THE EPIDEMIOLOGY OF GRAPEVINE POWDERY MILDEW IN MICHIGAN AND THE EFFECTS OF POWDERY AND DOWNY MILDEW ON VINE PHYSIOLOGY

By

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ABSTRACT

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Monitoring of the primary inoculum of the grape powdery mildew (*Erysiphe necator*) revealed that ascospores were caught almost continuously between bud break and fruit set, and release was best described as a function of degree-days. Monitoring of ascocarp production in the fall showed peaks in mid- to late September. This information can be used to optimize timing of fall eradicant sprays. A survey of fungicide sensitivity in *E. necator* in Michigan vineyards showed that strobilurin (QoI)-resistant isolates were most prevalent in research plots and some commercial wine grape vineyards and least prevalent in juice grape vineyards. The G143A mutation that confers resistance was detected in every isolate with an $EC_{50} \geq 1.01$ μg/ml trifloxystrobin. The effects of foliar downy and powdery mildew were evaluated under two disease levels (fungicide sprayed and unsprayed) and three cropping levels (33%, 67% and 100%) in juice grapes. Juice composition, cold hardiness, and starch content of canes were evaluated. At low to moderate levels of powdery mildew observed in 'Concord' grapes, fungicide applications did not appear to be cost-effective. However, reductions in starch reserves as well as sugar content and cane cold hardiness were observed in diseased, high-cropped 'Niagara' vines. This indicates that fungicide applications and/or crop thinning are important to protect the health of 'Niagara' vines with a high cropping level.

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TABLE OF CONTENTS

LIST OF TABLES ..... **vi**

LIST OF FIGURES ..... **ix**

CHAPTER 1
LITERATURE REVIEW **1**
Introduction 1
Grape production in Michigan 3
Optimizing grape production 4
Disease damage thresholds 5
Diseases of grapes 8
The grape powdery mildew 8
The grape downy mildew 16
Fungicide resistance 20
Strobilurins 24
Literature cited 26

CHAPTER 2
ASCOSPORE RELEASE BY *ERYSIPHE NECATOR* IN RELATION TO ENVIRONMENTAL CONDITIONS AND DISEASE DEVELOPMENT IN GRAPES IN MICHIGAN **36**
Abstract 36
Introduction 37
Materials and methods 40
Results 44
Discussion 58
Literature cited 67

CHAPTER 3
LATE-SEASON CHASMOTHECIUM PRODUCTION AND DISPERSAL OF *ERYSIPHE NECATOR* IN MICHIGAN **70**
Abstract 70
Introduction 71
Materials and methods 75
Results 78
Discussion 97
Literature cited 102

CHAPTER 4
SCREENING FOR STROBILURIN (QOI) RESISTANCE IN POPULATIONS OF *ERYSIPHE NECATOR* ON GRAPES IN MICHIGAN 106
Abstract 106
Introduction 108
Materials and methods 110
Results 117
Discussion 137
Literature cited 143

CHAPTER 5
EFFECTS OF DOWNY AND POWDERY MILDEW ON JUICE GRAPES (VITIS LABRUSCA L.) ADJUSTED TO DIFFERENT CROPPING LEVELS 147
Abstract 147
Introduction 149
Materials and methods 153
Results 163
Discussion 182
Literature cited 187

CHAPTER 6
CONCLUSIONS AND FUTURE DIRECTIONS 190

LIST OF TABLES

Table 1.1. List of global reports of fungicide resistance in the grapevine pathogens *Erysiphe necator* and *Plasmopara viticola* according to the specific active ingredients evaluated. Fungicide classes and the Fungicide Resistance Action Committee (FRAC) codes are also specified. Unless denoted, all correspond to field resistance reports 22

Table 2.1. Pearson correlation coefficients (r) for linear regressions between daily ascospore concentration and rainfall, leaf wetness, temperature, relative humidity, vapor pressure deficit, and growing degree-days at the Michigan State University research vineyards in Clarksville, MI and Traverse City, MI in 2009 and 2010 50

Table 2.2. Development of powdery mildew on potted bait plants of *Vitis vinifera* L. 'Chardonnay' placed on a weekly basis in an untreated vineyard of *Vitis* interspecific hybrid 'Chardonel' in Clarksville, MI and in an untreated vineyard of mixed *V. vinifera* in Traverse City, MI in 2009 and 2010. The total airborne inoculum concentration (*Erysiphe necator* ascospores per cubic meter of air), average temperature, average relative humidity, average vapor pressure deficit are shown for each 7-day sampling period..... 56

Table 3.1. Analysis of variance (ANOVA) of differences within and between sampling periods in *Erysiphe necator* chasmothecia collected in 3-L-bottle rain traps placed in vineyards of *Vitis* L. interspecific hybrid 'Chardonel' at the Michigan State University (MSU) Clarksville Research Center and *V. vinifera* 'Pinot Noir' at the MSU Northwest Michigan Research Center in 2008, 2009, and 2010, and a vineyard of *Vitis* L. interspecific hybrid 'Marechal Foch' in Suttons Bay, MI in 2009. *P*-values ≤ 0.05 were considered significant and are shown in bold font..... 79

Table 3.2. Cumulative total number of chasmothecia dispersed by rain and average ascocarp production of *Erysiphe necator* on leaves of *Vitis* L. interspecific hybrid 'Chardonel' and *V. vinifera* 'Pinot Noir' in 2008, 2009, and 2010, and *Vitis* L. interspecific hybrid 'Marechal Foch' in 2009 81

Table 3.3. Pearson correlation coefficients (*r*) between the number of chasmothecia and the amount of rainwater collected in 3-L-bottle traps placed in a vineyard of *Vitis* L. interspecific hybrid 'Chardonel' at the Michigan State University Clarksville Research Center and *V. vinifera* 'Pinot Noir' at the MSU Northwest Michigan Horticultural Research Center in 2008, 2009, and 2010, and a commercial vineyard of *Vitis* L. interspecific hybrid 'Marechal Foch' in 2009..... 87

Table 3.4. Analysis of variance (ANOVA) of differences within and between sampling periods of the number of *Erysiphe necator* chasmothecia observed on leaves of *Vitis* L. interspecific hybrid 'Chardonel' at the Michigan State University (MSU) Clarksville Research Center; *V. vinifera* 'Pinot Noir' at the MSU Northwest Michigan Research Center in 2008, 2009, and 2010; and a vineyard of *Vitis* L. interspecific hybrid 'Marechal Foch' in Suttons Bay, MI in 2009 89

Table 3.5. Pearson correlation coefficients (*r*) between the number of *Erysiphe necator* chasmothecia observed on the adaxial surface and the severity of powdery mildew in the leaves collected in a vineyard of *Vitis* L. interspecific hybrid 'Chardonel' at the Michigan State University (MSU) Clarksville Research Center in 2009 and 2010, and a vineyard of *V. vinifera* 'Pinot Noir' at the MSU Northwest Michigan Horticultural Research Center in 2010..... 96

Table 4.1. Percentage of *Erysiphe necator* isolates collected in Michigan vineyards in 2009 that germinated at each concentration of trifloxystrobin evaluated (0.001, 0.01, 0.1, 1, 10, and 100 µg/ml). Germination was considered to have occurred when the average number of germinated conidia at a certain fungicide concentration was at least two standard deviations above the mean in the control treatment 122

Table 4.2. Reproducibility of the *Erysiphe necator* conidium germination bioassays on agar slides amended with the fungicide trifloxystrobin and 100 mg/liter SHAM. The fungicide sensitivity assay was conducted three separate times for six different isolates..... 124

Table 4.3. Origin, G143A detection, and trifloxystrobin sensitivity for *Erysiphe necator* isolates collected in Michigan during 2009 126

Table 4.4. Chi-square analysis of the detection of G143A mutation in the cytochrome b gene of *Erysiphe necator* isolates according to type of site (baseline, commercial, or Michigan State University research vineyard), region in the state of Michigan (northwest, central, or southwest), and the type of grape (juice or wine grape) in 2009 131

Table 5.1. Test of significance of the fixed effects fungicide treatment (sprayed and unsprayed), cropping intensity level (33%, 67%, and 100% of the highest yielding potential), and their interaction on grape production, juice quality (Brix, pH, and titratable acidity) and vine physiology (starch content in canes, and cold hardiness of buds and canes) of *Vitis labrusca* L. 'Niagara' in 2008, 2009, and 2010 by two-way factorial ANOVA..... 167

Table 5.2. Test of significance of the fixed effects fungicide treatment (sprayed and unsprayed), cropping intensity level (33%, 67%, and 100% of the highest yielding potential), and their interaction on grape production, juice quality (Brix, pH, and titratable acidity) and vine physiology (starch content in canes, and cold hardiness of buds and canes) of *Vitis labrusca* L. 'Concord' in 2009 and 2010 by two-way factorial ANOVA 168

Table 5.3. Yield at harvest of fungicide-sprayed and unsprayed *Vitis labrusca* L. 'Niagara' vines cropped at three different intensity levels (33%, 67%, and 100% of the highest yielding potential) in 2008, 2009 and 2010 in Fennville, MI..... 170

Table 5.4. Yield at harvest of fungicide-sprayed and unsprayed *Vitis labrusca* L. 'Concord' vines cropped at three different intensity levels (33%, 67%, and 100% of the highest yielding potential) in 2009 and 2010 in Fennville, MI 171

Table 5.5. Effects of downy mildew on juice quality (Brix, pH, and titratable acidity) and vine physiology (starch content in canes, and cold hardiness of buds and canes) of *Vitis labrusca* L. 'Niagara' vines under three different imposed cropping intensity levels (33%, 67%, and 100% of the highest yielding potential) during 2008, 2009 and 2010 in Fennville, MI 174

Table 5.6. Effects of powdery mildew on juice quality (Brix, pH, and titratable acidity) and vine physiology (starch content in canes, and cold hardiness of buds and canes) of *Vitis labrusca* L. 'Concord' vines under three different imposed cropping intensity levels (33%, 67%, and 100% of the highest yielding potential) during 2009 and 2010 in Fennville, MI 175

Table 5.7. Pearson correlation coefficients (*r*) between downy and powdery mildew disease levels and the physiology of 'Niagara' and 'Concord' grapevines, respectively, during 2008, 2009, and 2010 in Fennville, MI..... 179

Table 5.8. Pearson correlation coefficients (*r*) between yield (kg/vine) and the physiology of 'Niagara' and 'Concord' grapevines during 2008, 2009 and 2010 in Fennville, MI 181

LIST OF FIGURES

Figure 1.1. Sporulating colonies of *Eryshipe necator* on (A) 'Concord' grape leaves and (B) a 'Chardonel' grape cluster, which also shows the split-berry symptom (white arrow). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis 10

Figure 1.2. Asexual and sexual stages of *Eryshipe necator*. (A) Conidial chains on a 'Chardonnay' grape leaf (bar = 50 μm). (B) Mature (black) and immature (light brown and yellow) chasmothecia developing on the abaxial surface of a 'Chardonel' grape leaf (bar = 100 μm) 13

Figure 1.3. Symptoms of downy mildew on 'Niagara' grapes. (A) Chlorotic, coalescing lesions on leaves. (B) Dying and shriveling downy mildew-infected leaves in top of canopy 18

Figure 2.1. Daily concentrations of airborne ascospores of *Erysiphe necator* (ascospores/m^3/day), daily total rainfall (mm/day), and daily average leaf wetness (% of the day) at the Michigan State University research vineyard in Clarksville during 2009 (A, C, and E, respectively) and 2010 (B, D, and F, respectively). Rainfall of more than 2.5 mm/day is noted by the breaks in the y-axis 45

Figure 2.2. Daily concentrations of airborne ascospores of *Erysiphe necator* (ascospores/m^3/day), daily total rainfall (mm/day), and daily average leaf wetness (% of the day) at the Michigan State University research vineyard in Traverse City during 2009 (A, C, and E, respectively) and 2010 (B, D, and F, respectively). Rainfall of more than 2.5 mm/day is noted by the breaks in the y-axis 46

Figure 2.3. Cumulative percentage of ascospores trapped at the research vineyards in Clarksville (A, B) and Traverse City (C, D), MI in 2009 (A, C) and 2010 (B, D). Black, down and up arrows indicate the occurrence of grapevine's bud break (Eichhorn-Lorenz stage 5) and first bloom (Eichhorn-Lorenz stage 21) in the phenology of the grapevine, respectively 48

Figure 2.4. Cumulative percentage of ascospores according to growing degree-days (calculated from 1 April, base 4°C) at the research vineyards in Clarksville, MI in (A) 2009 and (B) 2010, and Traverse City, MI in (C) 2009 and (D) 2010. The fitted Gompertz equations (solid line [−]) for each sampling period (A-D) are shown with their corresponding 95% confidence intervals (gray, dashed lines [--]). Parameter estimates and corresponding standard errors were (A) a = 101.7 ± 0.54, b = 144.3 ± 2.92, x_0 = 377.1 ± 1.97; (B) a = 99.7 ± 0.84, b = 100.8 ± 3.36, x_0 = 284.2 ± 2.04; (C) a = 96.2 ± 1.10, b = 46.7 ± 2.67, x_0 = 399.2 ± 1.81; (D) a = 95.6 ± 1.21, b = 116.6 ± 8.33, x_0 = 255.6 ± 4.48, respectively. R^2 and P-values of the regressions are shown for each site×year combination 53

Figure 3.1. The average number of *Erysiphe necator* chasmothecia (A, B, and C) and average amount of rainwater (D, E, and F) collected in 3-L-bottle traps (n = 4) attached to the trunk of untreated grapevines of *Vitis* L. interspecific hybrid 'Chardonel' planted at the Michigan State University Clarksville Research Center in Clarksville, MI during each sampling period in 2008 (A and D), 2009 (B and E), and 2010 (C and F). Asterisks (*) indicate trapping of less than five chasmothecia per bottle. Error bars indicate the standard error of the mean..... 83

Figure 3.2. The average number of *Erysiphe necator* chasmothecia (A, B, and C) and average amount of rainwater (D, E, and F) collected in 3-L-bottle traps (n = 4, except for 2009 when n = 2) attached to the trunk of untreated grapevines of *Vitis vinifera* 'Pinot Noir' planted at the Michigan State University Northwest Michigan Horticulture Center in Traverse City, MI during each sampling period in 2008 (A and D), 2009 (B and E), and 2010 (C and F). Asterisks (*) indicate trapping of less than five chasmothecia per bottle. Error bars indicate the standard error of the mean..... 84

Figure 3.3. (A) The average number of *Erysiphe necator* chasmothecia and (B) average amount of rainwater collected in 3-L-bottle traps (n = 2) attached to the trunk of untreated grapevines of *Vitis* L. interspecific hybrid 'Marechal Foch' in a commercial vineyard in Suttons Bay, MI during each sampling period in 2009. Error bars indicate the standard error of the mean..... 85

Figure 3.4. Average number of *Erysiphe necator* chasmothecia per square centimeter of leaf (A, B and C; n = 10 cm^2) and average number of chasmothecia found on the adaxial (D, E, and F; n = 5 cm^2) and abaxial (G, H, and I; n = 5 cm^2) leaf surfaces of untreated *Vitis* L. interspecific hybrid 'Chardonel' planted at the Michigan State University Clarksville Research Center in Clarksville, MI. Immature and mature chasmothecia are denoted with gray and black bars, respectively. Bars represent averages of four replicate samples of 20 leaves per vine on each sampling date during 2008 (A, D and G), 2009 (B, E and H), and 2010 (C, F and I)..... 92

Figure 3.5. Average number of *Erysiphe necator* chasmothecia per square centimeter of leaf (A, B and C; n = 10 cm^2) and average number of chasmothecia found on the adaxial (D, E, and F; n = 5 cm^2) and abaxial (G, H, and I; n = 5 cm^2) leaf surfaces of untreated *Vitis* vinifera 'Pinot Noir' planted at the Michigan State University Northwest Michigan Horticulture Center in Traverse City, MI. Immature and mature chasmothecia are denoted with gray and black bars, respectively. Bars represent averages of 5, 10, and 20 leaves on each sampling date during 2008 (A, D and G), 2009 (B, E and H), and 2010 (C, F and I), respectively 93

Figure 3.6. (A) Average number of *Erysiphe necator* chasmothecia per square centimeter of leaf (n = 10 cm^2) and average number of chasmothecia found on the (B) adaxial (n = 5 cm^2) and (C) abaxial (n = 5 cm^2) leaf surfaces of fungicide-untreated grapevines of *Vitis* L. interspecific hybrid 'Marechal Foch' planted in a commercial vineyard in Suttons Bay, MI. Immature and mature chasmothecia are denoted with gray and black bars, respectively. Bars represent averages of 20 leaves per sampling date during 2009 94

Figure 4.1. Detection of the single-nucleotide change in the cytochrome b (*CYTB*) gene that generates the G143A mutation in QoI resistant pathogens. Polymerase chain reaction (PCR) products obtained from the preliminary study with *Erysiphe necator* isolates in 2008 using the Amplification Refractory Mutation System (ARMS) mutant primer set and conditions standardized by Baudoin *et al*. (2008). Lanes 1-3, isolates from a commercial 'Concord' vineyard in Watervliet, MI; lanes 4-6, isolates from a 'Concord' vineyard at the Michigan State University (MSU) Trevor Nichols Research Center at Fennville, MI; lanes 7-9, isolates from a commercial 'Cabernet Sauvignon' vineyard in Traverse City, MI; lane 10, isolate from a 'Pinot Noir' vine at the MSU Northwest Michigan Horticultural Research Center, Traverse City, MI; lanes 11-12, isolates from 'Chardonnay' vines growing in a MSU greenhouse in campus, East Lansing, MI; lane L, 1-kb+ DNA ladder; lane W, negative control (water). The white arrow denotes the target amplicon *CYTB* (of less than 100 bp) 118

Figure 4.2. Amplification of a fragment of the mitochondrial cytochrome b (*CYTB*) gene containing the site for the G143A mutation in *Erysiphe necator* using the SYBR green Q-PCR Amplification Refractory Mutation System (ARMS) developed by Baudoin *et al*. (2008). Amplification plots (A and B) and product disassociation curves (C and D) obtained for an *E. necator* trifloxystrobin-sensitive isolate (A and C) and trifloxystrobin-resistant isolate (B and D) from grapes in Michigan using the ARMS wild-type (in blue) and G143A (in orange) primer sets are shown for each case 120

Figure 4.3. Distribution of effective concentration values of trifloxystrobin that inhibited 50% of the conidial germination (EC_{50} in micrograms per milliliter) in *Erysiphe necator* isolates collected in Michigan during 2009. Isolates were obtained from (A) baseline sites (n = 37), (B) commercial vineyards (n = 62), and (C) Michigan State University research vineyards (n = 26). Percentages were calculated according to the total number of isolates tested within each type of site 128

Figure 4.4. Detection of the G143A mutation according to EC_{50} ranges calculated for *Erysiphe necator* isolates collected in Michigan during 2009. The percentage of isolates with the mutation was calculated based on a total of 124 isolates tested by the *in vitro* germination bioassay as well as the Amplification Refractory Mutation System (ARMS)-SYBR Green Q-PCR developed by Baudoin *et al*. (2008) 133

Figure 4.5. Responses of a (A) trifloxystrobin-sensitive and (B) -resistant *Erysiphe necator* isolate from grapes in Michigan to different concentrations of salicylhydroxamic acid (SHAM; 0, 12, 25, 50, and 100 mg/liter) at different concentrations of trifloxystrobin (0, 0.001, 0.1, and 10 µg/ml)..... 136

Figure 5.1. Experimental layout for the evaluation of downy (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*) effects on juice quality and vine physiology of *Vitis labrusca* L. 'Niagara' and 'Concord', respectively, planted at the Michigan State University Trevor Nichols Research Center in Fennville, MI. The experiment was set up in a randomized complete block design with two factors (disease level and cropping intensity level) and six replicates. Each experimental unit consisted of three consecutive vines in the same row. Fungicide-sprayed and unsprayed vines are shown in green and orange, respectively. The three levels of cropping intensity were H = high (100% of the highest yielding potential), M = medium (67% of the highest yielding potential, and L = low (33% of the highest yielding potential). Guard vines are shown in dark gray..... 155

Figure 5.2. Representation of a grapevine dormant cane to indicate the division into basal (position 2 to 5) and medium (position 6 to 9) nodes for the analysis of cane and bud cold hardiness. The image was modified from the Des Moines Area Community College (DMACC) website 159

Figure 5.3. Rating scale used for the analysis of bud (A-C) and cane (D-F) cold hardiness of Labrusca grapevines. A value of "0" was assigned to buds or canes with no visible injury (A and D), "0.5" to dormant buds with partial damage to primary and secondary buds and canes with partial damage to the cambium (B and E), and "1" to completely damaged buds and canes with generalized tissue necrosis (C and F). White arrows indicate browning of the tissues in buds and canes..... 160

Figure 5.4. Overall disease severity (percentage of the leaf area infected) of (A) downy mildew in 'Niagara' grapes and (B) powdery mildew in 'Concord' grapes during the growing seasons of 2008, 2009, and 2010 in Fennville, MI..... 165

CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

For grape growers, the production of high-quality fruit is a continuous challenge. Participation in a highly competitive market requires the development of strategies that allow for this year's profit, but growers also need to consider long-term plant health. Due to the perennial nature of grapevines, weakening of vines due to ineffective horticultural practices or diseases can have detrimental effects in the years to come (20). In grape production, fungal diseases are a major problem (99) but disease incidence and severity can vary greatly from year to year and even from site to site, due to variations in environmental conditions conducive for disease development. In addition, not all grape cultivars have the same disease susceptibility profile, which means that for optimal disease management it is crucial to identify when effective control measures need to be put into. The application of fungicides as a primary management strategy has been employed for years, with a concomitant risk of fungicide resistance development in target fungi (23).

The epidemiology of grape powdery mildew, caused by the ascomycete fungus *Erysiphe necator* Schwein. has been studied extensively (18, 19, 29, 30, 33, 36, 42, 55, 56, 97, 98). In fact, currently there is a popular model available that predicts infection risk for grape growers in California and the Pacific Northwest, which is the University of California (UC) Davis Powdery Mildew Risk Assessment Model (43). In New York, Gadoury and Pearson (1990) established that ascospore release and infection follows periods with at least 2.5 mm of rain at a minimum temperature of 10^{o}C (30, 31, 34). However, the applicability of such forecasting models has

never been evaluated under Michigan weather conditions. Hence, the first and second objectives of this study were to determine the environmental factors that initiate primary inoculum release of powdery mildew in the spring and production of overwintering inoculum at the end of the grape growing season. By determining the conditions under which ascospores are released and chasmothecia are produced, we will be able to improve disease forecasting and management in Michigan. This has the potential to improve fungicide timing. By doing so, growers will be less prone to apply unnecessary fungicide sprays that not only have a negative impact on the environment but also increase the chance of fungicide resistance development in the field.

Fungicide resistance in fungal plant pathogens is a well-documented phenomenon (23). The grape powdery mildew pathogen, *E. necator*, has reportedly developed resistance to quinone outside inhibitors (QoI), demethylation inhibitors, methyl benzimidazole carbamates, and aza-naphthalenes (28). However, only recently failure to control powdery mildew started to be observed by some wine grape growers in Michigan. Development of fungicide resistance has been suspected but not yet proven in Michigan. For this reason, the third objective of this study was to evaluate the sensitivity of a representative population of powdery mildew in Michigan to a commonly used fungicide of the QoI class. When disease control measures fail as a consequence of fungicide resistance, the disease management schemes that growers have implemented would need to be modified to limit the exposure of the pathogen population to that particular fungicide class. The use of new fungicide chemistries and the adoption of cultural disease control practices would also be required.

Economic thresholds for foliar grape diseases are not yet available; therefore, there is a need for quantifying yield and quality losses in order to justify fungicide sprays. Therefore, the fourth objective of this study was to study the effects of foliar powdery and downy mildew on

aspects of the physiology of juice grapes, such as juice composition, vine cold hardiness, and starch accumulation in the canes. By getting these estimates, grape growers will be able to really know how much disease a vineyard can withstand before economic losses occur. The availability of disease damage thresholds will also allow growers to do a more reliable cost-benefit estimation that most likely will lower production costs by reducing the number of fungicide applications needed.

GRAPE PRODUCTION IN MICHIGAN

The grape industry in Michigan has incurred major changes since the 1970's, when the most popular cultivars were Concord, Niagara, and Delaware, and wine production was limited to dessert-type wines (52). Although the former two varieties are still the most commonly grown and are typically used in the juice market, wine grape cultivars have increased in importance. Currently, with 5,666 hectares of grapes planted, the state of Michigan ranks 5th in grape production and 13th in wine production in the United States (65, 95). Most of the grape hectarage is devoted to juice grapes, with about 809 hectares for wine grapes and 20 to 30 hectares for table grapes (Paolo Sabbatini, *personal communication*). Juice grapes are mostly located in the southwest region of the state, specifically Berrien and Van Buren counties. Wine grapes are primarily cultivated in southwest Michigan and in Allegan, Leelanau, and Grand Traverse counties (64).

Currently, three major categories of grapes are grown in Michigan: (i) Labrusca, which are varieties with genetic background of *Vitis labrusca* L. (common cultivars are Concord and Niagara); most of the fruit is used for making grape juice but a small percentage (3%) is still used for wine making and 'Concord' is also locally consumed as table grapes; (ii) Vinifera,

which are derived from *V. vinifera* L., which are native to the Middle East and Europe; 58% of Michigan's wine is made from these varieties; and (iii) hybrid varieties, which have genetic backgrounds from *V. vinifera* and one or more species native to North America, such as *V. labrusca* L., *V. riparia* Michx., *V. rupestris* Scheele, *V. aestivalis* Michx., *V. lincecumii* Buckley, or *V. longii* Prince, and are also used for wine production (52). Overall, total Michigan grape production in 2009 was 86,161 metric tons. Of these, 40,536 metric tons were 'Concord' and 24,554 metric tons were 'Niagara'. A total of 65,000 metric tons were utilized for juice production, 3,839 metric tons for wine production, and 1,161 metric tons for the fresh market. The estimated value of the production used in processed and fresh markets was $27,586 million (95).

OPTIMIZING GRAPE PRODUCTION

In any kind of crop production scenario, there is always the need to optimize yield and quality to achieve economic sustainability in a highly competitive market. Growers need to carefully manage the crop by adjusting nutrition and irrigation as needed and employing disease and pest management programs (89).

The concept of *vine balance* is the most important aspect of successful grape production. In order to obtain maximize grape yield and berry quality, growers need to optimize fruit production (reproductive growth) without detrimental effects on vegetative growth (51). Vine balance can be achieved by controlling the vine's growth with practices such as dormant pruning to reduce the number of buds to avoid overcropping, which can result in inadequate fruit ripening and reduced juice quality; and canopy management to minimize shading and increase the photosynthetic rate of the vines (89). The ratio of crop yield to pruning weight for a specific

vine, properly referred to as crop load, is widely used as a measure of vine balance, and ratios between 5 and 10 are usually considered most advantageous for high-quality grape production (12, 66). Likewise, the relationship between leaf area and crop weight is used as an indicator of a vine's potential to produce fully-ripened fruit; values of 0.7 to 1.4 m^2 of leaf area per kilogram of fruit have been reported as minimal requirements for ripening (51). Cultural practices such as thinning (the removal of clusters from the vine) are also employed by grape growers to adjust those parameters. However, studies showed no significant effect of fruit thinning on sugar content, titratable acidity, and pH unless the vines had a leaf area/crop weight ratio greater than or equal to 1 m^2/kg (26, 60).

DISEASE DAMAGE THRESHOLDS

Diseases are present in vineyards to some degree at all times, but the epidemic characteristics of a disease can drastically change from one year to another, and even from one region to another. Determining when a particular pathogen is more likely to cause severe economic losses is part of the objectives of integrated disease management, with scouting of vineyards being critical for providing information about the diseases that are present and how advanced they are. Chemical control is only justified when the disease epidemic reaches a level that would lead to economic losses. While it is relatively easy to see the relationship between disease on the fruit and direct economic losses, the relationship between foliar disease and corresponding yield loss is more difficult to determine and can be affected by various factors, including timing of disease onset, cultivar resistance or tolerance, physiological status of the vine, interactions with other stressors, and within- and between-plant compensation) (17). Consequently, quantitative estimates of disease levels that cause significant economic damage

are rare. Nevertheless, economic thresholds are critical for making significant advances in integrated disease management.

The concept of *economic threshold* was originally described for insect pests and was defined as "the density at which control measures should be determined to prevent an increasing pest population from reaching the economic injury level". Likewise, the concept of *economic injury level* was defined as "the lowest population density that will cause economic damage" (90). Both concepts are essential when developing a threshold for a specific pest. However, the issue becomes complicated when dealing with plant pathogens because microbial populations are not easy to enumerate and for some diseases, by the time symptoms become visible, economic injury has already occurred (54). Thresholds for pathogens are developed according to the progression of the disease, especially during the initial stages, which is the time when they can be more effectively controlled. However, the tolerance of a host to a specific pathogen needs to be thoroughly investigated under different scenarios because thresholds may vary as a result of differences in climatic conditions and cultural practices. In fact, Zadoks (1985) considered four critical aspects that strongly influence the damage threshold theory. First, the agronomical aspect that takes into account the expected yield of the crop, a factor that fluctuates every year. Second, the phytopathological aspect that identifies limitations of the threshold theory: (i) the fact that it cannot account for the damage caused by multiple pathogens, (ii) that there is a short window for disease monitoring, and (iii) that growers tend to overestimate disease severity. The third aspect focuses on the economics and incorporates market price fluctuations into the grower's management decision schemes. Lastly, the sociological aspect since growers tend to have different approaches for disease management based on their own experience (or lack of accurate information), national restrictions for fungicide usage, and even cultural origins and

socioeconomic status (104). Regardless of the limitations of disease thresholds, determining the direct effects of plant diseases on yield quantity and quality is necessary for well-informed cost/benefit estimations and form the basis of an integrated disease management program.

Rabbinge *et al*. (1985) studied the quantitative effects of powdery mildew on wheat and even though as little as 4% of leaf area covered significantly decreased the photosynthesis and transpiration rate, they failed to establish a single damage threshold because it depended on the location of the pathogen (low versus high leaf layers) and the crop development stage (82). McGrath (1996) however, was able to develop a disease threshold-based fungicide program for the management of powdery mildew in pumpkins. This program initiates fungicide applications of triadimefon and chlorothalonil as soon as one of 50 mature leaves have symptoms, and continues applications every 14 or 7 days, respectively (72). Another example of success in which the application of fungicides begins soon after a certain disease level is reached is the control of Botrytis leaf blight of onions; by initiating sprays as soon as an average of one lesion per 10 leaves is detected, the number of fungicide applications was reduced by 12-38% (88). In South Carolina, the integrated pest management program for early blight of tomato uses a threshold of 3 to 6% diseased leaf area to begin fungicide applications, however, many growers prefer to apply a protectant fungicide (59). With the development of the disease forecasting models BLITECAST, TOM-CAST and, later on, SIM-CAST (a modification of BLITECAST that includes cultivar resistance variability) growers have obtained robust tools that help them reduce the number of fungicide applications, not only for potato and tomato but also for a variety of other vegetable crops (10, 11, 59). Depending on the pathogen, most plants are able to tolerate a certain level of disease pressure without affecting crop productivity. For instance, *V*. *vinifera* 'Merlot' grapevines have the ability to compensate for the loss of photosynthetic tissue by

reallocating carbohydrate reserves (mainly starch) from woody tissues (e.g., roots, trunks, and shoots) in response to damage caused by the obligate oomycete *Plasmopara viticola* (57). Mansfield and Howell (1981) imposed a 50% defoliation treatment to *V. labrusca* 'Concord' vines by either removing all the leaves from one side of the bilateral cordon or removing all leaves on alternate shoots of the vine. Remarkably, the control vines defoliated to 100% and the vines with one defoliated cordon were still able to increase the soluble solids content from veraison to harvest by 57 and 29%, respectively, by mobilizing carbohydrates from other vegetative tissues (71).

DISEASES OF GRAPES

A wide range of microbial pathogens (fungi, bacteria, viruses, and nematodes), insect pests, and abiotic conditions can detrimentally affect the grape crop given the right environmental conditions. For the most part, reductions in yield, fruit quality, and even vine longevity are associated with fungal pathogens. Among these, powdery and downy mildew are still considered as the most important grape diseases worldwide, particularly because the most desirable wine grape varieties are highly susceptible to both diseases (80).

The grape powdery mildew.

Powdery mildew is the most destructive and widely distributed disease of grapes worldwide (14). It was first detected in North America in 1834 and, after being introduced into Europe, was first reported there in 1845 (80). The causal agent is the ascomycete *Erysiphe necator* Schwein. (syn. *Uncinula necator* [Schwein.] Burrill, order Erysiphales), which can be found in all grape-growing regions, causing severe reductions in yield, fruit quality and vine vigor, especially when weather conditions are dry and warm (58, 80). *Erysiphe necator* is a

biotrophic pathogen that can infect any type of grape tissue, colonizing the host epidermal cells where it develops a specialized nutrient-uptake structure called a haustorium (47). The grayish to whitish powdery appearance of the fungal colonies on leaves (Fig. 1.1A) and berries (Fig. 1.1B) give the disease its name.

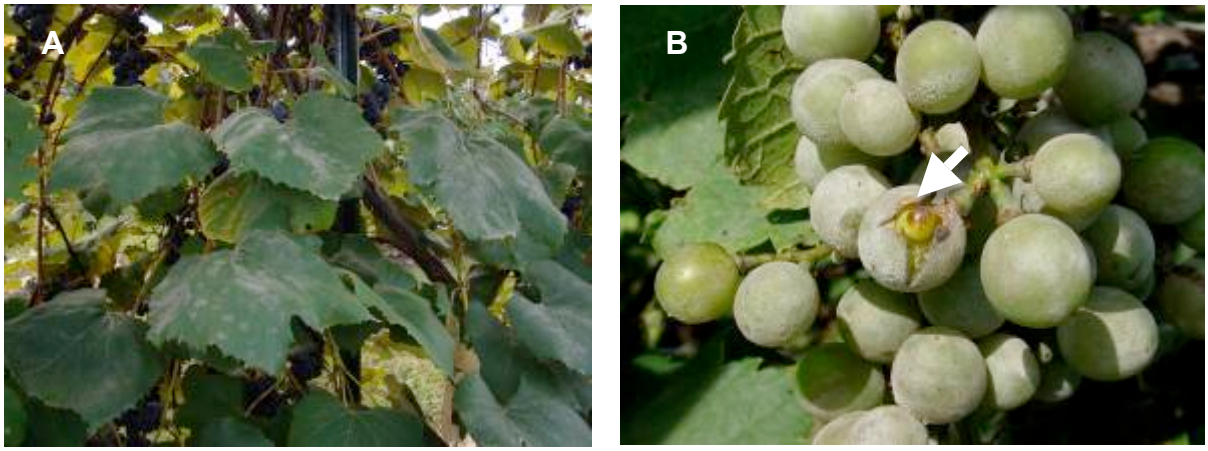

Figure 1.1 Sporulating colonies of *Eryshipe necator* on (A) 'Concord' grape leaves and (B) a 'Chardonel' grape cluster, which also shows the split-berry symptom (white arrow). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

The fungus causes indirect yield and quality losses by reducing the photosynthetic activity of the vine (67, 74). In an investigation on the effects of powdery mildew on photosynthesis, Lakso *et al*. (1982) found that the photosynthetic rate was reduced more in 'White Riesling' than in 'Concord' leaves. The authors also established that a visual estimation of powdery mildew infection does not correlate well with the effects on the physiology of 'Concord' leaves; their research demonstrated that 50% leaf disease severity corresponded to only 8% reduction in net photosynthesis (67). Moriondo *et al*. (2005) observed significant differences in the assimilation, stomatal conductance, and transpiration of healthy portions of leaves infected by *E. necator*. The authors determined that powdery mildew not only reduces the functional green leaf area but also reduces the functionality of the remaining healthy parts of the infected leaves, generating what they defined as a virtual lesion in the affected leaves (74).

