (Table 1). Reciprocally, NY populations of cleistothecia were overwintered at the aforementioned locations unless otherwise noted (Table 1). To

Table 1 Dates and locations for collection and assessment of *Erysiphe necator* cleistothecia 2005–2009.

Year/location ^a	Overwintering location	Date collected	Date in field	Sample date	
				First	Last
2005–2006					
Geneva, NY	Geneva, NY	24 Sept	29 Sept	12 Jan 06	30 May 06
	Prosser, WA	24 Sept	1 Nov	18 Jan 06	29 May 06
	Reedy Creek, NC	24 Sept	1 Nov	9 Jan 06	30 May 06
	Chatsworth, NJ	24 Sept	1 Nov	9 Jan 06	30 May 06
2006–2007					
Geneva, NY	Geneva, NY	9 Sept	15 Sept	17 Nov 06	6 June 07
	Prosser, WA	9 Sept	2 Nov	15 Nov 06	13 June 07
	Reedy Creek, NC	9 Sept	15 Sept	15 Nov 06	13 June 07
	Chatsworth, NJ	9 Sept	10 Oct	17 Nov 06	7 June 07
Prosser, WA	Geneva, NY	24 Oct	17 Oct	17 Nov 06	6 June 07
	Prosser, WA	24 Oct	2 Nov	15 Nov 06	13 June 07
Raleigh, NC	Geneva, NY	8 Sept	13 Oct	15 Nov 06	6 June 07
	Reedy Creek, NC	8 Sept	15 Sept	17 Nov 06	13 June 07
Chatsworth, NJ	Geneva, NY	10 Oct	13 Oct	17 Nov 06	6 June 07
	Chatsworth, NJ	10 Oct	10 Oct	17 Nov 06	7 June 07
2007–2008					
Geneva, NY	Geneva, NY	11 Sept	16 Sept	14 Dec 07	16 June 08
	Prosser, WA	11 Sept	20 Sept	10 Dec 07	16 June 08
	Reedy Creek, NC	11 Sept	3 Oct	10 Dec 07	16 June 08
Prosser, WA	Geneva, NY	17 Sept	26 Sept	10 Dec 07	16 June 08
	Prosser, WA	17 Sept	26 Sept	14 Dec 07	16 June 08
Raleigh, NC	Geneva, NY	25 Sept	2 Oct	14 Jan 08	16 June 08
	Reedy Creek, NC	25 Sept	3 Oct	10 Dec 07	16 June 08
2008–2009					
Geneva, NY	Geneva, NY	25 Sept	25 Sept	20 Jan 09	16 June 09

^aCleistothecia were collected from the following grape cultivars in each specified location: NY (*Vitis* interspecific hybrid Chancellor), NJ (*V. vinifera* Cabernet franc), WA (*V. vinifera* Chardonnay), and NC (*V. vinifera* Chardonnay). Leaves from NC and WA were shipped to NY for preparation. Cleistothecia from leaves collected in NJ were transferred to filter paper in that location.

accumulate sufficient ascocarps for maturity assessments, grapevine leaves from *Vitis vinifera* or *Vitis* interspecific hybrid cultivars bearing dense aggregations of mature ascocarps (Pearson and Gadoury 1987) were collected in late September or early October (before leaf fall) in vineyards at all locations.

Once collected, the leaves were rinsed with distilled water (dH₂O) over stacked Cobb sieves (US Standard Sieves, No. 50 over No. 120, with 0.297 and 0.125 mm mesh openings, respectively) to collect cleistothecia (Cortesi et al. 1995). Cleistothecia caught in the No. 120 sieve were suspended in dH₂O and transferred in 10 mL aliquots to a Büchner funnel containing a single 9 cm filter disc, under mild vacuum (-10 kPa). Additional 10 mL aliquots of suspension were sequentially added to the funnel until a final concentration of ~500 cleistothecia per disc was reached, concentrated to the center of the filter disc. Each filter disc was immediately air dried, folded into quarters (the face containing cleistothecia protected on the interior), and then affixed using push-pins to white-painted pine boards (25 cm wide by 60 cm high), with ~60 discs per board. Board paint color was selected to help reduce the artificial heat accumulation and radiation that is seen with darker colors (blackbody effect).

Boards bearing the folded discs were stored at 4°C for 1 to 4 days, and then placed at the NY vineyard site, or after overnight shipping, at vineyard sites facing south at NC, NJ, and WA. Boards were installed on posts within, or adjacent to, the vineyards, with the lowermost discs ~60 cm above the soil surface. Previous work using this method for overwintering indicated that the mean of cumulative distributions of ascospore maturity could be shifted in time by up to two weeks depending upon whether the boards faced north or south, but that initial ascospore release and the date at which >95% ascospore release occurred were not significantly affected (Pearson and Gadoury 1987). As the primary interests of this first study were site-to-site and season-to-season comparisons of the terminal points of the distribution of ascospore release, board orientation was standardized at each location so that the side bearing ascocarps was facing south.

