



**SUNLIGHT'S INFLUENCE ON GRAPEVINE POWDERY MILDEW: DIRECT  
EFFECTS ON PATHOGEN DEVELOPMENT AND ATTENDANT  
CONSEQUENCES OF CANOPY MANAGEMENT AND VINEYARD  
VARIABILITY**

by Craig Nathan Austin

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Wilcox, Wayne Frank (Chairperson)

Hodge, Kathie Therese (Minor Member)

Lakso, Alan Neil (Minor Member)

Seem, Robert Charles (Additional Member)

Cadle-Davidson, Lance E (Field Appointed Minor Member)

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In Partial Fulfillment of the Requirements for the Degree of  
Doctor of Philosophy

by

Craig Nathan Austin

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Craig Nathan Austin, Ph. D.

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Sunlight inhibits powdery mildew development through at least two mechanisms, i.e., UV radiation's damaging effects on the exposed conidia and thalli of the pathogen and through elevating the temperature of irradiated tissues to a level supraoptimal or inhibitory for pathogen development. Furthermore, these effects are synergistic at temperatures near the upper threshold for disease development. Improved understanding of the role UV-B exposure, surface temperature, and their interaction have on powdery mildew development may assist in better management of this disease through chemical and cultural means. Variability of sunlight distribution within vineyards was quantified via the enhanced point quadrat analysis technique (EPQA). Using EPQA the number of canopy shading layers and the fruitzone photon flux within individual vines were shown to have a significant correlation with fruit disease severity for those vines, i.e., less disease developed on clusters with more exposure to sunlight. Additionally, through use of a fluorescent tracer and EPQA assessments, deposition of spray materials upon clusters

was shown to be linearly related to their degree of exposure. Thus, canopy management practices designed to optimize sunlight exposure of grape clusters for fruit quality purposes should significantly assist in the management of powdery mildew as well. These results underscore the fact that viticultural practices targeted primarily at general vine growth and crop quantity/quality issues such as; vine vigor management, pruning level, training system, basal leaf removal, and irrigation regime, can also significantly affect the development of powdery mildew.

## BIOGRAPHICAL SKETCH

Craig Nathan Austin was born in Baltimore Maryland April 7, 1980 to William and Cathy Austin. After moving around as a child from Baltimore MD, to Lubbock TX, to Orlando FL, to Topeka KS, to East Greenville PA, by the time Craig was 6 years old, Craig was used to travelling. After an immediate interest in biology in science began at Upper Perkiomen High School under the guidance of Barb Ryan, Craig enrolled at Millersville University.

At Millersville Craig's interest in Biology became more refined to the field of Botany. Studying the segmented cambium of tropical lianas, Craig began to gain a specific interest in the interaction between plants and pathogens. Taking this interest, after graduation Craig worked for Dr. Ried Frederick at Fort Detrick Maryland in the Foreign Disease Weed Science Research Unit of the USDA.

At Fort Detrick Craig found the opportunity of a lifetime working on the select agent *Phakopsora pachyrhizi*, or soybean rust. Being a select agent, work on this pathogen required a government background check and security clearance, as well as working in a Bio-Safety Level III facility. As Craig's interest in plant pathogens was peaking, Craig began to find a passion for wine. As Craig was applying for plant pathology graduate programs at the time, he attempted to make his new found hobby his career. He was able to do so by being offered a position at Cornell University's New York State Agriculture Experiment Station working with Dr. Wayne Wilcox.

Working and studying in Dr. Wilcox's lab gave Craig the ability to work on a fungal pathogen of grapevines, and ideal situation for

Craig. Furthermore, Dr. Wilcox arranged a collaboration with Australian scientists for annual trips to the Barossa Valley and Adelaide for Craig during the New York winters. On Craig's last trip to Australia in February of 2009 Craig proposed to his beautiful girlfriend Rachel, and were subsequently married on October 3<sup>rd</sup> 2009.

At the time of this writing Rachel is pregnant with their first child, and likely the last official record of Craig and Rachel before parenthood.

This dissertation is dedicated to Mr. Reynolds' and his bottle of Châteauneuf-du-Pape. Without which my life would have never know the joys of vines and wines.

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## CHAPTER ONE

### EFFECTS OF SUNLIGHT EXPOSURE ON GRAPEVINE POWDERY MILDEW EXPOSURE ON GRAPEVINE POWDERY MILDEW DEVELOPMENT: UV RADIATION, TEMPERATURE ELEVATION OF EXPOSED TISSUES, AND THEIR INTERACTION

#### ABSTRACT

Both natural and artificially-induced shade increased grapevine powdery mildew (*Erysiphe necator*) severity in the vineyard. Responses were quantitative, with foliar disease levels up to 70-fold higher in the treatment with the greatest level of natural shade versus leaves in full sun. Cluster disease severities increased by 156 to 1090% relative to check vines when most ultraviolet (UV) radiation was filtered from sunlight reaching treated vines in artificial shading experiments. Surface temperatures of leaves in full sunlight averaged 5 to 8°C higher than those in natural shade, and in one experiment, filtering all solar radiation, including longer wavelengths responsible for heating irradiated tissues, increased disease more than filtering UV alone. Controlled environment experiments showed that UV-B radiation reduced viability of *E. necator* conidia and inhibited colony establishment (hyphal formation and elongation) and maturity (latent period), where the response was linear. Inhibitory effects of UV-B radiation were significantly greater at 30°C than at 20 or 25°C. Thus, sunlight appears to inhibit powdery mildew development through at least two mechanisms, i.e., UV radiation's damaging effects on the exposed conidia and thalli of the pathogen and through elevating the temperature of irradiated tissues to a level supraoptimal or inhibitory

for pathogen development; furthermore, these effects are synergistic at temperatures near the upper threshold for disease development. Improved understanding of the role UV-B exposure, surface temperature, and their interaction have on powdery mildew development may assist in better management of this disease through chemical and cultural means.