Additionally, *E. necator* causes direct effects on yield if the infection occurs prior to fruit maturation; the skin of the berries can become necrotic, preventing the epidermis from expanding, and the actively growing flesh causes the fruit to crack (Fig. 1.1B) (58, 80). Within grape varieties, there is a wide range of susceptibility to powdery mildew, from highly susceptible to resistant. Some of the most desirable wine grape varieties (*V. vinifera* L.) are susceptible, such as 'Chardonnay', 'Riesling', and 'Cabernet Sauvignon', while cultivars of the species *V. labrusca* tend to be more resistant (24, 35). Wine quality is significantly reduced in terms of taste and aroma even when as little as 1 to 5% of the berries in a cluster are infected, due to an increased concentration of total phenolics, hydroxycinnamates and flavonoids, and an enhanced viscous/oily texture (14, 92, 93). Powdery mildew has also been reported to reduce sugar accumulation in berries, juice color and acidity as well as wood maturity in 'Concord' grapes (35).

During the growing season, the anamorphic stage of *E. necator* (*Oidium tuckeri* Berk.) produces basipetal chains of airborne conidia (Fig. 1.2A) that cause secondary infections. At the end of the growing season, the fungus produces a resistant overwintering ascocarp, known as a chasmothecium (formerly cleistothecium, Fig. 1.2B), to withstand harsh environmental conditions and assure its viability the following growing season (18, 79). Since *E. necator* is a heterothallic fungus, the initiation of chasmothecia occurs only after compatible mating types (namely + and -) cross. During the growing season, disease incidence is positively correlated with ascocarp number in any given vineyard (32). Chasmothecium development has been studied under laboratory conditions to determine the effects of environmental factors and host biology (29). These studies demonstrated that temperatures below 10^{o}C hinder ascocarp maturation, and below 8^{o}C, ascocarp initiation.

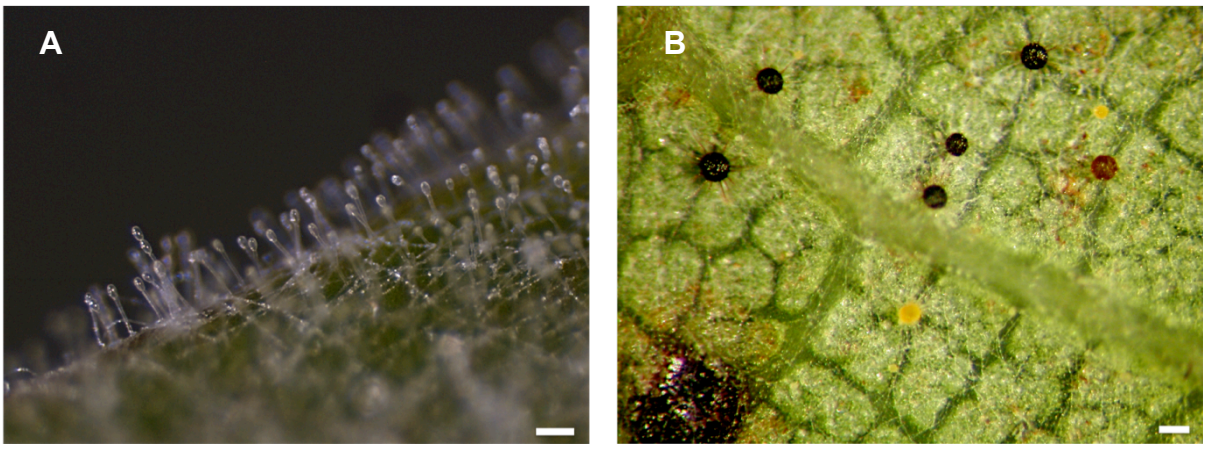

Figure 1.2. Asexual and sexual stages of *Eryshipe necator*. (A) Conidial chains on a 'Chardonnay' grape leaf (bar = 50 µm). (B) Mature (black) and immature (light brown and yellow) chasmothecia developing on the abaxial surface of a 'Chardonel' grape leaf (bar = 100 µm).

In addition, host susceptibility also influences the time required for ascocarp maturation, which is longer in less susceptible varieties (29). When chasmothecia are mature, they are readily detached from the leaves and are dispersed by rain to the vineyard soil or to the bark of the vines. Attachment of chasmothecia to the vine's trunk is assisted by appendages that act as hooks (29, 40, 50). Gadoury and Pearson (1988) showed that chasmothecia that remained attached to leaf tissues had not fully matured and were unable to survive the winter in New York, but those that were found in the bark crevices were all viable. However, in Australia, Italy, and eastern Washington, chasmothecia seem capable of surviving on senescent leaves on the vineyard soil (18, 42). Perennation of the pathogen can also occur as mycelium in dormant buds, especially of highly susceptible grape varieties grown in locations with mild winters (80, 87).

Although the conidial stage of *E. necator* is regarded as xerophytic and free water has been demonstrated to have deleterious effects on powdery mildew epidemics (30, 31), several studies have shown that wet periods (i.e. rainfall or leaf wetness) and temperatures above 4^{o}C are necessary for *E. necator* ascospore discharge. Laboratory studies on chasmothecium dehiscence revealed that as they mature, the strength of the ascocarp wall and the cytoplasmic water potential decrease so that, when in contact with water, the force produced by the water potential difference would result in the swelling of the ascocarp and its subsequent breaking (in the form of a chasm) (30). In the field, the same authors correlated a minimum precipitation of 2.5 mm and a minimum temperature of 4^{o}C with ascospore release. In New York state, this process is only initiated in spring when host tissues become available, with most of ascospores being released between bud break and bloom of grapevines (29, 79). In fact, ascospores that have not overwintered were rarely released (30, 31, 79). However, in Australia and Italy, the chasmothecia of *E. necator* were also able to mature and release ascospores at the end of the

growing season (i.e. fall and winter), probably due to a greater accumulation of degree-days and a milder winter season, both circumstances that allow ascocarp maturation at a faster rate (36, 86).

Primary infection occurs after bud break when mycelia overwintering in dormant buds become active or chasmothecium-released ascospores germinate on green tissues. Once released, ascospores are wind-dispersed to newly emerging leaves and other plant parts. Ascospores germinate within 24 h between 5^{o}C and 31^{o}C at 100% relative humidity (RH) (vapor pressure deficit [VPD] of 0 Pa) and are able to develop appressoria between 10^{o}C and 25^{o}C (31). From these experiments, Gadoury and Pearson (1990) suggest that ascosporic infection is likely to follow release if VPD is not greater than 1453 Pa (53% RH at 25^{o}C) and temperatures are not lower than 10^{o}C or higher than 31^{o}C. Under favorable conditions, shoots emerging from infected buds can be heavily covered with sporulating colonies (a symptom known as flag shoots). However, whitish colonies are more frequently seen and often develop first on the abaxial surface of leaves developing near the trunk of the vines, the place where chasmothecia overwinter. (80). Secondary infection occurs when conidia produced on these colonies are dispersed to other grape tissues where new infections will develop. Carroll and Wilcox (2003) determined that powdery mildew incidence and severity increase as relative humidity increases, reaching an optimum at 84% RH; however, disease development declined at higher RH values due to a sharp decrease in conidial germination (16). As a result of infection, young tissues can become distorted and stunted (80). Infection by overwintering mycelium and ascospores can be differentiated because different patterns of disease development are observed in vineyards. Ascosporic infections are randomly distributed throughout the vineyard whereas hot spots of

disease are created around flag shoots because they are concentrated sources of conidial inoculum (46, 103).

Although susceptibility to powdery mildew varies by cultivar, control of this disease mostly relies on the application of sulfur formulations, synthetic fungicides or combinations of both (80). Among synthetic fungicides, quinone outside inhibitors (QoI, also known as strobilurins) and sterol demethylation inhibitors (DMI) are widely used, and spray programs often commence as soon as grape shoots are 2.5 to 12.5 centimeters long (100). However, due to the single-site mode of action of many systemic fungicides, development of resistance remains a continuous concern.

The grape downy mildew.

Downy mildew, caused by the heterothallic oomycete *Plasmopara viticola* (Berk. et M. A. Curtis) Berl. et De Toni, is also a major disease of grapevines (80). It is responsible for substantial economic losses around the world, especially in warm and humid viticultural regions, where severe infections result directly in the destruction of young berry clusters, or indirectly when they reduce the photosynthetic rate by causing premature defoliation of the vines (13, 80). *Plasmopara viticola* is native to North America and was introduced into Europe in 1878 through the importation of native American grape rootstocks resistant to phylloxera (*Viteus vitifoliae* [Finch], grape lice) (41). As in the case of powdery mildew, *V. vinifera* cultivars are also the most susceptible to this disease (80). However, cultivar studies have shown that even within a grape species there is variation in susceptibility to *P. viticola*, depending on leaf age and pathogen genotype (13).

In the spring, when the soil is wet and temperatures reach 11^{o}C, the oospores (the sole source of primary inoculum) that have overwintered in infected leaves on the vineyard soil,

germinate and produce sporangia that contain zoospores; these are rain-splash dispersed to susceptible grape tissues and constitute the primary inoculum (80). When in contact with the host, zoospores position their ventral surface towards the plant surface and then encyst by detaching their flagella and secreting an adhesive matrix (48, 49). At 24 hours post infection (hpi), encystment of the released zoospores is observed on the stomata, which the pathogen penetrates with a germ tube (1). In susceptible cultivars and under optimal conditions (22 to 24°C), *P. viticola* becomes established in the substomatal cavity within 3.5 hpi (68, 94). The pathogen then grows intercellularly and produces intracellular haustoria for nutrient uptake (3).

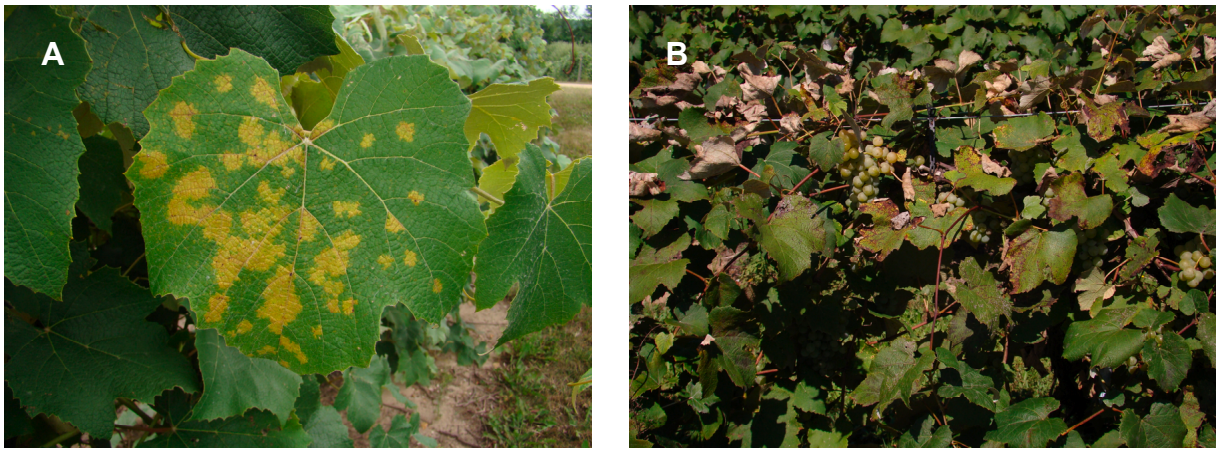

Figure 1.3. Downy mildew symptoms on 'Niagara' grapes. (A) Chlorotic, coalescing lesions on leaves. (B) Dying and shriveling downy mildew-infected leaves in top of canopy.

First lesions can appear on leaves as early as 5 days after infection, usually appearing as chlorotic spots (Fig. 1.3A), but these can also be red, depending on the age of the tissues and the grape variety. Primary infections are often called "oil spots" because of their greasy appearance (80). Sporangia are produced through the stomata on the underside of the leaves when relative humidity is above 90% and temperatures are between 18^{o}C and 22^{o}C. At least 4 hours of darkness are required for sporangium production (76, 84). Sporangia are dispersed by water or wind to new tissues. Primary infections are then followed by repeated cycles of asexual sporulation events, which continue as long as the required conditions are met (85). As the disease develops, the spots coalesce (Fig. 1.3A), turn into necrotic lesions, and whole leaves become compromised due to progressive tissue death (Fig. 1.3B). A single lesion can sporulate multiple times, but Kennelly *et al*. (2007) found that the longevity and productivity (number of sporangia per lesion) decrease as repeated cycles of sporulation occur, whereas under non-optimal conditions lesions remain viable and are able to sporulate for at least 22 to 24 days (61, 62). The disease can progress very rapidly, leading to premature defoliation and nearly total destruction of the crop (6). As the end of the growing season approaches, sexual reproduction takes place in infected leaves when two mating types (A1 and A2) fuse. The gametes from an antheridium and corresponding oogonium produce a thick-walled oospore (3, 80).

Control of downy mildew mostly relies on the application of protectant and systemic fungicides, which sometimes is coupled to disease prediction models, such as PLASMO and VINEMILD (both developed and validated in Italy) or DMCAST (available for use by Cornell University in New York state), to improve the timing of fungicide sprays (7, 76, 77, 84). In Ohio, fungicide applications begin as soon as flowers are fully developed (Eichhorn-Lorenz growth stage 17) when dealing with highly susceptible grape cultivars (70). In Michigan, fungicide

applications of copper formulations and systemic fungicides (e.g., quinone outside inhibitors, aza-naphthalenes [quinolines and quinazolinones], phthalimides, dithiocarbamates, and succinate dehydrogenase inhibitors) are recommended when shoots are 15 to 30 cm long (Eichhorn-Lorenz growth stage 12) and can continue after veraison, depending on the weather conditions and disease pressure. However, the pathogen has an incredible capacity to overcome the action of many commonly applied fungicide chemistries and now is considered a high-risk pathogen under the Fungicide Resistance Action Committee (FRAC) classification for likelihood of resistance evolution (39). Consequently, growing grape cultivars with more resistance to downy mildew would complement the aforementioned control strategies (13).

FUNGICIDE RESISTANCE

Fungicide resistance usually results from a natural mutation in a fungus, which results in reduced sensitivity to the fungicide in question. The term *fungicide resistance* may be used for different scenarios and researchers should be clear when referring to a certain type of resistance: (i) emergence of resistance *in vitro*, (ii) reduction of fungicide sensitivity in the field but chemical control is still effective, and (iii) field resistance, which implies a lack of disease control (22).

The agricultural industry began facing the problem of fungicide resistance in the 1970s when the rapid emergence of resistant strains to widely used fungicides of the pyrimidine and benzimidazole classes made traditional control strategies obsolete (15). Since then, fungicide failure due to resistance has been documented for almost half of the known chemical classes and over 100 diseases. Grape pathogens that have developed field resistance to fungicides include the causal agents of powdery mildew (*E. necator*), downy mildew (*P. viticola*), and gray mold

(*Botrytis cinerea* Pers.) (21). The specific mode of action of systemic fungicides and frequent field applications, along with the target pathogens' copious sporulation capacity, all favor selection of less-sensitive populations (15).

For the grape industry, a wide variety of protectant and systemic fungicides are available. The first report of resistance development in grape pathogens was published in 1977 when management of grape powdery mildew with benzimidazole systemic fungicides (e.g., benomyl, FRAC group 1) began to fail (75). Later, cases of resistance in grape powdery mildew to sterol 14α-demethylation inhibitor (DMI) fungicides (FRAC group 3) were also documented. Resistance to quinone outside inhibitor (QoI) fungicides (FRAC group 11) has been reported for both powdery and downy mildew in grape (5, 101). *Plasmopara viticola* has developed resistance to phenylamides (FRAC group 4), cyanoacetamide oximes (FRAC group 27), phosphonates (FRAC group 33), and carboxylic acid amides (FRAC group 40) (Table 1.1) (27, 28).

Table 1.1. List of global reports of fungicide resistance in the grapevine pathogens *Erysiphe necator* and *Plasmopara viticola* according to the specific active ingredients evaluated. Fungicide classes and the Fungicide Resistance Action Committee (FRAC) codes are also specified. Unless denoted, all correspond to field resistance reports.

Grape pathogen	**Fungicide**			**Country**	**Year (reference)**
	Common name	***Class***	***FRAC code***		
Erysiphe necator	Benomyl	Methyl benzimidazole carbamates	1	Romania USA	1977 (75) 1980 (78, 81)
	Triadimenol	Demethylation inhibitors	3	Portugal	1990 (91)
	Triadimefon Myclobutanil Penconazole Pyrifenox Triflumizole			Austria	1996 (83)
	Triadimefon			USA	2003 (73)
	Azoxystrobin Pyraclostrobin	Quinone outside inhibitors	11	USA	2003 (96)
	Proquinazid	Aza-naphthalenes	13	Europe	2009 (37)[a]

Table 1.1. (cont'd)

Plasmopara viticola	Metalaxyl	Phenylamides	4	France Switzerland	1981(69) 1983 (9)
	Azoxystrobin	Quinone outside inhibitors	11	Italy	2004 (44)
	Cymoxanil	Cyanoacetamide oximes	27	Italy	1997 (45)
	Fosetyl AL	Phosphonates	33	India	2003 (63)
	Mandipropamid Provalicarb Benthiavalicarb Dimethomorph Flumorph	Carboxylic acid amides	40	Europe	2007 (39)[b]
	Mandipropamid			Europe	2010 (8)[c]

[a] Cross-resistance *in vitro* studies.

[b] In a study on the inheritance of resistance.

[c] In a study on the mechanism of resistance.

Strobilurins.

Quinone outside inhibitor fungicides, commonly known as strobilurins, are derived from a chemical compound (strobilurin A and B) isolated from the basidiomycete *Strobilurus tenacellus* (Pers. ex. Fr.) Singer (2). Strobilurins were first sold in 1996 for agricultural purposes and are still widely used in numerous crops due to their broad-spectrum activity against Ascomycetes, Basidiomycetes, Deuteromycetes, and Oomycetes (4). Strobilurins act by blocking respiration in fungi and Oomycetes; they selectively bind to the ubiquinol oxidation center (Q_o site) of cytochrome b, obstructing the electron transfer to cytochrome c_1, which leads to a cellular energy deficiency due to an insufficient production of adenosine-5'-triphosphate (ATP) (4). For this reason, this class of fungicides has direct effects on spore germination, mycelial growth, and zoospore motility, because all of these require high inputs of energy (4). Due to the site-specific mode of action, target pathogens are prone to developing resistance. In fact, over 30 reports of strobilurin-resistant fungal pathogens have already been published (53). Specifically, Wong and Wilcox (2002) reported for the first time a reduction of sensitivity to azoxystrobin in a population of *E. necator* from a commercial vineyard in New York where a rotation spray program that included azoxystrobin (strobilurin) and myclobutanil (DMI) had been implemented (101).

The genetic basis of this type of fungicide resistance apparently lies in single-site mutations of the cytochrome b (*CYTB*) gene. The substitution of glycine with alanine at position 143 (mutation G143A), the change from phenylalanine to leucine at position 129 (mutation F129L), and the change from glycine to arginine at position 137 (mutation G137R) have all been correlated with strobilurin resistance in many plant pathogens (38, 53). However, the G143A mutation has been found consistently in highly resistant fungal isolates, whereas the other two

are present in less resistant isolates (25). This mutation has also been described for *E. necator* (5). However, resistance to strobilurins is not always due to mutations of the *CYTB* gene. When electron flow from ubiquinol to cytochrome bc_1 is blocked, fungi are able to use an alternative oxidase that accepts electrons directly from ubiquinol; this alternative oxidation pathway overcomes the electron blockage and allows for oxygen uptake (102). To rule out this mechanism of resistance, salicylhydroxamic acid (SHAM), a specific inhibitor of this pathway, must be added to any *in vitro* assays designed to test for fungicide resistance (102).

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CHAPTER 2

ASCOSPORE RELEASE BY *ERYSIPHE NECATOR* IN RELATION TO ENVIRONMENTAL CONDITIONS AND DISEASE DEVELOPMENT IN GRAPES IN MICHIGAN

ABSTRACT

The airborne ascospore concentration of *Erysiphe necator* was monitored during 2009 and 2010 in two vineyards in Michigan to verify the minimal environmental requirements for ascospore release (2.5 mm of rainfall at a temperature of 4°C) identified in an earlier study in New York. The aforementioned conditions were met only on a limited number of days. Instead, ascospores were detected on most days, including days with precipitation of less than 2.5 mm or a wetting period not initiated by rain, and even on days with 0 h of leaf wetness. Total daily ascospore counts were not correlated with the daily amount of rainfall, average temperature, average leaf wetness, or average vapor pressure deficit. However, a positive correlation with growing degree-days was detected. Also, the cumulative percentage of ascospores was described well by a Gompertz equation when expressed in terms of growing degree-days (calculated from 1 April, base 4°C). Ascospore release was almost complete (about 90%) by 600 GDD, which occurred around bloom of grapevines in our study sites. This information will be useful for the development of a regional forecasting model that will help growers decide when to initiate a fungicide program to prevent primary infections.

INTRODUCTION

Powdery mildew of grapes is caused by the ascomycete fungus *Erysiphe necator* Schwein. (formerly *Uncinula necator* [Schwein.] Burr.) and is a worldwide problem in grape production (17). Powdery mildew was first described in North America in 1834, but it only became a widespread and devastating problem after the fungal pathogen was introduced into European vineyards planted to *Vitis vinifera* L. cultivars that are highly susceptible to *E. necator* infection in 1845 (10, 20). Understanding the epidemiology of the disease is critical for the development of appropriate management strategies. In cold-climate regions, *E. necator* overwinters as chasmothecia (formerly called cleistothecia) in cracks of the bark of grapevines. Chasmothecia break open and release ascospores upon contact with free water in the spring (6). In regions with mild winters, the fungus is also able to survive as mycelium in infected dormant buds, which give rise to infected shoots called "flag shoots" in the spring (3, 19, 22). In Michigan, flags shoots have never been observed and chasmothecia are assumed to be the sole source of primary inoculum as in New York (18) and eastern Washington (11).

In the field, ascospore release has been associated with the occurrence of more than 2.5 mm of rainfall or after a leaf wetness period initiated by rain, at temperatures above 4^{o}C between the grapevine growth stages of bud break and bloom (6, 18). Although Gadoury and Pearson (1990) found a significant decline in the number of dehisced chasmothecia at 4^{o}C, about 4% to 10% still released ascospores at that temperature, depending on the length of the wetting period. Upon increasing the temperature from 10^{o}C to 32^{o}C, the number of chasmothecia that dehisced after 2 h of wetting increased from 7% to 20%, respectively (6). Jailloux *et al*. (1998) found that, under controlled conditions, fully developed chasmothecia maintained at either 5^{o}C or 10^{o}C

were equally able to release ascospores if subjected to an alternating dry-wet period for 110 days (with wetting occurring every 14 days). However, significantly more ascospores were released at 10°C after a dry-wet period of 140 and 170 days. Also, no ascospores were observed when chasmothecia were not wetted (12). The authors also suggest that, under natural conditions, chasmothecium dehiscence occurs during dry spells after a period of rain. Once released, ascospores are wind-dispersed to newly emerging leaves and other plant parts. Ascospores germinate within 24 h between 5°C and 31°C at 100% relative humidity (vapor pressure deficit [VPD] of 0 Pa) and are able to develop appressoria between 10°C and 25°C (7). From these experiments, Gadoury and Pearson (1990) suggest that ascosporic infection is likely to follow release if VPD is not greater than 1453 Pa (53% RH at 25°C) or temperatures are not lower than 10°C or higher than 31°C. Also, in a study by Grove (2004) in eastern Washington, the author found that ascospore release only occurs on days with rainfall of 3.9 to 9.7 mm between the grapevine phenophases of bud break and immediate postbloom (Eichhorn-Lorenz stages 5 to 27). However, in that study, no specific temperature requirements were indicated (11).

Even though the specific conditions for primary inoculum discharge and infection have been described, grape growers still experience difficulty in controlling this fungal pathogen. Currently, with 5,666 hectares of grapes planted, the state of Michigan ranks 5th in grape production and 13th in wine production in the United States (14, 23). The majority of grapes in Michigan are produced for juice, with about 809 hectares for wine grapes and 20 to 30 hectares for table grapes (Paolo Sabbatini, *personal communication*). Most of the grape hectarage is located in the southwest and northwest regions of Michigan's lower peninsula, along the shore of Lake Michigan. Control of grape powdery mildew in Michigan mainly relies on the application

of fungicides throughout the growing season (20). Despite a range of fungicides being registered for powdery mildew control, powdery mildew control is variable, either due to poor fungicide timing or coverage or to a reduction in fungicide sensitivity of powdery mildew populations (1).

The onset of powdery mildew epidemics in grapes varies greatly from year to year. While most grape growers take a preventative approach to powdery mildew control, sometimes unexpectedly severe fruit infections incur economic losses. Once the disease has gained a foothold in a vineyard it becomes very difficult to control, particularly on the fruit. Considering that the environmental effects on chasmothecium dehiscence have been demonstrated elsewhere (6, 11), the aim of this research was to determine the timing of ascospore release in relation to Michigan weather conditions in order to validate previous findings. In Michigan, and other grape-growing regions where the weather conditions do not allow the fungus to overwinter in dormant buds, the control of ascosporic infection is crucial to delay the epidemic development of the disease. Once the pathogen successfully infects its host and switches to asexual reproduction, its ability to produce large amounts of secondary inoculum makes it more difficult to control. Therefore, determining when *E. necator* inoculum is available will be useful in predicting the risk of primary infection. This information will aid growers in optimizing fungicide application timing, thereby reducing the number of necessary sprays and improving disease control efficacy.

MATERIALS AND METHODS

Ascospore sampling.

A Burkard 7-day volumetric spore trap (Burkard Manufacturing Ltd., Rickmansworth, Hertfordshire, UK) was placed in established research vineyards of Michigan State University (MSU) in 2009 and 2010. One vineyard of *Vitis* interspecific hybrid 'Chardonel' was located at the Clarksville Research Center (CRC, 42° 52' 18'' N, 85° 15' 31'' W) in Clarksville, MI. The other vineyard of mixed *V. vinifera* cultivars was located at the Northwest Michigan Horticultural Research Center (NWMHRC, 44° 52' 59'' N, 85° 40' 28'' W) in Traverse City, MI. In 2009, the Burkard spore traps were placed in the vineyards at bud swell (7 May in Clarksville and 29 May in Traverse City.

The spore traps were placed between untreated rows with the sampling orifice at a height of 60 cm. The airflow was adjusted to sample 10 liters of air per minute and was calibrated every week when the tapes were changed. Tapes were placed in the trap drum and were coated with a thin layer of a gelvatol mixture (gelvatol 35 g [Burkard Manufacturing Ltd., Rickmansworth, UK], phenol 2 g, glycerol 50 ml, and distilled water 100 ml) that was allowed to dry before a thin layer of petroleum jelly was placed. Every tape was changed after a 7-day sampling period, cut into 48-mm segments corresponding to each 24-hour period, and mounted on glass slides with 0.05% cotton blue in lactophenol. The slides were examined using a compound light microscope, and ascospore numbers were recorded after identification at 400× magnification based on morphology. Counts were converted to numbers of ascospores per cubic meter of air sampled per hour. Daily ascospore concentrations were calculated as the sum of 24 hourly sampling periods.

In 2010, ascospore release monitoring was initiated several days before bud burst in Clarksville (15 April to 1 May) and Traverse City (28 April to 7 May). Airborne ascospore

concentration was monitored until fruit set (Clarksville: 16 July 2009 and 16 June in 2010; Traverse City: 2 July 2009 and 6 July in 2010), a time when the first signs of powdery mildew infection had started to be noticed in field-grown grapevines. In Clarksville, ascospores were trapped from 7 May until 16 July in 2009 (trapping period of 71 days) and 15 April until 16 June in 2010 (trapping period of 62 days). In 2010, ascospore concentrations could not be monitored at Clarksville during the following days (time interval in hours) due to malfunctioning of the spore trap: 27 May (0000 to 1000 h), 30 May (0000 to 2300 h), 2 June (1400 to 2300 h), 3 June (0000 to 1000 h), 9 June (1000 to 2300 h), and 10 June (0000 to 0900 h). In Traverse City, sampling periods started on 29 May and finished on 2 July in 2009 (trapping period of 35 days) and 28 May finishing on 6 July in 2010 (trapping period of 70 days). Malfunctioning of the spore trap did not allow data collection in Traverse City in 2010 on 3 May (1500 to 2300 h) and 27 June (0000 to 2300 h).

Environmental data.

Weather stations (Campbell Scientific, Inc., Logan, UT, USA) from the Enviro-weather Automated Weather Station Network (Michigan State University, East Lansing, MI, USA, http://www.agweather.geo.msu.edu/mawn) located on-site at the CRC and NWMHRC within 200 m from the Burkard spore traps were used to collect detailed weather data (temperature, rainfall, relative humidity, and leaf wetness) every 5 minutes. Vapor pressure deficit (VPD) data were calculated per hour from air temperature and relative humidity. Daily means were calculated for air temperature, VPD, and leaf wetness, and daily totals were calculated for rainfall. Growing degree-day data for each research site and year were obtained from the Enviro-weather website and were calculated on a daily basis from the beginning of the grape growing season, defined to start on 1 April (24), using the Baskerville-Emin method from the wensite

with a base temperature of 4°C. This temperature was chosen because ascocarp dehiscence is almost nil at this temperature) (6). Both the starting date and base temperature were chosen in order to match host tissue availability with ascospore release, which would allow the formulation of a more stringent ascospore release model. Base temperatures of 0°C and 10°C were also evaluated but the results were not considered further because the best fits were obtained at 4°C.

Bait plants.

Potted, 1-m tall *V. vinifera* L. 'Chardonnay' vines were used as bait plants to assess *E. necator* infection at the CRC 'Chardonel' vineyard during 2009 and 2010. The plants were grown in BACCTO soil (Michigan Peat Company, Houston, TX, USA) from three-node, dormant cuttings obtained from field-established 'Chardonnay' vines. Prior to potting, cuttings were surfaced sterilized by immersion in a 20% solution of sodium hypochlorite (Clorox, Oakland, CA, USA) for 2 min and then washed three times with sterile distilled water for 1 min. Cuttings were allowed to grow in the greenhouse at 25°C, with a photoperiod of 16 h light and 8 h darkness. When plants had rooted and had a 15-cm shoot, they were transplanted into 16-cm diameter pots. In 2010, bait plats were also used at the NWMHRC vineyard to detect ascospore activity. These plants were two-year-old, bare-root, grafted 'Chardonnay' vines (Double A vineyards Inc., Fredonia, NY, USA) that were planted directly into 16-cm pots containing the above-mentioned potting soil.

Four bait vines were placed near the trunks of untreated vines in four different locations in the vineyard, from before bud break (Eichhorn-Lorenz stage 5) until the first powdery mildew colonies were visible in the field-grown vines. At that point, it was assumed that the production of conidia would confound detection of ascosporic infections. The potted vines were changed

after each 7-day exposure period, transported back to the laboratory, and incubated in a growth chamber at 25^{o}C with a 16/8-hour light/dark cycle to promote the development of powdery mildew colonies. For each of the four replicate bait vines, symptoms were rated as the number of leaves on each plant that showed signs of infection (disease incidence) and the number of colonies per infected leaf (disease severity). For each sampling period, the percentage of infected leaves and the average number of colonies on infected leaves were calculated.

Statistical analysis.

Correlations between daily ascospore concentrations and daily total rainfall (mm), daily average leaf wetness (percentage of the day that the leaf wetness sensor was wet), daily average temperature (^{o}C), daily average vapor pressure deficit (kPa), and growing degree-days (base 4^{o}C calculated from 1 April) were examined using the PROC CORR procedure of SAS (SAS version 9.2, SAS Institute Inc., Cary, NC, USA). The cumulative percentage of ascospores per cubic meter of sampled air was log-transformed to accomplish a linearization of the models according to the accumulation of growing degree-days using the PROC GLM procedure of SAS. Log-transformations were performed according to the monomolecular, Gompertz, and logistic models. Comparisons of the statistical parameters (i.e. coefficient of determination [R^2], mean square error, standard deviation of the intercept and slope, and residual distribution) also included a simple regression model. The program SigmaPlot (version 11.0, Systat Software Inc., San Jose, CA, USA) was used to graph the Gompertz equation in the form $y = a \times \exp(-\exp[-(x-x_0)/b])$ (with 95% confidence intervals) that was the best fit to the cumulative ascospore numbers during each sampling period. Additionally, diurnal patterns in the release of ascospores were investigated using the PROC LOESS procedure of SAS.

RESULTS

Ascospore discharge in spring.

The number of ascospores detected per hour was very low in both research vineyards and years. The ascospore concentration fluctuated between 0 and 8.3 ascospores/m^3/h. In Clarksville, ascospores were detected daily during most of the sampling period. Days when ascospores were not trapped in the Clarksville vineyard were 1 July, 5 July, 7-11 July, and 14-15 July in 2009; and 15 April, 20 May, 30 May, 2 June, 9-10 June, 13-14 June, and 16 June in 2010 (Figs. 2.1A and 2.1B). In Traverse City, ascospores were not trapped on 17 June, 19 June, 23 June, 26-27 June, 30 June, and 1-2 July in 2009 (Fig. 2.1C). A total of 968 and 412 ascospores/m^3 were trapped in Clarksville in 2009 and 2010, respectively. In Traverse City, 162 and 63 ascospores/m^3 were trapped in 2009 and 2010, respectively. Ascospores were detected even before bud break (Eichhorn-Lorenz stage 5), when host tissues were not yet available. In 2010, about 18 and 26% of the total number of ascospores sampled at Clarksville and Traverse City, respectively, were released during this period. It is possible that ascospores were released even before then.

At Clarksville, peaks in ascospore release (> 30 ascospores/m^3/day) occurred on 15 May, 22 May, 25-26 May, 2 June, 14 June, and 16 June in 2009, and 5 May, 16 May, and 29 May in 2010 (Fig. 2.1A and 2.1B). In Traverse City, ascospore peaks occurred on 10 June 2009 (28 ascospores/m^3/day, Fig. 2.2A) and 13 May 2010 (10 ascospores/m^3/day, Fig. 2.2B).

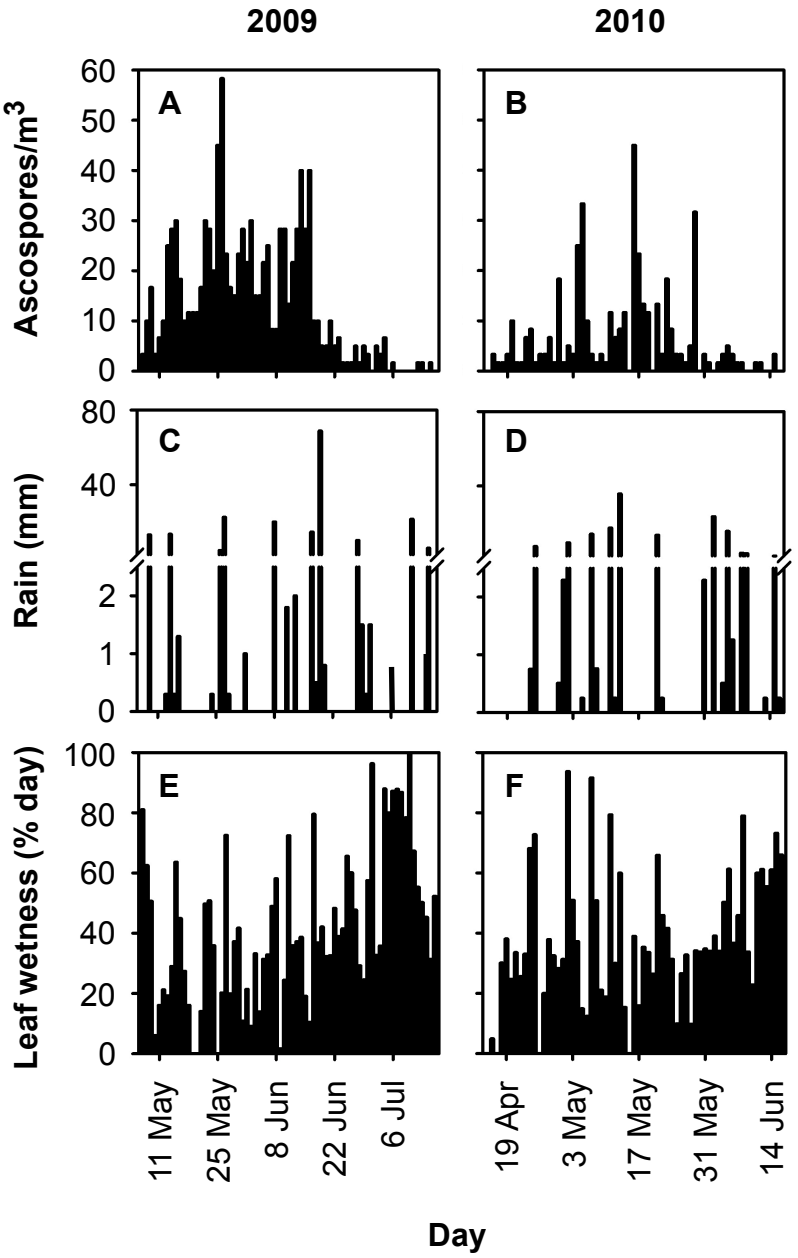

Figure 2.1. Daily concentrations of airborne ascospores of *Erysiphe necator* (ascospores/m^3/day), daily total rainfall (mm/day), and daily average leaf wetness (% of the day) at the Michigan State University research vineyard in Clarksville during 2009 (A, C, and E, respectively) and 2010 (B, D, and F, respectively). Rainfall of more than 2.5 mm/day is noted by the breaks in the y-axis.