Beginning in November to January in each year of this first study (2005 to 2009, Table 1), and at 1 to 2 week intervals thereafter, three filter discs were collected from each site on each sampling date. Prior periodic sampling indicated an inability to dehiscence between in-field collection and date of first sampling; therefore, the specific timeframe of sampling was chosen to increase the likelihood of nonzero responses (i.e., observation of released ascospores). For sites other than NY, the filter discs were shipped overnight to the NY State Agricultural Experiment Station in Geneva (APHIS permit P526P-07-04968). Using a cork borer, a 1-cm circle was cut from each filter disc and affixed to the inner lid of a 9-cm petri plate that was lined with two 9-cm filter discs. All filter discs were then moistened with distilled water, and a glass microscope slide was placed in the base of the petri plate, directly below the cleistothecia to collect released ascospores. Combined plates (lids + base) were incubated at room temperature (22 to 25°C) for 24 hr, with a light regime of 16 hr

light/8 hr dark. After 24 hr, slides were removed, stained with 0.05% Cotton Blue in lactoglycerol, and examined at 100x under a compound microscope (Leica DMLB, Wetzlar, Germany) to enumerate total ascospores released and ascospore germination frequency. Germination was defined as the presence of a germ tube at least equal in length to the spore's width. The total number of cleistothecia per associated filter disc was enumerated. The foregoing method does not precisely describe a cumulative normal distribution, as it can lead to double sampling of ascospores not released between sampling dates and exclusion of ascospores that are released from the field-stored samples between sampling dates. Nonetheless, repeated sampling of a fixed number of field-stored ascocarps does allow the accurate identification of the time of initial, peak, and exhaustion of the ascospore supply (Gadoury et al. 1992). These cardinal points (beginning, peak, and end) have a practical importance in disease management and are used in similarly derived and applied models of ascospore maturity and release (Eikemo et al. 2011).

Model development and validation. For each year, the cumulative percentage of the season-total ascospore release from each site was calculated by normalizing the average number of released ascospores per sampling date (average of three discs per site:population combination) to the average number of cleistothecia present on the filter discs ($n = 3$) and summing those normalized values for each consecutive sampling date. Normalization was done to reduce variability due to survival rates of the cleistothecia.

Cumulative season-total ascospore release was compared to several weather variables to screen for potential contributing or predictive factors. These included: (1) the number of putative ascospore release events, defined with the conservative threshold of ≥ 2.5 mm of rain coincident with a daily maximum temperature $> 0^\circ\text{C}$ (Gadoury and Pearson 1990); (2) degree days (base 0°C , 5°C , 10°C , using daily T_{max} and T_{min} for calculation) accumulated since 1 Dec, 1 Jan, or 1 Feb; (3) biological days, a ranking of average daily temperature on a 0 to 1 scale reflecting developmental favorability as defined by ascocarp maturation responses (Gadoury and Pearson 1990); or (4) a combination thereof. Biological days were determined by regressing cleistothecia dehiscence relative to average daily temperature (Gadoury and Pearson 1990). Temperature with the maximum level of dehiscence was ranked as 1, and other levels were defined as the fraction of 1, from periods starting on 1 Dec, 1 Jan, or 1 Feb through 30 June for each year. The biological day scores were summed over the time period, similar to degree day summation/accumulation.

For model development, only data from NY-originating populations that were overwintered in NY were used; remaining populations overwintered in NY and in other locales were reserved for model testing. For all potential predictors, regression was used to determine the relationship between the aforementioned weather scales and the duration of ascospore release. Logit transformation (complementary log-log transformation link) was used for data fitting. Transformations of this form, for investing ascospore release, have also been used

with success in past studies (Rossi et al. 2010). The complementary log-log transformation takes the generic form of:

$$\pi(x) = 1 - \exp [-\exp (\alpha + \beta x)]$$
 Eq. 1

Additional parameters can be added, in the case of multiple inputs, in the form of adding γz , and so on. Model performance was assessed by regressing predicted versus actual percent season-total ascospore release and evaluated based on minimizing RMSE while maximizing R^2 values. Model evaluations were done on a site basis (i.e., NC, NJ, and WA individually), using all years of the study and all populations overwintered at those sites. An additional evaluation was done on non-NY populations (i.e., NC, NJ, and WA) overwintered in NY.

Weather and phenological data collection. Temperature at the NY site was recorded at 1 min intervals and averaged over 1 hr using a CR10X datalogger (Campbell Scientific, Logan, UT) located within 6 m of the overwintering boards. Precipitation was recorded at 1 min intervals and summed over 1 hr. Daily temperature and precipitation data were obtained from similar dataloggers within 1 km of all other sites; data were downloaded from September through June of each year. Due to changes in data quality control (i.e., removal of faulty weather readings that can be common in remotely uploaded weather data; depending on location, “improved” data from weather modeling or extrapolation from nearby stations were used to fill in missing data), weather data from sites outside of NY were redownloaded from their respective web-based depositories and reevaluated in 2013 (Table 2).

Maturation and ascocarp release from summer (early) versus autumn (late) cohorts. In 2009, populations of cleistothecia representing two cohorts were collected at each of the two NY vineyard sites: a 30-year-old *Vitis* interspecific hybrid Chancellor vineyard and a 5-year-old *V. vinifera* Chardonnay vineyard. Leaves bearing abundant cleistothecia from multiple nodes, shoots, and vines were collected on 9 Sept (early cohort) and 20 Oct (late cohort). Eight rain events ≥ 2.5 mm occurred between collection dates, providing potential ascocarp dispersal events (Gadoury and Pearson 1988). Cleistothecia were harvested from collected leaves using Cobb sieves and transferred to filter discs, as described previously. Discs bearing cleistothecia were placed on white, south-facing boards at their respective collection sites and ascospore discharge tests were performed, as previously described. Regression analysis comparing cohort cumulative ascospore release was performed for both locations to compare potential shifts in ascospore-release dynamics. For both locations, regression analysis was limited between 10 and 90% ascospore release. This method was favored over logit transformation for sim-

plicity of analysis (Madden et al. 2007) and has been successfully used in previous studies of ascospore release in related pathosystems (Eikemo et al. 2011, Stensvand et al. 2005).