## INTRODUCTION

Powdery mildew, caused by the fungus *Erysiphe necator* (Schw.) Burr., is a disease ubiquitous to grape growing regions worldwide. Foliar infections negatively affect photosynthesis, can cause premature defoliation, crop reduction, and in severe cases can even lead to vine mortality (Lakso *et al.* 1982, Gadoury *et al.* 2001). Fruit infections on highly susceptible cultivars can cause complete crop loss if left uncontrolled, but severity levels as low as 3% can negatively alter wine flavor (Pool *et al.* 1984) and even inconspicuous levels of disease can promote the development of Botrytis bunch rot and growth of wine-spoilage microorganisms on the berry skins (Gadoury *et al.* 2007). Thus, powdery mildew control is an essential component of any vineyard management program.

Numerous observations have associated an increased development of grapevine powdery mildew with shaded conditions (e.g., Zahavi *et al.* 2001). Sources of shade can vary within a vineyard, and may include various external (e.g., adjacent wood lots, persistent cloud cover) and/or internal (e.g., tall weeds, dense canopy growth)

entities. The light microclimate within the canopy can be further influenced by vine training system, slope, pruning level, row orientation, geographic location of the vineyard, fruit zone leaf removal, or time of year (Reynolds and Vanden Heuvel 2009, Caldwell *et al.* 1983, Dokoozlian and Kliewer, 1995a, Dokoozlian and Kliewer, 1995b, Calonnec *et al.* 2009). Although a significant amount of research has been conducted on the light environment within grapevine canopies, these studies have focused primarily on yield and quality components of the fruit. Sunlight exposure on fruit is often associated with an improvement in fruit quality, and allowing sunlight penetration into and distribution within the fruit zone of the canopy is an important concept in vineyard management (Lee *et al.* 2007, Smart 1985).

Inherent with increases in direct sunlight exposure are increases in exposure to ultraviolet (UV) radiation (290-400 nm). This component of sunlight (in particular, the UV-B segment, i.e., 290-320 nm) has numerous effects on biological systems. UV-B has been shown to alter fungal population ratios on plant leaves (Moody *et al.* 2001); change grapevine secondary metabolite production (Kolb *et al.* 2001, Keller and Torres-Martinez 2004); and generally increase fungal mortality (Bjorn 2007, Isard *et al.* 2006, Arabi and Jawhar 2003, Ulevicius *et al.* 2004, Rotem *et al.* 1985). Recent reports have further stressed the importance of studying the effects of UV-B radiation in agricultural systems in relation to the observed increases caused by reduced stratospheric filtering (Björn 2007, Manning and von

Tiedemann 1995, Paul 2000). Although the response to UV-B radiation is host/pathogen specific (Roberts and Paul 2006), the vast majority of reported studies can be characterized as demonstrating a negative correlation between disease development and UV-B exposure. A second component associated with sunlight that can alter the phylloplane environment is an increase in surface temperature of exposed tissue. Increased surface temperature due to sunlight exposure has been documented on grapes (Kliewer *et al.* 1968, Smart and Sinclair 1976, Bergqvist *et al.* 2001, Millar 1972, Downey *et al.* 2006) and other fruit (Chen *et al.* 2009, Ferguson *et al.* 1998, Schroeder 1965), where temperatures of exposed surfaces are often observed to be as much as 5-15°C above ambient. The effect of leaf exposure on leaf temperature is also accentuated in water-stressed leaves that cannot evaporatively cool when stomata close. These studies, however, have focused on the physiological effects of these temperature differences on the leaves or fruit, not on the consequence that they may have on microorganisms growing on the sunlight-exposed surfaces.

Several researchers have reported specific impacts that sunlight exposure can have on the development of grapevine powdery mildew. Chellemi and Marois (1992) reported that the cultural practice of basal leaf removal around fruit clusters, intended to benefit fruit quality, resulted in reduced powdery mildew severity on these berries. They concluded that this effect was due to improved fungicide coverage and inferred that removing basal leaves produced a berry microclimate less

conducive for disease development. Calonnec *et al.* (2009) found that the spatial spread of powdery mildew was strongly related to the leaf density of individual vines. They hypothesized that vigorous vines may either (i) allow a greater production of secondary inoculum due to their greater volume of diseased leaves; or (ii) provide tissues of greater physiological susceptibility. Zahavi *et al.* (2001) demonstrated that training systems and row spacing that reduced sunlight exposure on fruit were associated with increased powdery mildew severity on fruit. They attributed this effect on disease development to differences in sunlight distribution within the fruit zone, as there was no measurable difference in air temperature or ambient humidity between the treatments. Keller *et al.* (2003) found that potted Chardonnay and Cabernet Sauvignon vines protected from the sun's UV-B radiation had significantly greater levels of foliar powdery mildew than did vines afforded full sun exposure, and attributed this effect to differences in vine physiology between the two treatments. Willocquet *et al.* (1996) demonstrated a reduced frequency of germination of *E. necator* conidia, and weaker mycelial growth thereafter, when inoculated leaf discs were exposed to UV-B radiation.