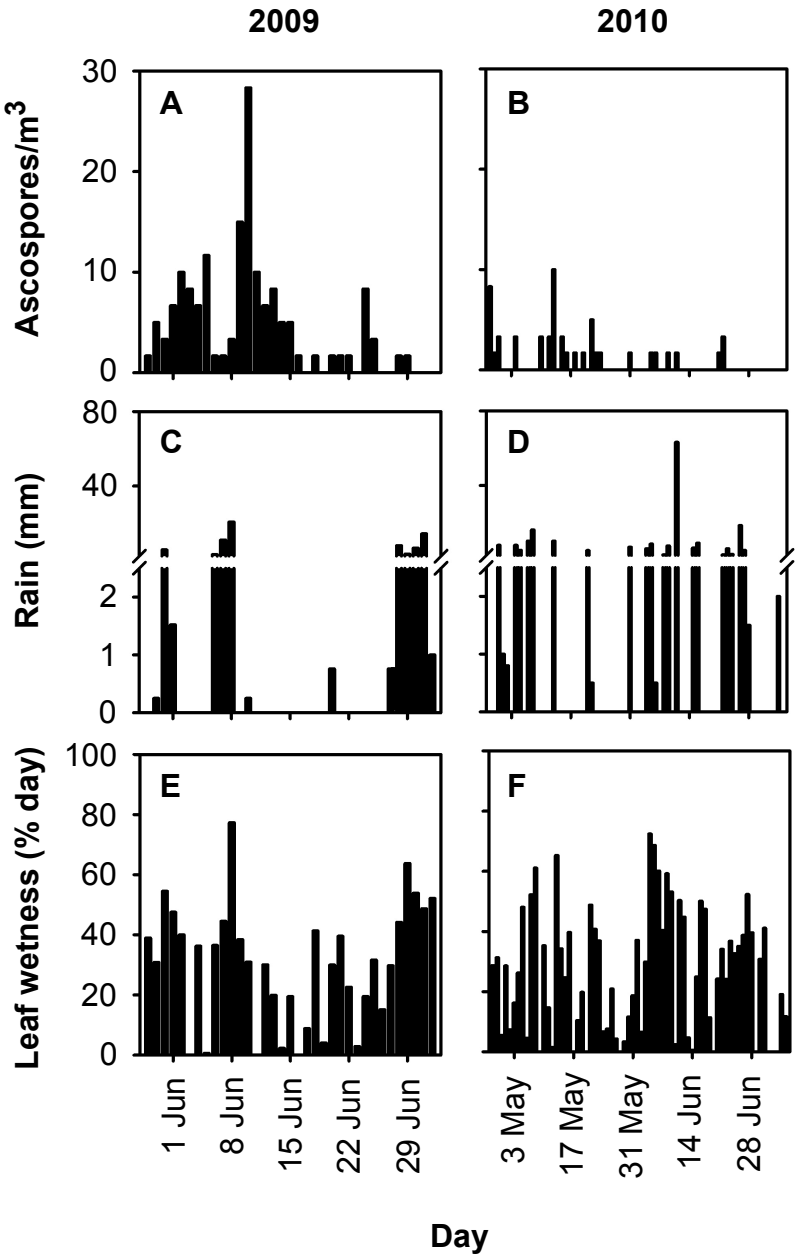

Figure 2.2. Daily concentrations of airborne ascospores of *Erysiphe necator* (ascospores/m^3/day), daily total rainfall (mm/day), and daily average leaf wetness (% of the day) at the Michigan State University research vineyard in Traverse City during 2009 (A, C, and E, respectively) and 2010 (B, D, and F, respectively). Rainfall of more than 2.5 mm/day is noted by the breaks in the y-axis.

According to the grapevine's growth stages, most of the ascospores were trapped between the stages of bud break and first bloom (Eichhorn-Lorenz stages 5 and 21, Fig. 2.3). By first bloom, about 80-90% of the ascospores had been released at each vineyard site (Fig. 2.3). The remaining percentage of detected ascospores was released before bud break and after bloom in both research vineyards. When Burkard spore traps were removed from the vineyards, ascospore release had considerably diminished; ascospores were still detected during some days but in very low numbers (Figs. 2.1A, 2.1B, 2.2A, 2.2B, and 2.3).

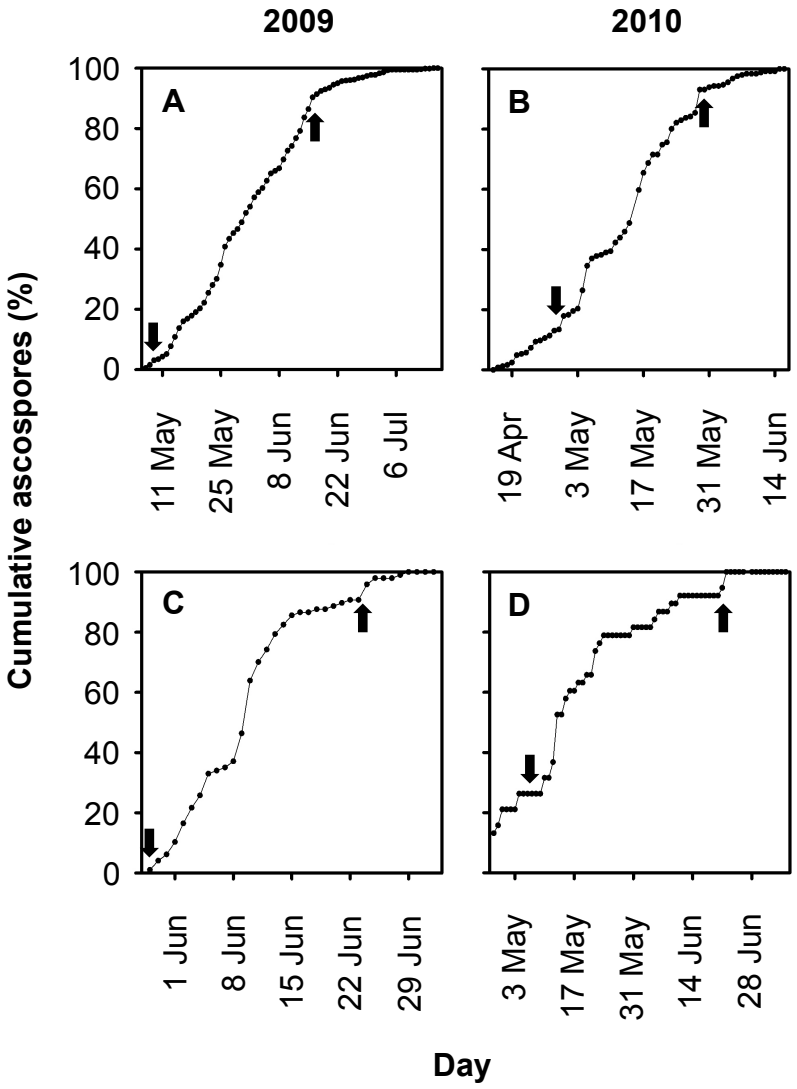

Figure 2.3. Cumulative percentage of ascospores trapped in the research vineyards in Clarksville (A, B) and Traverse City (C, D), MI in 2009 (A, C) and 2010 (B, D). Black, down and up arrows indicate the occurrence of grapevine's bud break (Eichhorn-Lorenz stage 5) and first bloom (Eichhorn-Lorenz stage 21) in the phenology of the grapevine, respectively.

Relationship between weather conditions and airborne ascospore concentration.

In the Pearson correlation analyses, only the growing degree-day (GDD) measurement (calculated from 1 April, base 4^{o}C) was consistently and negatively correlated with daily ascospore concentration in both years and sites, although the correlation was not significant at the Clarksville site in 2010 (Table 2.1). Leaf wetness was negatively correlated with daily ascospore concentration only in Clarksville in 2009. Temperature was also negatively correlated with ascospore concentration in Clarksville in 2009 and Traverse City in 2010. No significant correlations were found with rainfall, relative humidity or vapor pressure deficit (Table 2.1).

Table 2.1. Pearson correlation coefficients (*r*) for linear regressions between daily ascospore concentration and rainfall, leaf wetness, temperature, relative humidity, vapor pressure deficit, and growing degree-days at the Michigan State University research vineyards in Clarksville, MI and Traverse City, MI in 2009 and 2010.

Environmental factors†	Pearson correlation coefficient (*r*)			
	Clarksville		Traverse City	
	2009	*2010*	*2009*	*2010*
Growing-degree days	-0.56725 *	-0.19314 ns	-0.46822 *	-0.36297 *
Leaf wetness	-0.47529 *	-0.08945 ns	-0.13547 ns	0.21150 ns
Rainfall	-0.06254 ns	-0.02789 ns	-0.23061 ns	0.10993 ns
Relative humidity	-0.19851 ns	-0.17495 ns	-0.23188 ns	-0.00176 ns
Temperature	-0.28490 *	0.01024 ns	-0.26048 ns	-0.27404 *
Vapor pressure deficit	-0.03849 ns	0.16105 ns	0.00658 ns	-0.16015 ns

† Environmental factors: Rainfall = daily total rainfall (mm), Leaf wetness = daily average leaf wetness (% of the day), Temperature = daily average temperature (^{o}C), Relative humidity = daily average relative humidity (%), Vapor pressure deficit = daily average vapor pressure deficit (kPa), and Growing-degree days = calculated from 1 April, base 4^{o}C.

* Significant at $P \leq 0.05$; ns = not significant.

In addition, ascospore counts did not reveal any diurnal patterns of release; ascospores were trapped during all hours of a 24-hour period (data not shown). Ascospores were mostly trapped on days with rainfall of more than 2.5 mm or days having a leaf wetness period of more than 2 h. However, it is worth mentioning that several of these leaf wetness periods (Figs. 2.1E, 2.1F, 2.2E, and 2.2F) were not always initiated by precipitation. In Clarksville, rain fell on 23 days of the trapping period during both years (Figs. 2.1C and 2.1D) and in Traverse City on 14 days in 2009 (Fig. 2.2C) and 26 days in 2010 (Fig. 2.2D).

Ascospores were also detected on days with rainfall of less than 2.5 mm and even on dry days (no rain and less than 2 h of leaf wetness). In Clarksville, dry days with ascospore detection occurred on 10 May, 19-20 May, 25 May, and 9 June in 2009; and 16 April, 17 April, and 26 April in 2010. In Traverse City, dry days with ascospore detection were recorded on 3 June, 5 June, 11 June, 14 June, and 16 June in 2009 and 12 May, 20 May, and 24 May in 2010. The total number of ascospores trapped per day on dry days varied between 1.7 and 45 ascospores/m^3 in Clarksville and 1.7 and 11.7 ascospores/m^3 in Traverse City. Dry days with the highest ascospore concentrations occurred at Clarksville on 25 May and 9 June 2009, but several hours of wetness were recorded during the previous day by the weather station (a total of 8 and 13 h, respectively); ascospores started to be detected after 17 and 15 h, respectively, since the last wet period and detection continued for more than 30 h (data not shown). The daily average temperature during all of our sampling periods was always higher than 4^{o}C; no ascospore release patterns were detected in relation to temperature (Table 2.1).

Ascospore accumulation in both vineyards exhibited exponential growth between late bud swell and first bloom (Eichhorn-Lorenz stages 3 and 19) but reached a plateau at the end of

the sampling periods, showing a trend of depletion of the primary inoculum source when the grapevines were blooming (Figs. 2.3). When comparing models to best describe the dynamics of *E. necator* primary inoculum release, the Gompertz model resulted in a better fit for the cumulative percentage of ascospores according to the accumulation of GDD (base 4^{o}C, Fig. 2.4). The values of the determination coefficients were 0.995 and 0.986 for ascospore accumulation in Clarksville in 2009 (Fig. 2.4A) and 2010 (Fig. 2.4B), respectively, and 0.982 and 0.9999 for Traverse City in 2009 (Fig. 2.4C) and 2010 (Fig. 2.4D), respectively.

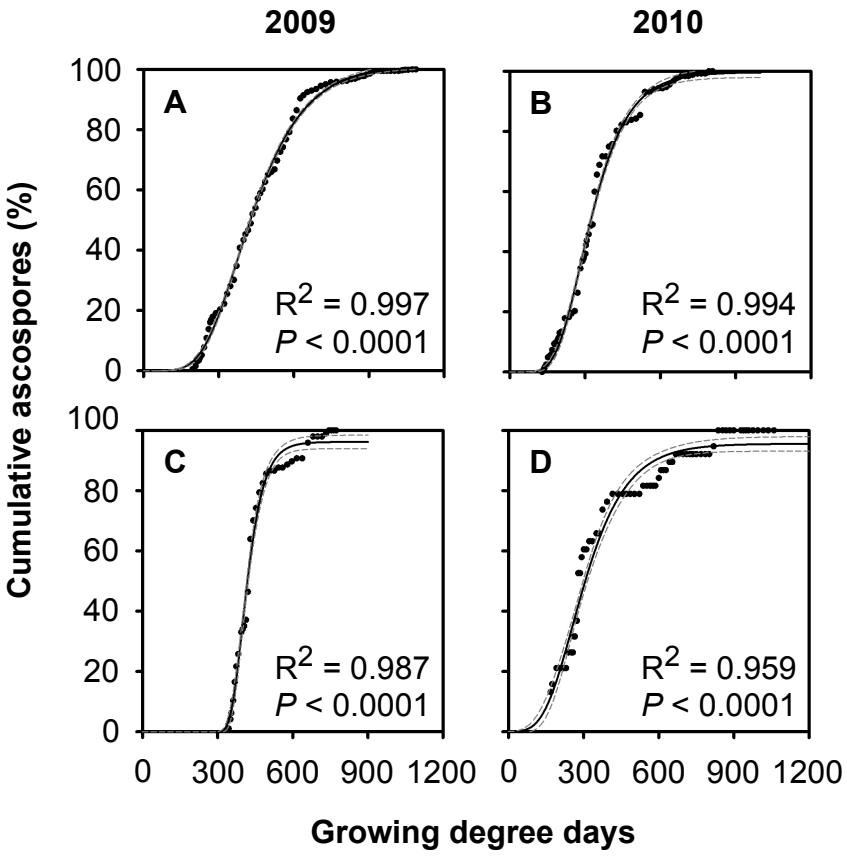

Figure 2.4. Cumulative percentage of ascospores according to growing degree-days (calculated from 1 April, base 4°C) at the research vineyards in Clarksville, MI in (A) 2009 and (B) 2010, and Traverse City, MI in (C) 2009 and (D) 2010. The fitted Gompertz equations (solid line [−]) for each sampling period (A-D) are shown with their corresponding 95% confidence intervals (gray, dashed lines [--]). Parameter estimates and corresponding standard errors were (A) a = 101.7 ± 0.54, b = 144.3 ± 2.92, x_0 = 377.1 ± 1.97; (B) a = 99.7 ± 0.84, b = 100.8 ± 3.36, x_0 = 284.2 ± 2.04; (C) a = 96.2 ± 1.10, b = 46.7 ± 2.67, x_0 = 399.2 ± 1.81; (D) a = 95.6 ± 1.21, b = 116.6 ± 8.33, x_0 = 255.6 ± 4.48, respectively. R^2 and P-values of the regressions are shown for each site × year combination.

Disease development on bait plants.

The infectivity of *E. necator* ascospores released during the sampling periods was evaluated on potted *Vitis vinifera* L. 'Chardonnay' that were used as bait plants in the 'Chardonel' vineyard in Clarksville during 2009 and 2010 and the NWMHRC vineyard during 2010. During 2009, all bait plants in Clarksville developed powdery mildew symptoms from late bud swell (7 May 2009) onwards, indicating that the released ascospores were viable and infective during the entire sampling period. On the bait plants, disease incidence on the leaves ranged from 21.9% to 58.6% and average severity from 0.7 to 11.3 colonies per infected leaf (Table 2.2). The peak of *E. necator* infection took place during the sampling period of 4 to 11 June 2009, when field-grown 'Chardonel' vines were at Eichhorn-Lorenz stage 15. Disease incidence higher than 50% also occurred during the periods of 18 to 25 June and 2 to 9 July. In 2010, bait plants at Clarksville developed infection only during the last two sampling periods (3 to 10 and 10 to 16 June); by that time, airborne ascospore concentrations had notably decreased. During these two periods, disease incidence was 37.1% and 44.4%, respectively (Table 2.2). On the field-grown 'Chardonnay' vines, powdery mildew symptoms were first detected at full bloom (Eichhorn-Lorenz stage 23; 25 June) in 2009 and at the buckshot berry growth stage (Eichhorn-Lorenz stage 29; 23 June) in 2010. Considering that the growing season started earlier in 2010, disease onset in the field was delayed during this year.

At Traverse City, 'Chardonnay' bait plants from two (of seven) sampling periods (25 May to 1 June and 29 June to 6 July) developed powdery mildew symptoms. During those periods, values for disease incidence were 4.2% and 4.7% and disease severity was 0.7 and 0.5 colonies per infected leaf, respectively (Table 2.2). Early disease development on bait plants occurred in a week after high temperatures and high ascospore concentration were recorded.

Afterward, a sharp decline in temperature was experienced from 1 to 15 June. Powdery mildew did not develop on bait plants placed in the vineyard between 1 June and 29 June; however, airborne inoculum was still detected. The other infection period took place at the end of the study interval, when the average air temperature began to rise (Table 2.2). On field-grown *V. vinifera* vines, powdery mildew was initially observed at the end of July, after fruit set (Eichhorn-Lorenz stages 31 and 33).

Table 2.2. Development of powdery mildew on potted bait plants of *Vitis vinifera* L. 'Chardonnay' placed on a weekly basis in an untreated vineyard of *Vitis* interspecific hybrid 'Chardonel' in Clarksville, MI and in an untreated vineyard of mixed *V. vinifera* in Traverse City, MI in 2009 and 2010. The total airborne inoculum concentration (*Erysiphe necator* ascospores per cubic meter of air), average temperature, average relative humidity, and average vapor pressure deficit are shown for each 7-day sampling period.

Sampling periods	**Powdery mildew detection†**			**Environmental factors††**		
	Incidence (%)	Severity (Number of colonies/leaf)	Inoculum (Number of ascospores/m^3)	Temp (^oC)	RH (%)	VPD (kPa)
Clarksville						
2009						
May 7-14	39.9	1.4	86.0	12.5	61.6	0.5
May 14-21	32.2	1.2	115.0	13.7	67.7	0.8
May 21-28	28.4	0.9	223.3	16.6	51.6	0.7
May 28 - June 4	29.8	1.0	151.7	13.1	72.4	0.5
June 4-11	58.6	11.3	130.0	15.5	61.3	0.6
June 11-18	21.9	0.7	181.7	17.5	77.7	0.7
June 18-25	52.3	3.2	40.0	23.2	75.0	0.8
June 25 - July 2	38.1	2.0	21.0	18.9	70.2	0.7
July 2-9	52.3	6.9	13.3	17.1	80.3	0.6
2010						
April 29 - May 6	0.0	0.0	90.0	16.4	52.2	0.7
May 6-13	nd	nd	43.3	6.8	60.8	0.3
May 13-20	0.0	0.0	106.7	13.3	61.2	0.6
May 20-27	nd	nd	50.0	20.8	70.3	0.8
May 27 - June 3	0.0	0.0	43.3	21.1	73.6	1.0
June 3-10	37.1	2.0	16.7	16.7	67.6	0.5
June 10-16	44.4	3.7	6.7	20.0	65.4	0.4
Traverse City						
2010						
May 18-25	0.0	0.0	11.7	19.2	65.4	0.9
May 25 - June 1	4.2	0.7	1.7	21.0	63.0	1.1
June 1-8	0.0	0.0	3.3	15.1	76.0	0.5
June 8-15	0.0	0.0	3.3	16.8	80.3	0.4
June 15-22	0.0	0.0	5.0	19.8	74.5	0.7
June 22-29	4.7	0.5	3.3	18.8	79.9	0.5
June 29 - July 6	nd	nd	0	20.6	62.5	1.0

Table 2.2 (cont'd)

† Powdery mildew activity was evaluated as: Incidence = mean percentage of infected leaves [(number of infected leaves) × 100/total number of leaves]; Severity = mean number of colonies on the infected leaves; and Ascospores = total ascospore concentration for the sampling period (ascospores/m^3/week).
†† The environmental factors considered were: Temp = mean temperature calculated for the sampling period (^oC); RH = mean relative humidity for the sampling period (%); and VPD = mean vapor pressure deficit calculated from the temperature and relative humidity data recorded per hour during the sampling period (kPa).
nd = No data were collected due to frost (May 6-13, 2010) and herbicide application (May 20-27, 2010) in Clarksville, and plant availability (June 26-July 6, 2010) in Traverse City.

DISCUSSION

The release of *E*. *necator* airborne ascospores in spring has been studied in the eastern and western United States (6, 11, 18), France (12, 13), Italy (21), and southern Australia (9). In the studies by Gadoury and Pearson (1990) in New York, the environmental conditions that trigger chasmothecia dehiscence and ascospore discharge were first identified; their work has been the foundation for the epidemiology and forecasting of the grape powdery mildew in viticultural regions where the pathogen's only source of primary inoculum is overwintering chasmothecia.

In our study, we found that the environmental conditions for ascospore release in *E*. *necator* as determined by Gadoury and Pearson (1990) did not sufficiently explain ascospore discharge patterns in Michigan. In particular, the amount of precipitation needed for ascospore release did not agree with their report for New York or the findings of Grove (2004) in Eastern Washington, in which ascospore release was restricted to 24-h periods with rainfall of 3.9 to 9.7 mm taking place between bud burst and immediate postbloom of grapevines. Although not significant, we observed a negative correlation between the amount of rainfall and the concentration of airborne ascospores (Table 2.1) while in other studies, the availability free water has been directlyassociated with chasmothecium dehiscence (6, 11, 12). In Traverse City, it was more evident that after the periods of heavy rainfall in 2009, more ascospores were detected (Fig. 2.2A and 2.2C). The results from Jailloux *et al*. (1998) indicated that when chasmothecia are subjected to alternating periods of wetness and dryness, dehiscence seems to be associated with dry spells that follow rainfall (12). Also, Gadoury and Pearson (1990) observed that as chasmothecia age, the ascocarp wall weakens, promoting ascospore release. Together, these findings would explain the occurrence of such trends in our correlation analysis.

Ascospores were mostly trapped during 24-h periods with an average temperature higher than 4°C. Average temperatures lower than 4°C were recorded in Traverse City in 2009, and ascospores were trapped on only two days with an average temperature of 2.5 (31 May) and 2.9°C (4 June). However, the numbers were very low (1 ascospore/day corresponding to a concentration of 1.7 ascospores/m^3). In Clarksville, the average daily temperature was always higher than 4°C during both years of the study (data not shown). Likewise, in Traverse City, the average temperature was higher than 4°C, except on 8 May 2010 (2.3°C) but no ascospores were detected in the air during that day. At both sites the sampling periods were started several days before bud break in 2010; the fact that ascospores of *E. necator* were detected before host tissues became available might indicate that release was at least responding to the minimal temperature requirement during 15 April to 1 May 2010 at Clarksville (Fig. 2.3B) and 28 April to 7 May at Traverse City (Fig. 2.3D), periods between the placement of the Burkard spore trap and bud break of grapevines. During those sampling periods the daily average temperature ranged from 4.3°C to 20.2°C and 2.3°C to 19.3°C (data not shown).

Several authors have observed ascospore release before bud break. For example, although Rossi *et al*. (2010) reported ascospore release peaks between bud break and bloom in Italy, 8% of the total number of ascospores were released before bud break (21). In a study by Gee *et al*. (2000), chasmothecia were collected from Australian vineyards to study their ability to release ascospores; however, ascospore release was not related to the timing of bud break of grapevines as chasmothecia collected during fall and winter also dehisced (9). Furthermore, Moyer *et al*. (2008) suggest that, under the right environmental conditions, chasmothecia will release ascospores before bud break and for 100 degree-days (base 10°C) continuously until depletion (16). In most cases, lower average temperatures occur while grapevines are dormant, and

inoculum availability is irrelevant for the development of the disease. However, from a disease standpoint, knowing how much inoculum has been released during dormancy of grapevines would allow for an estimation of remaining primary inoculum during the growing season, particularly during fruit set when grape berries are very susceptible to infection. In fact, primary inoculum could be exhausted before bud break if favorable wet periods occur early in the growing season (16). Grape growers can then use this information to control powdery mildew epidemics with a more effective disease management program.

Interestingly, ascospores were present regardless of the total amount of rainfall in all sampling periods. Although most of the ascospores were definitely trapped between bud break and bloom of grapevines, the minimal environmental requirements ($\geq$ 2.5 mm of rain at $\geq 4^{o}$C) outline in previous studies (6) would only predict ascospore release for a limited number of days. In Clarksville, these conditions would have only been met on 10 days (of a 71-day sampling period, Fig. 2.1C) in 2009 and 11 days (of a 62-day sampling period, Fig. 2.1D) in 2010, thereby underestimating inoculum availability. In Traverse City, these conditions were met during 8 days (of a 35-day sampling period, Fig. 2.2C) in 2009 and on 20 days (of a 70-day sampling period, Fig. 2.2D) in 2010, accounting for 16% and 13% in Clarksville, and 8% and 42% in Traverse City, of the total number of ascospores trapped per year, respectively.

From these results, precipitation might not be a suitable environmental factor for predicting *E. necator* primary inoculum availability in Michigan. However, the highest ascospore release peaks observed in Clarksville (26 May 2009 and 16 May 2010) seem to have been boosted by the rainfall events on 26 May 2009 (4.8 mm) and 13 May 2010 (35.8 mm), respectively (Fig. 2.1). In Traverse City, the peak observed on 10 June 2009 may have occurred as a consequence of the heavy rainfall of 8 June 2009 (20.8 mm) and light rain (0.3 mm) during

10 June 2009 of the release (Fig. 2.2A and 2.2C). The peak of 13 May 2010 occurred on a day with rainfall of 10.2 mm (Fig. 2.2B and 2.2D).

Since ascospore discharge has also been correlated to a minimum cumulative rainfall of 2 mm followed by at least 2.5 hours of leaf wetness at a temperature above 11°C by Jailloux *et al*. (1999) in the Bordeaux region (13), and Gadoury and Pearson (1990) observed chasmothecium dehiscence at 4°C and 2 h of wetness in the laboratory, we defined a day as dry when less than 2 h of wetness occurred. Leaf wetness data for our sampling sites indicated several days with wetting periods (Figs. 2.1E-F and 2.2E-F). In most cases, the recorded wetting periods were not initiated by rainfall. We assumed that all of these wetting periods were due to the formation of dew because they mostly occurred at night. Since the availability of free water is necessary for chasmothecia to dehisce, overnight dew exposure may explain the detection of ascospores on days with no precipitation. However, some ascospores were detected even on days with 0 h of wetness. In a study by Rossi *et al*. (2010) in northern Italy, the authors detected 4% of the total airborne ascospores during periods with no rain but with several hours of leaf wetness due to dew, and also during a 7-day period with just 3.5 h of wetness (21). In our study, the observed peaks in ascospore release in Clarksville and Traverse City always occurred on wet days (i.e. a day having more than 2 h of leaf wetness) or after at least 4 wet days had accumulated.

Our study demonstrates that airborne primary inoculum of *E. necator* in Michigan is present on most days during spring and early summer. Days when ascospores were not trapped started to be more frequent at the end of the sampling periods. However, ascospore availability does not imply that successful infections will occur in the field. For example, even though the 'Chardonnay' bait plants developed powdery mildew symptoms during all exposure periods of 2009, infection was not as successful in 2010. In 2009, symptoms developed as early as the late

bud swell stage of the field-grown vines but in 2010, powdery mildew colonies were only observed on the bait plants that were exposed during immediate pre-bloom and bloom of grapevines in Clarksville (3 to 10 June and 10 to 16 June, respectively). The higher concentration of airborne ascospores during 2009 might have facilitated the infection, but also the environmental conditions that the bait plants were exposed to while in the vineyard could have played an important role in determining success of infection.

Gadoury and Pearson (1990) stated that free water is not only critical for ascospore release but also favors ascosporic infection, as does a saturated atmosphere. The authors determined that *E. necator* ascospores are more sensitive to dry conditions than conidia because ascospore germination and appressorium formation were significantly reduced when increasing VPD from 0 kPa to 0.6 kPa, 0.8 kPa, and 1.5 kPa (i.e. 81%, 76%, and 54% RH at 25^{o}C, respectively). However, conidia are relatively insensitive to VPD at 25^{o}C. Also, ascospore germination was significantly reduced at 5^{o}C and temperatures higher than 31^{o}C when comparing to germination at 10^{o}C to 25^{o}C. Furthermore, appressorium formation was significantly reduced at 10^{o}C and 15^{o}C, and completely inhibited at 5^{o}C or temperatures higher than 31^{o}C when comparing to the 20^{o}C, 22^{o}C, and 25^{o}C treatments (7).

In Clarksville, RH ranged between 51.6% and 80.3% in 2009 and 52.2% and 73.6% in 2010, and the average temperature ranged between 12.5^{o}C and 23.2^{o}C in 2009 and 6.8 and 21.1^{o}C in 2010 (Table 2.2). From these values, the calculated VPD ranged between 0.53 kPa and 0.82 kPa and 0.27 kPa and 0.98 kPa, respectively, indicating a greater fluctuation in the saturation of the atmosphere during 2010. Due to the variation in humidity and temperature in the vineyard, the effects of these environmental conditions on the establishment of successful infections are expected to be higher. However, to our knowledge, no information is available

regarding the survival span of already released ascospores and the progression of the infection by *E. necator* during interrupted periods of wetness and fluctuating temperatures. Moyer *et al*. (2010) studied the effect of low temperatures on the conidial stage of powdery mildew and found a significant reduction in conidium infection efficiency and colony expansion when leaves were inoculated after a cold treatment of 2°C to 8°C for 2 h or 8 h. When evaluating the effects of cold temperatures on already infected leaves, the authors also observed reductions in colony density due to death of hyphae and an increase in the duration of latent periods (15). Aside from the differences in water tolerance between ascospores and conidia (the latter are considered xerophytic), both types of spores have displayed similar responses to temperature. Conidial germination was studied by Delp (1954) and was found to occur at a minimum of 6°C (only 3% germination), optimal at 25°C (100% germination), and at a maximum of 33°C (only 3% germination) (4). The only difference with ascospore germination was a higher germination percentage at lower temperatures (20% at 5°C), probably due to an adaptation of the spores to develop during spring (7). However, these attributes were not examined in our study.

During 2010, unusually high temperatures induced vine development in April, giving an early start to the growing season in Michigan, followed by severe spring freezes. These cold periods in the spring of 2010 may explain the failure to observe powdery mildew infection on the bait plants prior to temperature stabilization, as it was also observed for the bait plants placed in the Traverse City vineyard during that year (Table 2.2). Furthermore, the observed delay in the onset of powdery mildew on field-grown vines compared to 2009 may also indicate that the environmental conditions conducive for disease development where less than optimal in 2010. A deeper insight into the environmental features underlying the development of primary infections

under natural conditions would allow more accurate estimations of powdery mildew risk before epidemic development.

Due to the low daily concentration of airborne ascospores of *E. necator* in this study, a more in-depth analysis of the environmental conditions that affect ascospore release did not yield consistent results. The correlations between daily ascospore concentration and total rainfall or daily average temperature were neither significant (Table 2.1) nor consistent with the studies on chasmothecium dehiscence that indicate the role of precipitation in the form of rain. However, the weather stations recorded several wetting periods (more than 2-h of wetness, not initiated by rainfall events) that were several hours long (usually between 7 and 9 h). Dew as a source of free water may have provided the necessary moisture for chasmothecia to dehisce in our study. Gadoury and Pearson (1990) clearly illustrated the importance of water potential for the rupture of the ascocarps and ascospore discharge. In their *in-vitro* assays, chasmothecia dehisced only when wet and the proportion of ascocarps that dehisced considerably decreased when dropping the temperature from 10^{o}C to 4^{o}C (6). A PCR-based technique to specifically detect *E. necator* in air samples was developed by Falacy *et al*. (2007), and the authors were able to detect DNA extracted from as little as one conidium in 46% of the reactions (5). If necessary, perhaps an optimization of their detection protocol could help circumvent the ascospore trapping limitations of a Burkard spore trap, and a more detailed analysis of the effects of Michigan weather conditions on ascospore discharge would be allowed in the future to better predict and assess risk periods of infection.

Since the growing degree-day calculation was the only environmental variable that consistently showed a significant linear correlation with the daily ascospore concentration (Table 2.1), GDD data could be used as a predictor of the percentage of released ascospores. Even

though the Pearson correlation coefficients disclosed a negative relation between GDD and the daily concentration of the inoculum (Table 2.1), this explains the dynamics of ascospore release in terms of a source that is being depleted.

When plotting the ascosporic release dynamics, it became evident that a linear model was not the best to explain the observable trends. The Gompertz model that described the cumulative percentage of ascospores as a function of GDD was a good fit. The curves fitted for each of the sampling periods showed that approximately at 600 GDD (from 1 April, base 4^{o}C), about 90% of the ascospores were released (Fig. 2.4). By that time, grapevines went through the phenological stages of immediate prebloom and first bloom, respectively, which agrees with previous research (6, 11, 13). This information can also be used to develop an adequate disease management program to protect the vines from primary infections. Reducing the risk of ascosporic infections could not only considerably delay the onset of powdery mildew but it could also slow the disease progress rate. In fact, lime sulfur applications to dormant grapevines planted in viticultural regions where *E. necator* can only overwinter as chasmothecia were proven to kill the ascocarps of this pathogen, delay the development of powdery mildew epidemics, and reduce the infection severity in fruit clusters (8). However, due to the polycyclic nature of powdery mildew, an integrated control scheme of the disease would also need to include the management of the secondary infection cycles by reducing the number of conidial infections. Carisse *et al*. (2009) developed a degree-day model to initiate fungicide applications according to the concentration of airborne conidia. Their model was able to reduce the number of fungicide applications by half, but was unable to predict the best time to begin the fungicide program in some of their trials (2). By considering ascospore availability and subsequent infection periods, the estimation of the initiation date for fungicide programs could be made

more accurate. Then again, forecasting models for *E. necator* require the validation and subsequent adjustment of parameters to the local environmental scenario in order for management schemes to effectively keep the disease pressure below an economic threshold.

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LITERATURE CITED

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CHAPTER 3

LATE-SEASON CHASMOTHECIUM PRODUCTION AND DISPERSAL OF *ERYSIPHE NECATOR* IN MICHIGAN

ABSTRACT

The production and dispersal of the overwintering inoculum of *Erysiphe necator* were monitored on grapevines in Michigan during 2008, 2009, and 2010 to determine the timing of *E. necator* chasmothecium production. Differences in inoculum production were observed between sites and years of the study that are possibly related to the inherent susceptibility of the grape cultivars evaluated and to year-to-year climate variation. However, independently of the host or disease pressure, peaks of chasmothecium production as well as major dispersal events were repeatedly observed in mid- to late September. Every year, the amount of chasmothecia produced on the abaxial surface was considerably higher than on the adaxial surface of the leaves. Correlations between the amount of rainfall and number of chasmothecia trapped were not always conclusive due to the occurrence of heavy rain in periods when ascocarps were still undergoing maturation or to light rain in periods when ascocarps were fully mature and ready for dispersal. Likewise, a lack of correlation was observed between ascocarp quantity and disease severity because chasmothecia on the adaxial surface of the leaves are more exposed to the rain. The survival and epidemiological role of chasmothecia that landed on the ground with senescent leaves is still unknown. These findings could be used to determine the timing of eradication sprays and to estimate the potential source of inoculum for the following growing season in Michigan vineyards.