Effects of ascosporic inoculum density on disease development. *Artificial infestation.* Vines at the Chardonnay vineyard in NY were artificially infested with cleistothecia immediately preceding budbreak in 2002 and 2003 in order to assess the carryover effects of primary inoculum load on subsequent disease severity and to determine the practical impact of primary inoculum dose on the efficacy of subsequent fungicide sprays. Prior to infestation with captured, overwintered cleistothecia, the basal level infestation (cleistothecia per kg of exfoliating bark) at the site was estimated by sampling bark from 10 vines immediately after leaf fall the previous autumn (Cortesi et al. 1997). The basal level of infestation was estimated to be 2.2 and 1.7 cleistothecia per vine in 2002 and 2003, respectively, based upon the measured ascocarps per kg bark and an assumption of 100 g of exfoliating bark per vine at the site (D.M. Gadoury, author’s unpublished data).

Cleistothecia used for infestation were collected from a severely mildewed Chancellor vineyard in Feb 2002 and Mar 2003. Approximately 2 kg of exfoliated bark was placed in the tub of a top-loading clothes washing machine, which was operated on cold cycle and agitated for 5 min. Wash water expelled during the rinse cycle was poured through stacked Cobb sieves as described above, and cleistothecia that were collected were backwashed into 2 L beakers. The concentration of the suspended ascocarps was determined, the suspension was divided into 100 equal aliquots, and each aliquot was successively transferred to a Büchner funnel containing filter discs and placed under vacuum as described previously. The filter discs were removed, dried, folded twice, and pinned to vineyards posts at the Chardonnay vineyard in NY to complete overwintering. During the subsequent spring, cleistothecia density was augmented by a factor of 10, 100, or 1000 by adding an appropriate number of collected cleistothecia to designated vines. A suspension containing cleistothecia was prepared by removing ascocarps from the overwintered filter discs and placing them in a water suspension. Cleistothecia concentration was determined, and the appropriate volume of suspension was transferred by pipette to the head and cordons of vines to achieve the desired level of infestation (i.e., 10, 100, or 1000x the basal level of infestation). The vines were infested approximately one week before budbreak in both years of this second study.

Infestation treatments were assigned to six vines by two row plots and were replicated four times in a completely random design. Plots were separated by a sprayed buffer row and treated vines alternated with sprayed buffer vines within that

Table 2 Weather station location, website, and date of most recent download.

Location	Source	Station name	Website	Download
Reedy Creek, NC	CRONOS	REED-ECONET	www.nc-climate.ncsu.edu/cronos	30 May 2013
Oswego Lake, NJ	NJWXnet	Oswego Lake	http://www.njweather.org/data/daily	30 May 2013
Geneva, NY	NYSAES	Veg Farm	http://weather.nysaes.cals.cornell.edu/reports/	20 Jan 2014
Prosser, WA	AWN	ROZA	http://weather.wsu.edu	3 June 2013

row. In 2003, the infestation treatments were shifted within rows to use the previously sprayed buffer vines, thereby standardizing the basal inoculum levels.

Three different fungicide-timing treatments were imposed upon selected clusters within each infestation treatment by covering them with 1 L food-grade plastic bags during specific spray applications (Gadoury et al. 2003). Clusters were either exposed to or protected from spray applications to create the following disease management treatments at each infestation level: (1) unsprayed control, (2) critical period spray program (three sprays total, at 10-day intervals beginning immediately before bloom), and (3) a full-season spray program (eight sprays total; starting ~15 cm shoot growth and continuing through veraison). There were 10 clusters per treatment replicate within each infestation level; all clusters were assigned their respective treatments at the time that the first spray occurred. The remaining vines in the vineyard were sprayed with a rotational program of sulfur and kresoxim-methyl to suppress powdery mildew, applied at 10 to 14 day intervals from the 10 to 15 cm shoot growth stage until veraison. Applications were made with a hooded-boom, single-row sprayer at a volume of 936 L/ha.

Grape clusters from each treatment were collected at veraison, and disease severity was recorded as the percentage of cluster surface bearing macroscopically visible colonies of *E. necator*. Treatment means were regressed against log-transformed inoculum doses, and resulting slopes were compared (Student's *t* test) between the spray programs to determine impact of inoculum density on program effectiveness.

Naturally derived infestation. In order to relate the effects of different imposed primary inoculum levels with those that develop naturally under variable management practices, fungicide sprays were terminated on additional vines in the planting during summer 2002, either (1) two weeks after fruit set (early July), (2) preveraison (early August), or (3) pre-harvest (early September). This provided vines with three levels of foliar mildew at the end of the season, resulting in a 21-fold range of cleistothecia densities on the bark just before the start of growth in 2003 (Table 3), determined as described above. The identical “critical-period” spray program described previously was imposed across all three treat-

ments in 2003, and 20 clusters from the center of each plot were rated for powdery mildew severity before harvest, based on the percentage of the surface area that was symptomatic. Individual plots were three rows wide and six vines long and were arranged in a randomized complete block design with four replications. Mean differences were determined using the Waller–Duncan k-ratio *t* test.