Although these studies have shown that sunlight exposure is deleterious to grapevine powdery mildew development, the magnitude of this effect has not been well characterized nor have its mechanisms been well established. Thus, our objective was to manipulate sunlight distribution in vineyards through natural and artificial shading and directly observe and quantify the effects of variable exposure levels on

powdery mildew severity. Furthermore, using controlled environment conditions, we refined our vineyard observations and investigated the effects that UV-B radiation, elevated surface temperature, and their interaction have on *E. necator* development. Abstracts of this work have been published previously (Austin *et al.* 2009a, Austin *et al.* 2009b, Austin *et al.* 2008).

## MATERIALS AND METHODS

*Natural Shading.* The effects of two different types of natural shading were investigated in a Umbrella Kniffen-trained Chardonnay vineyard planted in 1988 located near Dresden, NY (approximately 20 km south of the NY State Agricultural Experiment Station in Geneva), utilizing the easternmost row in a north-south oriented vineyard. The first type of shade was the transient shade associated with a specific location in the vineyard. This was examined by comparing vines immediately west of a 20-m long grouping of 15-m tall pine trees--which completely shaded the vines until approximately 11:30 am during the summer--versus those 50 m down the same row, well removed from any external source of shade (Illustration 1.1). The second type of shade was the constant shade that occurs within the interior of a dense canopy. This was examined by comparing shoots fully exposed to the sun on the outer edge of the canopy versus others that were tucked into the heavily-shaded interior of the canopy and tied to remain within. Weather parameters were monitored within and



Illustration 1.1. Chardonnay vineyard located near Dresden NY. Pine trees immediately east of one section of the vineyard shaded adjacent vines until approximately 11:30 am each day during the summer.

above the canopy both adjacent to and away from the shade trees using CR-10X data loggers (Campbell scientific, Logan, UT) with HMP-45C temperature and relative humidity probes fitted with radiation shields, and LI-200 pyranometers (400-1100nm) (LICOR,, Lincoln, NE).

During each of two seasons, three separate inoculations were performed on the adaxial surface of the 4<sup>th</sup> fully expanded leaf from the terminus of 10 shoots per treatment. Cultures used for inoculum were sourced from field populations of *E. necator* collected from local vineyards and maintained year round on 1- to 3-month-old Riesling seedlings in the greenhouse. In May of each year, when frost no longer posed a threat to the fungus or host, conidia for inoculations were multiplied on 2-month-old Riesling seedlings maintained in a covered shadehouse with mesh walls to allow for free air movement. Sporulating leaves were transported to the vineyard, where inoculum was prepared by first agitating the diseased leaves in 50-ml falcon tubes with 0.005% Tween solution in dH<sub>2</sub>O, then bringing the spore suspension to a final volume necessary to provide 1 ml per inoculated leaf, with additional 0.005% Tween solution. Inoculum was applied using a small spray-paint apparatus (Preval; Yonkers, NY). Subsequent quantification in the lab with the aid of a hemocytometer revealed concentrations of  $5 \times 10^4$  to  $1 \times 10^5$  conidia per ml. Foliar disease severity was assessed 2 weeks after each inoculation, using a 0-100 scale based on the percentage of leaf tissue visibly diseased. Leaf surface temperatures were measured regularly with a Mikron MI-N14 portable infrared thermometer (LumaSense Technologies Inc., Santa Clara, CA), arbitrarily choosing 10 leaves per treatment at solar noon on days with clear skies.

*Artificial shading experiments.* The effects of individual sunlight components were investigated in two vineyards. One consisted of a

north-south oriented planting of cordon-trained, own-rooted vines of the interspecific hybrid cv. Chancellor established in 1970 near Geneva, NY. During the 2005-2009 seasons, selected vines were draped with a double layer of black neutral density shade cloth (Griffin Greenhouse & Nursery Supplies, Tewksbury, MA) 3 weeks after budbreak. The frame constructed to support the shade cloth suspended the material approximately 1m away from the contour of the entire vine canopy, and was designed to have minimal effect on air movement or rainfall reaching the vine. Environmental parameters were monitored with Campbell CR-10X data loggers as described above. Pyranometers established in the vineyard showed that the shade cloth, which was meant to simulate levels of natural shade levels often found within grapevine canopies, filtered out 80% of radiation in the range of 400-1100nm.

In order to filter UV radiation but not the longer wavelengths responsible for heating sun-exposed tissues, in the 2006 through 2009 seasons, sheets of poly(methyl methacrylate) plastic (Plexiglas®; Altuglas International, Philadelphia, PA) were affixed 1 m above the tops of other selected vines, via a modified metal framing assembly. For each of two vines, two sheets of 3.1-mm thick Plexiglas®, 2.4 m long x 1.2 m wide, were mounted atop support beams placed at the four corners of the plot. Each sheet was inclined towards the center with an apex support beam between them, providing a structure similar to a gabled roof over the vines. The sides below the UV-filtering Plixiglas remained open to allow for free air movement and

equilibration with the atmosphere of the surrounding environment (Illustration 1.2). With assistance from the USDA-ARS UV-B



Illustration 1.2. Shade cloth and UV-filtering Plexiglas® gabled roofs over Chancellor vines near Geneva, NY.