INTRODUCTION

The ascomycete fungus *Erysiphe necator* Schwein. is the causal agent of powdery mildew in grapevines, one of the most devastating diseases in worldwide grape production (24). European wine grape cultivars (*Vitis vinifera* L.) are highly susceptible whereas American cultivars (*Vitis Labrusca* L.) are regarded as more tolerant (7, 12). Currently, with 5,747 hectares of grapes planted in southwest Michigan (mostly in Berrien and Van Buren counties) and northwest Michigan (mostly in Leelanau and Grand Traverse counties), Michigan ranks 5th in grape production and 13th in wine production in the United States (19, 34). Most of the acreage has been designated for juice grape production and is located in southwest Michigan. However, the wine industry in Michigan has been growing; from 57 hectares of wine grapes (*V. vinifera* L.and *Vitis* L. interspecific hybrids) planted in 1973 to over 800 hectares currently (19, 20, 34).

The most popular grape varieties are highly susceptible to powdery mildew and even at low levels, e.g., 3% infected berries in a cluster, wine quality is significantly reduced in terms of taste and aroma (33). Due to the importance of powdery mildew in grapes worldwide, the epidemiology of *E. necator* has been studied mostly in *V. vinifera cultivars* over the past 30 years in order to discern the pathogen's biology and develop more effective disease management strategies. However, in a nationwide survey conducted in the United States, 38% of grape growers reported unsatisfactory powdery mildew control (32). Lack of control may occur when fungicide sprays are not applied in a timely manner or when commonly used fungicides become ineffective due to the development of fungicide resistance (1, 15, 35). Knowledge of the pathogen and its disease cycle is essential when developing more effective strategies for disease control, especially in light of recent evidence of a response of the powdery mildews to climate change (13). Cook *et al*. (2006) found a newly introduced species of powdery mildew (*E. elevata*

[Burrill] U. Braun & S. Takam.) in Europe on Catalpa (*Catalpa bignonioides* Walter), an ornamental tree species native to the southeastern United States. *Erysiphe elevata* seems to have rapidly adapted to the current climatic conditions of Europe (4).

Powdery mildew of grape is a polycyclic disease, which makes the management of the disease very challenging (2). In regions where the host is dormant during the winter, a delay in disease epidemics (and consequent reduction in disease severity) can be accomplished by preventing the formation of overwintering structures in the fall or by targeting the initial infections caused by the overwintering inoculum in spring (11, 18, 27). When the host is dormant, *E. necator* has the ability to overwinter either in the form of inactive mycelium in dormant buds or as ascocarps called chasmothecia (sexual form, formerly known as cleistothecia) (24, 25, 29). The term chasmothecium has been coined for grape powdery mildew ascocarps due to the way they break open transversely upon dehiscence, which is different from cleistothecia of other fungi (9, 13). In areas with mild winters where the fungus also overwinters in live buds, mycelial growth in infected buds is reactivated soon after bud break of grapevines (i.e. Eichhorn-Lorenz stage 5), and infection spreads to the newly emerging shoots, which are rapidly covered by a mat of mycelia and conidia. This type of symptom in early spring is known as a "flag shoot"(24, 29). Flag shoots are concentrated sources of secondary inoculum in the form of conidia, but their incidence in the vineyards is usually very low (5, 16).

Perennation of the pathogen in live buds has only been observed in viticultural locations with mild winters, such as Fresno, CA, USA (30), the Western Cape province, South Africa (16), and southern Germany (26, 28). In locations where flag shoots have never been observed, such as some Italian vineyards and grape-growing regions in Quebec, Canada and the states of New York and Washington, USA, chasmothecia represent the sole source of primary inoculum (3, 5,

14, 25). Most likely, this is also the case for Michigan, as flag shoots have not been observed in Michigan vineyards (Annemiek Schilder, *personal communication*). Since *E. necator* is a heterothallic organism, the production of chasmothecia requires compatible mating types (namely + and -) of the fungus to cross. Therefore, as the growing season progresses, ascocarp production is positively correlated to powdery mildew incidence in any given vineyard because the chances for out-crossing increase (10).

Chasmothecium development has been studied under laboratory conditions to determine the effects of environmental factors and host biology. These studies demonstrated that temperatures below 10^{o}C hinder ascocarp maturation, and below 8^{o}C, ascocarp initiation (8). In addition, host susceptibility also influences the time required for maturation, which is longer in less susceptible varieties (8). When chasmothecia mature, they are readily detached from the leaves and are dispersed by rain to the vineyard soil or to the bark of the vines, where they have a better chance to survive the cold temperatures of winter (8). Attachment of chasmothecia to the vine's trunk is assisted by appendages that act as hooks (8, 13, 17). Gadoury and Pearson (1988) showed that chasmothecia that remained attached to leaf tissues had not gone through an appropriate maturation process, hence they were unable to survive the winter in New York, USA. However, in Australia, Italy, and the eastern region of Washington, USA, chasmothecia seem capable of surviving on senescent leaves on the vineyard soil (5, 14).

Research done by Gadoury *et al*. (1994) demonstrated that a single dormant application of lime sulfur at a rate of 120 ml/L in 2,800 L water per hectare in the spring significantly reduced primary inoculum and delayed powdery mildew development and disease severity. However, the applications costs were too high to be commercially viable (11). Once the conidial stage of *E. necator* has developed in the field, the disease becomes very difficult to control and

the application of systemic fungicides with a site-specific mode of action carries a high risk of selection for fungicide-resistant strains (22). Therefore, prevention of the initial infections caused by ascospores would be beneficial for powdery mildew management, which could be accomplished by prevention of ascocarp development during the previous growing season. Preliminary studies have shown that contact fungicides like JMS Stylet Oil (paraffinic oil) and Sulforix (calcium polysulfide), when applied in early fall, can drastically reduce the formation of chasmothecia on grape leaves infected with powdery mildew (31).

The objectives of this study were to determine the timing of *E. necator* overwintering inoculum production in established vineyards in relation to climatic conditions in Michigan. By studying the dynamics of chasmothecium development in the fall, Michigan grape growers will be able to apply more timely fungicide applications to eradicate overwintering inoculum and, possibly, fewer applications will be needed to control powdery mildew during the following growing season.

MATERIALS AND METHODS

Research sites.

The production of *E. necator* chasmothecia was studied in central and northwest Michigan during 2008, 2009, and 2010. Studies were conducted in an established *Vitis* L. interspecific hybrid 'Chardonel' research vineyard located at the Michigan State University (MSU) Clarksville Research Center (CRC, 42° 52' 17'' N, 85° 15' 30'' W) in Clarksville, MI and on *V. vinifera* L. 'Pinot Noir' grapevines planted in a mixed-cultivar trial of *V. vinifera* located at the MSU Northwest Michigan Horticultural Research Center (NWMHRC, 44° 52' 58'' N, 85° 40' 28'' W) in Traverse City, MI. Due to low powdery mildew severity at the NWMHRC in 2009, and additional study site was chosen in a commercial vineyard of *Vitis* L. interspecific hybrid 'Marechal Foch' located near Suttons Bay, MI.

Sampling of chasmothecia in rainwater.

For this study, four unsprayed grapevines were chosen at each vineyard site. A plastic 18-cm-diameter collection funnel, connected with a rubber hose to a 3-L plastic soda bottle, was placed below the canopy of each vine at a height of 1 m above ground level. All funnels were attached to the trunk and trellis of the vines and remained in the field during the whole sampling period. Plastic bottles were covered with aluminum foil and contained two pulverized tablets of tetraglycine hydroperiodide (Potable Aqua, Wisconsin Pharmacal Co., LLC, Jackson, WI, USA) to prevent sample deterioration. Plastic bottles containing rain samples were replaced after each weekly sampling period and stored at 4°C until analyses were performed. Rainwater samples were vacuum-filtered through a Whatman No.1 filter mounted in a Buchner funnel. The amount of rain collected was recorded for each replicate sample and sampling period. Filter papers were

observed under a dissection microscope (model SMZ-2B, Nikon, Tokyo, Japan) at 40× magnification to determine the number of trapped chasmothecia. The number was recorded for each separate sample. In 2009 and 2010, only two bottle traps per site were placed at the NWMHRC 'Pinot Noir' and the commercial 'Marechal Foch' vineyards.

Production of chasmothecia on leaves.

Eight additional unsprayed grapevines were chosen in the 'Chardonel' vineyard for this study. From these vines, four replicate samples of 20 randomly selected leaves were collected weekly starting on 4 September in 2008 and 2009. Samples were collected two weeks earlier in 2010 (19 August) to assess early chasmothecium development in this vineyard. The sampling period ended with the occurrence of the first frost in the month of October, as grape leaves were no longer available for sampling. Leaves were placed in plastic resealable bags and kept at 4^{o}C until processing. For chasmothecium quantification, leaves were observed under a dissection microscope at 40× magnification using a grid with a preset area of one square centimeter. Counts were obtained for five 1-cm^2 areas on both adaxial and abaxial surfaces of the leaves.

In northern Michigan, samples were also collected in 2008, 2009 and 2010 from untreated 'Pinot Noir' vines at the NWMHRC and in 2009 from 'Marechal Foch' vines in a commercial vineyard. In 2008, a sample of five leaves was randomly collected from 'Pinot Noir' vines on two dates only (9 and 17 September). In 2009, samples of 10 leaves were collected from each cultivar on a weekly basis starting on 24 August and ending on 8 October in Traverse City and 15 October in Suttons Bay. In 2010, a sample of 20 'Pinot Noir' leaves was randomly collected every week from 16 August to 29 October. All samples were observed for chasmothecium quantification as described above.

Environmental data.

Detailed weather data on temperature and rainfall were collected every five minutes by weather stations (Campbell Scientific, Inc., Logan, UT, USA) from the Michigan Automated Weather Network (Michigan State University, East Lansing, MI, USA) located at the CRC and NWMHRC within 200 m from the research vineyards. Daily averages and totals were calculated for air temperature and rainfall, respectively.

Statistical analysis.

Differences in the amount of chasmothecia trapped with 3-L-bottle traps and observed on leaves were analyzed by two-way analysis of variance (ANOVA). Differences within and between the sampling periods were analyzed further using Fisher's protected LSD as the all-pairwise multiple comparison procedure. Treatment effects were declared significant at $P < 0.05$. Data that violated the ANOVA assumption of normality, even after data transformation, were analyzed using a non-parametric ANOVA, the Kruskal-Wallis test, for each factor. All analyses were performed using the statistics package of SigmaPlot (version 11.0, Systat Software Inc., San Jose, CA, USA).

The correlation between the number of chasmothecia captured in bottle traps and the amount of rainwater collected (in milliliters) was examined using the PROC CORR procedure of SAS (SAS version 9.2, SAS Institute Inc., Cary, NC, USA). In the same way, the correlation between the number of chasmothecia on the adaxial surface of the leaves (per square centimeter of leaf area) and disease severity (percentage of infected leaf area) was investigated.

RESULTS

Chasmothecia in rainwater samples.

The overwintering structures of *E. necator* were found in every bottle trap that had collected rainwater. Similar numbers of chasmothecia were found in the traps within each sampling period (all *P*-values were higher than a significance level of $P = 0.05$, Table 3.1). However, significant differences were found in the number of chasmothecia trapped between sampling periods ($P < 0.05$, Table 3.1).

Table 3.1. Analysis of variance (ANOVA) of differences within and between sampling periods in *Erysiphe necator* chasmothecia collected in 3-L-bottle rain traps placed in vineyards of *Vitis* L. interspecific hybrid 'Chardonel' at the Michigan State University (MSU) Clarksville Research Center and *V. vinifera* 'Pinot Noir' at the MSU Northwest Michigan Research Center in 2008, 2009, and 2010, and a vineyard of *Vitis* L. interspecific hybrid 'Marechal Foch' in Suttons Bay, MI in 2009. *P*-values ≤ 0.05 were considered significant and are shown in bold font.

Factors	Year	ANOVA *P*-values		
		Central Michigan	Northern Michigan	
		'Chardonel'	'Pinot Noir'	'Marechal Foch'
Within sampling periods†	2008	0.096	0.448	nd
	2009	0.285	0.128	0.358
	2010	0.073	0.645	nd
Between sampling periods††	2008	**0.007**	**0.003**	nd
	2009	**0.019**	**< 0.001**	**0.027**
	2010	**0.035**	0.141	nd

† Differences in the number of chasmothecia collected among bottle traps (four per vineyard, except in northern Michigan in 2009, when there were two per vineyard).
†† Differences in the mean number of chasmothecia collected among different sampling periods
nd = No data were collected.

The total number of chasmothecia collected per vine over the trapping period in the CRC 'Chardonel' vineyard was 731, 210, and 530 in 2008, 2009, and 2010, respectively (Table 3.2). In the NWMHRC 'Pinot Noir' vineyard the total number of chasmothecia per vine over the trapping period was 9,916 in 2008, 3,595 in 2009, and 380 in 2010 (Table 3.2). Lastly, a total of 257 chasmothecia per vine were collected in the 'Marechal Foch' vineyard in Suttons Bay, MI in 2009 (Table 3.2).

Table 3.2. Cumulative total number of chasmothecia dispersed by rain and average ascocarp production of *Erysiphe necator* on leaves of *Vitis* L. interspecific hybrid 'Chardonel' and *V. vinifera* 'Pinot Noir' in 2008, 2009, and 2010, and *Vitis* L. interspecific hybrid 'Marechal Foch' in 2009.

Sampling method	Year	Central Michigan	Northern Michigan	
		'Chardonel'	'Pinot Noir'	'Marechal Foch'
Bottle traps (per vine)†	2008	731	9,916	nd
	2009	210	3,595	257
	2010	530	380	nd
Leaves (per cm^2)††	2008	15.3	65.5	nd
	2009	1.6	10.6	2.6
	2010	7.6	4.6	nd

† Trapping periods in 'Chardonel': 29 August to 9 October, 2008 (41 days); 21 August to 1 October, 2009 (41 days); 14 August to 1 October, 2010 (48 days); in 'Pinot Noir': 19 August to 2 October, 2008 (44 days); 24 August to 8 October (45 days); 16 August to 12 October (57 days); in 'Marechal Foch': 24 August to 8 October (45 days).
†† Chasmothecium averages on leaves collected weekly over two weeks ('Pinot Noir' in 2008), five weeks ('Chardonel' in 2008 and 2009), seven weeks ('Pinot noir' in 2010; 'Marechal Foch' in 2009; 'Chardonel' in 2010) and eight weeks ('Pinot noir' in 2009).
nd = No data were collected.

In the ‘Chardonel’ vineyard located in central Michigan, the highest number of chasmothecia was trapped in the sampling periods of 11-18 September in 2008 (Fig. 3.1A), 18-25 September in 2009 (Fig. 3.1B), and 17-23 September in 2010 (Fig. 3.1C). Peak captures usually coincided with the heaviest rain events of the sampling periods (Figs. 3.1D to 3.1F). In 2009, the peak occurred after two weeks of no rain (Fig. 3.1E). In the ‘Pinot Noir’ vineyard in northwest Michigan, high numbers of chasmothecia were trapped in the period of 12-18 September in 2008 (Fig. 3.2A) and 1-8 October in 2009 (Fig. 3.2B). In 2010, the peaks were observed in the sampling periods of 13-21 September and 21-24 September (Fig. 3.2C); however, due to a high variability among the samples taken during those periods, the differences between sampling periods were not significant (Table 3.1). As observed in the CRC vineyard, chasmothecium peak capture coincided with the occurrence of heavy rain (Figs. 3.2D to 3.2F).

In the ‘Marechal Foch’ commercial vineyard sampled in 2009, a peak in chasmothecium capture occurred in the sampling period of 1-8 October (Fig. 3.3A), which also coincided with the highest amount of rain collected (Fig. 3.3B).

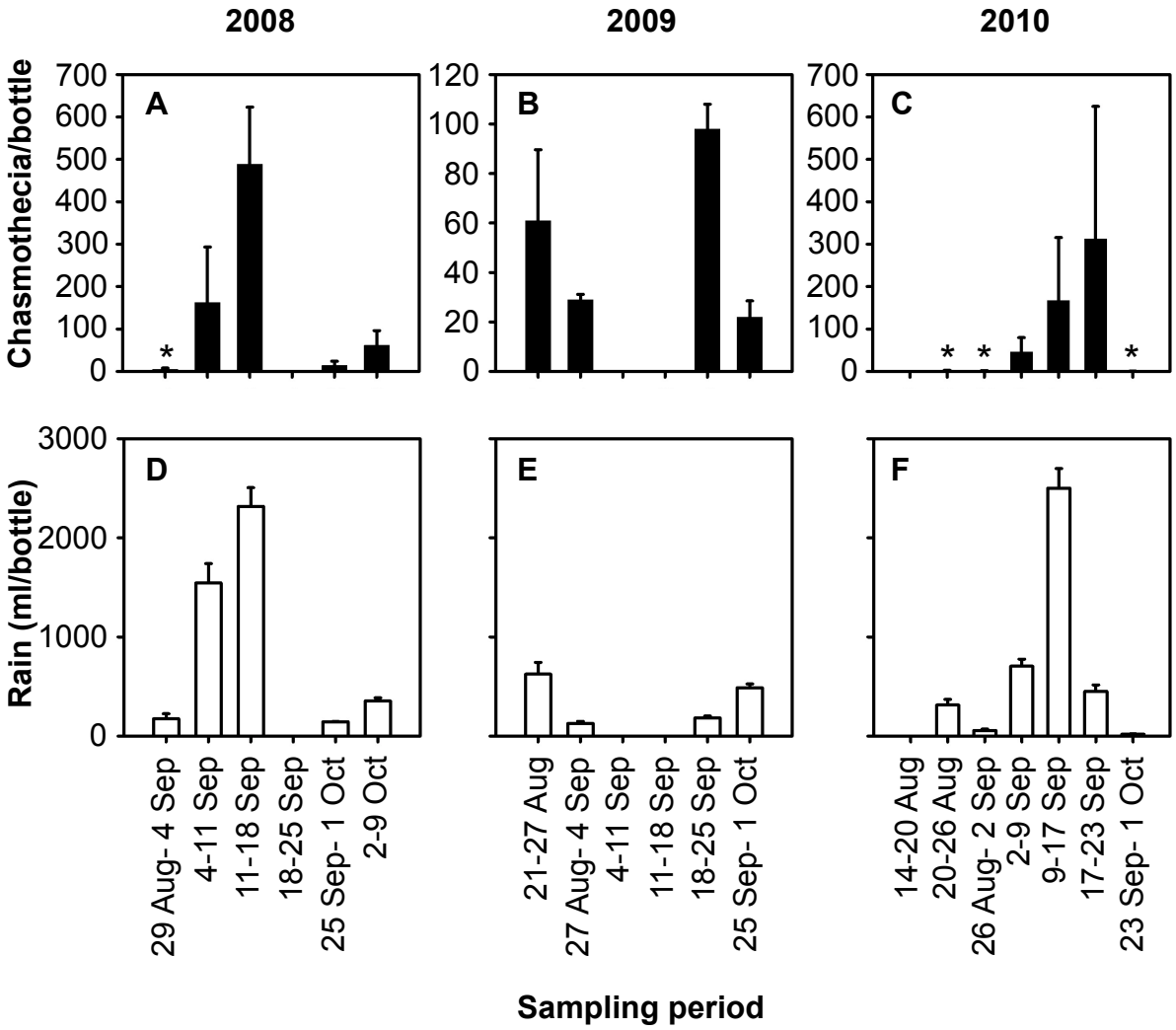

Figure 3.1. The average number of *Erysiphe necator* chasmothecia (A, B, and C) and average amount of rainwater (D, E, and F) collected in 3-L-bottle traps (n = 4) attached to the trunk of untreated grapevines of *Vitis* L. interspecific hybrid 'Chardonel' planted at the Michigan State University Clarksville Research Center in Clarksville, MI during each sampling period in 2008 (A and D), 2009 (B and E), and 2010 (C and F). Asterisks (*) indicate trapping of less than five chasmothecia per bottle. Error bars indicate the standard error of the mean.

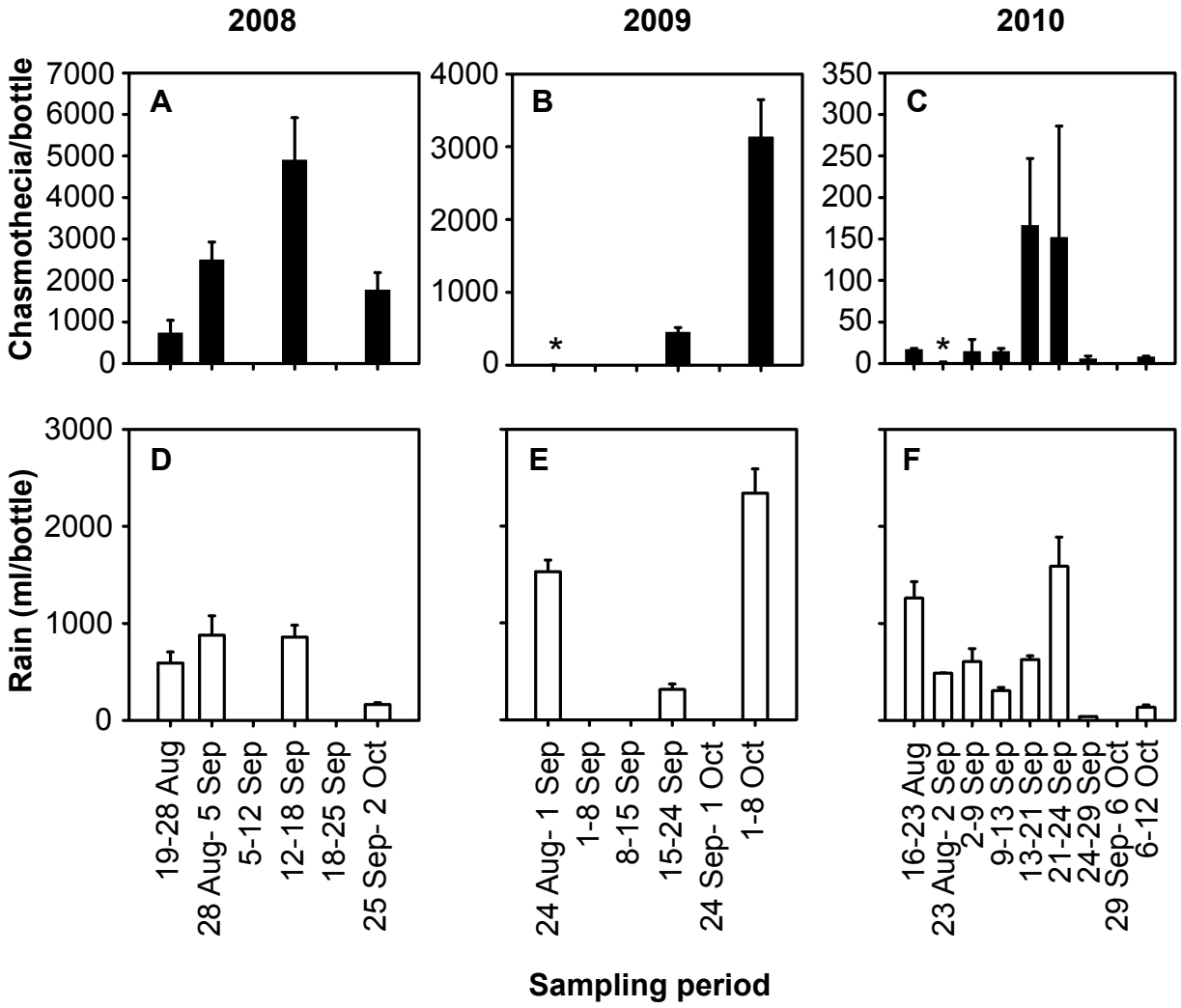

Figure 3.2. The average number of *Erysiphe necator* chasmothecia (A, B, and C) and average amount of rainwater (D, E, and F) collected in 3-L-bottle traps (n = 4, except for 2009 when n = 2) attached to the trunk of untreated grapevines of *Vitis vinifera* 'Pinot Noir' planted at the Michigan State University Northwest Michigan Horticulture Center in Traverse City, MI during each sampling period in 2008 (A and D), 2009 (B and E), and 2010 (C and F). Asterisks (*) indicate trapping of less than five chasmothecia per bottle. Error bars indicate the standard error of the mean.

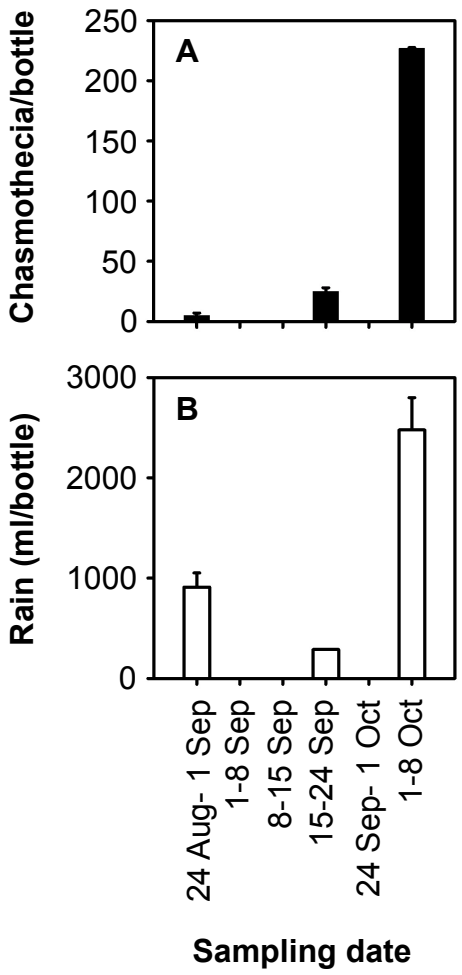

Figure 3.3. (A) The average number of *Erysiphe necator* chasmothecia and (B) average amount of rainwater collected in 3-L-bottle traps (n = 2) attached to the trunk of untreated grapevines of *Vitis* L. interspecific hybrid 'Marechal Foch' in a commercial vineyard in Suttons Bay, MI during each sampling period in 2009. Error bars indicate the standard error of the mean.

In the Pearson correlation analysis, a positive correlation was found between the number of chasmothecia trapped and the amount of rain collected in the 3-L-bottle traps for every site×year combination. However, the correlation was not statistically significant in the 'Chardonel' vineyard in 2009 and 2010 and the 'Pinot Noir' vineyard in 2010 (Table 3.3).

Table 3.3. Pearson correlation coefficients (*r*) between the number of chasmothecia and the amount of rainwater collected in 3-L-bottle traps placed in a vineyard of *Vitis* L. interspecific hybrid 'Chardonel' at the Michigan State University Clarksville Research Center and *V. vinifera* 'Pinot Noir' at the MSU Northwest Michigan Horticultural Research Center in 2008, 2009, and 2010, and a commercial vineyard of *Vitis* L. interspecific hybrid 'Marechal Foch' in 2009.

Year	**Pearson's correlation coefficient (*r*)**		
	Central Michigan	**Northern Michigan**	
	'Chardonel'	'Pinot Noir'	'Marechal Foch'
2008	**0.950 ****	**0.778 ***	nd
2009	0.414 ns	**0.793 ***	**0.938 ****
2010	0.317 ns	0.581 ns	nd

Values in **bold** indicate a significant correlation at $P = 0.05$ (**) or $P = 0.10$ (*); ns = not significant.
nd = No data were collected in the 'Marechal Foch' vineyard in 2008 and 2010.

Production of chasmothecia on leaves.

The replicate samples of leaves randomly collected from powdery-mildew infected 'Chardonel' grapevines for ascocarp quantification had variable numbers of chasmothecia per square centimeter of leaf area but numbers were similar for the samples taken from 'Pinot Noir' and 'Marechal Foch' vines ($P > 0.05$, Table 3.4). It is worth mentioning that more leaves were collected from the 'Chardonel' vineyard (4 samples of 20 leaves every week) than for the other two cultivars (samples of either 5, 10 or 20 leaves, see Materials and Methods). However, we were still able to find significant differences in the number of chasmothecia observed per square centimeter of leaf area between sampling periods for most site×year combinations ($P < 0.05$). At the CRC, differences between sampling periods in 2009 were significant at $P = 0.10$. At NWMHRC, no significant differences were found in 2008 ($P = 0.921$, Table 3.4).

Table 3.4. Analysis of variance (ANOVA) of differences within and between sampling periods of the number of *Erysiphe necator* chasmothecia observed on leaves of *Vitis* L. interspecific hybrid 'Chardonel' at the Michigan State University (MSU) Clarksville Research Center; *V. vinifera* 'Pinot Noir' at the MSU Northwest Michigan Research Center in 2008, 2009, and 2010; and a vineyard of *Vitis* L. interspecific hybrid 'Marechal Foch' in Suttons Bay, MI in 2009.

Factors	Year	ANOVA *P*-values		
		Central Michigan	Northern Michigan	
		'Chardonel'	'Pinot Noir'	'Marechal Foch'
Within sampling periods†	2008	< 0.001	0.909	nd
	2009	0.025	0.651	0.842
	2010	0.001	0.802	nd
Between sampling periods††	2008	0.014	0.921	nd
	2009	0.085	< 0.001	0.001
	2010	0.040	< 0.001	nd

† Differences in the number of chasmothecia observed per square centimeter of leaf area between replicate leaf samples within a sampling period.
†† Differences in the number of chasmothecia observed per square centimeter of leaf area among sampling periods.
nd = No data were collected.

During the season, the average numbers of chasmothecia produced per square centimeter of 'Chardonel' leaf area were 15.3, 1.6, and 7.6 in 2008, 2009, and 2010, respectively (Table 3.2). Similarly, on leaves of 'Pinot Noir', on average, 65.5, 10.6, and 4.6 chasmothecia/cm^2 were produced in 2008, 2009, and 2010, respectively (Table 3.2). In the 'Marechal Foch' vineyard, the average for the 2009 season was 2.6 chasmothecia/cm^2 of leaf area (Table 3.2). Chasmothecia were always more abundant on the abaxial surface of the leaves (Figs. 3.4G-I, 3.5G-I, and 3.6C) than on the adaxial surface (Figs. 3.4D-F, 3.5D-F, and 3.6B). The highest number of chasmothecia on the adaxial surface of 'Chardonel' leaves was observed on 11 September 2008 with 5.9 chasmothecia/cm^2 (Fig. 3.4D) while the highest number observed on the abaxial surface was 32.5 chasmothecia/cm^2 on 2 October of the same year (Fig. 3.4G). On 'Pinot Noir', peaks on the adaxial and abaxial leaf surfaces were 30.6 chasmothecia/cm^2 on 1 October 2009 (Fig. 3.5E) and 118.3 chasmothecia/cm^2 on 17 September 2008 (Fig. 3.5D), respectively. Similarly, on the adaxial and abaxial surface of 'Marechal Foch' leaves, production peaks were 4.6 chasmothecia/cm^2 on 1 October 2009 (Fig. 3.6B) and 8.2 chasmothecia/cm^2 on 16 September 2009, respectively (Fig. 3.6C).

The overwintering structures of *E. necator* were first observed on leaves as early as 21 August in the CRC vineyard in 2010 (Fig. 3.4C), August 16 in the NWMHRC vineyard in 2010 (Fig. 3.5C), and 24 August 2009 at the commercial 'Marechal Foch' vineyard (Fig. 3.6A). The number of chasmothecia produced before 4 September of 2008 and 2009 in the MSU CRC vineyard was not estimated because leaves were not collected prior to that date (Figs. 3.4A-B).

Likewise, at the NWMHRC, leaf sampling began on 9 September 2008 (Fig. 3.5A). However, no chasmothecia were observed on the 'Pinot Noir' leaves collected on 24 August 2009 (Fig. 3.5B).

Over the sampling periods, the proportion of immature chasmothecia (yellow- or brown-colored ascocarps) decreased in relation to mature chasmothecia (black colored ascocarps) (Figs. 3.4, 3.5, and 3.6). The abrupt decreases in the total number of chasmothecia/cm^2 that were observed in the 'Chardonel' vineyard on 18 September 2008 (Fig. 3.4A) and 25 September 2010 (Fig. 3.4C) occurred after episodes of heavy rain in Clarksville, MI (Figs. 3.1D and 3.1F). In 2009, the decrease observed on 17 September (Fig. 3.4B) occurred during a dry period; in Clarksville, no rain was recorded between 1 to 20 September (Fig. 3.1E). Similarly, in the 'Pinot Noir' vineyard, the significant reductions in the total number of chasmothecia/cm^2 of 8 October 2009 (Fig. 3.5B) and 29 September 2010 (Fig. 3.5C) took place after rainfall events (Figs. 3.2E and 3.2F). Peaks in total chasmothecium production were observed on 4 September 2008 (18.4 chasmothecia/cm^2), 1 October 2009 (2.3 chasmothecia/cm^2), and 17 September 2010 (12.9 chasmothecia/cm^2) in the 'Chardonel' vineyard (Figs. 3.4A-C). At the NWMHRC, peaks in production occurred on 9 September 2008 (66.1 chasmothecia/cm^2), 1 October 2009 (30.5 chasmothecia/cm^2), and 24 September 2010 (7.7 chasmothecia/cm^2) (Figs. 3.5A-C). In the 'Marechal Foch' vineyard, a peak was observed on 16 September 2009 (5.8 chasmothecia/cm^2) (Fig. 3.6A).

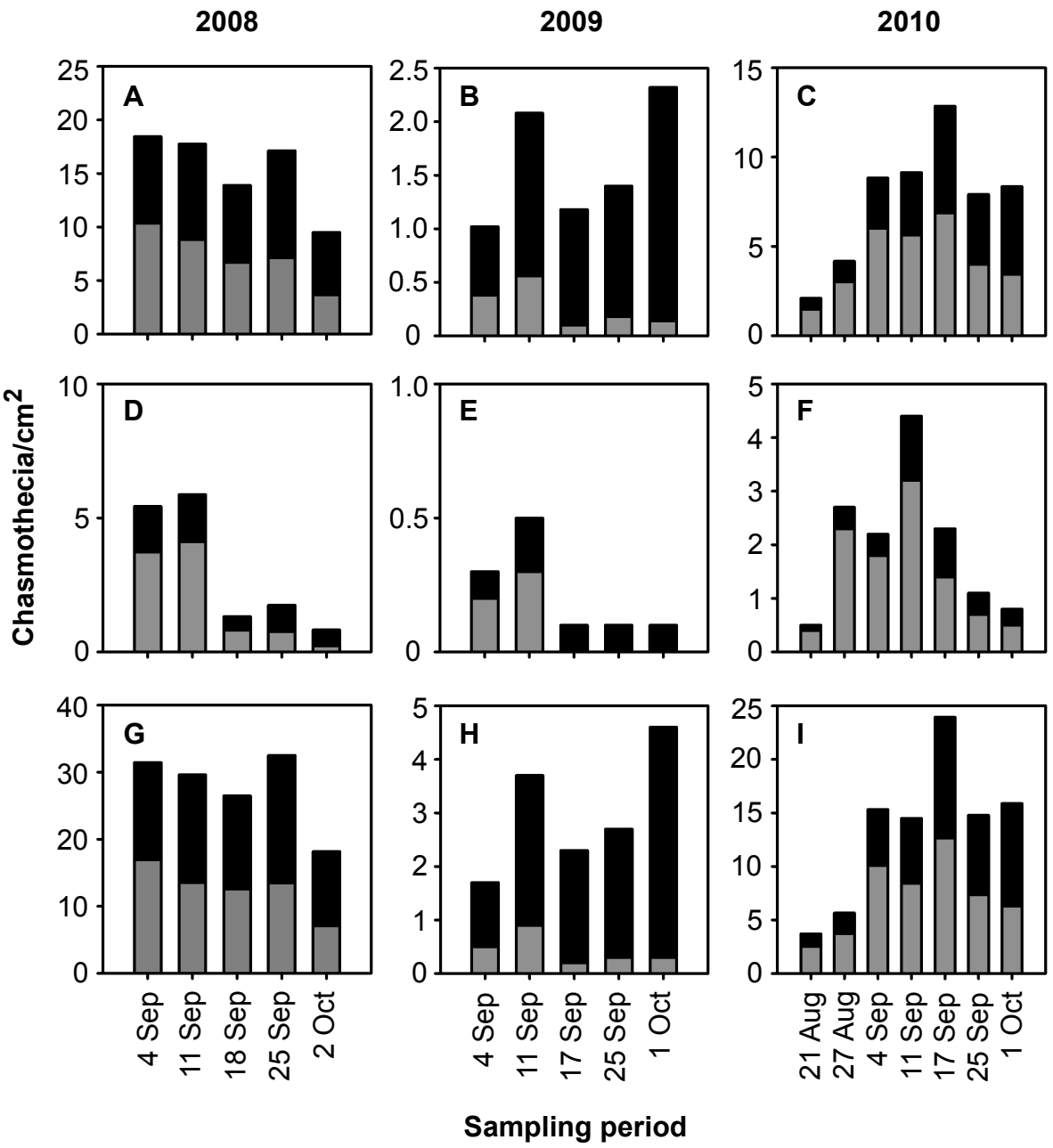

Figure 3.4. Average number of *Erysiphe necator* chasmothecia per square centimeter of leaf (A, B and C; n = 10 cm^2) and average number of chasmothecia found on the adaxial (D, E, and F; n = 5 cm^2) and abaxial (G, H, and I; n = 5 cm^2) leaf surfaces of untreated *Vitis* L. interspecific hybrid 'Chardonel' planted at the Michigan State University Clarksville Research Center in Clarksville, MI. Immature and mature chasmothecia are denoted with gray and black bars, respectively. Bars represent averages of four replicate samples of 20 leaves per vine on each sampling date during 2008 (A, D and G), 2009 (B, E and H), and 2010 (C, F and I).