Statistical analyses. Unless otherwise indicated, all statistical analyses were performed using JPM Statistical Software (ver. 9; SAS Institute, Cary, NC). Ascospore release model curves were developed using the generalized linear model platform in JMP, with the CompLogLog link function. Regression analyses were performed using the bivariate procedure in JMP. Comparison of slope and intercepts of ascospore cohort release and disease severity under different inoculum doses and intercepts between two lines was done using Student's *t* test. Mean comparison of disease levels in fungicide control programs was conducted using the Waller–Duncan k-ratio *t* test.

Results

Cleistothecia collection, overwintering, and discharge assessments. *Distribution of ascospore release.* For all site/year combinations, putative discharge events (PDE), defined as days with rainfall ≥ 2.5 mm coincident with a daily maximum temperature $> 0^{\circ}\text{C}$ from 1 Jan, combined with accumulation of degree days (DD_0 , base = 0°C) from 1 Jan were the best predictors for determining season-total ascospore release. Best predictors are defined here as maximizing R^2 values, while minimizing RMSE when predicted results were compared to actual observations. The model, developed from NY populations overwintered in NY, takes the form of:

$$\% \text{ Ascospore release} = 1 - \exp [-\exp (-3.335 + 0.00222 (\text{DD}_0) + 0.150287 (\text{PDE}))] \quad \text{Eq. 2}$$

Regression of predicted versus actual results yielded $R^2 = 0.96$ and $\text{RMSE} = 0.08$ (Figure 1); effects tests on the individual parameters showed significant contribution to the model at $\chi^2 < 0.001$ for DD_0 and $\chi^2 < 0.001$ for PDE. Other parameters, including various DD base temperatures, rainfall temperatures, starting dates for accumulation of DD, and intensity of rain events, in singular or interactive form, did not

Table 3 Naturally derived foliar powdery mildew levels in *Vitis vinifera* Chardonnay by artificially shortening in-season fungicide treatments in 2002 resulting in different levels of cleistothecia overwintering to 2003. During the 2003 growing season, the fungicide program was again adjusted and occurred only during the critical period for fruit infection.

October 2002		April 2003	September 2003
Foliar disease ^{a,b}			
% Leaves (incidence)	% Leaf area (severity)	Cleistothecia/kg bark ^b	Cluster disease (% area) ^{b,c}
45.0 a	1.0 a	133 a	11.4 a
92.0 b	17.0 b	534 b	21.9 b
97.0 b	28.0 c	2,867 c	47.7 c

^aFungicide treatments were terminated on select vines in July, Aug, or Sept 2002, to provide three levels of foliar disease severity by the end of the season. Five leaves on each of 10 shoots per plot were rated for disease incidence and severity on 2 Oct 2002. Four replicate plots (3 rows x 6 vines) per treatment.

^bMean values for four replicate measures per plot. Means within a column not followed by a common letter are significantly different ($p \leq 0.05$) according to the Waller–Duncan k-ratio *t* test.

^cFungicides (kresoxim-methyl and sulfur) were applied to all vines at the start of bloom and twice more at 10- to 14-day intervals; 20 clusters per plot were rated for disease severity on 29 Sept.

significantly improve the fit of expected to observed values (data not shown).

The model had varying levels of performance when applied to cleistothecia populations overwintered in other locations (NC, NJ, and WA) and nonlocal populations overwintered in NY (Figure 2). The model performed well in all locations with local and NY populations: NJ ($R^2 = 0.87$, RMSE = 0.14), WA ($R^2 = 0.75$, RMSE = 0.15), and NC ($R^2 = 0.80$, RMSE = 0.19). It also performed well in NY with WA, NJ, and NC populations ($R^2 = 0.71$, RMSE = 0.22).

For all overwintering sites, varying degrees of ascospore release preceded local budbreak of *Vitis vinifera*. For populations overwintered in NY from 2006 to 2009, 50% of the season-total ascospore release occurred 42, 24, and 35 days before and 9 days after budbreak of Chardonnay, respectively. For populations overwintered in WA in 2006, 2007, and 2008, 50% season-total ascospore release occurred 3, 7, and 15 days before Chardonnay budbreak, respectively. In 2006 and 2007 in NJ, 50% season total ascospore release occurred 55 and 3 days before estimated Cabernet franc budbreak, respectively. In 2006, 2007, and 2008, 50% season total ascospore release for populations overwintered in NC occurred 10, 30, and 10 days before Chardonnay budbreak, respectively.

Maturation and ascocarp release from summer versus autumn cohorts. Cleistothecia collected in the early (summer) cohort reached the point of 50% ascospore release up to 21 days before those collected in the late (autumn) cohort (Figure 3A, B). Models fit to early and late cohorts at both sites yielded significantly different y-intercepts ($p < 0.001$, both locations) and statistically similar slope coefficients ($p = 0.35$ and 0.14 for the Chardonnay and Chancellor vineyards, respectively) (Figure 3C, D).