monitoring research program at the Natural Resource Ecology Laboratory (Ft. Collins, CO), an Ultraviolet Multifilter Rotating Shadowband Radiometer was used to measure direct, total horizontal, and diffuse UV irradiance at 300, 311, 317, 325, 332 and 368 nm. This sensor showed that these units filtered 87% of all UV radiation reaching the vines; measurements with pyranometers showed that they filtered only 9% of wavelengths longer than 400nm. This UV radiometer further demonstrated that the shade cloth treatment filtered 88% of UV radiation. Plexiglas® shelters were also erected in a

second vineyard consisting of an east-west planting of Chardonnay vines on 3309C rootstock, established in 2007 approximately 5 km from the Chancellor vineyard. In this vineyard, two contiguous UV shelters of the dimensions given above were erected over each of two different four-vine plots. Fruit clusters were inoculated in all experiments 1 week after 50% anthesis. Inoculum for the 2005-2008 seasons was produced as described above. In 2009, inoculum was produced on detached Chardonnay leaves sourced from 7-yr-old potted vines, maintained on 1.5% water agar in a petri dish and incubated at 25°C in a growth chamber. Final inoculum preparation and delivery was as described for foliar inoculations. Fungal and oomycete diseases on these vines were managed with minimal applications of mancozeb and a highly refined petroleum oil (JMS Stylet Oil, JMS Flower Farms, Vero Beach, FL), chosen for their lack of vapor activity. Test clusters were protected from the sprays within individual plastic bags during each application. Cluster disease severity was assessed on a 0-100% scale weekly, beginning 2 weeks post-inoculation. Final disease severity ratings were made near veraison each year due to rapid degradation of the most heavily infected clusters and the frequent onset of *Botrytis* bunch rot by that time. Leaf surface temperatures were collected from both vineyards, as described above.

*Controlled environment experiments.* The effect of UV-B dosage, and its interaction with temperature, was assessed on detached cv. 'Chardonnay' leaves sourced from 7-yr-old potted vines maintained in

a greenhouse. The fourth expanded leaf from the shoot tip was surface disinfested for 10 min in a 10% bleach solution, triple rinsed with sterile type 1 water, and blotted dry. The petiole of a single leaf was then inserted through a small hole drilled into the bottom of a 9-cm-diameter petri dish, which was fused atop a second petri dish filled with distilled water. This created a vessel that maintained a water supply to the detached leaf, which remained uncovered with its adaxial surface exposed. Fourteen-day-old powdery mildew colonies from the detached leaf inoculum source, as described above, were brushed against the disinfested leaves, which were immediately transferred to a growth chamber maintained at 20, 25, or 30°C. In each growth chamber, leaves were exposed to one of two different light treatments: (i) 3.0 W/m<sup>2</sup> of UV-B radiation, supplied by two narrowband fluorescent light bulbs with peak emission at 312 nm (Amjo Corp. West Chester, OH), which were covered with a 0.0762mm thick acetate film (Du All Drafting and Art Supply. Madison Heights, MI) to prevent UV-C (200-290nm) radiation from reaching the sample, plus photosynthetically-active radiation supplied by low-mercury cool white 20W fluorescent bulbs (Grainger, Lake Forest, IL); or (ii) the cool white fluorescent light alone, to which the leaves were otherwise exposed when not subjected to individual UV-B treatments. UV-B intensity was measured at the beginning and end of each experiment with a hand-held UVX radiometer fitted with a 310nm calibrated sensor (UVP LLC, Upland, CA). The UV-B radiation intensity of 3.0 W/m<sup>2</sup> was chosen as representative of peak values that we had measured previously in Geneva, NY on sunny mid-summer afternoons.

For “acute” dose experiments, three replicate leaves per treatment were subjected to the UV-B light source for 0, 6, 12, or 24 h immediately after inoculation, providing a total radiation dosage of 0, 21.6, 43.2 or 86.4 kJ/m<sup>2</sup>, respectively. Leaves were then transferred to a paired growth chamber maintained at the same temperature and relative humidity (70-80% dependent on chamber cycling) without UV-B radiation. For “chronic” dose experiments, three replicate leaves in all but the check treatment were subjected to a 6-h dose of UV-B immediately after inoculation, then transferred to the paired growth chamber without UV-B. At daily intervals thereafter, selected leaves were transferred back to the UV-B chambers for additional exposure, so that the final treatments consisted of 6-h exposures on each of 0, 1, 2, or 4 consecutive days, providing the same total radiation dosage of 0, 21.6, 43.2 or 86.4 kJ/m<sup>2</sup>, respectively, as applied acutely.

A small portion of leaf tissue was removed with a scalpel from each inoculated leaf 48 h after inoculation and the leaves were returned to their controlled environment chambers. The excised tissue was cleared overnight in a 3:1 ethanol/acetic acid solution, subsequently stained with Coomassie blue, and mounted on slides for assessment of conidia via light microscopy at 100X magnification. Conidia were classified into one of three progressive, exhaustive developmental categories: (i) no germination; (ii) formation of an appressorium; or (iii) production of one or more hyphae, indicating successful establishment of an infection. A second small portion of each leaf was removed 96 h post-inoculation and conidia were