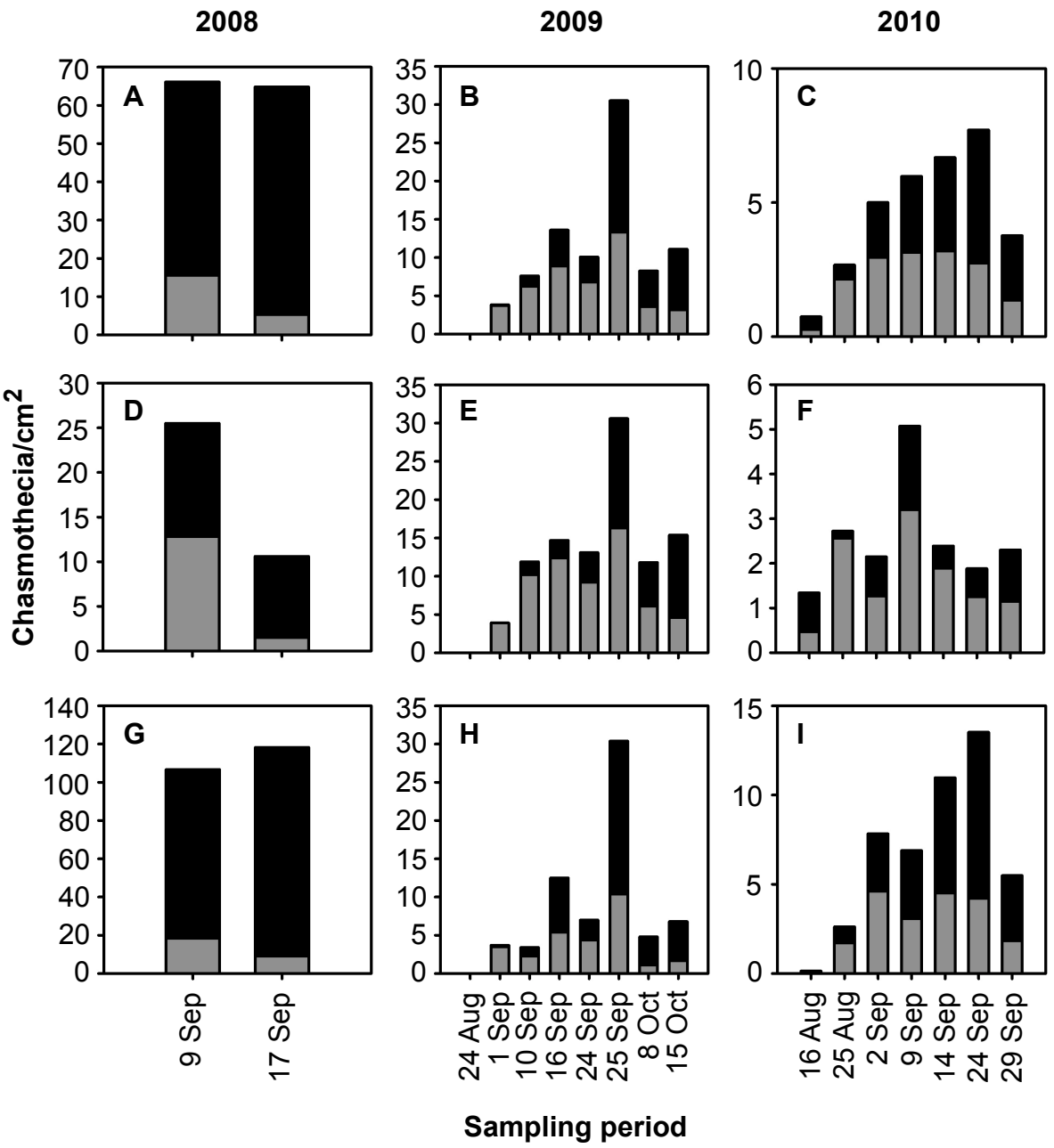

Figure 3.5. Average number of *Erysiphe necator* chasmothecia per square centimeter of leaf (A, B and C; n = 10 cm^2) and average number of chasmothecia found on the adaxial (D, E, and F; n = 5 cm^2) and abaxial (G, H, and I; n = 5 cm^2) leaf surfaces of untreated *Vitis* vinifera 'Pinot Noir' planted at the Michigan State University Northwest Michigan Horticulture Center in Traverse City, MI. Immature and mature chasmothecia are denoted with gray and black bars, respectively. Bars represent averages of 5, 10, and 20 leaves on each sampling date during 2008 (A, D and G), 2009 (B, E and H), and 2010 (C, F and I), respectively.

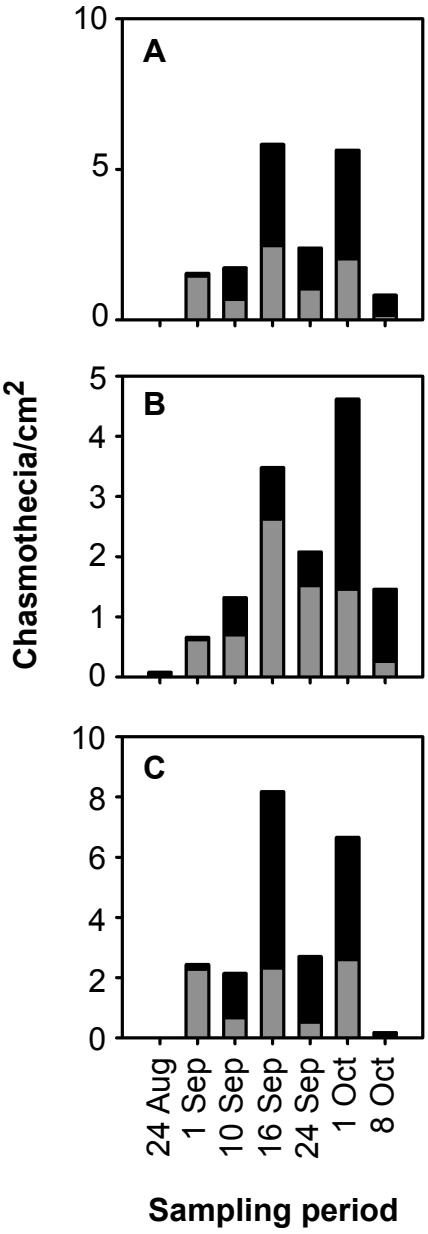

Figure 3.6. (A) Average number of *Erysiphe necator* chasmothecia per square centimeter of leaf (n = 10 cm^2) and average number of chasmothecia found on the (B) adaxial (n = 5 cm^2) and (C) abaxial (n = 5 cm^2) leaf surfaces of fungicide-untreated grapevines of *Vitis* L. interspecific hybrid 'Marechal Foch' planted in a commercial vineyard in Suttons Bay, MI. Immature and mature chasmothecia are denoted with gray and black bars, respectively. Bars represent averages of 20 leaves per sampling date during 2009.

In the Pearson correlation analyses, little or no association was found between the number of *E. necator* chasmothecia observed on the adaxial surface of the leaves and foliar severity of powdery mildew in the 'Chardonel' and 'Pinot Noir' vineyards when all the data from the different sampling dates in 2009 and 2010 were pooled together (Table 3.5). However, when looking at the specific sampling dates, significant positive correlations were found in the 'Chardonel' vineyard on 19 August and 2 September 2010 (Table 3.5), possibly because little or no rain fell during 14 to 20 August and 26 August to 2 September 2010 (Fig. 3.1F).

Table 3.5. Pearson correlation coefficients (*r*) between the number of *Erysiphe necator* chasmothecia observed on the adaxial surface and the severity of powdery mildew in the leaves collected in a vineyard of *Vitis* L. interspecific hybrid 'Chardonel' at the Michigan State University (MSU) Clarksville Research Center in 2009 and 2010, and a vineyard of *V. vinifera* 'Pinot Noir' at the MSU Northwest Michigan Horticultural Research Center in 2010.

Cultivar	Year†	Sampling date	*r*
'Chardonel'	2009	September 4	**0.198 ***
		September 11	0.179 ns
		September 17	0.122 ns
		September 25	-0.172 ns
		October 1	0.133 ns
		Overall††	0.037 ns
	2010	August 19	**0.452 ****
		August 26	0.094 ns
		September 2	**0.395 ****
		September 9	0.091 ns
		September 17	0.007 ns
		Overall	**0.186 ****
'Pinot Noir'	2010	September 2	-0.014 ns
		September 9	**-0.403 ***
		September 14	-0.061 ns
		September 24	-0.109 ns
		Overall	-0.066 ns

Values in **bold** indicate a significant correlation at $P = 0.05$ (**) or $P = 0.10$ (*); ns = not significant.

† Disease severity data were not collected for the 'Chardonel' leaves in 2008 and 'Pinot Noir' leaves in 2008 and 2009.

†† Overall = the analysis was performed with all sampling dates in the year.

DISCUSSION

The overwintering inoculum of *E. necator* was produced on infected leaves at all sites in the three years of our study. Chasmothecia were found from mid-August until the freezing temperatures of fall killed the remaining leaves on the vines (early October). Flag shoots were never observed during the study, corroborating the assumption of chasmothecia being the sole source of primary inoculum in Michigan, as determined in New York and eastern Washington (14, 25). This was not unexpected as Michigan's winters are similar to or more severe than New York. After many decades of extensive research, the epidemiological role of chasmothecia as a source of primary inoculum, particularly in viticultural regions where *E. necator* cannot overwinter as mycelium in dormant buds, has been widely accepted (3, 5, 8, 16, 25).

The highest numbers of chasmothecia on leaves were always observed in mid- to late September, and were also reflected in rainwater samples. The differences observed in the number of chasmothecia produced in each vineyard may be related to the inherent susceptibility of the hosts. Both 'Chardonel' and 'Marechal Foch' are hybrid cultivars that are moderately susceptible to powdery mildew. On the other hand, 'Pinot Noir' is a European Vinifera grape variety that is highly susceptible to the disease. The differences that were observed between the years of the study at each site can be explained by variations in the disease pressure during each growing season; a late start and overall cooler temperatures characterized the growing season of 2009.

We have shown that a large number of chasmothecia are produced per square centimeter of leaf area. For example, considering that the total area of a 'Chardonel' leaf was 79.1 cm^2 (average of 100 leaves, standard error 4.4) and taking the highest and lowest number of chasmothecia observed during our study (15.3 and 1.6 chasmothecia/cm^2 in 2008 and 2009, respectively, Table 3.2), we estimate that a maximum of 1,210 chasmothecia and a minimum of

126 chasmothecia can be produced per infected 'Chardonel' leaf. However, since not all ascocarps develop simultaneously under field conditions, those ascocarps that develop later in the season will not be able to reach maturity by the time the growing season ends. Also, the estimation of production on leaves is modified by the effect of rainfall, which washes off mature chasmothecia, resulting in a dynamic continuum. Under controlled temperatures, Gadoury and Pearson (1988) determined that 50% or more of the chasmothecia in a sample would mature provided that 528, 500, and 625 degree days (base 0^{o}C) had accumulated at 16^{o}C, 20^{o}C, and 25^{o}C, respectively (8). In Clarksville, the degree-day accumulation was 737, 677, and 711 from 21 August to 1 October in 2008, 2009, and 2010, respectively. During the same periods, the degree-day accumulation at Traverse City was 737 in 2008, 694 in 2009, and 695 in 2010. Although these values are higher than the minimum accumulation estimated by Gadoury and Pearson (1988), they do not exceed the reported values by much. Therefore, this would explain the presence of immature chasmothecia at the end of the growing season, since the decreasing temperatures experienced later in the fall would slow down the development of any chasmothecia produced after the first week of September. Based on a proposed temperature requirement for ascocarp maturation, Legler *et al*. (2011) developed a set of equations to predict the development of immature and mature chasmothecia in terms of time and temperature. Aside from an overestimation of the number of mature ascocarps (which is related to inoculum dispersal after rain events), the prediction models developed by Legler *et al*. represent a step forward in the development of strategies for powdery mildew inoculum eradication (21).

When chasmothecia are fully developed, they detach from the anchoring fungal colony to be readily dispersed by the rain (8). As expected, only black, fully-mature chasmothecia were found in the rainwater samples collected in the 3-L bottle traps. Furthermore, the fact that mature

chasmothecia were found (generally in very low numbers) in most of the traps from the first sampling period at all sites, indicates that the development of the overwintering inoculum in those vineyards had begun even earlier. In a study by Cortesi *et al*. (1995), a lack of correlation was observed between the amount of rainfall and the number of chasmothecia trapped. The authors argued that variations in the number of chasmothecia at the detached stage might be responsible for such an outcome (6). In the present study, the Pearson correlation analyses only revealed a positive correlation between the two variables in four of the seven site×year combinations (Table 3.3). The lack of correlation in Clarksville in 2009 and 2010 may be due to the occurrence of rainfall after a long dry period when a large number of chasmothecia were ready to be dispersed, meaning that not much rain was needed to cause a major dispersal. On the other hand, several rainy days were observed early in the sampling season at Traverse City in 2010, a time when most chasmothecia were probably still in the process of maturation. Additionally, the low correlation found between the number of ascocarps on the adaxial surface of the leaves and disease severity is indicative of the role of rain events in the dispersal of *E. necator*.

Rain-dispersed chasmothecia can be either land on the vineyard soil or the trunk of the vines. However, in New York vineyards, chasmothecia could not survive in the soil but only in the bark of the vines (8). Survival on senescent leaves on the vineyard soil has been demonstrated in Australia and Italy but also in the eastern region of the state of Washington (5, 14, 23). Survival of chasmothecia on leaves on the soil surface is likely a function of how fast leaves break down; in dry climates, leaves and chasmothecia may remain intact for a much longer time than in the humid midwestern and eastern United States. Earthworms may also play a role in breakdown of leaf tissue and concomitant destruction of chasmothecia in the fall and

spring (David Gadoury, *personal communication*). In Michigan, we assume that *E. necator* survival is also restricted to chasmothecia in the bark crevices of the vines, but this has not been confirmed. Interestingly, every year, we found considerably larger amounts of chasmothecia on the abaxial than on the adaxial surfaces of the leaves. Chasmothecia developing on the adaxial surfaces are more exposed to wash-off by rain. Furthermore, Willocquet *et al*. (1996) demonstrated that mycelial growth of *E. necator* was significantly reduced by UV exposure, hence radiation might also have a negative effect on the development of chasmothecia on the adaxial surfaces of the leaves (36). There may also be differences in temperature and humidity experienced by chasmothecia on either side of the leaf. By the time of leaf drop, mature chasmothecia still remained attached to the leaves; therefore, this inoculum would also fall to the ground with senescent leaves. Gadoury and Pearson (1988) concluded that the reason why chasmothecia that remained attached to leaf tissues are unable to survive the winter in New York is that the ascocarps were not fully mature (8). However, considering the large numbers of black, apparently mature chasmothecia observed on the abaxial surfaces of leaves in our study, the fate of these chasmothecia may require further investigation.

The present research is part of an effort to discern the epidemiology of powdery mildew under Michigan weather conditions in order to improve available disease management schemes. The information gathered on the timing of ascocarp development and the density of potential overwintering inoculum at the end of the growing season, together with our investigation on ascospore release in spring, would allow better protection of grapevines by reducing the risk of primary infections. The application of eradication measures before a large percentage of the developing chasmothecia reach full maturation appears to be a suitable approach to control powdery mildew epidemics during the following growing season, since fungicide applications do

not affect ascocarps at this final developmental stage (21). However, since powdery mildew is a polycyclic disease, an integrated approach for disease control is still required because it is very unlikely that all chasmothecia would be eliminated from the vineyard, especially in years with specific environmental conditions that favor disease development.

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CHAPTER 4

SCREENING FOR STROBILURIN (QOI) RESISTANCE IN POPULATIONS OF *ERYSIPHE NECATOR* ON GRAPES IN MICHIGAN

ABSTRACT

Powdery mildew, caused by *Erysiphe necator*, is the most common and destructive disease of grapes worldwide. In Michigan, it is primarily controlled with fungicides, including strobilurins (Quinone outside Inhibitors [QoIs]). Within the United States, resistance to this class of fungicides has been reported in *E. necator* populations in New York and Virginia. In a preliminary survey to determine whether QoI resistance is present in Michigan vineyards, 12 *E. necator* isolates were collected from five vineyards in 2008 and evaluated for the G143A single-nucleotide mutation responsible for QoI resistance. The mutation was detected in one isolate, which was confirmed to be resistant in a conidium germination assay on water agar amended with trifloxystrobin at 0.001, 0.01, 0.1, 1, 10, or 100 μg/ml and salicylhydroxamic acid (100 mg/liter). The mutant was able to germinate on 100 μg/ml trifloxystrobin amended media, whereas a representative wild-type isolate did not germinate at concentrations higher than 0.1 μg/ml. In 2009, 172 isolates were collected from a total of 21 vineyards (juice and wine grapes): three vineyards with no fungicide application history (baseline sites), six research vineyards, and 12 commercial vineyards. EC_{50} values were determined for each isolate by linear regression analysis of their dose-response curves. QoI resistance was defined as an $EC_{50} \geq 1.01$ μg/ml. Isolates from baseline sites had EC_{50} values mostly below 0.01 μg/ml, while isolates that were highly resistant to trifloxystrobin ($EC_{50} > 100$ μg/ml) occurred in five research and three

commercial wine grape vineyards at frequencies of 40 to 100% and 25 to 75% of the isolates, respectively. Detection of the G143A mutation was positive for every isolate with an $EC_{50} \geq$ 1.01 μg/ml. These results suggest that fungicide resistance may play a role in poor control of powdery mildew observed in some Michigan vineyards.

INTRODUCTION

Powdery mildew (*Erysiphe necator* Schwein.) is the most destructive and widely distributed disease of grapes worldwide (16). Wine grape cultivars (*Vitis vinifera* L. and many *Vitis* L. interspecific hybrids) are highly susceptible. Considerable reductions in fruit and wine quality can occur even when infections are relatively mild, e.g., 1 to 5% of the berries infected in a cluster (4, 20, 21). Juice grapes (*V. labrusca* L.) are more tolerant to powdery mildew but reductions in juice quality in terms of sugar content, color, and acidity have also been reported (10). Traditionally, management of powdery mildew has relied on the application of sulfur, synthetic fungicides, or a combination of both (16). The sterol demethylation inhibitor (Fungicide Resistance Action Committee [FRAC] code 3) and Quinone-outside-Inhibitor (QoI, strobilurin) fungicides (FRAC code 11) are widely used around the world. In Michigan, QoI fungicides may be applied throughout the growing season but the total number of applications per hectare per season is limited to reduce the risk of fungicide resistance (23). Since the introduction of fungicides with a site-specific mode of action, the development of resistance has become an important issue in crop protection because the repeated use of these products selects for those rare fungicide-resistant isolates with genetic mutations that survive and proliferate in the population (13).

Fungicides in the QoI class are site-specific fungicides that act by blocking mitochondrial respiration in a wide range of fungi (Ascomycetes, Basidiomycetes, and Deuteromycetes) as well as Oomycetes; they specifically bind to the ubiquinol oxidation center (Q_o site) of cytochrome b, obstructing the electron transfer to cytochrome c_1, which then prevents the synthesis of adenosine-5'-triphosphate (ATP) (2). In total, 11 point mutations have been identified in the cytochrome b (*CYTB*) gene that confer different levels of resistance to QoI fungicides in a

variety of organisms (11). In plant-pathogenic fungi, three single-nucleotide mutations of the *CYTB* gene (G143A, F129L, and G137R) and the activation of the alternative oxidation (AOX) pathway have been reported as the means through which these fungi acquire resistance to QoI fungicides (12, 13). In the United States, QoI resistance in *E. necator* has been previously reported in New York (22) and Virginia (3). However, only the nucleotide change that leads to the G143A mutation was associated with the resistant phenotype (3, 6).

The objectives of this study were to screen a subpopulation of *E. necator* isolates collected from Michigan vineyards for resistance to trifloxystrobin, a synthetic fungicide in the QoI class that is commonly used in disease management programs throughout the state. This survey was designed in response to complaints from grape growers regarding poor control of powdery mildew in their vineyards. The baseline sensitivity of *E. necator* to trifloxystrobin was determined for a subsample of individuals isolated from sites with no history of strobilurin fungicide applications.

MATERIALS AND METHODS

Collection of *Erysiphe necator* isolates.

Preliminary study of QoI resistance. In October 2008, a total of 12 single-conidium isolates were collected in Michigan from two commercial vineyards (three isolates from a 'Concord' vineyard in Watervliet and three from a 'Cabernet Sauvignon' vineyard in Traverse City), two Michigan State University (MSU) research vineyards (three isolates from 'Concord', in Fennville and one isolate from 'Pinot Noir' in Traverse City) and a MSU greenhouse (two isolates from potted 'Chardonnay' plants in East Lansing).

Main study of QoI resistance. A more in-depth sampling was performed in September and October 2009; isolates were collected from 21 juice and wine grape vineyards in Michigan. The vineyards were located in southwest, central, and northwest Michigan. Three sites with no history of fungicide applications (from this point forward referred to as "baseline sites"), six MSU research vineyards, and 12 commercial grape vineyards were included in the sample. Baseline sites were located in East Lansing (abandoned 'Concord' vineyard) and Traverse City ('Pinot Gris' and 'Vignoles' vineyards farmed with the unique organic principles of biodynamic agriculture). MSU research vineyards were located at the Clarksville Research Center (CRC, 42° 52' 18'' N, 85° 15' 31'' W) in Clarksville; Northwest Michigan Horticultural Research Center (NWMHRC, 44° 52' 59'' N, 85° 40' 28'' W) in Traverse City; Southwest Michigan Research and Extension Center (SWMREC, 42° 5' 22'' N, 86° 21' 36'' W) in Benton Harbor; and Trevor Nichols Research Center (TNRC, 42° 35' 38'' N, 86° 9' 19'' W) in Fennville, Michigan. Commercial vineyards were mostly located in Southwest Michigan (two 'Chancellor', six 'Concord', one 'Frontenac', and one mixed *V. vinifera* vineyard). One 'Frontenac' vineyard in

Highland was also sampled as well as a young 'Chardonnay' vineyard near Traverse City (Table 4.1).

Each vineyard in 2009 was sampled following a diagonal pattern. Two leaves were collected from a single vine in 10 different rows evenly spaced and proportional to the size of the vineyard. In vineyards with less than 10 rows, more vines were sampled per row. A total of 20 powdery-mildew-infected leaves were collected per site. Samples were cold-transported to the laboratory in plastic resealable bags.

Maintenance of *Erysiphe necator* isolates.

An ethanol-sterilized eyelash attached with Parafilm M to a glass Pasteur pipet was used to transfer a single conidium from each field sample onto a single, detached, disease-free *V. vinifera* 'Chardonnay' leaf placed in a Petri dish containing 25 ml of water agar as previously described (8). A total of 20 transfers were attempted per site. The leaves were taken from potted, 1-year old, bare-root vines grown in 16-cm plastic pots containing commercial potting soil (BACCTO, Michigan Peat Company, Houston, TX, USA), selecting young leaves at the 3rd and 4th node positions from the shoot tip. The vines were started and kept in a controlled-environment growth chamber (model E15, Conviron, Winnipeg, Manitoba, Canada) at 25^{o}C, with a photoperiod of 16 h light (fluorescent light intensity of 230 mE at 30 cm height) and 8 h darkness. In total, 172 single-conidium isolates were obtained in 2009 from both juice (*Vitis labrusca* L.) and wine (*V. vinifera* L. and interspecific hybrids) grapevines (Table 4.1). Powdery mildew isolates were maintained on 'Chardonnay' leaves by transferring two to three conidial chains every three weeks onto three separate areas of a leaf (in duplicate), until fungicide sensitivity and molecular analyses were performed. To prevent cross contamination between

isolates, all transferring utensils were dipped in ethanol for 1 min and allowed to dry before the next inoculation. All transfers were conducted in a laminar flow hood, which was also sprayed with ethanol between isolates. Non-inoculated leaves served as controls of contamination. Between three to seven transfers were made per isolate before DNA extraction and bioassays were performed.

Detection of the G143A mutation.

For every *E. necator* isolate collected in 2008 and 2009, DNA was extracted from conidia scraped from 3-week-old colonies growing on detached 'Chardonnay' leaves. Conidia were suspended in 1 ml of lysis buffer (2% CTAB, 3% SDS, 25 mM EDTA, 200 mM Tris-HCl pH 8.5, and 250 mM NaCl) heated at 65°C for 1 h. DNA in the supernatant fractions was extracted and stored as previously described (18).

Preliminary study of QoI resistance. In the study of isolates collected in 2008, 5 µl of DNA were used in 25-µl PCR reactions amplifying the ribosomal internal transcribed spacer (ITS) region (as a control of fungal DNA amplification) and a fragment of the mitochondrial cytochrome b (*CYTB*) gene containing the G143A single-nucleotide mutation responsible for strobilurin resistance (13). The primer sets used in each case were ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), and the Amplification Refractory Mutation System (ARMS) primer sets developed by Baudoin *et al*. (2008) to detect the wild-type and mutant allele G143A (3), respectively. To detect the G143A mutation, PCR was performed under the following conditions: an initial denaturation step at 95°C for 15 min, followed by 40 amplification cycles (denaturation at 94°C for 15 s, annealing at 60°C for 30 s, extension at 72°C for 30 s), and a final elongation step at 72°C for 10 min. PCR

products were then run in a 1% agarose Tris-acetate-ethylenediaminetetraacetic acid (TAE) gel at 100 V for 1 h in 1X TAE buffer, and stained with ethidium bromide (0.5 µg/ml). To verify *CYTB* gene amplification, the products obtained with both the wild type and mutant primer sets were cloned for two representative isolates using the pGEMT-T Easy Kit (Promega, Madison, WI, USA). Then, 5 µl of the ligation reaction was used to transform DH5α chemically competent cells (Invitrogen, Carlsbad, CA, USA) using the manufacturer's protocols. Inserts were sequenced using T7 (5′-AATACGACTCACTATAG-3′) and Sp6 (5′-ATTTAGGTGACACTATAG-3′) primers in the MSU Genomics Technology Support Facility (GTSF). Contiguous sequences were constructed with the Lasergene Core Suite (DNASTAR Inc., Madison, WI, USA) and were analyzed using the GenBank BLASTn program. The total DNA of these two *E. necator* isolates was diluted to a concentration of 5 ng/µl and used as a reference to standardize the detection of the G143A mutation for the 2009 powdery mildew collection by quantitative PCR. DNA concentrations were determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). Detection of the point mutation was performed using the ARMS-SYBR Green Q-PCR standardized by Baudoin *et al*. (2008) and the Applied Biosystems 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, CA, USA) in the MSU GTSF. This was done at three different concentrations: the standard concentration (5 ng/µl) and two tenfold serial dilutions (1:10 and 1:100) of the DNA extracts of each reference isolate. Amplification plots and disassociation curves were inspected for each pair of primers to analyze the quality of each overall reaction and final PCR product, respectively.

Main study of QoI resistance. The DNA extracts of the 2009 powdery mildew collection were diluted to a concentration of 5 ng/µl for Q-PCR. Detection of the G143A mutation was

performed as previously described using the ARMS-SYBR Green Q-PCR standardized by Baudoin *et al*. (2008). The percentage of G143A in each isolate was calculated using the cycle threshold (Ct-value) obtained from the amplification with both wild-type and mutant primer sets in the formula as described by Colcol (2008): $100 \times (1 / (1 + 2^{[\text{mutant Ct} - \text{wild type Ct}]}))$ (5). Amplification plots and disassociation curves were also inspected for every isolate that was evaluated.

Differences in the detection of the G143A mutation were also assessed by performing Chi-square analysis. Chi-square (χ^2) values were determined for the hypothesis that the total number of positive detections was evenly distributed within type of site, region in Michigan, and type of grape.

Fungicide sensitivity.

A conidium germination bioassay was performed on microscope slides covered with 3 ml of water agar amended with the commercial formulation of the fungicide Flint (trifloxystrobin, 50% active ingredient; Bayer Crop Sciences, Kansas, MO, USA) at a trifloxystrobin concentration of 0.001, 0.01, 0.1, 1, 10, or 100 μg/ml. The medium was also supplemented with 100 mg/liter of salicylhydroxamic acid (SHAM, 99%, Sigma-Aldrich, St. Louis, MO, USA) dissolved in methanol to block the alternative oxidation (AOX) pathway (25). Germination controls included powdery mildew conidia on slides with water agar only and water agar amended with SHAM. Conidia were transferred from a 2-week-old *E. necator* colony onto the agar slides with an ethanol-sterilized, fine-hair paintbrush. Inoculated slides were placed inside 150x15 mm Petri dishes. After 24 h of incubation under fluorescent lights at ambient temperature (22 to 24^{o}C), 50 conidia were microscopically observed for germination in each of

three replicate samples per treatment. A conidium was considered germinated if the germ tube was equal in length to at least half the width of the conidium. From the preliminary study in 2008, two representative isolates with differential patterns of amplification using the G143A primer set for the detection of the G143A mutation (Fig. 4.1) were tested to corroborate the results obtained using the molecular approach. A total of 125 isolates were evaluated in 2009 using this germination assay.

Qualitative analysis of trifloxystrobin sensitivity. For this analysis, an isolate was declared resistant if the average number of germinated conidia in a particular treatment was at least two standard deviations above the mean in the control treatment (i.e. agar slides amended with SHAM). At each trifloxystrobin concentration evaluated, either a "1" (germination occurred hence the isolate was considered resistant) or a "0" (germination did not occur hence the isolate was considered sensitive) was assigned to every isolate tested.

Quantitative analysis of trifloxystrobin sensitivity. Inhibition percentages were calculated relative to germination of conidia in the SHAM control. The effective concentration of trifloxystrobin that inhibited 50% of the conidial germination (EC_{50}) was calculated by linear regression analysis of the germination percentage of *E*. *necator* conidia according to the logarithm of the active ingredient concentration. When the control treatment with SHAM only had a larger inhibitory effect than the lowest fungicide concentration (i.e. agar slides amended with trifloxystrobin at 0.001 μg/ml and SHAM 100 mg/liter), the latter was used as the reference point for calculating the EC_{50}. The reproducibility of the bioassay for trifloxystrobin sensitivity was evaluated by testing six isolates (three from baseline sites and three from MSU research vineyards) three times. For every repetition of the bioassay, new stock solutions of both SHAM and trifloxystrobin were prepared.

Responses of *E. necator* to trifloxystrobin and SHAM.

Due to an inhibitory effect of SHAM on some of the isolates, the responses of *E. necator* to SHAM were further characterized for a sensitive isolate (collected from a baseline vineyard) and a resistant isolate (collected from an MSU research vineyard) isolate. Different concentrations of SHAM (12.5, 25, 50, and 100 mg/liter) were evaluated on agar slides amended with 0, 0.001, 0.1, and 10 μg/ml trifloxystrobin. Treatment differences were analyzed by one-way analysis of variance (ANOVA) using the statistical computer package SigmaPlot (version 11.0, Systat Software Inc., San Jose, CA, USA). Differences were analyzed further using Fisher's protected LSD as the all-pairwise multiple comparison procedure, and treatment effects were declared significant at $P \leq 0.05$.

RESULTS

Preliminary study of QoI sensitivity in 2008.

The ARMS primer sets and conditions standardized by Baudoin *et al*. (2008) allowed for the amplification of a cytochrome b (*CYTB*) gene fragment by traditional PCR, which contained the single-nucleotide change that generates the G143A mutation. A distinct amplification pattern was observed for a single isolate (out of 12 total) in a 1% agarose TAE gel. Background amplification was observed in reactions with all other isolates (lanes 1-7 and 9-12) and in the water control (lane W) with bands at approximately 400, 350, and a faint band at 100bp. However, in lane 8 a strong PCR band of about 100 bp was observed with no background amplification (Fig. 4.1). This *E*. *necator* isolate was collected from a commercial 'Cabernet Sauvignon' vineyard in Traverse City, MI.

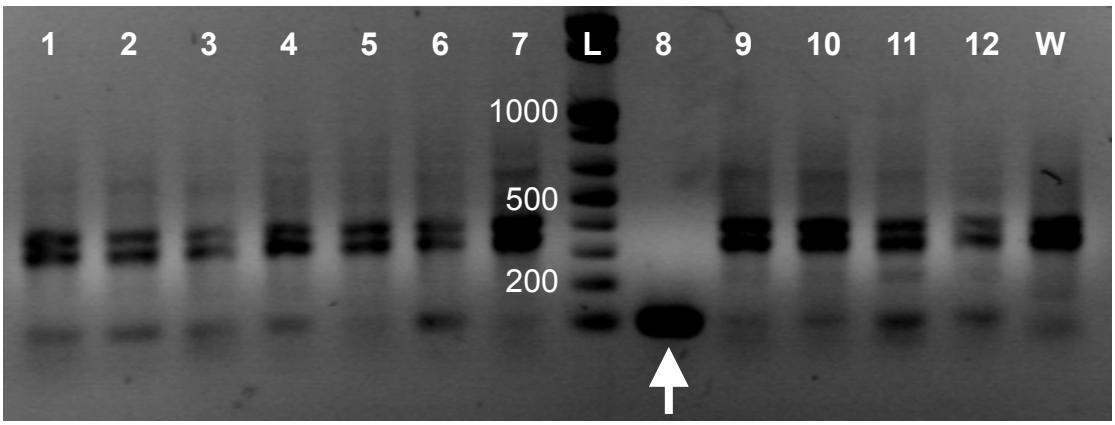

Figure 4.1. Detection of the single-nucleotide change in the cytochrome b (*CYTB*) gene that generates the G143A mutation in QoI resistant pathogens. Polymerase chain reaction (PCR) products obtained from the preliminary study with *Erysiphe necator* isolates in 2008 using the Amplification Refractory Mutation System (ARMS) mutant primer set and conditions standardized by Baudoin *et al*. (2008). Lanes 1-3, isolates from a commercial 'Concord' vineyard in Watervliet, MI; lanes 4-6, isolates from a 'Concord' vineyard at the Michigan State University (MSU) Trevor Nichols Research Center at Fennville, MI; lanes 7-9, isolates from a commercial 'Cabernet Sauvignon' vineyard in Traverse City, MI; lane 10, isolate from a 'Pinot Noir' vine at the MSU Northwest Michigan Horticultural Research Center, Traverse City, MI; lanes 11-12, isolates from 'Chardonnay' vines growing in a MSU greenhouse in campus, East Lansing, MI; lane L, 1-kb+ DNA ladder; lane W, negative control (water). The white arrow denotes the target amplicon *CYTB* (of less than 100 bp).

The detection of the G143A mutation was further confirmed by ARMS-SYBR Green Q-PCR with final DNA concentrations of 500, 50, and 5 pg/µl. The G143A primers led to earlier amplicon detection in the reaction with the resistant isolate as compared to the wild-type primers (Fig. 4.2B). In a susceptible isolate, detection occurred earlier while using the wild-type primers (Fig. 4.2A).