Effects of primary inoculum dose on consequent severity of berry infection. *Artificial infestation.* Severity (Y [%]) of fruit infection at harvest was linearly related to the

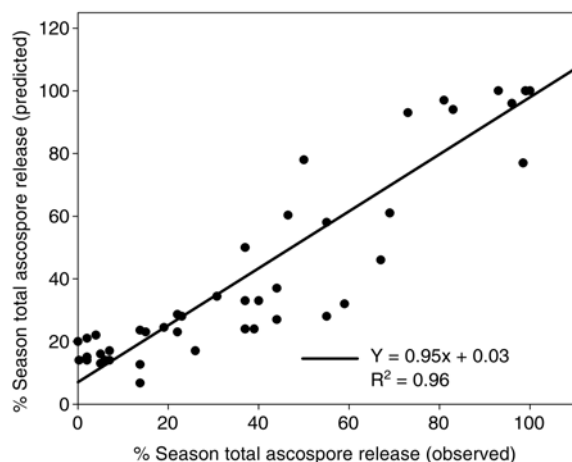


Figure 1 Predicted versus observed season ascospore release for NY populations of *Erysiphe necator* cleistothecia overwintered in New York. Predicted values based on the following equation: all combined populations and overwintering sites, based on the prediction equation: % Ascospore release = $1 - \exp[-\exp(-3.335 + 0.00222 (DD_0) + 0.150287 (PDE))]$. PDE: putative discharge events (rainfall ≥ 2.5 mm coincident with temperatures $> 0^\circ\text{C}$); DD_0 : degree day accumulation, base 0°C .

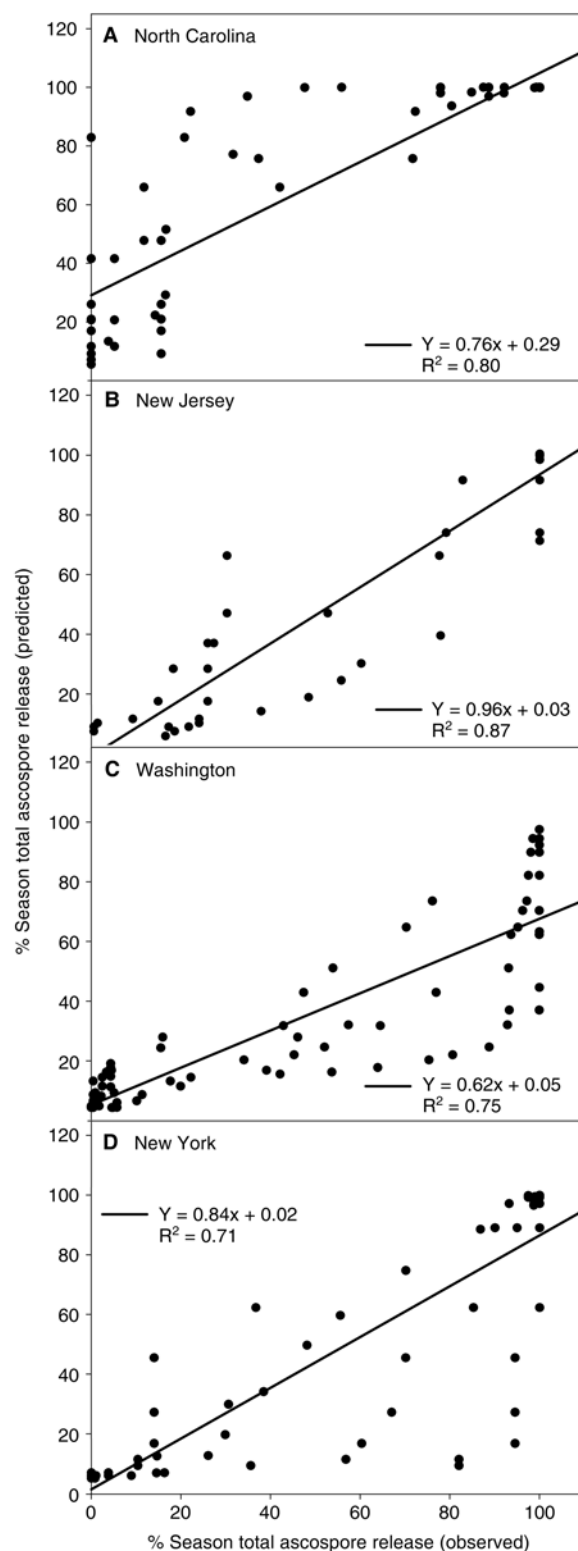


Figure 2 Performance of the New York ascospore release model (Equation 2, in text), in other regions and with other populations of origin of *Erysiphe necator* cleistothecia. Model performance in (A) North Carolina with three years of data with NY cleistothecia populations, and two years of data with NC cleistothecia populations; (B) New Jersey with three years of data with NY cleistothecia populations, and one year of data with NJ cleistothecia populations; (C) Washington with three years of data with NY cleistothecia populations, and two years of data with WA cleistothecia populations; and (D) New York with one year of data with a NC cleistothecia population, one year of data with a NJ cleistothecia population, and two years of data with WA cleistothecia populations.

\log_{10} of cleistothecia per vine (X) in both years of this second study (Figure 4). For the year 2002, linear regression of the \log_{10} of cleistothecia per vine yielded the following equations for the full season, critical period, and control spray programs, respectively: $Y = -0.88 + 1.85 \log_{10}(X)$, $R^2 = 0.96$; $Y = 2.63 + 4.13 \log_{10}(X)$, $R^2 = 0.89$; and $Y = -4.13 + 10.75 \log_{10}(X)$, $R^2 = 0.87$ (Figure 4A). Slope coefficients decreased ($p = 0.02$) between the untreated control and critical period treatments, and between the critical period and full season treatments ($p = 0.06$). For the year 2003, linear regression of the \log_{10} of cleistothecia per vine yielded the following equations for the full season, critical period, and control spray programs, respectively: $Y = 2.51 + 0.47 \log_{10}(X)$, $R^2 = 0.87$; $Y = 9.32 + 11.99 \log_{10}(X)$, $R^2 = 0.97$; and $Y = 34.68 + 8.16 \log_{10}(X)$, $R^2 = 0.85$ (Figure 4B). Slope coefficients were similar ($p = 0.12$) between the control and critical period treatments, but the slope coefficient of the full season treatment was lower than that of the control and critical period treatments ($p < 0.02$, Figure 4B). The effect of logarithmic increases in inoculum dose was reduced in proportion to the intensity of spraying in 2002 (Figure 4A). In 2003, only the full season treatment effectively negated the impact of logarithmic increased in inoculum dose across the full range of the factor (Figure 4B).