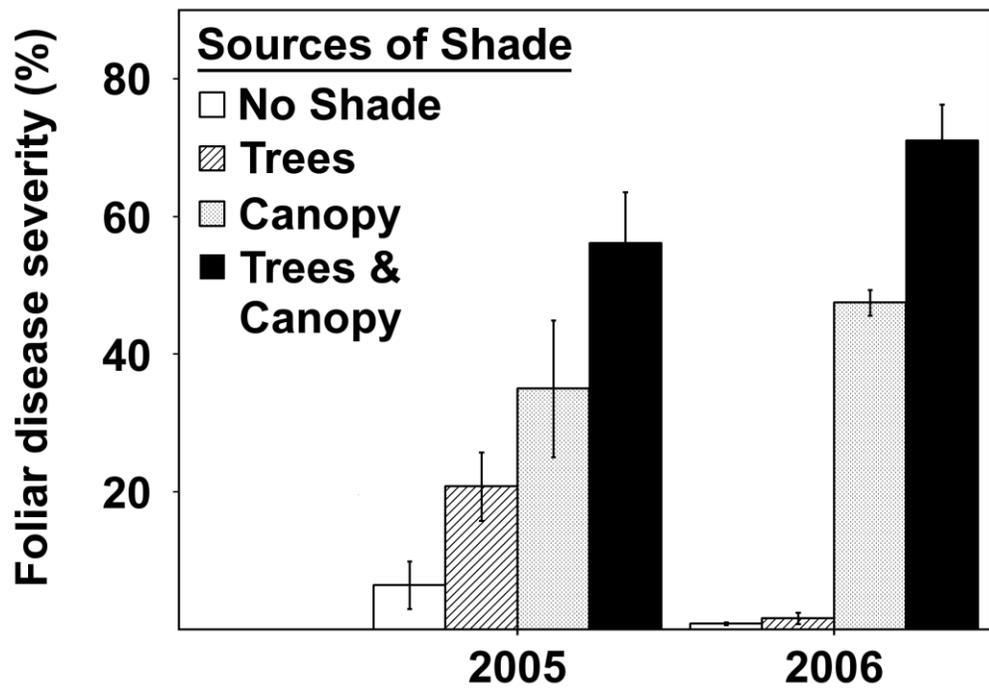
similarly assessed. The hyphal lengths of 10 arbitrarily-chosen conidia from each replicate leaf in the “chronic” experiment were measured 48 and 96 h after inoculation, using an ocular micrometer. Following these assessments, all leaves were maintained in their respective growth chambers and the entirety of each leaf was examined twice daily at 5X magnification under a stereoscope beginning 4 days post-inoculation, to determine the latent period for these infections. The latent period was considered complete when the production of the first new conidium was observed. For each run of the experiment, both the acute and chronic exposure treatments were examined at a single temperature, with three repeated runs per temperature.

Data were analyzed for significance using generalized linear regression in JMP statistical software (SAS Institute, Cary, NC). Latent period slope comparisons were made by comparing sum of squares error for regression equations with and without interaction terms to determine F-statistics.

## RESULTS

*Natural shade experiments.* In both seasons, shading from both sources, external and internal, increased foliar disease development, with leaves in the most heavily shaded treatment (inner canopy adjacent to trees) exhibiting nine- and 70-fold higher disease severity ratings than those afforded full sun exposure in 2005 and 2006, respectively (Figure 1.1). For leaves within the canopies, disease

Figure 1.1. Percent foliar area infected with powdery mildew on Chardonnay grapevine shoots (i) receiving full solar radiation, i.e., located on the outer canopy edge on vines well away from a bordering group of trees (No Shade); (ii) on the outer canopy edge but otherwise shaded by an adjacent group of pine trees until 11:30am each day (Trees); (iii) located within the interior part of the dense canopies of vines away from the trees (Canopy); and (iv) subjected to both internal canopy shading plus that provided by the adjacent trees (Trees & Canopy). Disease severity was assessed 14 days after inoculation, using a 0-100 scale, based on the percent leaf area visibly covered with signs of the pathogen. Values represent the means of four independent repeats of the experiment, with 10 inoculated leaves per treatment in each, error bars represent one standard error of the mean.



severity was 38 and 32% lower for vines away from the trees relative to those adjacent to them in these same respective years. However, the effect of the morning shade supplied by the trees varied substantially between years with respect to disease severity on the outside of the canopy, i.e., it was threefold higher on vines adjacent to the trees than away from them in 2005, whereas there was virtually no difference between these two treatments in 2006. Relative to the full sun treatment, daily interception of solar radiation, as indicated by pyranometer readings, averaged 74, 24, and 12% for the tree, canopy, and tree & canopy treatments, respectively, across the two seasons (Table 1.1). There were no measurable differences in ambient temperature or RH adjacent to the foliage within each of the treatments during the experiments (Table A1.1), but mean leaf temperatures were elevated as much as 5°C-8°C on the sun-exposed versus shaded leaves (Table 1.2).