The *in vitro* germination bioassay on agar slides amended with trifloxystrobin and SHAM showed that the isolate with the G143A mutation was able to germinate at the highest trifloxystrobin concentration tested (89% germination at 100 µg/ml, relative to the SHAM control). A representative wild-type isolate was chosen based on the *CYTB* genotype screening. This isolate was collected from a 'Concord' MSU research vineyard in Fennville and was unable to germinate at a trifloxystrobin concentration greater than 0.1 µg/ml. The estimated EC_{50} value for the sensitive isolate was 0.03 µg/ml, while the value for the resistant isolate could not be calculated because it exceeded the highest concentration tested. The DNA from these two *E. necator* isolates were used as reference controls for the ARMS-SYBR Green Q-PCR detection of the G143A mutation in the 2009 powdery mildew collection.

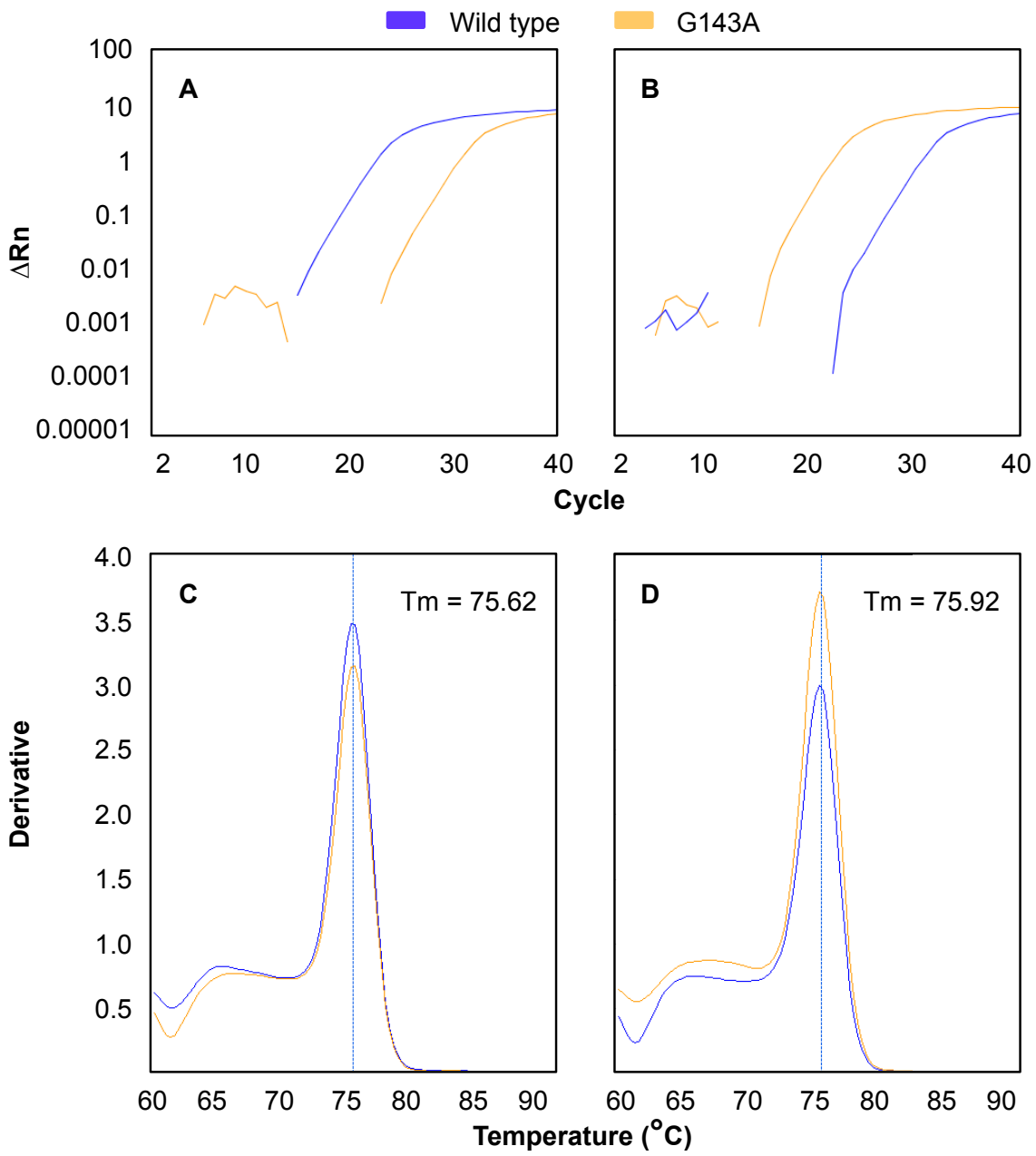

Figure 4.2. Amplification of a fragment of the mitochondrial cytochrome b (*CYTB*) gene containing the site for the G143A mutation in *Erysiphe necator* using the SYBR green Q-PCR Amplification Refractory Mutation System (ARMS) developed by Baudoin *et al*. (2008). Amplification plots (A and B) and product disassociation curves (C and D) obtained for an *E. necator* trifloxystrobin-sensitive isolate (A and C) and trifloxystrobin-resistant isolate (B and D) from grapes in Michigan using the ARMS wild-type (in blue) and G143A (in orange) primer sets are shown for each case.

Qualitative analysis of QoI sensitivity in 2009.

Twenty-one vineyards were sampled in Michigan during 2009. In total, 125 isolates were evaluated for sensitivity to trifloxystrobin in spore germination bioassays. Overall, the qualitative germination analysis demonstrated that 74.4% of the 125 isolates evaluated germinated at 0.001 μg/ml trifloxystrobin. At 0.01, 0.1, and 1 μg/ml trifloxystrobin, 36.8, 27.2, and 22.4% of the isolates were able to germinate. At the highest fungicide concentrations (10 and 100 μg/ml trifloxystrobin), only 20.8% of the isolates germinated (Table 4.1).

None of the *E. necator* isolates collected from the abandoned 'Concord' vineyard in East Lansing (n = 14 isolates, one of the baseline sites) were able to germinate at trifloxystrobin concentrations higher than 0.001 μg/ml. Among the 23 baseline isolates collected from the 'Pinot Gris' and 'Vignoles' biodynamic vineyards near Traverse City, 35% were able to germinate at 0.01 μg/ml trifloxystrobin and 9% germinated at 0.1 μg/ml trifloxystrobin, which corresponds to 21.6 and 5.4% of all baseline isolates, respectively (Table 4.1). At the highest trifloxystrobin concentration evaluated (100 μg/ml), only 11.3% of the isolates from commercial vineyards germinated whereas 73.1% of the isolates from research vineyards did (Table 4.1).

When comparing isolates from juice grapes (*V. labrusca* L.) and wine grapes (*V. vinifera* L. and *Vitis* L. interspecific hybrids), 32.1% of the isolates from wine grapes (26 of 81 isolates) were able to germinate at 100 μg/ml trifloxystrobin (Table 4.1) whereas no isolates from juice grapes were able to germinate at concentrations higher than 0.01 μg/ml trifloxystrobin. In fact, only 6.8% of the juice grape isolates (3 of 44 isolates) germinated at 0.01 μg/ml trifloxystrobin (Table 4.1).

Table 4.1. Percentage of *Erysiphe necator* isolates collected in Michigan vineyards in 2009 that germinated at each concentration of trifloxystrobin evaluated (0.001, 0.01, 0.1, 1, 10, and 100 µg/ml). Germination was considered to have occurred when the average number of germinated conidia at a certain fungicide concentration was at least two standard deviations above the mean in the control treatment.

		Total number of isolates	**Percentage of isolates that germinated at each trifloxystrobin concentration (µg/ml)**					
			0.001	**0.01**	**0.1**	**1**	**10**	**100**
Type of site	Baseline	37	86.5	21.6	5.4	0.0	0.0	0.0
	Commercial	62	61.3	25.8	16.1	12.9	11.3	11.3
	Research	26	88.5	84.6	84.6	76.9	73.1	73.1
Type of grape	Juice[a]	44	63.6	6.8	0.0	0.0	0.0	0.0
	Wine[b]	81	80.2	53.1	42.0	34.6	32.1	32.1
All		*125*	*74.4*	*36.8*	*27.2*	*22.4*	*20.8*	*20.8*

[a] Juice grapes are *Vitis labrusca* L. cultivars.

[b] Wine grapes are cultivars of *V. vinifera* L. and *Vitis* L. interspecific hybrids.

Quantitative analysis of QoI sensitivity in 2009.

Reproducibility of the conidium germination bioassay. The effective concentrations of trifloxystrobin at which conidial germination was inhibited by 50% (EC_{50}) were estimated for six isolates (three from baseline sites and three from research vineyards) in three repetitions of the germination bioassay on agar slides amended with trifloxystrobin and SHAM. The calculated coefficients of variation ranged from 23 to 141% (Table 4.2). All three *E. necator* isolates from baseline sites had a mean EC_{50} value lower than 0.001 μg/ml while two of three isolates from research vineyards had a mean EC_{50} value higher than 100 μg/ml. Due to potential errors that may result from extrapolating beyond the range of concentrations tested, no EC_{50} values are reported here for those *E. necator* isolates with a value lower than 0.001 or higher than 100 μg/ml, but they were grouped in the EC_{50} categories of "≤ 0.001 μg/ml" or "≥ 100 μg/ml", respectively. The mean EC_{50} value for the third isolate from research vineyards was estimated as 1.2 μg/ml (Table 4.2).

Table 4.2. Reproducibility of the *Erysiphe necator* conidium germination bioassays on agar slides amended with the fungicide trifloxystrobin and 100 mg/liter SHAM. The fungicide sensitivity assay was conducted three separate times for six different isolates.

Isolate	Origin of isolate		Mean EC_{50} (µg/ml)[a]	CV (%)[b]
	Type of site	Host cultivar		
1	Baseline	Vignoles	< 0.001	37
2	Baseline	Vignoles	< 0.001	75
3	Baseline	Pinot Gris	< 0.001	23
4	Research	Aurora	1.2	129
5	Research	Vignoles	> 100	138
6	Research	Chardonel	> 100	141

[a] Mean EC_{50} value of trifloxystrobin (Flint, 50% active ingredient). The values "< 0.001 µg/ml" or "> 100 µg/ml" denote cases were the numerical value lay beyond the resolution range of the test but numbers were considered to calculate the coefficient of variation.

[b] Coefficient of variation (CV = [standard deviation/mean] × 100) for the EC_{50} values, based on estimates of three separate bioassays.

Trifloxystrobin sensitivity ranges in the subpopulation of 2009. The calculation of EC_{50} values generated a series of fungicide sensitivity ranges (Table 4.3, Fig. 4.3). *Erysiphe necator* isolates from the baseline sites had EC_{50} values of less than 0.001 μg/ml to 0.159 μg/ml (Fig. 4.3). Isolates collected from commercial vineyards and MSU research centers had EC_{50} values that ranged from less than 0.001 μg/ml to more than 100 μg/ml (Fig. 4.3). However, isolates with EC_{50} values higher than 100 μg/ml were found in 5 out of 6 MSU research vineyards and 3 out of 12 commercial vineyards (Table 4.3). EC_{50} values were less than 0.001 μg/ml for isolates from a newly established MSU vineyard at Benton Harbor and ranged from less than 0.001 μg/ml to 0.003 μg/ml in commercial vineyards from central, southwest, and northwest Michigan (Table 4.3).

The percentage of *E. necator* isolates that were highly sensitive to trifloxystrobin (EC_{50} values lower than 0.001 μg/ml) was 73 and 79% in the baseline and commercial vineyards, respectively (Figs. 4.3A and 4.3B). This proportion dropped to 8% in the MSU research vineyards, where 62% of the isolates had EC_{50} values higher than 100 μg/ml (Fig. 4.3C).

Table 4.3. Origin, G143A detection, and trifloxystrobin sensitivity for *Erysiphe necator* isolates collected in Michigan during 2009.

Site[a]		Location[b]		Grape host[c]		G143A detection[d]		Trifloxystrobin sensitivity[e]	
No.	**Type**	**City**	**Region**	**Species**	**Cultivar**	**Total tested**	**Total positive**	**Total tested**	**EC_{50} range (µg/ml)**
1	Baseline	East Lansing	C	Labrusca	Concord	14	0	14	< 0.001 to 0.005
2	Baseline	Traverse City	NW	Vinifera	Pinot Gris	8	0	8	< 0.001 to 0.159
3	Baseline	Traverse City	NW	Hybrid	Vignoles	17	0	15	< 0.001 to 0.006
4	Research	Fennville	SW	Hybrid	Aurora	8	8	4	57.3 to > 100
5	Research	Fennville	SW	Hybrid	Chancellor	8	8	7	22.2 to > 100
6	Research	Clarksville	C	Hybrid	Chardonel	4	4	3	> 100
7	Research	Traverse City	NW	Vinifera	Mixed	9	9	5	0.1 to > 100
8	Research	Benton Harbor	SW	*Vitis* spp.	Mixed	7	0	2	< 0.001
9	Research	Clarksville	C	Hybrid	Vignoles	7	7	5	0.251 to > 100
10	Commercial	Lawton	SW	Hybrid	Chancellor	5	5	4	0.316 to > 100
11	Commercial	Lawton	SW	Hybrid	Chancellor	6	6	2	1.7 to > 100
12	Commercial	St. Joseph	SW	Labrusca	Concord	4	0	4	< 0.001
13	Commercial	Lawton	SW	Labrusca	Concord	8	1	3	< 0.001
14	Commercial	Berrien Springs	SW	Labrusca	Concord	5	0	5	< 0.001
15	Commercial	Lawton	SW	Labrusca	Concord	9	0	7	< 0.001 to 0.002
16	Commercial	Lawton	SW	Labrusca	Concord	10	0	7	< 0.001
17	Commercial	Lawton	SW	Labrusca	Concord	8	0	4	< 0.001 to 0.002
18	Commercial	Highland	C	Hybrid	Frontenac	10	0	10	< 0.001 to 0.003
19	Commercial	Lawton	SW	Hybrid	Frontenac	6	6	4	0.088 to > 100
20	Commercial	Berrien Springs	SW	Vinifera	Mixed	10	0	8	< 0.001
21	Commercial	Traverse City	NW	Vinifera	Chardonnay	9	0	4	< 0.001
						Total tested = 172		**Total tested = 125**	

Table 4.3. (cont'd)

[a] Site = number (1 to 21) and type of site (baseline = no history of fungicide applications, research = Michigan State University research vineyard, commercial = Michigan grape grower's vineyard).
[b] Location = city and region where vineyards were located (SW = Southwest Michigan, C = Central Michigan, NW = Northwest Michigan).
[c] Grape host = species (Labrusca = *Vitis labrusca* L., Vinifera = *V. vinifera* L., hybrid = *Vitis* L., interspecific hybrid) and cultivar of the host.
[d] A total of 172 isolates were tested using the Amplification Refractory Mutation System (ARMS)-SYBR Green Q-PCR standardized by Baudoin *et al*. (2008). The results for each site are shown as the number of isolates tested and the number of positive detections of G143A mutation in the *CYTB* gene. A positive detection was defined as an isolate having more than 95% of the mutant allele.
[e] A total of 125 isolates were tested in an *in vitro* sensitivity assay to trifloxystrobin (Flint, 50% active ingredient). The number of isolates tested and the range of EC_{50} values are shown for every site.

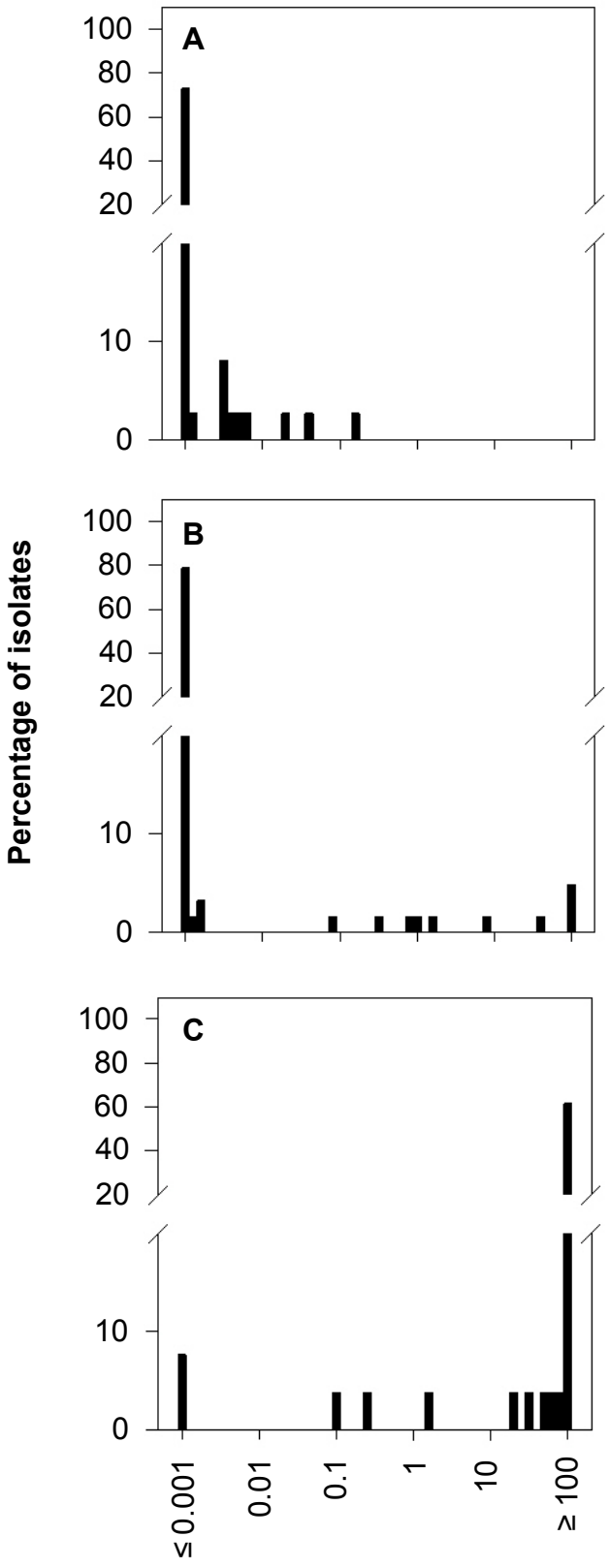

Figure 4.3. Distribution of effective concentration values of trifloxystrobin that inhibited 50% of the conidial germination (EC_{50} in micrograms per milliliter) in *Erysiphe necator* isolates collected in Michigan during 2009. Isolates were obtained from (A) baseline sites (n = 37), (B) commercial vineyards (n = 62), and (C) Michigan State University research vineyards (n = 26). Percentages were calculated according to the total number of isolates tested within each type of site.

Detection of G143A mutation in the 2009 collection.

Total DNA was extracted from 172 *E. necator* isolates collected in Michigan during 2009. All of these isolates were analyzed for the presence of the G143A mutation in the *CYTB* gene using the ARMS-SYBR Green Q-PCR detection system developed by Baudoin *et al*. (2008). The mutant allele was detected at very low percentage ranges (between 0.005 and 1.75%) in the isolates obtained from all baseline sites. These low percentages were considered as negative detection of the G143A mutation. However, in the majority of MSU research vineyards (5 of 6 sites) and in some commercial vineyards (4 of 12 sites), isolates having a high percentage of the G143A allele were detected (ranging between 95.3 and 99.99%); for this reason the detection of mutation G143A was considered positive (Table 4.3). Calculations of the %G143A allele in each isolate showed that isolates that tested positive for the mutation probably possessed residual wild-type mitochondria because the proportion of the mutant allele was close but never estimated at 100% (Anton Baudoin, *personal communication*).

Within the site types, the proportion of isolates that exhibited the mutation (%G143A > 95%) was significantly different ($\chi^2 = 77.9$, $P < 0.0001$, Table 4.4). No positive detections were obtained in isolates from baseline vineyards. In research vineyards, 84% of the isolates (36 out of 43) tested positive, whereas in commercial vineyards, 20% of the isolates (18 out of 90 isolates) tested positive (Table 4.4). By region, no significant differences in the proportion of isolates having the G143A mutation were observed ($\chi^2 = 3.2$, $P = 0.2038$, Table 4.4). The mutation was detected in 21, 31 and 36% of the *E. necator* isolates from the northwest, central, and southwest regions of Michigan, respectively (Table 4.4). By type of grape, the mutation was more frequently detected in isolates collected from wine (i.e. *V. vinifera* and hybrids) than juice grapes ($\chi^2 = 37.8$, $P < 0.0001$, Table 4.4). In fact, 47% (53 of 112) of the powdery mildew isolates from

wine grapes tested positive whereas only 2% (1 of 60) of isolates from juice grapes tested positive (Table 4.4). The G143A mutation was detected in only one of eight isolates from a 'Concord' vineyard in Lawton, MI (Table 4.3). However, this isolate was lost before a QoI resistant phenotype could be corroborated by the *in vitro* germination assay. This vineyard was within a mile from a wine grape vineyard where resistant isolates were found.

Table 4.4. Chi-square analysis of the detection of G143A mutation in the cytochrome b gene of *Erysiphe necator* isolates according to type of site (baseline, commercial, or Michigan State University research vineyard), region in the state of Michigan (northwest, central, or southwest), and the type of grape (juice or wine grape) in 2009.

		Number of isolates			Statistical test[a]		
		Positive	**Negative**	**Total**	χ^2	**df**	***P*-value**
Site	Baseline	0	39	39	77.9	2	< 0.0001
	Commercial	18	72	90			
	Research	36	7	43			
Region	Northwest	9	34	43	3.2	2	0.2038
	Central	11	24	35			
	Southwest	34	60	94			
Grape	Juice	1	59	60	37.8	1	< 0.0001
	Wine	53	59	112			

[a] The null hypothesis was that the proportions of isolates with (positive: > 95% G143 allele) and without (negative: < 2% G143A allele) the G143A mutation are the same within each of the three categories (type of site, region in Michigan, and type of grape).

Relationship between the bioassay and Q-PCR G143A detection.

A total of 124 isolates from the 2009 *E. necator* collection were evaluated for QoI resistance using both the conidial germination assay and the molecular detection by ARMS-SYBR Green Q-PCR. The proportion of isolates with the G143A mutation progressively increased at higher EC_{50} values of trifloxystrobin (Fig. 4.4). None of the 87 isolates that had an EC_{50} value of less than 0.001 to 0.01 μg/ml tested positive for the G143A mutation. However, 1 out of 4 isolates with EC_{50} values of 0.0101 to 0.1 μg/ml and 4 out of 5 isolates with EC_{50} values in the range of 0.101 to 1.0 μg/ml displayed the G143A mutation. The mutation was also detected in all of the 28 *E. necator* isolates with estimated EC_{50} values equal to or higher than 1.01 μg/ml (Fig. 4.4).

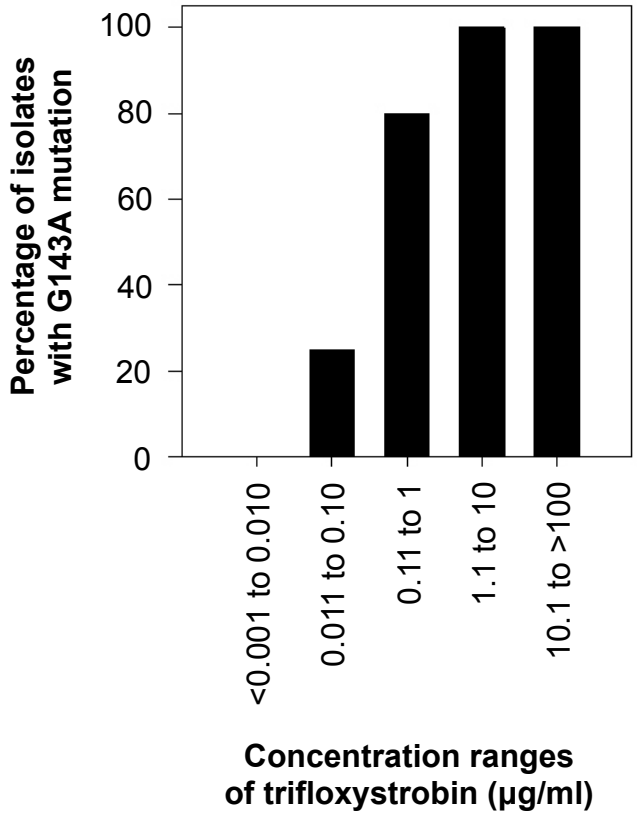

Figure 4.4. Detection of the G143A mutation according to EC_{50} ranges calculated for *Erysiphe necator* isolates collected in Michigan during 2009. The percentage of isolates with the mutation was calculated based on a total of 124 isolates tested by the *in vitro* germination bioassay as well as the Amplification Refractory Mutation System (ARMS)-SYBR Green Q-PCR developed by Baudoin *et al*. (2008).

***Erysiphe necator* responses to SHAM.**

The calculation of EC_{50} values was complicated as some *E. necator* isolates were inhibited by SHAM at 100 mg/liter in the agar slides amended with SHAM only. The conidial germination rate of 35% (13 of 37) of the isolates from baseline sites was reduced by 31-98% compared to the germination on slides with non-amended water agar. Likewise, germination of 20% (7 out of 35) of the isolates from MSU research vineyards was reduced by 21-65% and the germination of 23% (14 out of 62) of the isolates from commercial vineyards was reduced by 19-97% (data not shown). The remaining percentages in each category correspond to isolates that were not inhibited by the addition of SHAM to the media.

To further analyze the inhibitory effect of SHAM on the germination of *E. necator*, two representative isolates (one resistant, one sensitive, as determined from the *in vitro* bioassay) were tested on water agar slides amended with 0, 12.5, 25, 50, and 100 mg/liter SHAM. In the treatments with SHAM only (trifloxystrobin 0 μg/ml), an inhibitory effect of the chemical was observed at all concentrations (Fig. 4.5). However, it was only significant at 100 mg/liter SHAM for the sensitive isolate (Fisher's protected LSD, $P < 0.001$). Germination was reduced to less than 50% in this treatment (Fig. 4.5A). For the resistant isolate, although the inhibition of SHAM was not as pronounced as for the sensitive isolate, it was significant at 50 and 100 mg/liter (Fisher's protected LSD, $P < 0.001$). The actual conidial germination of the resistant isolate in these SHAM treatments was 81 and 84%, respectively (Fig. 4.5B).

The combined effect of SHAM and trifloxystrobin was further analyzed for these two *E. necator* isolates with contrasting fungicide-sensitivity phenotypes. Depending on the amount of SHAM utilized for the germination assay, different responses to the fungicide were observed (Fig. 4.5). The greatest inhibitory effect was observed at 100 mg/liter SHAM for both isolates.

Conidium germination of the sensitive isolate was inhibited to different degrees at 0.001 μg/ml trifloxystrobin depending on the amount of SHAM added to the media (Fisher's protected LSD, $P < 0.001$), but germination was completely inhibited at higher trifloxystrobin concentrations regardless of the amount of SHAM in the media (Fig. 4.5A). In the case of the resistant isolate, the two highest SHAM concentrations inhibited germination the most (Fig. 4.5B). Germination was reduced by 11 and 45%, respectively, in the 50 mg/liter SHAM plus 0.1 and 10 μg/ml trifloxystrobin treatments when compared to the 0 mg/liter SHAM treatment (Fisher's protected LSD, $P < 0.001$). Similarly, in the 100 mg/liter SHAM plus 0.1 and 10 μg/ml trifloxystrobin treatments, the germination of the resistant *E. necator* isolates was reduced by 47 and 80%, respectively, compared to the respective controls (Fisher's protected LSD, $P < 0.001$, Fig. 4.5B).

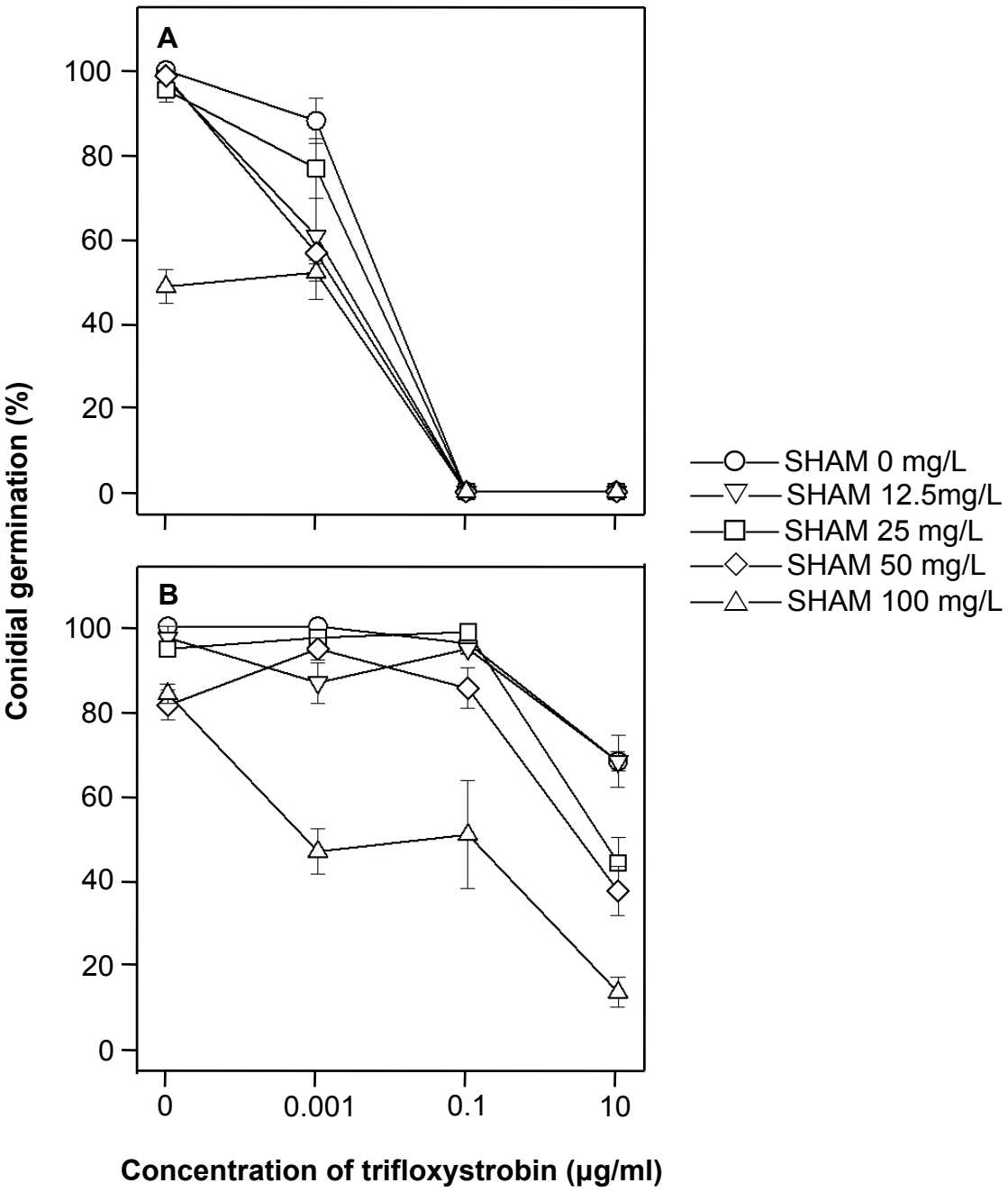

Figure 4.5. Responses of a (A) trifloxystrobin-sensitive and (B) -resistant *Erysiphe necator* isolate from grapes in Michigan to different concentrations of salicylhydroxamic acid (SHAM; 0, 12, 25, 50, and 100 mg/liter) at different concentrations of trifloxystrobin (0, 0.001, 0.1, and 10 µg/ml).

DISCUSSION

Strobilurin (QoI) fungicides have been used for disease management in grapes in the United States for more than a decade (7). Due to their broad-spectrum activity and good weathering ability, growers adopted them relatively quickly. Resistance to QoI fungicides was detected in certain plant pathogens, notably *Blumeria graminis* (DC.) Speer in cereals, within a few years of first use (2, 13). In addition, resistant isolates of *E. necator* have been detected before in grapes in the United States (3, 22). In most cases, QoI resistance has been associated with the G143A mutation (2, 13). An outbreak of powdery mildew in Michigan vineyards in 2006 despite regular fungicide applications led growers to question whether fungicide resistance had developed. However, poor control may also result from high disease pressure, use of ineffective materials, improper fungicide timing, poor spray coverage or a combination of these. This study represents the first assessment of fungicide (QoI) sensitivity in the *E. necator* population on grapes in Michigan using conidium germination (phenotypic approach) and Q-PCR assays to detect the G143A mutation (genotypic approach).

In grapes in Michigan, *E. necator* isolates with reduced sensitivity to trifloxystrobin were mostly found in research vineyards and to a lesser extent in commercial vineyards. Of the latter, wine grape vineyards were more likely to have resistant strains than juice grape vineyards. All of these isolates appear to have acquired resistance to trifloxystrobin through the G143A mutation. Detection of resistant strains does not necessarily equate to field-scale resistance with a concomitant loss of control. However, targeted sampling vineyards with severe powdery mildew problems despite regular fungicide spray programs in the Traverse City area in 2010 resulted in the detection of resistant isolates in two out of three vineyards. Observations in MSU research vineyards over several years also exposed lower-than-expected powdery mildew control with

rotational programs of strobilurins and sterol inhibitors. Adding a protectant fungicide like ziram usually improved control (19).

In a study by Wong and Wilcox (2002), the range of EC_{50} values determined for single-conidial-chain *E. necator* isolates collected from two baseline vineyards in the state of New York was 0.0058 to 0.052 μg/ml trifloxystrobin (mean 0.015 μg/ml, n = 26), which falls into the same EC_{50} range that we estimated for the baseline sites in Michigan. However, our powdery mildew baseline subpopulation included several isolates with EC_{50} values of less than 0.001 μg/ml (27 of 37 isolates, corresponding to 73%). The authors also found a strong correlation between azoxystrobin and trifloxystrobin EC_{50} values and proposed a single discriminatory dose of 0.031 μg/ml of azoxystrobin for the characterization of isolates within the range that was determined for sensitive isolates (24). From our results, 95, 84 and 8% of isolates from the baseline, commercial, and research vineyards, respectively, had an EC_{50} value below the above-mentioned dose. Wong and Wilcox (2002) also established an additional discriminatory dose of 2 μg/ml of azoxystrobin for the characterization of resistant isolates. When using this cut-off point, 0, 8, and 81% of the isolates from the baseline, commercial, and research vineyards in Michigan, respectively, were resistant to the QoI fungicide.

In another QoI sensitivity study, 35 *E. necator* isolates were collected from vineyards producing table grapes, raisins, and wine grapes in California and tested for trifloxystrobin sensitivity (among other fungicides) (14). EC_{50} values ranged from 0.00003 to 0.343 μg/ml, with a mean value of 0.021 μg/ml for the state of California. The range that we have established for baseline isolates in Michigan was < 0.001 to 0.159 μg/ml, with a mean value of 0.007 μg/ml

trifloxystrobin (Table 4.2), demonstrating that our isolates were more sensitive to the QoI fungicide than those from California. However, the Californian isolates were collected from commercial vineyards throughout the state. In all, the EC_{50} values calculated for isolates from commercial vineyards in Michigan varied greatly (< 0.001 to > 100 μg/ml of trifloxystrobin) (Table 4.2).

In this study, the calculation of EC_{50} values was complicated by the inhibitory effect of SHAM on conidial germinatin of *E. necator*. However, since the alternative respiration pathway is not normally active *in planta* (1, 15, 25) blocking the alternate oxidation pathway with SHAM would give a better estimation of the real EC_{50} value because under natural conditions pathogens would be more sensitive to QoI fungicides (15). In some fungi, the synthesis of the alternative oxidase is constitutive; however, in many other fungi, the synthesis is induced when the main pathway has been inhibited (25). Inhibitory effects of SHAM were also reported for several foliar fungal pathogens of citrus, including *Colletotrichum acutatum* J. H. Simmonds, *Elsinoe fawcettii* Bitanc. & Jenkins, *Diaporthe citri* (Faw.) Wolf, and *Mycosphaerella citri* Whiteside (15). The authors also observed an increase in colony diameter in treatments that included a low rate of the fungicide (i.e. 0.01 μg/ml pyraclostrobin or azoxystrobin plus SHAM versus SHAM alone). In our study, the response to 100 mg/liter SHAM was stronger in the sensitive (50% inhibition) than the resistant isolate (16% inhibition), which is not surprising as mutations in the *CYTB* gene would be able to circumvent the blockage of the Q_o site. This provides evidence that *E. necator* utilizes the alternative oxidation (AOX) pathway. In that sense, our results disagree with the study by Mondal *et al*. (2005) in which no evidence was found of AOX occurring in the mycelium of fungal pathogens of citrus. However, this is probably because QoI fungicides affect

mycelial growth less than spore germination (15). Wood and Hollomon (2003) also discussed that, *in planta,* the energy efficiency for a pathogen to sporulate and germinate is higher than for mycelial growth. Therefore, the AOX pathway is less likely to compensate for the high-energy requirements during early stages of infection (25).