Naturally derived infestation levels. Termination of the in-season fungicide programs in 2002 resulted in statistically

different ($p < 0.05$) foliar disease severity and cleistothecia amounts the following spring (Table 3). Similar to the artificially infested treatments, a stepwise increase in severity of fruit infection was observed in 2003 across the three distinct levels of inoculum dose, even when vines were treated only during the critical period of fruit susceptibility.

Discussion

A general model based upon both accumulated rainfall and degree days has been developed to describe the distribution of ascospore release potential through the late winter/early spring dormancy period of the vine. Similar studies in warmer climates have also indicated a relationship between dormant season heat unit accumulation and timing of ascospore release (Rossi et al. 2010).

Previous investigations have not focused on ascospore release during the months preceding local budbreak of *Vitis vinifera* (Gadoury and Pearson 1990, Grove 2004, Jailloux et al. 1999, Pearson and Gadoury 1987). Another study reported on the dynamics and timing of ascospore maturation, but not specifically as to how conditions during vine dormancy might influence subsequent ascospore release (Legler et al. 2012a). As a consequence, extant advisory systems have not accounted for the potential impact of pre-budbreak weather conditions on inoculum survival or depletion (Caffi et al. 2011, Gubler et al. 2009).

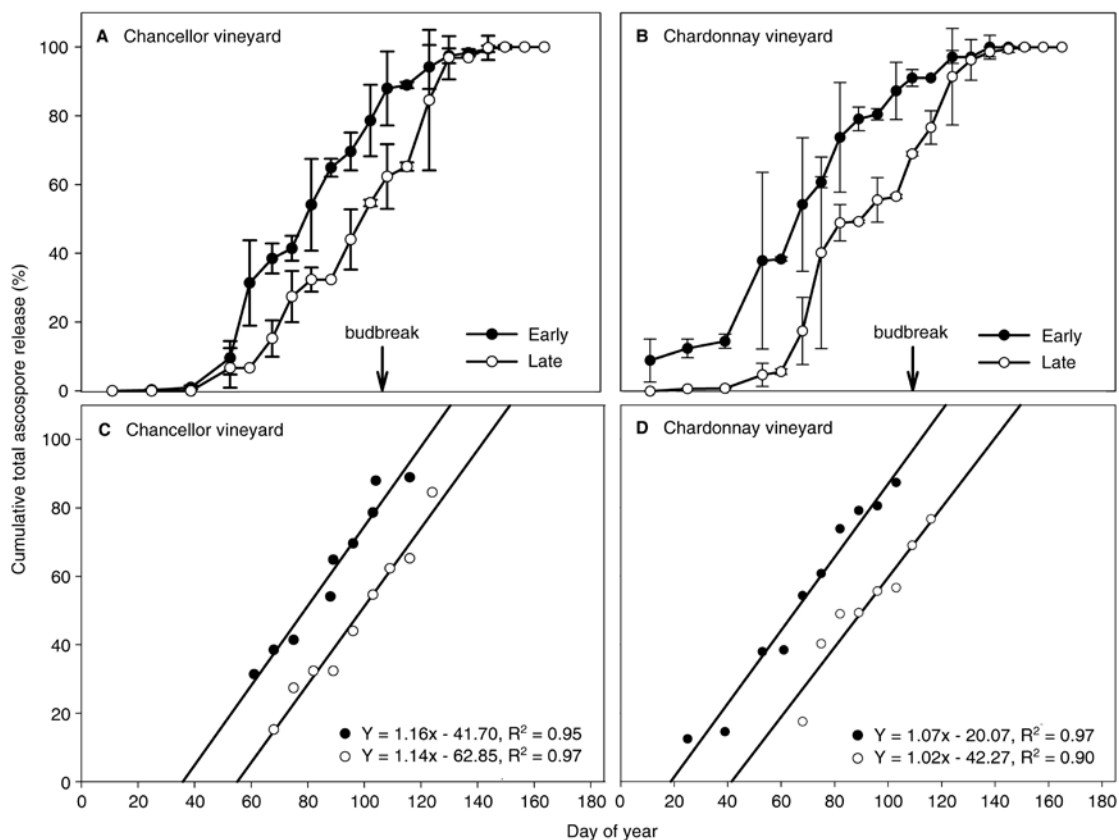


Figure 3 Ascospore release distribution curves for *Erysiphe necator* cleistothecia collected from two vineyard sites on either 9 Sept (early) or 20 Oct (late) 2009, using the collecting method as described in text. Data points represent the cumulative mean ascospore release events as calculated from three replications; error bars represent one standard error. Budbreak of *Vitis* hybrid Chancellor in 2010 began on DOY 105 (15 April); budbreak of *V. vinifera* Chardonnay in 2010 began on DOY 111 (21 April) and are indicated by arrows in **A** and **B**.