*Artificial Shading.* In all seasons and at both locations, cluster disease severity was substantially lower in the full sun treatment than in those from which UV radiation was filtered. The Plexiglas® increased disease severity by 156 to 1090% relative to the full sun treatment in the Chancellor vineyard, depending on the year, and by 250 and 191% in the Chardonnay vineyard in 2007 and 2008, respectively (Fig. 1.2). Environmental monitoring within each treatment showed no differences in air temperature or relative humidity surrounding clusters (Table A1.1) among the different treatments in a given experiment. Midday leaf temperatures were similar to the ambient air

Table 1.1. Average leaf temperature increase (°C) relative to ambient in each shade treatment, for all vineyards locations				
Experiment, years	Treatment	n <sup>a</sup>	Mean <sup>b</sup>	Std. Dev.
Chardonnay (Dresden), 2005-06	Full Sun	170	6.7	± 1.49
	Trees	170	6.4	± 2.15
	Canopy	170	1.0	± 0.99
	Trees & Canopy	170	0.8	± 0.98
Chancellor, 2005-09	Full Sun	410	4.7	± 1.60
	UV filter	410	4.9	± 1.78
	Shade Cloth	410	1.6	± 0.85
Chardonnay (Geneva), 2007- 08	Full Sun	130	7.8	± 1.50
	UV filter	130	7.5	± 1.78

<sup>a</sup>Number of leaf temperatures measured for this treatment over all seasons. Temperatures were determined at solar noon on clear days with no wind throughout the growing season.

<sup>b</sup>Mean temperature above ambient.

Table 1.2. Relative sun exposure (as reflected by pyranometer readings, 400-1100 nm wavelengths) from 2005 to 2007 for Chancellor and Chardonnay (Dresden) vines subjected to different shading treatments, compared to those in full sun expressed as a percent					
	Chancellor <sup>a</sup>		Chardonnay (Dresden) <sup>b</sup>		
	Low UV	Shade Cloth	Tree	Canopy	Tree & Canopy
2005	na	20%	74%	26%	14%
2006	97%	24%	74%	22%	10%
2007	91%	25%			

<sup>a</sup> Treatments as identified in Fig. 2.

<sup>b</sup> Treatments as identified in Fig. 1.

temperature for leaves in the shade cloth treatment, but were on average 5°C higher on the exposed leaves, with or without UV filtering (Table 2). Cluster disease severity was significantly greater on Chancellor vines beneath the shade cloth than on those beneath the Plexiglas® filters in 2006, although there was little difference between these two treatments in 2008 and 2009 (Fig. 2).

*Controlled environment experiments - Colony establishment.* Increases in acute UV-B radiation dosage reduced both the frequency of germinating conidia and the rate of their subsequent development (Fig. 3). At 25°C and 30°C, there was a significant decrease in the percentage of non-germinated spores from 48 to 96 h in both the nil exposure and lowest dosage (6-h) treatment, indicating that some of the slowest-developing conidia were nevertheless able to germinate 2 to 4 days after contact with the leaf (Fig 3 E-F, I-J). In contrast, the percentage of non-germinated conidia subjected to immediate 12- and 24-h irradiation treatments remained uniformly high both 48 and 96 h after inoculation. The percentage of conidia apparently able to establish a successful infection (i.e., to produce a hypha) within 96 h after inoculation was reduced significantly by progressive increases in UV-B radiation, at all temperatures (Table 1.3). At the coolest temperature of 20°C, the frequency of conidia developing hyphae after 96 compared to 48 h increased for all UV-B dosages; a similar result was observed for the nil and lowest-dosage treatments at 25 and 30°C (Figures 3A-B, E-F, and I-J). In contrast, relatively few conidia established successful infections within 96 h following the initial 12-

Figure 1.2. Percent cluster area infected with powdery mildew on Chancellor and Chardonnay vines receiving (i) full solar radiation (Full Sun); (ii) sunlight reduced to 13% of ambient UV radiation by Plexiglas® shelters (Low UV); or (iii) sunlight reduced to 20% of ambient intensity for 400-1100nm and 12% of ambient UV radiation via two layers of neutral density shade cloths (Shade Cloth). Disease severity was assessed weekly on a 0-100 scale based on the percent cluster area visibly covered with signs of the pathogen, and the final ratings (made near veraison) are presented. Values represent the means for 10 clusters in each of four replicate plots per treatment, and error bars represent one standard error of the mean. \* No Low UV treatment in 2005. \*\* Less than 10% fruit set occurred in the Shade Cloth treatment in 2007, and no fruit disease ratings were obtainable.