Despite the inhibitory effects of SHAM on germination of *E. necator*, the detection of the G143A mutation corroborates our findings. Overall, the mutant allele was always found in isolates with EC_{50} values higher than 1 μg/ml trifloxystrobin, a dose that could be used as a discriminatory dose for future trifloxystrobin sensitivity screenings. A higher dose, such as the one proposed by Wong and Wilcox (2002) for azoxystrobin, would allow a better discrimination of resistant isolates (24). Compared to the results obtained by Reuveni (2001), powdery mildew isolates from grapes in Michigan tolerate higher concentrations of this chemical compound than isolates from the Golan region in Israel. The isolates from Israel were collected at a time when QoI fungicides were first introduced in the market, a fact that explains the high fungicide sensitivity observed. Reuveni (2001) found that a concentration of 0.1 μg/ml of trifloxystrobin completely inhibited the conidial germination of *E. necator in vitro* (17). However, the fungicide sensitivity assays in the Reuveni study did not test single-conidium isolates and mixtures of individuals that can occur within a single lesion might have obscured their results. In this study, most of the isolates from baseline sites in Michigan (35 of 37 isolates) were also completely inhibited at a concentration of 0.1 μg/ml trifloxystrobin. The two isolates that were still able to germinate at that concentration belonged to the baseline sites in Traverse City; these sites were in close proximity to commercial wine-grape vineyards that are farmed according to conventional farming practices. It is possible that airborne inoculum from those commercial vineyards reached the baseline sites.

Overall, our results provide evidence that QoI resistant *E. necator* isolates are easily detectable and may be contributing to decreased efficacy of fungicide programs in some Michigan vineyards. This emphasizes the need for continued vigilance and fungicide resistance management. Though QoI resistance does not appear to be widespread in commercial vineyards, the survey exposed different scenarios in which QoI resistance is more likely to develop. For example, adequate management programs that aim to delay the development of fungicide resistance are not normally followed in MSU research vineyards because for many years these sites have been utilized for fungicide efficacy trials that included QoI fungicides. Research plots would have been exposed to multiple strobilurin sprays per season over the past 10 years, which would have imposed relatively continuous selection pressure on local powdery mildew populations. Grape growers tend to be more conservative when applying strobilurin fungicides due to label restrictions and application costs. This is especially true for juice grapes that are grown with a very small economic margin. Consequently, the selection pressure in commercial vineyards would be lower than in research vineyards. We observed that resistant isolates were more commonly found in wine grape than in juice grape vineyards. Cultivars of the American *V. labrusca* are more tolerant to powdery mildew than the European *V. vinifera* cultivars (16); this intrinsic characteristic of the vines allows growers to considerably reduce the number of fungicide applications to juice grapes. In addition, due to the relatively higher cost of strobilurins compared to sterol inhibitor fungicides, juice grape growers would be less likely to use them. In many wine grape vineyards in Michigan, a total of 20 to 30 applications of strobilurins are estimated to have been made since these fungicides first became available.

At this time, field-scale loss of powdery mildew control due to QoI resistance appears to be limited in Michigan, as growers rely on multiple chemical classes to control powdery mildew.

This information will be used to develop more effective disease management programs to control powdery mildew and manage fungicide resistance in Michigan vineyards, including frequent rotation of fungicides with different modes of action. Sole reliance on strobilurins during periods of high fruit susceptibility, i.e. the first 3 to 4 weeks after bloom, is discouraged. Newer fungicides like quinoxyfen and metrafenone have unique modes of action (9) and can be used in rotation or tank mixes with strobilurins. Older protectant fungicides, like sulfur, oils and bicarbonate salts may take on a more important role (23). Further research is needed to confirm suspected resistance to sterol inhibitor fungicides in Michigan to improve recommendations for use of these materials in fungicide programs as well.

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LITERATURE CITED

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CHAPTER 5

EFFECTS OF DOWNY AND POWDERY MILDEW ON JUICE GRAPES (*VITIS LABRUSCA* L.) ADJUSTED TO DIFFERENT CROPPING LEVELS

ABSTRACT

Foliar diseases of grapes caused by *Plasmopara viticola* and *Erysiphe necator* require frequent applications of fungicides throughout the growing season in humid viticultural areas of the world. However, in Michigan juice grapes (*Vitis labrusca*) these diseases often do not appear until later in the season and it was not clear whether they negatively affect juice quality, reserves, and vine cold hardiness at that point. To evaluate the effects of downy mildew on 'Niagara' and powdery mildew on 'Concord', we relied on natural disease development and established two disease levels (low and high) by either applying a selective fungicide program or not. Three cropping intensity levels (33%, 67%, and 100% of the highest yielding potential) were also imposed by manual cluster removal at 660 growing-degree days (base 10^{o}C, calculated from 1 April 1) after crop estimation. Downy mildew reached 53%, 57% and 73%, and powdery mildew < 1%, 32% and 20% of the leaf area infected in 2008, 2009 and 2010, respectively. Brix, pH, and titratable acidity were mostly affected by the cropping intensity. The lowest Brix and pH values were observed in diseased, high-cropped 'Niagara' vines. Downy mildew significantly reduced cold hardiness of 'Niagara' canes in 2008 and 2010 and cropping intensity reduced it in 2009. Cane cold hardiness was not significantly reduced in 'Concord' infected by powdery mildew. Bud cold hardiness was comparable between sprayed and unsprayed vines. Starch content was reduced in both cultivars but only significantly in diseased 'Niagara' vines. At the levels of powdery mildew observed in 'Concord', fungicide applications did not appear to be cost-

effective. However, ‘Niagara’ needs protection against downy mildew to avoid a reduction in sugar content and cold hardiness, particularly under a high cropping intensity level.

INTRODUCTION

Downy mildew, caused by the oomycete *Plasmopara viticola* (Berk et Curt.) Berlese et de Toni, and powdery mildew, caused by the ascomycete fungus *Erysiphe necator* Schwein., are the most devastating diseases of grapes worldwide (21). Traditional wine grape varieties (*Vitis vinifera* L.) are highly susceptible to both diseases whereas juice grapes (*V. labrusca* L.) are regarded as more tolerant (7). These grape pathogens are able to infect all plant tissues, including leaves, flowers, clusters, and shoots, and can cause great reductions in yield and fruit quality under inadequate disease control strategies (3, 6, 11). Grape yield and wine quality were significantly reduced by powdery mildew (3, 28). Changes in juice composition and sensory characteristics can be detected when as little as 1 to 5% of the berries in a cluster are infected (29). Likewise, downy mildew was directly associated with crop losses when clusters were infected, but also indirectly when leaf infections affected photosynthetic assimilation (2). However, the effects of downy and powdery mildew on vine physiology have been more widely documented on Vinifera (3, 10-12, 28, 29) than Labrusca grapes (7).

In Michigan and the northeastern United States, juice grape cultivars (*V. labrusca* L. 'Concord' and 'Niagara') are the most widely planted varieties although wine grape cultivars (both *V. vinifera* and hybrid varieties) are starting to become more commercially attractive to growers (15). Currently, Michigan ranks 5th in grape production with 5,666 hectares planted in the lower peninsula, mostly distributed in the southwest and northwest regions along the shore of Lake Michigan. Most of the hectarage is comprised of juice grapes, with about 800 ha of wine grapes and 20 to 30 ha of table grapes (30). In Michigan, the control of powdery and downy mildew relies on a combination of protectant and systemic fungicides that are applied during the growing season; however, the application costs are high and Michigan grape growers struggle to

maintain profitability in a highly competitive market. The price of juice grapes has declined steadily from the mid 1970's up to now, and current grape prices are below the break-even point for some Michigan growers. Growers also have to deal with the severe climatic conditions during the growing season in Michigan (e.g., spring frost, humid and cool weather, and early autumn frost). In challenging years, growers must reduce vineyard maintenance costs to the minimum, but it is not clear whether a reduction in the number of fungicide applications would negatively affect vine physiology. The utility of damage thresholds is facilitated when pests can be monitored (e.g., assessing the growth of insect populations in the field by counting individuals); however, microbial plant pathogen populations tend to be visible only after injury to the crop has already occurred and may be too advanced for control measures to be effective (22). In general, there is a lack of published information on the lowest disease levels that can cause economic losses, and grape growers usually rely on their own experience to make decisions on timing and number of fungicide applications throughout the growing season. Mostly, they tend to be risk-averse and will apply more fungicides than necessary to ensure a harvestable crop of high-quality grapes.

In the cases of downy and powdery mildew of grapes, most of the detrimental effects of both pathogens might develop from foliar infections, since infection by *P. viticola* and *E. necator* is not commonly observed on *V. labrusca* fruit clusters. In *V. vinifera* L. 'Merlot', Jermini *et al*. (2010b) determined that the leaf damage caused by downy mildew primarily affected the sugar content of berries but the reduction was not proportional to the decrease of leaf area because of the potential capacity of grapevines to compensate for the ripening demands (11). The results from this study also indicated that the number of fungicide applications could be reduced if the first treatments are applied in a timely manner to delay the epidemics of downy mildew and limit

disease severity between 1 to 5% of the leaf area during the first ripening phase (11). However, this hypothesis has not been validated for *V. labrusca* grapes. Moriondo *et al*. (2005) found that green leaf tissues of downy- and powdery mildew-infected leaves of *V. vinifera* L. 'Sangiovese' had a reduced gas exchange rate, but leaves infected with powdery mildew exhibited a more pronounced reduction (19). Also, Lehman (2005) determined that the photosynthetic capability and carbon allocation of downy mildew-infected, potted *V. labrusca* L. 'Niagara' vines was negatively affected by *P. viticola* (17), but the relationship to yield and quality of juice grapes and, in derivation, the levels of downy or powdery mildew that *V. labrusca* vines can withstand before economic losses take place is still unknown.

Gadoury *et al*. (2001a) determined that the berries of *V. labrusca* L. 'Concord' vines are susceptible to powdery mildew for only 2 weeks after fruit set (5). Similarly, Kennelly *et al*. (2005) found that 'Niagara' berries are highly susceptible to downy mildew infection until one week after bloom (14). These short windows for infection, along with a shortage of inoculum, would restrict the direct losses caused by both pathogens in juice grape production. Another study by Gadoury *et al*. (2001b) evaluated the effects of powdery mildew on 'Concord', pointing out the importance of fungicide applications to prevent reductions in sugar accumulation below the minimal industrial standards, especially in vines that were heavily cropped (6). However, the authors did not evaluate the disease effects under a low cropping intensity scenario (less than 5 metric tons/ha) or establish a threshold level for powdery mildew.

Therefore, the objective of this study was to evaluate the individual impacts of downy and powdery mildew on yield, juice quality, starch accumulation, and cold hardiness of 'Niagara' and 'Concord' grapevines, respectively. The focus was to relate our results to the work done by Gadoury *et al*. (2001b), but expanding to lower cropping intensity levels. The effects

were studied during three consecutive years on grapevines that were subjected to two disease levels (fungicide sprayed and unsprayed) and three cropping intensity levels (33%, 67%, and 100% of the highest yielding potential) to simulate different Michigan viticulture scenarios.

MATERIALS AND METHODS

Experimental site.

Research vineyards were located at the Michigan State University (MSU) Trevor Nichols Research Center (TNRC, 42° 35' 38'' N, 86° 9' 19'' W) in Fennville, MI, USA from 2008 through 2010. Own-rooted 'Concord' and 'Niagara' grapes (*Vitis labrusca* L.) were planted in 1999, spaced 1.8 m between vines and 3.0 m between rows, and trained to a bilateral cordon at the top wire (Hudson River Umbrella) 1.5 m from the ground. Each research vineyard was composed of two ranges of 11 rows, with 7 vines per row, equivalent to 1,703 vines/ha. All grapevines were manually pruned to a total of 80 nodes per vine every year. Fertilization consisted of annual application of post-bloom nitrogen (calcium nitrate or ammonium nitrate at 30 kg/ha). No irrigation was provided and standard summer vine canopy management practices were used. Temperature data were recorded for the duration of the experiment by a weather station (Campbell Scientific, Inc., Logan, UT, USA) from the Enviro-weather Automated Weather Station Network (Michigan State University, East Lansing, MI, USA, http://www.agweather.geo.msu.edu/mawn) located on site within 100 m from the 'Concord' vineyard and 500 m from the 'Niagara' vineyard. Recommended weed control practices were followed and the insect pest management program was based on scouting and weather conditions. A maintenance spray program to control Phomopsis fruit rot and black rot was started in each experimental vineyard at Eichhorn-Lorenz stage 9, and continued until a third post-bloom treatment was applied. In the 'Niagara' vineyard, the fungicides triflumizole (Procure 50WS at 0.6 kg/ha, Chemtura USA Corporation, Middlebury, CT, USA) and ziram (Ziram 76DF at 3.3 kg/ha, Cerexagri, Inc., King of Prussia, PA, USA) were applied whereas captan (Captan 80WDG at 2.8 kg/ha, Arysta LifeScience North America LLC, Cary, NC, USA)

and phosphorous acid (Phostrol at 3.5 L/ha, Nufarm Americas Inc., Burr Ridge, IL, USA) were applied in the 'Concord' vineyard.

Treatments and experimental design.

In 2008, the experiment was set up in two different juice grape ('Niagara' and 'Concord') vineyards in a randomized complete block design with two factors (disease level and cropping intensity level) and six replicates. An experimental unit consisted of three consecutive vines in the same row, and each seven-vine row contained two experimental units separated by a guard vine. Data were collected from the two vines on the inside of the row, excluding the end vine to avoid edge effects. Each plot was flanked by guard rows (Fig. 5.1).

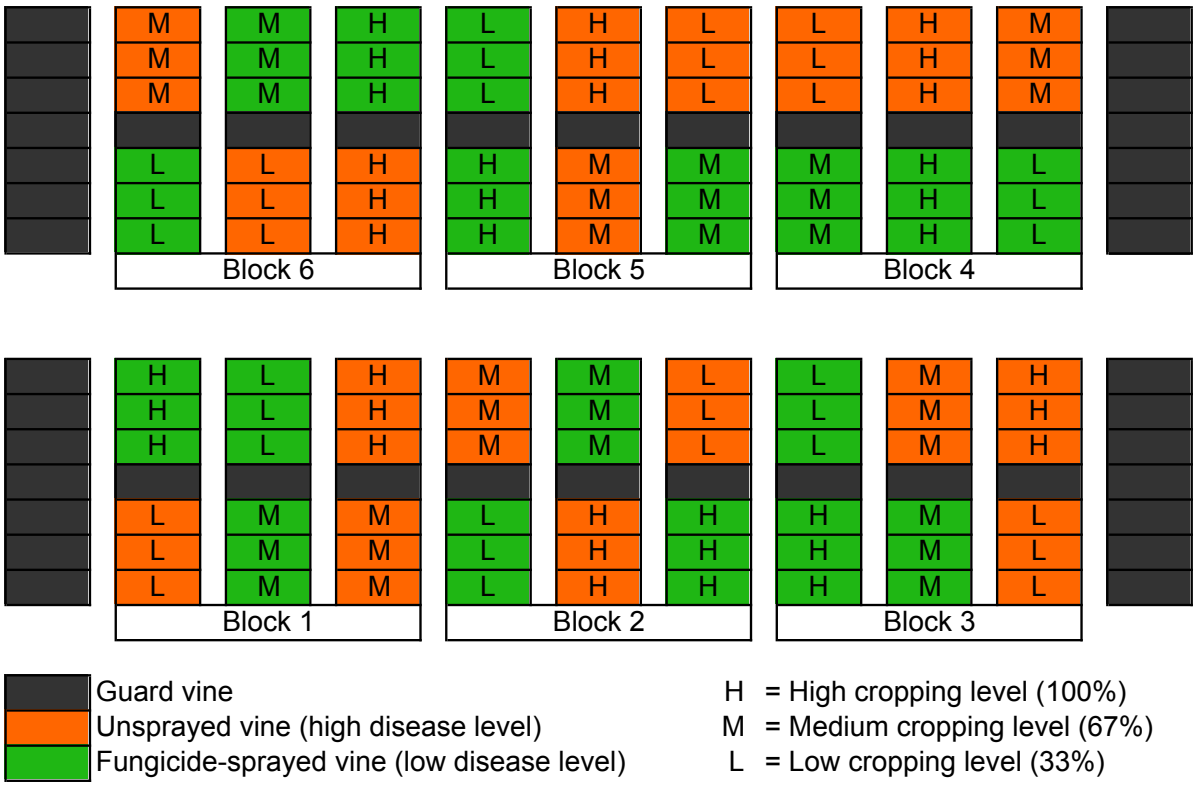

Figure 5.1. Experimental layout for the evaluation of downy (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*) effects on juice quality and vine physiology of *Vitis labrusca* L. 'Niagara' and 'Concord', respectively, planted at the Michigan State University Trevor Nichols Research Center in Fennville, MI. The experiment was set up in a randomized complete block design with two factors (disease level and cropping intensity level) and six replicates. Each experimental unit consisted of three consecutive vines in the same row. Fungicide-sprayed and unsprayed vines are shown in green and orange, respectively. The three levels of cropping intensity were H = high (100% of the highest yielding potential), M = medium (67% of the highest yielding potential, and L = low (33% of the highest yielding potential). Guard vines are shown in dark gray.

Two disease levels were imposed in 'Niagara' grapes (downy mildew) and 'Concord' grapes (powdery mildew) by applying five post-bloom fungicide treatments at two-week intervals (low level of disease) or leaving plots unsprayed during the whole growing season (high level of disease). To specifically control downy mildew in 'Niagara' grapes, mefenoxam (Ridomil Gold Copper or Ridomil Gold MZ, Syngenta Crop Protection, Greensboro, NC, USA) was applied at 2.2 kg/ha. Powdery mildew in 'Concord' grapes was controlled with tebuconazole (Elite at 0.3 kg/ha, Bayer Crop Sciences, Kansas, MO, USA) and ziram (Ziram at 3.4 kg/ha, Bayer Crop Sciences, Kansas, MO, USA).

Every year, at 660 growing degree-days (GDD, base temperature 10^{o}C (32), calculated from 1 April), the potential yield per vine was estimated after counting and weighing all clusters in the guard row vines. At that time, berries are considered to be at 50% of their final berry weight (24). Three levels of cropping intensity were established: 33%, 67%, and 100% of the highest yielding potential. The cropping levels were intended to simulate a low crop (e.g., after spring freeze), a moderate crop, and a potential overcropping situation, respectively. Crop adjustments were done by manually removing grape clusters from the vines to 33% and 67% of the estimated yield for each year. Vines that were assigned a 100% cropping intensity level were left untouched. Sprayed and unsprayed plots remained the same during the three years of the study, but cropping level was assigned based on vine size (as measured by the weight of all cane prunings per vine).

In 2010, an additional experimental plot was set up in a 'Concord' vineyard that was planted at the TNRC in June 2005. This plot had to be established because the fungicide trifloxystrobin (which is specific for the control of powdery mildew and is phytotoxic to 'Concord' grapes) was mistakenly applied to the main 'Concord' vineyard.

Disease rating.

Incidence and severity of powdery and downy mildew were visually assessed on 25 randomly selected leaves per experimental unit and plot, starting when diseases were first observed and continued on a weekly basis. Disease incidence (percentage of infected leaves) and severity (percentage of the leaf area infected) ratings were collected eight times each year starting at 660 GDD and continuing until the week before harvest.

Juice analysis.

Soluble solid content (Brix) measurements were taken to determine the grape harvest date. At harvest, all clusters from the two inside vines of each experimental unit were harvested, counted, and weighed. Cluster weight was calculated by dividing the total yield by the number of clusters. The three to five bottom berries of each cluster were sampled from 15 clusters per vine. Samples were placed in plastic resealable bags and stored at -20^{o}C until juice analysis was performed. For the analysis, berries were thawed and pressed to obtain juice. Brix, pH, and titratable acidity were measured for each juice sample. Brix (%) was measured using a digital refractometer (model PAL-1, Atago U.S.A., Inc., Kirkland, WA, USA). Measurements of pH were taken at room temperature using a pH meter (Orion PerpHecT LogR model 370, Thermo Scientific Inc., Beverly, MA, USA). The titratable acidity of the juice was determined by titration against a standardized 0.1 N sodium hydroxide solution using an automated titrator coupled to an autosampler and control unit (Titroline 96, Schott, Mainz, Germany) and the acidity was expressed as grams of tartaric acid per liter.

Bud and cane cold hardiness.

Each winter in February, three dormant shoots were cut from the two inside vines of the experimental unit. Shoots were cut into one-node pieces and separated into basal (position 2-5

from the base) or medium (position 6-9 from the base) node groups (Fig. 5.2). Six replicate cane pieces per treatment combination were placed inside a Super Cold Chest Freezer model 40-12A (Scientemp Corporation, Adrian, MI, USA) programmed to decrease the temperature from -18°C to -36°C at intervals of 3°C per hour. At each 3°C interval, a replicate of each treatment was taken out and placed in a cold room at 4°C. Control samples were kept at 4°C at all times. At the conclusion of the freeze run, canes were placed in a humid chamber at room temperature for 1 week to allow freeze-injury symptoms to develop, such as oxidative browning of tissues (26). For injury assessment, buds and canes were sectioned by hand using a single edge razor blade. A value of "0" was assigned to buds or canes with no visible injury and "1" to canes and buds with necrotic cambium and dead primary and secondary bud. An intermediate value of "0.5" was assigned for canes with partial damage to the cambium and buds (Fig. 5.3). The temperature at which 50% of the buds or canes were killed (LT_{50}) was calculated as previously described by Bitterbender and Howell (1974).

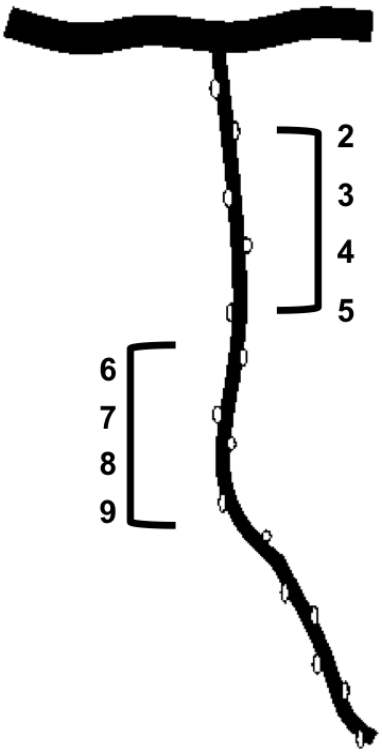

Figure 5.2. Representation of a grapevine dormant cane to indicate the division into basal (position 2 to 5) and medium (position 6 to 9) nodes for the analysis of cane and bud cold hardiness. The image was modified from the Des Moines Area Community College (DMACC) website.

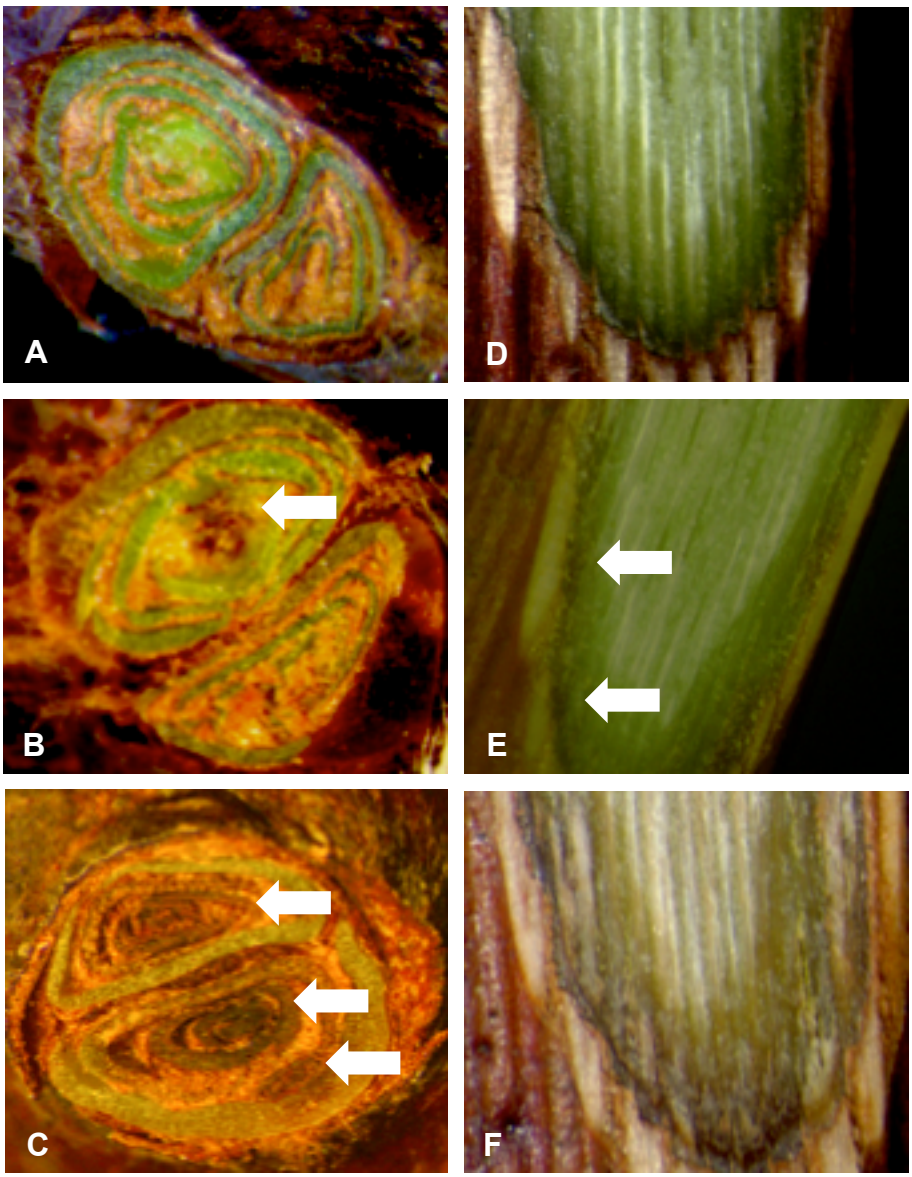

Figure 5.3. Rating scale used for the analysis of bud (A-C) and cane (D-F) cold hardiness of Labrusca grapevines. A value of "0" was assigned to buds or canes with no visible injury (A and D), "0.5" to dormant buds with partial damage to primary and secondary buds and canes with partial damage to the cambium (B and E), and "1" to completely damaged buds and canes with generalized tissue necrosis (C and F). White arrows indicate browning of the tissues in buds and canes.

Starch analysis.

For starch analysis, 5-mm pieces were cut from the cane samples described above before the freeze run. Pieces were stored at -80^{o}C until analysis. Cane pieces were then lyophilized and ground through a 40-mesh screen of a Thomas Wiley Mini-Mill (Thomas Scientific, Swedesboro, NJ, USA). Soluble carbohydrates were extracted three times from 100 mg of dried, ground tissue with 2-ml aliquots of 80% ethanol. Glucose levels were then determined for the pellets using the spectrophotometric glucose oxidase assay as previously described by Ebell (1969) and modified by Keller and Loescher (1989). Starch content was calculated based on the concentration of glucose and expressed as milligrams per gram of sample following the equation of Keller and Loescher (1989).

Statistical analysis.

The statistical model included fungicide treatment, cropping intensity level and the interaction between them as fixed factors and blocks as a random factor. Basic statistics and analysis of variance were conducted using the PROC UNIVARIATE and PROC MIXED procedures of SAS statistical package version 9.1.3 (SAS Institute Inc., Cary, NC, USA). The residuals were checked for normality using normal probability plots and normality tests. The homogeneity of variances was assessed using plots of residuals and Levene's test. When treatment variances were found to be unequal, the analysis was performed using the REPEATED/GROUP option in the PROC MIXED procedure. Residual plots were assessed for the presence of outliers. Mean separation was performed using the SAS least square means (LSMEANS) test (probability of difference [PDIFF]). The statistical significance of the fungicide treatment at different levels of cropping intensity, and vice versa was obtained using the LSMEANS/SLICE option in PROC MIXED. When the main, interaction or slicing effects

were found to be statistically significant, the comparisons between individual factor levels or treatments were conducted using t-tests. Factor effects and differences among treatments were declared statistically significant at $P \leq 0.05$.

The PROC CORR procedure of SAS was used to determine correlations between disease level and all the parameters measured as indicators of the physiological status of the vine, such as yield per vine, number of clusters per vine, juice chemistry (soluble solids content, pH and titratable acidity), cane and bud cold hardiness, and starch accumulation. Disease level was evaluated in terms of incidence, severity, and the area under the disease progress curves (AUDPC). AUDPC values for each combination of disease level and cropping intensity level were calculated following the equation of Shaner and Finney (1977). Likewise, correlations were evaluated against the yield obtained per vine.

RESULTS

Development of foliar diseases.

Downy and powdery mildew onset in the *V. labrusca* juice grape vineyards always occurred after fruit set and the infection was limited to the foliage; fruit infection was never observed during the study. Disease levels (in terms of incidence and severity) were not significantly different among the three different cropping intensity levels. However, the application of selective fungicides significantly reduced disease incidence and severity in the sprayed grapevines (t-test, $P < 0.05$). Downy mildew was first observed in the 'Niagara' vineyard at the beginning of August in 2008 and 2009 and the last week of July in 2010 (Fig. 5.4). On average, final downy mildew severity in the unsprayed and sprayed vines reached 53% and 29% of the leaf area infected in 2008, 57% and 1% in 2009, and 73% and 8% in 2010, respectively, before harvest (Fig. 5.4A). The final average incidence of downy mildew in the unsprayed vines was 97% in both 2008 and 2009, and 100% in 2010. Although severity was lower in the sprayed vines, disease incidence reached an average of 90% in 2008 and 69% in 2010 when Ridomil Gold Copper was applied. In 2009, incidence was 13% with Ridomil Gold MZ.

Final powdery mildew severity in the unsprayed 'Concord' vineyard was less than 1% of the leaf area during 2008; therefore, yield and fruit composition data were not collected for that year. During 2009 and 2010, the onset of powdery mildew occurred in early September (Fig. 5.4B). However, in the additional 'Concord' plot established in 2010, powdery mildew was observed earlier (in the last week of July) and disease severity reached an average of 20% of the leaf area in the unsprayed vines and 1% in the sprayed vines (Fig. 5.4B). In the main plot, powdery mildew severity in the unsprayed and sprayed vines reached 32% and < 1% in 2009;

and 18 and 1% in 2010, respectively (Fig. 5.4B). The incidence of powdery mildew was also significantly reduced in the sprayed vines (t-test, $P < 0.05$). Powdery mildew incidence in sprayed and unsprayed vines was respectively 5% and 95% in 2009; 14% and 73% in the main plot in 2010; and 18% and 72% in the additional plot in 2010.

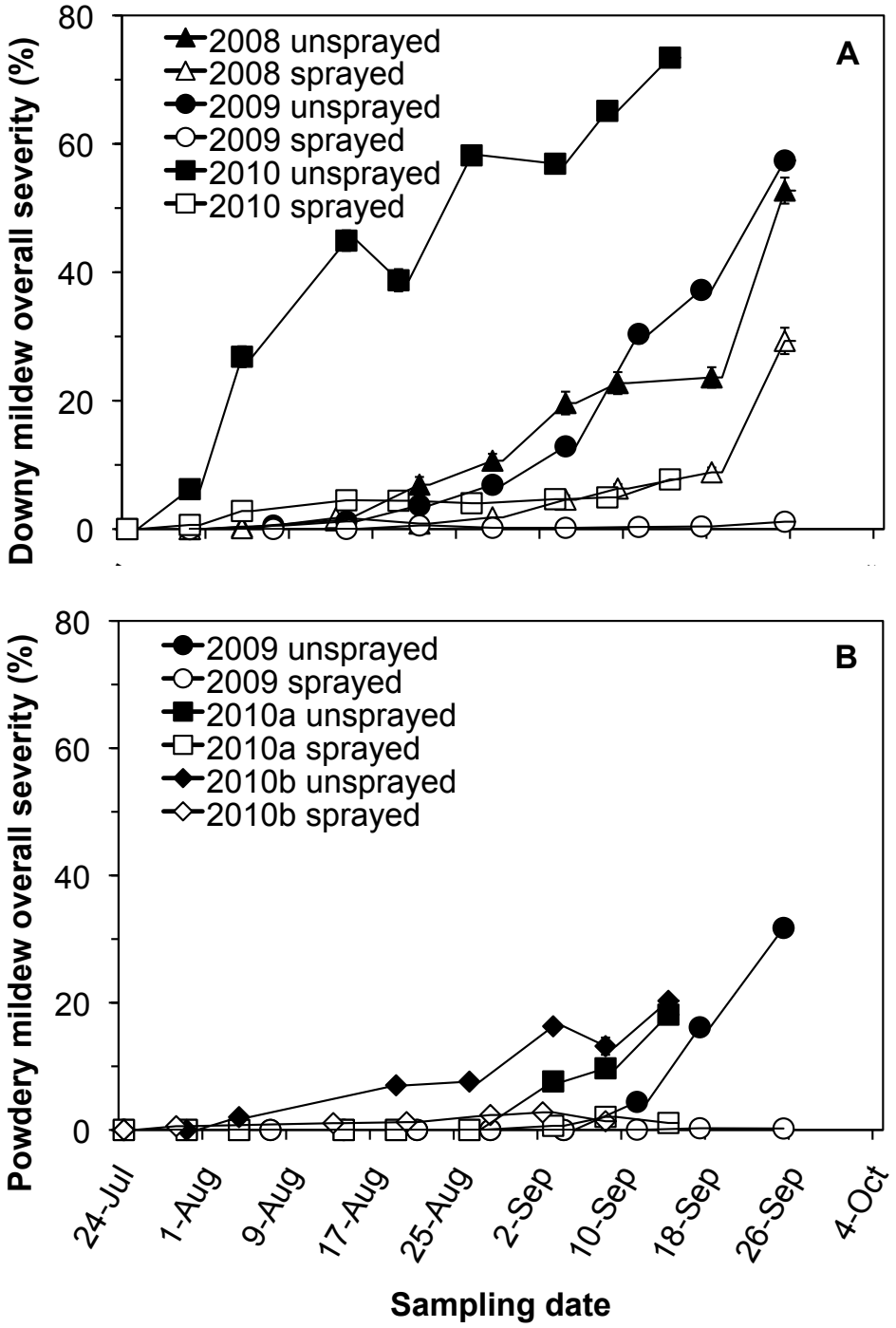

Figure 5.4. Overall disease severity (percentage of the leaf area infected) of (A) downy mildew in 'Niagara' grapes and (B) powdery mildew in 'Concord' grapes during the growing seasons of 2008, 2009, and 2010 in Fennville, MI.

Grape production.

The yield per vine varied greatly in both research plots, even within the imposed levels of cropping intensity, most likely due to intrinsic natural variation of the vines planted in the vineyards. Overall, the manual crop adjustment achieved a significant yield differentiation between the lowest imposed level of cropping intensity (33%) and the highest (100%) ($P < 0.05$, Tables 5.1-5.4). Also, at the disease levels observed during the study, the application of fungicides did not have a significant effect on grape yield ($P > 0.05$, Tables 5.1 and 5.2).

Table 5.1. Test of significance of the fixed effects: fungicide treatment (sprayed and unsprayed), cropping intensity level (33%, 67%, and 100% of the highest yielding potential), and their interaction on grape production, juice quality (Brix, pH, and titratable acidity) and vine physiology (starch content in canes, and cold hardiness of buds and canes) of *Vitis labrusca* L. 'Niagara' in 2008, 2009, and 2010 by two-way factorial ANOVA.