Observations of lab-induced release of ascospores from field-overwintered cleistothecia in this study indicated that ascocarps are often sufficiently mature to allow discharge of a substantial proportion of the season's total supply of primary inoculum before budbreak of *V. vinifera*, across a broad range of environments. Historical weather data from within the study region also indicated that there is often ample opportunity for pre-budbreak ascospore release to occur under field conditions. For example, at the NY, NJ, NC, and WA sites, for the period between 1 Jan and the average date of local budbreak, there were an average of 21.0, 19.0, 21.75, and 6.5 rain events of ≥ 2.5 mm on days with an average temperature $\geq 0^\circ\text{C}$ for the years 2006 through 2009, respectively (M. Moyer, author's unpublished data). This potential depletion of primary inoculum would be realized in climates substantially colder than those previously studied (Grove 2004, Jailloux et al. 1998, Rossi et al. 2010). Compared to these previous reports, the present study indicated that up to 50% of the seasonal total of ascospores could have been released in 85% of our site-year combinations by the time of local *V. vinifera* budbreak.

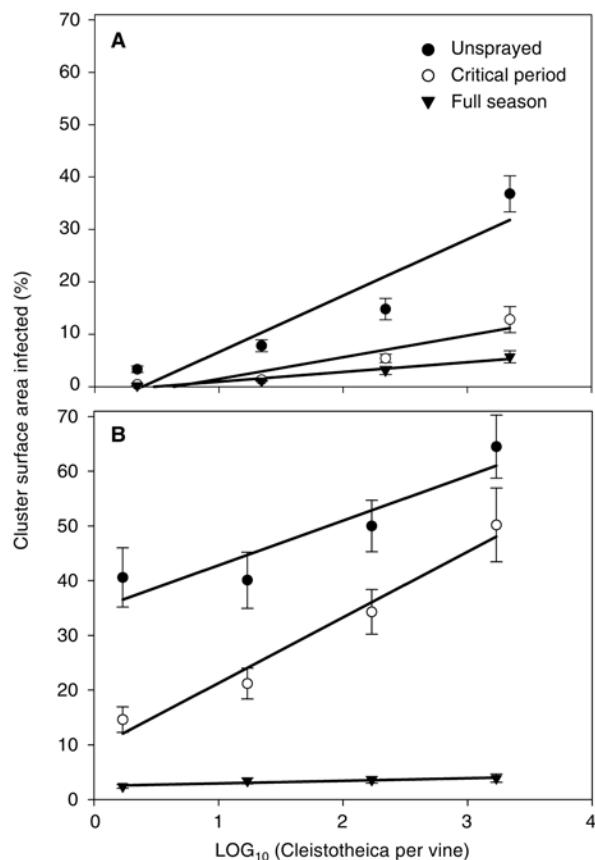


Figure 4 Relationship between increasing dose of *Erysiphe necator* cleistothecia and subsequent development of disease on *V. vinifera* Chardonnay clusters under different intensities of fungicidal-derived disease suppression in (A) 2002 and (B) 2003. Infestation levels were artificially derived by inoculating known levels of cleistothecia onto the vines in spring of 2002 or 2003. During in-season management, selected clusters were bagged during fungicide applications to provide spray timing treatments: unsprayed (control); critical period (3 sprays total); and full season (8 sprays total). Clusters were rated for % disease severity at harvest; data points are representative of the average four treatment replicates (10 clusters per replicate). Error bars represent standard error (n = 4).

The presumed center of origin for *E. necator* is eastern North America (Brewer and Milgroom 2008). Results presented here demonstrate asynchrony in ascospore release in eastern North America compared to the phenological development of the European winegrape (*V. vinifera*) after dormancy. The alignment of the peak period of ascospore release to grapevine budbreak is considerably improved when compared to phenology of the indigenous *V. riparia* in the same region. Phenological development of *V. riparia* is approximately three weeks in advance of *V. vinifera*. In Geneva, NY, budbreak of *V. riparia* preceded that of *V. vinifera* Chardonnay by 20 days in 2011 and 27 days in 2012. Perhaps developmental asynchrony of *E. necator* and *V. vinifera* is related to the recent (in an evolutionary sense) introduction of *V. vinifera* to the geographic range and climate of an endemic pathogen adapted to native host species. Further examples of asynchrony can be seen where *E. necator* has spread beyond its native range to the Mediterranean climates of South Australia (Gee et al. 2000), California (Gubler et al. 1994), and Italy (Rossi et al. 2010), where ascospore release can occur in autumn before leaf abscission. This phenomenon may be related to the exposure of early formed cleistothecia to heat unit accumulations sufficient for physiological maturity well before grapevine leaf senescence and abscission (Gee et al. 2000).

The initial appearance of ascocarps in vineyards can vary by several weeks to months, as disease incidence and severity determine the probability compatible mating type pairing for this heterothallic pathogen (Gadoury and Pearson 1988). Previous reports showed that once initiated, cleistothecia of *E. necator* required ~ 500 DD₀ to mature and survive apart from a living mildew colony. This was accomplished in as few as 20 to 25 days at temperatures from 20 to 25°C (Gadoury and Pearson 1988). Thereafter, progress toward physiological maturity and eventual dehiscence of ascocarps under the prevailing overwintering conditions in central New York (Gadoury and Pearson 1988) or South Australia (Gee et al. 2000) occurred over several months. In the present study, cohorts of ascocarps that were collected approximately one month apart in autumn matured and released ascospores at the same rate the following spring, but the temporal distributions of the early versus late collections were shifted by nearly three weeks (Figure 3). Our early collected cohorts yielded between 51 and 60% of the season's total ascospore release by 1 March, nearly two months before local budbreak of *V. vinifera*, whereas the late collections provided only 10 to 13% release by the same date.