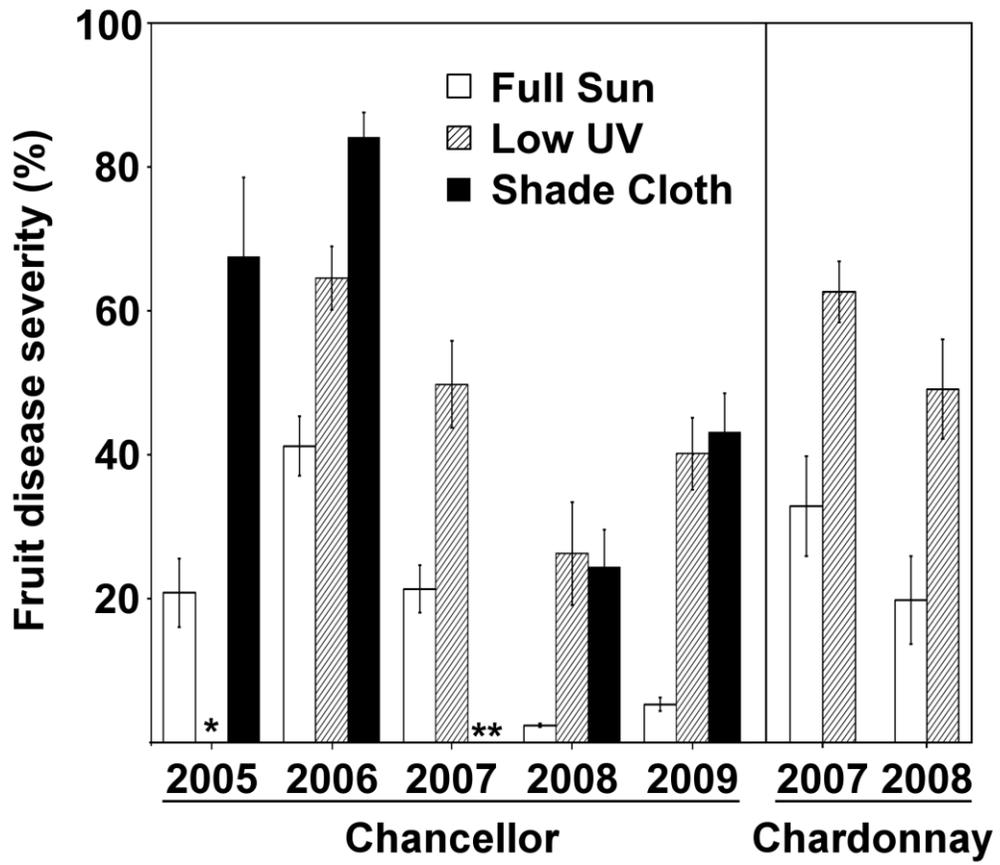


Figure 1.3. Percent of non-germinated conidia, germinated conidia forming only an appressorium, or germinated conidia forming at least one hypha at both 48 and 96 h after inoculation with *Erysiphe necator* conidia, in response to acute and chronic exposures to UV-B irradiation at three different temperatures. For each temperature, conidia were exposed to 3.0 W/m<sup>2</sup> UV-B radiation, either (i) continuously for 0, 6, 12, or 24 h immediately after inoculation (panels A, B, E, F, I, and J); or (ii) for 6 h on each of 0, 1, 2, or 4 consecutive days, beginning immediately after inoculation (panels C, D, G, H, K, and L), thereby receiving the same total energy doses of 0, 21.6, 43.2, or 86.4 kJ/m<sup>2</sup>, respectively. For each of three replicate leaves, 100 conidia were assessed for all temperature x UV-B combinations. Experiments were repeated three times and mean values for all are reported. Error bars represent one standard error of the mean.