Parameter†	Sources of variation	*P*-values		
		2008	***2009***	***2010***
Yield (kg/vine)	Fungicide treatment	0.8431	0.6968	0.0614
	Cropping level	**0.0034**	**0.0046**	**0.0006**
	Interaction	0.1945	0.7611	0.4399
Brix (%)	Fungicide treatment	0.2397	0.0938	0.9869
	Cropping level	**0.0043**	**0.0108**	0.4451
	Interaction	0.3247	0.6263	0.8816
pH	Fungicide treatment	0.0866	0.2014	0.2405
	Cropping level	0.0025	0.1321	0.2936
	Interaction	0.6316	0.1517	0.0759
TA (g/L)	Fungicide treatment	0.1621	0.7857	0.2956
	Cropping level	0.4869	0.1200	0.2788
	Interaction	0.0978	0.1699	0.7491
Starch (mg)	Fungicide treatment	**0.0053**	**0.0418**	**0.0491**
	Cropping level	0.2158	0.2398	0.6301
	Interaction	0.1319	**0.0416**	0.0744
Bud (^{o}C)	Fungicide treatment	0.6062	0.5629	0.9076
	Cropping level	0.0227	0.1427	0.4712
	Interaction	0.0915	**0.0415**	0.2982
Cane (^{o}C)	Fungicide treatment	**0.0228**	0.5015	**0.0272**
	Cropping level	0.9282	**0.0482**	0.2703
	Interaction	0.2775	0.2025	0.7763

† The parameters evaluated were: Yield = grape production per vine (kg/vine); Brix = juice soluble solid content (%); pH = juice pH; TA = juice titratable acidity (g/L of tartaric acid); Starch = starch content in dormant grape canes (mg per gram of sample); Bud = the temperature at which 50% of the buds were killed (LT_{50} in ^{o}C); and Cane = the temperature at which 50% of the canes were killed (LT_{50} in ^{o}C).
Values in **bold** or underlined indicate a significant effect at $P = 0.05$ or $P = 0.10$, respectively.

Table 5.2. Test of significance of the fixed effects: fungicide treatment (sprayed and unsprayed), cropping intensity level (33%, 67%, and 100% of the highest yielding potential), and their interaction on grape production, juice quality (Brix, pH, and titratable acidity) and vine physiology (starch content in canes, and cold hardiness of buds and canes) of *Vitis labrusca* L. 'Concord' in 2009 and 2010 by two-way factorial ANOVA.

Parameter†	Sources of variation	*P*-values		
		2009	***2010***	***2010+***
Yield (kg/vine)	Fungicide treatment	**0.0085**	0.1978	0.3408
	Cropping level	**< 0.0001**	**< 0.0001**	**0.0237**
	Interaction	0.3002	0.6440	0.3525
Brix (%)	Fungicide treatment	0.7165	0.9070	0.8389
	Cropping level	**0.0004**	**0.0016**	0.5823
	Interaction	0.2044	0.8166	0.0780
pH	Fungicide treatment	**0.0208**	**0.0002**	**0.0301**
	Cropping level	0.1430	0.0613	0.5071
	Interaction	0.9016	0.1082	0.1286
TA (g/L)	Fungicide treatment	0.1083	0.8294	0.1148
	Cropping level	0.6191	0.2371	0.0583
	Interaction	0.3198	0.5622	0.7026
Starch (mg)	Fungicide treatment	0.3579	0.5470	0.9313
	Cropping level	0.4665	**0.0249**	0.3039
	Interaction	0.1919	0.2440	0.9679
Bud (^{o}C)	Fungicide treatment	0.8586	0.2416	0.4606
	Cropping level	0.8988	0.7708	0.2574
	Interaction	0.5367	0.7164	0.2618
Cane (^{o}C)	Fungicide treatment	0.6392	0.3992	0.3909
	Cropping level	0.8089	0.0814	0.3310
	Interaction	0.4625	0.3400	0.6978

† The parameters evaluated were: Yield = grape production per vine (kg/vine); Brix = juice soluble solid content (%); pH = juice pH; TA = juice titratable acidity (g/L of tartaric acid); Starch = starch content in dormant grape canes (mg per gram of sample); Bud = the temperature at which 50% of the buds were killed (LT_{50} in ^{o}C); and Cane = the temperature at which 50% of the canes were killed (LT_{50} in ^{o}C).
Values in **bold** or underlined indicate a significant effect at $P = 0.05$ or $P = 0.10$, respectively.
+ An additional experiment in a 'Concord' research vineyard was established at MSU TNRC in 2010.

Within each cropping intensity level, sprayed and unsprayed vines exhibited equivalent yields (Tables 5.3 and 5.4). However, the yield of ‘Niagara’ unsprayed vines cropped to 67% was similar to the unsprayed vines cropped to 33% during all years (Table 5.3). This trend was also observed in the ‘Concord’ vineyard, but only in 2009 (Table 5.4). During 2010, all vineyards suffered severe spring frost injury and the production was reduced to about 10% of the yield obtained in the previous years.

Table 5.3. Yield at harvest of fungicide-sprayed and unsprayed *Vitis labrusca* L. 'Niagara' vines cropped at three different intensity levels (33%, 67%, and 100% of the highest yielding potential) in 2008, 2009 and 2010 in Fennville, MI.

Year	Fungicide treatment	Cropping intensity (%)	Yield (kg/vine)	
2008	Sprayed	33	5.10	bc§
		67	7.21	ab
		100	7.95	ab
	Unsprayed	33	3.02	c
		67	5.88	bc
		100	10.70	a
2009	Sprayed	33	7.12	c
		67	10.05	abc
		100	13.03	ab
	Unsprayed	33	8.36	bc
		67	9.17	bc
		100	14.27	a
2010	Sprayed	33	0.48	c
		67	1.10	ab
		100	1.28	ab
	Unsprayed	33	0.93	bc
		67	1.14	ab
		100	1.55	a

§ Within each cultivar and year combination, least-square means having the same letter are not significantly different from each other (t-test, $P \leq 0.05$).

Table 5.4. Yield at harvest of fungicide-sprayed and unsprayed *Vitis labrusca* L. 'Concord' vines cropped at three different intensity levels (33%, 67%, and 100% of the highest yielding potential) in 2009 and 2010 in Fennville, MI.

Year	Fungicide treatment	Cropping intensity (%)	Yield (kg/vine)
2009	Sprayed	33	7.41 d§
		67	11.18 bc
		100	13.65 b
	Unsprayed	33	9.71 cd
		67	12.39 bc
		100	18.61 a
2010	Sprayed	33	1.19 c
		67	1.75 b
		100	2.66 a
	Unsprayed	33	0.89 c
		67	1.66 b
		100	2.40 a
2010+	Sprayed	33	0.80 bc
		67	1.19 b
		100	2.03 a
	Unsprayed	33	0.61 c
		67	1.15 b
		100	1.70 a

§ Within each cultivar and year combination, least-square means having the same letter are not significantly different from each other (t-test, $P \leq 0.05$).
+ An additional experiment in a 'Concord' research vineyard was established at MSU TNRC in 2010.

Grape juice analysis.

The application of fungicides did not significantly impact the basic fruit chemistry of 'Niagara' juice in terms of soluble solids content (Brix), pH, or titratable acidity (Tables 5.1 and 5.5). However, Brix reductions and higher acidity were usually observed in the grape juice obtained from downy mildew-infected vines (Table 5.5). The cropping intensity level had a significant effect on Brix and pH levels of 'Niagara' juice in 2008 and 2009 (Table 5.1) as indicated by the reduced sugar content and higher acidity in the juice from heavily-cropped vines (Table 5.5). No treatment effects were observed in the low-yielding vines of 2010 (Table 5.1).

On the other hand, the ANOVA revealed a significant main effect for the fungicide treatment on 'Concord' juice pH ($P = 0.0208$, Table 5.2). However, no significant differences were observed between the sprayed and unsprayed vines within each cropping intensity level in 2009 (Table 5.6). The *P*-values for 33%, 67%, and 100% cropping intensity levels were 0.0927, 0.1224, and 0.3308, respectively. In 2010, the year of the severe spring frost, the juice of all sprayed 'Concord' vines (from both main and additional plot) had a lower pH than that of the unsprayed vines (Table 5.6). Brix levels and titratable acidity were not affected by the development of powdery mildew. For Brix, the *P*-values were 0.7165 in 2009 and 0.9070 in 2010. In the additional plot, the *P*-value was 0.8389; for titratable acidity the P-values were 0.1083, 0.8294, and 0.1148 (Table 5.2). However, the cropping intensity level in the main plot had a significant effect on Brix level in 2009 and 2010 (*P*-values were 0.0004 and 0.0016, respectively, Table 5.2). Significantly higher sugar levels were mostly obtained in vines cropped to 33% (Table 5.6). In 2009, the sugar content of unsprayed 'Concord' vines cropped to the 67% level was similar to the vines cropped to 33% whereas the sprayed vines cropped to 67% had a similar Brix level as the vines cropped to a 100% crop (Table 5.6). However, the low yields of

the vines did not allow this differentiation during 2010. Also, in 2009 the pH level of unsprayed, high-cropped vines was significantly lower than sprayed vines cropped to 33% ($P = 0.0111$, Table 5.6).

Table 5.5. Effects of downy mildew on juice quality (Brix, pH, and titratable acidity) and vine physiology (starch content in canes, and cold hardiness of buds and canes) of *Vitis labrusca* L. 'Niagara' vines under three different imposed cropping intensity levels (33%, 67%, and 100% of the highest yielding potential) during 2008, 2009 and 2010 in Fennville, MI.

Year	Cropping intensity (%)	Fungicide treatment	Parameter†					
			Brix (%)	pH	TA (g/L)	Starch (mg/g)	Bud (^{o}C)	Cane (^{o}C)
2008	33	Sprayed	13.93	3.20	6.85	**22.85**	-28.22	-28.65
		Unsprayed	14.31	3.26	7.06	12.13	-29.22	-27.15
	67	Sprayed	13.57	3.15	**6.81**	18.66	-28.15	-28.21
		Unsprayed	12.34	3.16	7.31	17.72	-27.39	-27.97
	100	Sprayed	12.47	3.11	6.97	17.13	-27.83	**-29.15**
		Unsprayed	11.71	3.15	6.78	11.36	-27.02	-26.46
2009	33	Sprayed	14.24	2.96	7.24	**31.85**	-25.38	-26.15
		Unsprayed	13.17	3.01	7.62	26.37	-24.77	-25.77
	67	Sprayed	13.48	2.97	7.66	31.27	-24.52	-25.50
		Unsprayed	13.31	3.00	7.12	34.24	-24.40	-24.25
	100	Sprayed	12.53	2.96	7.73	**34.06**	**-23.77**	-24.27
		Unsprayed	11.31	2.94	8.08	24.32	-25.03	-25.00
2010	33	Sprayed	13.35	3.13	12.85	**40.12**	-27.40	-27.27
		Unsprayed	13.53	3.13	12.24	30.15	-27.28	-28.52
	67	Sprayed	13.87	3.13	12.14	35.05	-26.65	-27.03
		Unsprayed	13.93	3.15	11.98	36.00	-27.40	-28.15
	100	Sprayed	14.01	**3.21**	12.12	34.77	-27.78	-27.77
		Unsprayed	13.78	3.12	11.97	32.07	-27.27	-29.92

† The parameters evaluated were: Brix = juice soluble solid content (%); pH = juice pH; TA = juice titratable acidity (g/L of tartaric acid); Starch = starch content in dormant grape canes (mg per gram of sample); Bud = the temperature at which 50% of the buds were killed (LT_{50} in ^{o}C); and Cane = the temperature at which 50% of the canes were killed (LT_{50} in ^{o}C).
Values in **bold** indicate a significant difference between sprayed and unsprayed grapevines at $P = 0.05$ (t-test); values that are underlined indicate a significant difference between sprayed and unsprayed grapevines at $P = 0.10$ (t-test).

Table 5.6. Effects of powdery mildew on juice quality (Brix, pH, and titratable acidity) and vine physiology (starch content in canes, and cold hardiness of buds and canes) of *Vitis labrusca* L. 'Concord' vines under three different imposed cropping intensity levels (33%, 67%, and 100% of the highest yielding potential) during 2009 and 2010 in Fennville, MI.

Year	Cropping intensity (%)	Fungicide treatment	Parameter†					
			Brix (%)	pH	TA (g/L)	Starch (mg/g)	Bud (^{o}C)	Cane (^{o}C)
2009	33	Sprayed	14.18	3.05	8.92	23.32	-25.60	-27.90
		Unsprayed	14.06	3.01	8.99	26.98	-25.78	-29.03
	67	Sprayed	13.08	3.03	8.99	26.32	-25.55	-28.40
		Unsprayed	13.68	2.99	9.08	18.53	-25.90	-28.90
	100	Sprayed	13.10	3.01	**8.85**	22.90	-26.03	-28.53
		Unsprayed	12.84	2.98	9.42	20.00	-25.65	-27.78
2010	33	Sprayed	12.33	**2.93**	15.50	12.58	-29.15	-29.90
		Unsprayed	12.60	2.98	15.26	11.53	-28.40	-27.90
	67	Sprayed	11.63	2.97	15.52	14.83	-28.28	-30.28
		Unsprayed	11.78	2.99	15.88	12.10	-28.17	-30.65
	100	Sprayed	11.75	**2.95**	16.13	15.00	-29.15	-30.77
		Unsprayed	12.13	3.04	15.83	16.80	-27.78	-30.65
2010+	33	Sprayed	12.70	3.18	13.42	23.57	-27.90	-27.40
		Unsprayed	13.37	3.18	13.51	23.47	-27.78	-25.65
	67	Sprayed	13.45	3.17	12.76	23.48	-28.28	-28.27
		Unsprayed	12.94	3.21	13.13	22.88	-26.02	-27.90
	100	Sprayed	13.31	**3.16**	12.58	20.75	-26.02	-27.15
		Unsprayed	13.28	3.24	13.10	21.07	-26.77	-27.05

† The parameters evaluated were: Brix = juice soluble solid content (%); pH = juice pH; TA = juice titratable acidity (g/L of tartaric acid); Starch = starch content in dormant grape canes (mg per gram of sample); Bud = the temperature at which 50% of the buds were killed (LT_{50} in ^{o}C); and Cane = the temperature at which 50% of the canes were killed (LT_{50} in ^{o}C).
Values in **bold** indicate a significant difference between sprayed and unsprayed grapevines at $P = 0.05$ (t-test); values that are underlined indicate a significant difference between sprayed and unsprayed grapevines at $P = 0.10$ (t-test).
+ An additional experiment in a 'Concord' research vineyard was established at MSU TNRC in 2010.

Starch content in grape canes.

Downy-mildew-infected 'Niagara' vines had a lower starch content in the dormant canes; however, the reduction was only consistently significant in vines cropped to an intensity level of 33% (Tables 5.1 and 5.5). In 2009, the high-cropped sprayed vines had significantly more starch than the high-cropped unsprayed vines ($P = 0.0065$, Table 5.5). The reduction of carbohydrate reserves in unsprayed vines was also observed in the canes of 'Concord'; however, the effect was not significant (Tables 5.2 and 5.6). Additionally, in 2010, the cropping intensity level had a significant effect on the starch content of unsprayed vines in the main 'Concord' plot, with high-cropped vines having the highest starch concentrations (Tables 5.2 and 5.6). This was not observed in the additional 'Concord' plot.

Cold hardiness of grape buds and canes.

In 2008, there were no significant differences between medium and basal nodes regarding the cold hardiness of 'Niagara' buds or canes (data not shown). Hence, data were averaged to produce one single data set. The application of fungicides did not affect bud cold hardiness but we found significant differences in the bud LT_{50} values within the unsprayed vines, ($P = 0.0228$, Table 5.1). The buds from unsprayed, low-cropped vines resisted lower temperatures better than the buds from unsprayed vines cropped to 67% or 100% (Table 5.5). On the other hand, the fungicide treatment significantly increased the cane LT_{50} value of sprayed, high-cropped vines (Table 5.5). Moreover, the unsprayed, high-cropped vines had the poorest cold tolerance.

In 2009, the interaction between the fungicide application and the cropping intensity level revealed a significant effect on the cold hardiness of 'Niagara' buds ($P = 0.0415$, Table 5.1). The interaction was also significant in 2008 but at the 90% confidence level ($P = 0.0915$, Table 5.1). Individual factor effects were not found in 2009. Within the 'Niagara' sprayed vines, the bud

LT_{50} values were significantly lower in the vines cropped to a level of 100% (Table 5.5). Also, the buds from unsprayed, high-cropped vines tolerated lower freezing temperatures better than the sprayed counterpart (Table 5.5). Differences between basal and medium nodes were not found in the 'Niagara' bud LT_{50} data set; however, the cane LT_{50} was significantly different in the sprayed vines cropped to the 33% level (-25°C for basal and -27°C for medium node, P = 0.043, data not shown). When all basal and medium node data were pooled together, a significant effect of the cropping intensity level on cane cold hardiness was revealed (P = 0.0482, Table 5.1). Within the sprayed 'Niagara' vines, the cane LT_{50} value was significantly higher for the vines cropped to the 33% level (Table 5.5). In the 'Concord' vineyard, no significant effects were found (Table 5.2) and differences between the LT_{50} values for canes and buds of sprayed and unsprayed vines were not significant (Table 5.6).

In 2010, the year with severe spring freezes, no significant effects were observed in the bud LT_{50} values of 'Niagara' vines but there were significant differences between the cane LT_{50} values of medium and basal nodes (P = 0.0014). Canes from medium nodes of 'Niagara' vines resisted colder temperatures better than canes from basal nodes. The effect of the fungicide treatment was significant when pooling all the data together (P = 0.0272, Table 5.1); however, no significant differences at the 95% confidence level were found between 'Niagara' sprayed and unsprayed pairs (Table 5.5). The P-values for 33%, 67%, and 100% cropping intensity levels were 0.2714, 0.3247, and 0.0644, respectively. The cane LT_{50} value of 'Niagara' unsprayed vines cropped to 100% was significantly higher than the values of sprayed vines cropped to 33% and 67%. The difference between sprayed and unsprayed vines cropped to 100% was significant

at the 90% confidence level ($P = 0.0644$). The treatments did not have a significant effect on the cold hardiness of 'Concord' vines from the main plot in 2009 and 2010, or additional vineyard established in 2010 (Tables 5.2 and 5.6).

Linear correlation analyses.

We calculated a Pearson's correlation coefficient (r) between quantitative measures of disease (i.e. final disease severity and incidence, and respective area under the disease progress curves as descriptors of the disease epidemics) and all the parameters that measured the physiological status of the vines (juice quality, starch content in dormant canes, and cold hardiness of canes and buds) (Table 5.7). Likewise, a Pearson's correlation coefficient was also calculated between yield and the aforementioned vine physiology parameters to determine bivariate associations (Table 5.8).

Of all the evaluated parameters in 'Niagara' grapevines, disease severity was consistently and negatively correlated with the starch content of dormant canes (Table 5.7) and the correlation was more significant in 2008 and 2009 than in 2010, when the vineyards were affected by spring freezes. This correlation was not evident in the 'Concord' vineyards, most likely due to the limited development of powdery mildew. On the other hand, a significant correlation between disease level and the pH of 'Concord' juice was observed (Table 5.7). In 2009, these two variables were negatively correlated, particularly with measures of powdery mildew incidence (i.e. final rating and AUDPC), whereas in 2010 this correlation was positive (Table 5.7). However, no significant correlations were found with titratable acidity as it was observed for 2009 (Table 5.7). These discrepancies might be consequences of the great yield reduction due to spring freeze damage in 2010 (about 10% of the crop was left on the vines).

Table 5.7. Pearson correlation coefficients (*r*) between downy and powdery mildew disease levels and the physiology of 'Niagara' and 'Concord' grapevines, respectively, during 2008, 2009, and 2010 in Fennville, MI.

Parameter†	'Niagara'			'Concord'		
	2008	***2009***	***2010***	***2009***	***2010***	***2010+***
Brix (%)	ns§	ns	ns	ns	ns	ns
	ns§§	ns	ns	ns	0.3047	ns
	ns§§§	ns	ns	ns	ns	ns
	ns§§§§	ns	ns	ns	ns	ns
pH	ns	ns	ns	ns	**0.4515**	**0.3680**
	ns	ns	ns	-0.2875	**0.4693**	**0.4572**
	ns	ns	ns	**-0.3491**	0.3207	ns
	ns	ns	ns	**-0.3499**	**0.3673**	**0.3735**
TA (g/L)	ns	ns	ns	0.3138	ns	ns
	ns	ns	ns	**0.3404**	ns	0.3196
	ns	ns	ns	**0.3427**	ns	ns
	0.2948	ns	ns	**0.3382**	ns	ns
Starch (mg)	**-0.4060**	**-0.4408**	-0.3091	ns	ns	ns
	-0.4332	**-0.4707**	-0.3133	ns	ns	ns
	-0.4819	**-0.4229**	ns	ns	ns	ns
	-0.4322	**-0.4366**	ns	ns	ns	ns
Bud (^{o}C)	ns	ns	ns	ns	ns	ns
	ns	ns	ns	ns	ns	ns
	ns	ns	ns	ns	ns	ns
	ns	ns	ns	ns	ns	ns
Cane (^{o}C)	**0.4020**	ns	**-0.3415**	ns	ns	ns
	ns	ns	**-0.3785**	ns	ns	ns
	ns	ns	**-0.3902**	ns	ns	ns
	ns	ns	**-0.4018**	ns	ns	ns

† The parameters evaluated were: Brix = juice soluble solid content (%); pH = juice pH; TA = juice titratable acidity (g/L of tartaric acid); Starch = starch content in dormant grape canes (mg per gram of sample); Bud = the temperature at which 50% of the buds were killed (LT_{50} in ^{o}C); and Cane = the temperature at which 50% of the canes were killed (LT_{50} in ^{o}C).
§ Final disease severity rating (%); §§ Area under disease severity curve (AUDPC); §§§ Final disease incidence rating (%); §§§§ Area under disease incidence curve (AUDPC).
Values in **bold** indicate a significant correlation at $P = 0.05$; values that are underlined indicate a significant correlation at $P = 0.10$; ns = not significant.
+ An additional experiment was established in a 'Concord' vineyard at the MSU TNRC in 2010.

With respect to grape yield, the *r* coefficients showed a more consistent negative correlation with Brix and pH levels (Table 5.8). The relationships with other parameters were not reproducible or significant. In 2010, data from the 'Niagara' vineyard showed that the quality of the juice was not affected by disease pressure or cropping intensity level (Table 5.7 and 5.8). However, in the main 'Concord' vineyard, lower Brix and higher acidity were observed as the cropping intensity level increased (Table 5.8). In the additional 'Concord' vineyard, the correlations between those juice quality parameters and yield were opposite (Table 5.8). This vineyard was planted in 2005 and the vines were not balanced-pruned to a total of 80 nodes per vines in winter because this plot was only started later in the season.

Table 5.8. Pearson correlation coefficients (*r*) between yield (kg/vine) and the physiology of 'Niagara' and 'Concord' grapevines during 2008, 2009 and 2010 in Fennville, MI.

Parameter†	**'Niagara'**			**'Concord'**		
	2008	***2009***	***2010***	***2009***	***2010***	***2010+***
Brix (%)	**-0.5242**	**-0.7368**	ns	**-0.7424**	**-0.4474**	**0.4973**
pH	**-0.5683**	**-0.4070**	ns	**-0.4444**	ns	**-0.5675**
TA (g/L)	ns	**0.4249**	ns	ns	0.3078	**-0.5077**
Starch	-0.3117	ns	ns	ns	ns	ns
Bud (^{o}C)	**0.5294**	ns	ns	ns	ns	ns
Cane (^{o}C)	**0.4709**	ns	ns	ns	ns	ns

† The parameters evaluated were: Brix = juice soluble solid content (%); pH = juice pH; TA = juice titratable acidity (g/L of tartaric acid); Starch = starch content in dormant grape canes (mg per gram of sample); Bud = the temperature at which 50% of the buds were killed (LT_{50} in ^{o}C); and Cane = the temperature at which 50% of the canes were killed (LT_{50} in ^{o}C)
Values in **bold** indicate a significant correlation at $P = 0.05$; values that are underlined indicate a significant correlation at $P = 0.10$; ns = not significant.
+ An additional experiment was established in a 'Concord' research vineyard at MSU TNRC in 2010.

DISCUSSION

This study evaluated the effects of downy and powdery mildew on the physiology of own-rooted *Vitis labrusca* 'Niagara' and 'Concord' grapevines, respectively, under conditions with naturally occurring inoculum and three different cropping intensity levels in the field. In a similar study by Gadoury *et al*. (2001b) the effect of powdery mildew on vine growth, yield, and quality of 'Concord' grapes was evaluated at three levels of cropping intensity. The authors observed powdery mildew infection in the clusters (3% to 52% disease severity) and the average disease severity on the leaves ranged between 20% and 70% in the unsprayed vines, which is a higher range than that observed in this study. However, Gadoury *et al*. (2001b) did not find significant differences between sprayed and unsprayed 'Concord' vines during two years when powdery mildew severity on the leaves was below 30%, even when cluster infection reached 52% (6). The results from our study confirm their findings and also suggest that fungicide applications to 'Concord' vineyards are not cost-effective at the powdery mildew levels observed (up to 32% final disease severity in the leaves), especially in years when spring freezes reduce the crop to very low levels.

In contrast, Stummer *et al*. (2003) reported a considerable increase in titratable acidity and decrease in total soluble solids in *V. vinifera* 'Chardonnay' grape juice from clusters with 30% powdery mildew infection and reductions of total soluble solids in *V. vinifera* 'Cabernet Sauvignon' juice from clusters with 1% to 20% infection (28). Wines made from grape clusters that have 1% to 5% of the berries infected have undesirable changes in their sensory characteristics (28, 29). Since wine grape varieties are more susceptible to powdery mildew infection than juice grape varieties, a higher disease pressure can be tolerated in *V. labrusca* vineyards before fungicide applications are needed.

In a study by Howell *et al*. (1978), 100% defoliation of 'Concord' vines at veraison significantly reduced berry soluble solids and primary bud cold hardiness (9). In a second experiment by Mansfield and Howell (1981) in 'Concord' vines, 50% defoliation imposed by either removing all the leaves from one side of the bilateral cordon or removing all leaves on alternate shoots of the vine also significantly reduced the soluble solids content and the cold hardiness of primary buds. The control vines defoliated to 100% and the vines with one defoliated cordon were still able to increase the soluble solids content from veraison to harvest to 57% and 29%, respectively, by mobilizing carbohydrates from other vegetative tissues (18). The absence of significant effects of powdery mildew on 'Concord' physiology probably can be explained by the low disease development during our study and the fact that powdery mildew is not a disease that causes defoliation of the host; in fact, *E*. *necator* infection is restricted to the epidermal cells (20). Additionally, the amount of observable disease is not a good predictor of the detrimental effects on the vines since Lakso *et al*. (1982) found that visual estimation of powdery mildew infection did not correlate well with the effects on the physiology of 'Concord' leaves. The authors found that 50% foliar disease severity corresponded to only an 8% reduction in net photosynthesis (16).

On the other hand, downy mildew development on 'Niagara' vines reached higher levels than powdery mildew on 'Concord' vines. Final disease severity ranged between 53% and 73% on the leaves of unsprayed vines and 1% to 30% in the sprayed vines. Also, in 2010, defoliation by downy mildew in the 'Niagara' vineyard was about 35% in the unsprayed vines at harvest (data not shown). Better control of downy mildew was achieved in 2009 and 2010. Disease pressure did not have a significant effect on the quality of 'Niagara' juice; however, it appears that unsprayed grapevines compensated for the disease pressure by reallocating the

carbohydrates reserves from the canes to the grape clusters since no significant differences were observed in the soluble solid content (Brix) but higher starch contents were observed in the canes of sprayed vines. Every year this effect was more pronounced in vines cropped to a low intensity level (33%). The reduction in starch content of 'Niagara' canes was also confirmed with Pearson correlation analyses. Nevertheless, the reduction in carbohydrates reserves did not have a significant effect on the cold hardiness of the grape canes. Jermini *et al*. (2010a) studied this compensation mechanism in *V. vinifera* 'Merlot' vines. The authors observed a mobilization of the starch reserves stored in the woody parts of the vines to compensate for the carbohydrate demands during the ripening phase of the fruit (10). In their study, this mobilization did not exhaust the reserves of such tissues, which would explain why in this study the cold hardiness of canes and buds was not significantly affected when comparing the sprayed and unsprayed treatment combinations.

Since stressed grapevines have proven to have a remarkable ability to mobilize carbohydrate reserves from available tissues to sustain fruit ripening demands, the level of infection observed might have just caused a mobilization of carbohydrates that resulted in insignificant changes to the juice sugar content. However, the starch reduction in the canes was not large enough to change the cold hardiness of the vines. Stergios and Howell (1977) suggested that the combination of leaf-area loss, light pruning, and non-thinning delayed vine acclimation in fall, which had a negative effect on the cold hardiness of 'Concord' grapevines (27). Therefore, greater effects would be expected when disease-stressed vines are cropped to higher levels year after year because a reduced photosynthetic rate would not allow the allocation of enough carbohydrates for a proper wood maturation, which is a critical requirement for grapevine survival in cool-climate conditions.

In the ‘Concord’ vineyards a relationship between powdery mildew pressure and the acidity of the juice was observed, which points out an alteration of the ripening process, particularly in 2009. As fruit ripens, the sugar content and pH of the berries increases, and the concentration of organic acids (e.g., malic and tartaric acids) decreases (8, 23). The data collected from this vineyard in 2009 showed that powdery mildew disrupted such process, because the vines produced a juice of higher acidity (low pH and high titrable acidity) as disease levels increased in the field. However, reductions in the Brix levels were not significant.

The effects on ripening were more apparent when yield was taken into account. At higher cropping intensity levels, the juice soluble solids content and pH decreased, indicating a delay in the ripening process. Detrimental effects of overcropping other than maturation delays and the consequent effects on juice quality also include lowering or depletion of reserves (particularly in the root system), and even yield reductions because the vines grow weaker every year (31).

The results from this research highlight the need to reassess disease management in juice grape vineyards under Michigan growing conditions and low disease pressure (powdery mildew and downy mildew foliar severity of less than 30%), especially if dealing with low-yielding vines. Although the main effect of the foliar diseases under study (particularly of downy mildew on ‘Niagara’ grapes) was on carbohydrate reserves of the vines and not on the characteristics of the juice, a reduction in the number of fungicide applications without compromising fruit quality or vine health seems feasible.

This project represents a step towards the development of disease damage thresholds for downy and powdery mildew in juice grapes. The availability of damage thresholds will help growers develop more effective disease management programs, which most likely will decrease the farming costs, increase profitability, and reduce the impact to the environment by minimizing

the use of fungicides and achieving optimal fruit yield, quality, and vine health in an environmentally and economically sustainable manner.

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CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

This thesis has been mainly a study of epidemiological and biological aspects of the causal agent of the grape powdery mildew (*Erysiphe necator* Schwein.). We also evaluated the individual effects of powdery and downy mildew (caused by the oomycete *Plasmopara viticola* [Berk. et M. A. Curtis] Berl. et De Toni) on juice grapes (*Vitis labrusca* L.). In worldwide grape production, these diseases are a major problem and the application of fungicides is a management strategy that has been employed for years, regardless of the frequently recorded emergence of fungicide resistance. In Michigan, powdery and downy mildew are still highly destructive diseases that need further study. Both diseases were first described at the beginning of the 19th century and today, 176 years later, we are still struggling to decipher the biology of these pathogens and develop a long-term solution for every grape-growing area in the world, including Michigan.

Our investigations into the environmental factors that initiate primary inoculum dispersal of *E. necator* in the spring in Michigan suggest that dew formation (measured as leaf wetness during hours without rainfall) triggers spore release and that, even more strikingly, during long dry periods, ascospores are still released in Michigan. These findings contrast with research done in the state of New York that suggests ascospore release only after 2.5 mm of rainfall at a temperature of at least 4^{o}C. We also observed a discrepancy between the presence of primary inoculum and disease development that may be related to the exceptionally cool growing season In Michigan. This suggests that ascospore trapping, while it provides useful information on the

availability of initial inoculum, overestimates the risk of infection in Michigan and other cool-climate regions because not every ascospore release event leads to a successful infection.

Our study of the timing of *E. necator* overwintering inoculum (chasmothecium) production in relation to fall climatic conditions in Michigan demonstrated that chasmothecium development on grape leaves begins at mid summer, reaching a production peak in the middle of September. The proportion of mature vs. immature chasmothecia increased as the season progressed, and higher numbers were observed on the abaxial surfaces of the leaves. A fair number of mature chasmothecia remained on the leaves at the end of the season, when freezing temperatures led to leaf drop, and the fate of these ascocarps is still unknown and may require further investigation. Previous research in our laboratory evaluated post- infection eradicant sprays and found that paraffinic oil and calcium polysulfide were effective at eradicating existing colonies and preventing chasmothecium formation. Fall and spring dormant sprays also significantly reduced powdery mildew development throughout the growing season. Together, these results suggest mid to late season eradicant sprays to reduce primary inoculum of *E. necator* and the number of sprays needed for the following growing.

We also investigated the sensitivity of *E. necator* to strobilurins (Quinone outside Inhibitors [QoI], FRAC group 11), specifically trifloxystrobin (Flint, Bayer CropScience). Grape production in Michigan greatly depends on the frequent application of fungicides, including strobilurins. Our goal was to determine if reduced sensitivity of *E. necator* to strobilurins may be to blame for disease control failures in Michigan vineyards. Powdery mildew isolates were recovered from vineyards with no history of fungicide applications (baseline sites), commercial vineyards, and Michigan State University research vineyards. By means of conidium germination experiments, highly resistant isolates were only found at research sites and some

commercial (mostly wine grape) vineyards. The calculated EC_{50} values for isolates from baseline sites ranged from < 0.001 to 0.159 μg/ml. Calculated EC_{50} values for isolates from commercial and research vineyards varied greatly and ranged from < 0.001 to > 100 μg/ml trifloxystrobin; however, a lesser number of isolates sensitive to trifloxystrobin were found in research vineyards. We corroborated our results using a molecular biology approach to detect the G143A mutation in the cytochrome b gene, linked to a loss of strobilurin sensitivity. There was a close correlation between the mutation and reduced sensitivity observed in the conidium germination assay ($EC_{50} \geq 1.01$ μg/ml). Our findings suggest that the observed reduction in powdery mildew control in some commercial Michigan vineyards may be due to the development of strobilurin resistance in *E. necator.* This research will help design more effective fungicide programs for grape growers in Michigan.

Viticulture in Michigan is limited by a cool and humid climate and foliar diseases, such as powdery and downy mildew that require frequent fungicide applications. In juice grapes (*V. labrusca* L.), these diseases often appear after fruit set and their impact on vine physiology is still under study. To evaluate their effects, we established two contrasting disease levels by not spraying fungicides throughout the growing season or applying selective fungicides to control downy mildew in 'Niagara' and powdery mildew in 'Concord' vines cropped at three levels (33%, 67%, and 100% of the highest yielding potential). Downy mildew reached 53%, 57% and 73%, and powdery mildew < 1%, 32% and 20% of the leaf area infected in 2008, 2009 and 2010, respectively. Fruit composition (Brix, pH and titratable acidity) was affected more by cropping intensity than by disease, with the lowest Brix and pH values observed in diseased, high-cropped 'Niagara' vines. Cold hardiness of 'Niagara' canes was significantly reduced by downy mildew

in 2008 and 2010 and by high cropping level in 2009. Cane cold hardiness was not significantly reduced in 'Concord'. Bud cold hardiness was unaffected in both cultivars. Starch content of the canes was reduced in both cultivars but only significantly in downy mildew-infected 'Niagara' vines. At the levels of powdery mildew seen in 'Concord', fungicide applications did not appear to be cost-effective. However, 'Niagara' needs protection against downy mildew to avoid a reduction in cold hardiness, particularly under high cropping levels. The results from this research highlight the need to reassess disease management in juice grape vineyards under low disease pressure (powdery mildew and downy mildew foliar severity of less than 30%). Although a reduction in the number of fungicide applications without compromising fruit quality or vine health seems feasible, the cumulative effects of both diseases on juice grapes still need further investigation.

The availability of information on the epidemiology and fungicide sensitivity of the grape powdery mildew fungus in Michigan as well as damage thresholds for downy and powdery mildew will help grape growers make decisions on fungicide applications and likely reduce the number of fungicide applications needed. Reducing the number of sprays and cost of spray programs while maintaining fruit yield, quality, and vine health is key to reducing production costs, which will promote economic viability of family farms in the region. It will also reduce the risks of pesticide exposure for growers, farm workers, consumers, and the environment.