Consequently, the date when ascocarp initiation begins, and the length of time over which ascocarp formation continues during the growing season, can affect several aspects of primary inoculum potential (e.g., time of initial release, duration of the release period, and proportion of the ascospores released before budbreak). A later date of cleistothecia initiation in the autumn, followed by a brief period of maturation before leaf abscission (as might occur in vineyards in colder climates with effective disease suppression), would produce a common cohort of cleistothecia with perhaps greater potential to retain ascospores until after budbreak. An earlier date of

initiation and a longer period of formation before leaf abscission (possible in a warmer climate vineyard if combined with less effective disease suppression) would create several cohorts and a more variable and protracted distribution of ascospore release the following spring. An additional source of variation due to cohorts in a natural system would be the differential opportunities for passive dispersal of cleistothecia to the bark of the vine during rain events. Ineffective dispersal would lead to increased mycoparasitism or burial by detritivores (Pearson and Gadoury 1987). Consequently, the most abundant cohorts would not necessarily represent the greatest inoculum source.

The observed and potential variation in the distributions of ascospore release, and the consequent impacts upon inoculum dose, may partially explain the wide year-to-year variations observed in the incidence and severity of powdery mildew on grapevine berry clusters in New York State (Moyer et al. 2010). The dose of ascosporic inoculum significantly influences disease severity on grapevine berries in years with moderate disease pressure (Figure 4A), but it is less of an influence on final disease levels in years with conducive weather for disease development (Figure 4B). Conducive weather patterns that favor powdery mildew disease development on fruit include warmer temperatures the preceding fall (cleistothecia development) and low evapotranspiration (ET_0) during the weeks surrounding bloom (Moyer 2011). While fall heat accumulations in 2001 and 2002 were similar (502 and 510 growing degree days base 10°C from 1 Aug to 15 Sept), average ET_0 during bloom for 2002 was 6.13 mm versus 5.4 mm in 2003 (Moyer 2011). These effects can be offset by an appropriately timed early season spray regime focused on a critical period of fruit susceptibility (Figure 4, Table 3). Thus, inoculum dose may set the potential for severe fruit infection, but the degree to which that potential is realized may be affected more by environmental conditions after budbreak and further mitigated by either the suppressive action of fungicides or the lack of an effective chemical control program. In situations where weather is not conducive for powdery mildew development on fruit, a critical period spray regime may effectively control disease severity (Figure 4A); however, in conducive years that provide ideal conditions for fungal growth, a shortened spray program may not be enough to suppress development of escaped ascospores during the transition period from susceptible to resistant. It remains to be demonstrated how the levels of inoculum deployed in our experiments relate to the range of inoculum dose in commercial vineyards under stringent management programs. The ascosporic dose levels of the current study spanned several orders of magnitude and at the uppermost levels likely surpass the primary inoculum levels encountered in commercial vineyards, based upon previously reported densities of cleistothecia on bark of unsprayed vines (Gadoury and Pearson 1988, Pearson and Gadoury 1987).

Effective early season disease control would delay powdery mildew epidemics and reduce the available time for cleistothecia production and maturation (Gadoury and Pearson 1988). However in seasons like 2003, an extended spray

program past the critical period of fruit susceptibility may help reduce cleistothecia production from “escaped” powdery mildew colonies (i.e., inoculum that was not suppressed due to lack of complete spray coverage). These sprays need to be considered with regard to their proximity to harvest day, product type (i.e., residual sulfur impacts on fermentation), and associated weather conditions (i.e., hot and dry suppresses powdery mildew development, negating the need for a spray application). Extended spray programs should be viewed as a secondary means for cleistothecia development control, not the primary means. While data presented here show that cleistothecia that develop later in the autumn also release their ascospores later in the spring (Figure 3), the later-maturing cleistothecia are typically the product of poor disease management directly after bloom. If onset of disease occurs much after bloom, then there is not sufficient time for a powdery mildew colony to achieve mating and then have sufficient maturation of the ascocarp (Gadoury and Pearson 1988, 1991). This time is earned either through poor early season disease control (i.e., an earlier onset of disease development) or through an extended fall frost-free period (i.e., a later natural “stop” to disease development).

Conclusion

The release of ascospores from cleistothecia of *E. necator* appears to lack a high degree of synchrony with regrowth of *V. vinifera* after the period of host dormancy. A substantial proportion of the primary inoculum has the ability to release while the host is dormant. The consequent depletion of primary inoculum may be related to observed year-to-year variation in severity of fruit infection across broad geographic regions. It is still unclear how climates with mild winter conditions (e.g., Mediterranean) might affect this potential loss of inoculum. The longer growing season could conceivably allow ascocarp formation over longer spans of time, thereby producing cohorts of ascocarps that mature and release ascospores over a protracted period, perhaps offsetting the lack of synchrony with host growth. Irrespective of these unknown factors, these results demonstrate that primary inoculum dose has an impact on the consequent severity of fruit infection. The model presented in this study may be useful to describe potential depletion of primary inoculum across a range of environmental conditions and would therefore seem relevant to analysis of potential risks in epidemic development. It can also be used as an early season forecast system (integrated into regional weather stations) to allow growers to determine potential inoculum-dose-based risks going into the growing season and before spray programs that match in-season weather-driven disease pressure.

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