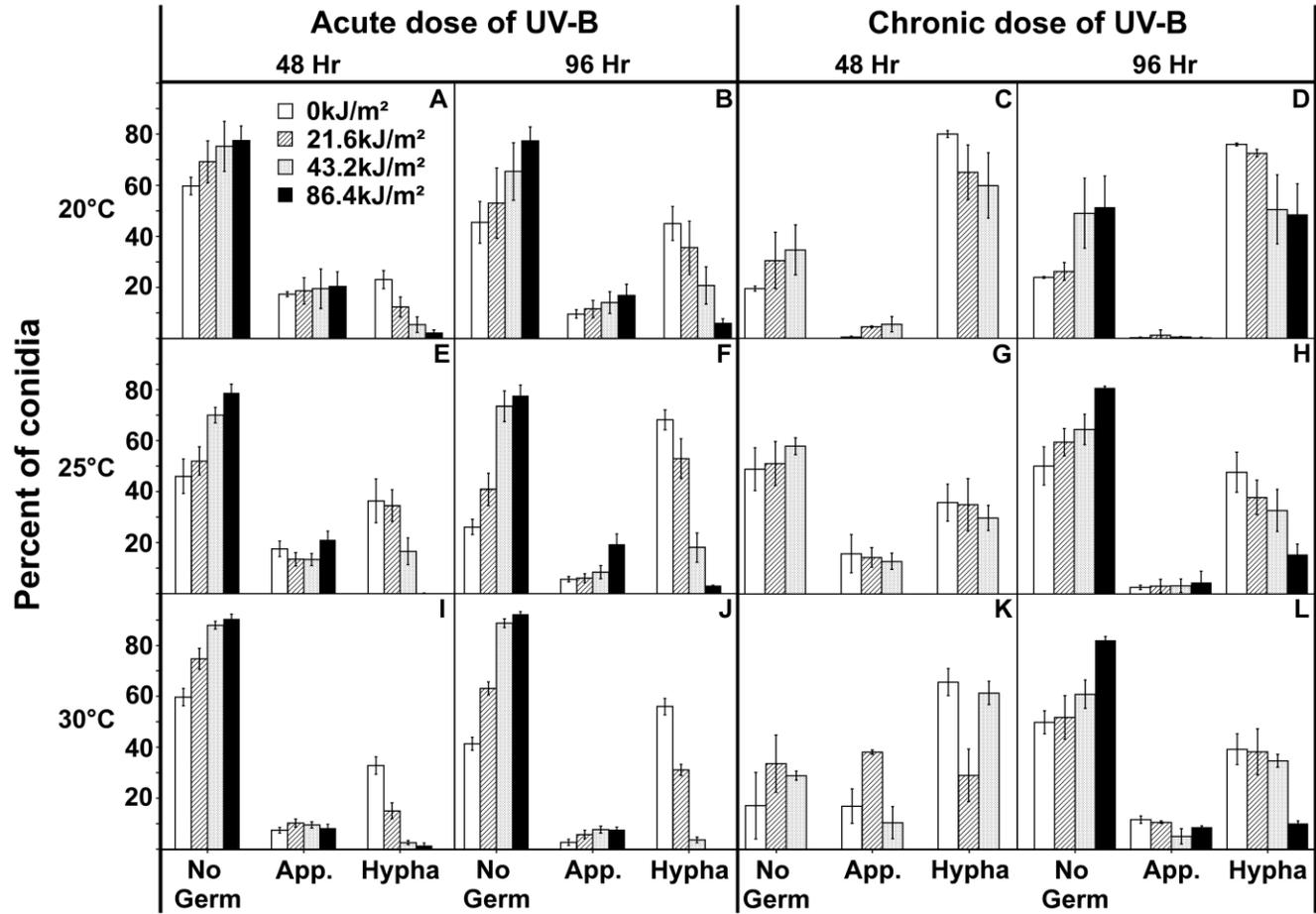


Table 1.3. Analysis of variance for acute UV-B exposure effects on <i>Erysiphe necator</i> conidial development at variable temperatures						
	Temperature		UV-B Dose		Interaction	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Colony Establishment <sup>a</sup>						
48h No Germination	1.52	0.22	2.73	0.05	1.27	0.27
48h App	2.77	0.07	0.46	0.71	0.54	0.78
48h Hypha	5.23	0.007	8.96	<0.0001	3.27	0.006
96h No Germination	2.29	0.11	5.06	0.003	1.31	0.26
96h App	1.79	0.17	1.27	0.29	1.32	0.26
96h Hypha	5.74	0.005	12.5	<0.0001	3.04	0.01
Colony Maturity						
Latent Period	7.50	0.001	3.52	0.02	4.45	0.0007

<sup>a</sup> Analyses for the percentage of conidia that had germinated, formed appressoria, or formed a primary hypha (indicating establishment of a successful infection) 48 and 96 h after inoculation.

and 24-h irradiations for the highest temperature of 30°C, with frequencies of 4% and 0% associated with those two respective doses versus 56% for the no UV-B check. Temperature and UV-B dosage were significantly interactive with respect to their effects on hyphal development ( $P < 0.01$ ) but did not interact with respect to their effects on conidial germination or appressorium formation ( $P = 0.26$  to  $0.78$ , depending on measure) (Table 1.3).

For the chronic exposure treatments, temperature generally had a more significant effect than UV-B dosage on influencing early conidial development (Table 1.4). However, UV-B exposure did significantly reduce germination and hyphal development 96 h post-inoculation. As with acute UV-B doses, the only significant interaction between temperature and chronic UV-B exposure was on hyphal development, albeit only at 48 h post-inoculation. There was little evidence of fungal development between 48 and 96 h post-inoculation at either 20°C or 25°C, based upon changes in the frequencies of germinated conidia or those that had formed hyphae between these two time points. Nevertheless, frequencies of both conidial germination and hyphal formation were affected similarly by progressive UV-B doses applied in a chronic manner as they were by acute exposures at all temperatures, although the responses were somewhat less intense following chronic exposure. For example, the frequency of hyphal formation after 4 days at 20°C following a dosage of 86.4 kJ/m<sup>2</sup> was nine-fold lower (relative to the check) when it was applied acutely but less than a two-fold lower when applied chronically

Table 1.4. Analysis of variance for chronic UV-B exposure effects on <i>Erysiphe necator</i> conidial development at variable temperatures						
	Temperature		UV-B Dose		Interaction	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Colony Establishment <sup>a</sup>						
48h Germination	9.40	0.0005	0.89	0.42	0.56	0.69
48h App	2.76	0.08	0.21	0.81	2.28	0.08
48h Hypha	12.3	<0.0001	1.41	0.26	2.57	0.05
96h Germination	4.03	0.02	3.64	0.02	0.39	0.88
96h App	6.10	0.005	0.06	0.98	0.63	0.70
96h Hypha	5.21	0.01	3.25	0.03	0.43	0.86
Colony Growth						
48h Hyphal Length	143.6	<0.0001	37.2	<0.0001	5.27	0.0005
96h Hyphal Length	586.3	<0.0001	248	<0.0001	10.3	<0.0001
Colony Maturity						
Latent Period	27.47	<0.0001	6.41	0.001	54.6	<0.0001

<sup>a</sup> Analyses for the percentage of conidia that had germinated, formed appressoria, or formed a primary hypha (indicating establishment of a successful infection) 48 and 96 h after inoculation.

(Figure 3B-D); similarly, this same dosage allowed modest hyphal formation when applied in a chronic manner at 25 and 30°C, versus minimal to no such development when applied acutely (Figs. 3F-H and FJ-L). Similar to the acute dose experiments, the negative effect on conidia establishing infections and producing hyphae was strongest at 30°C and weakest at 20°C.

*Colony growth.* Increasing the number of 6-h daily UV-B doses reduced the length of those hyphae that did develop from germinating conidia, at all temperatures (Figure 1.4). In the absence of UV-B, conidia at 20°C and 25°C developed the longest hyphae after 48 h, whereas those at 30°C were 41 and 50% shorter, respectively. At each temperature, regression analysis indicated there was a linear reduction in maximum hyphal length in response to increasing UV-B exposure ( $P < 0.0001$ ). Compared to the nil controls, four daily UV-B exposures (86.4 kJ/m<sup>2</sup> in total) at 20, 25, and 30°C, resulted in mean reductions of 66, 54, and 65% in hyphal length, respectively. There was a highly significant interaction ( $P < 0.0005$ ) between temperature and UV-B exposure on hyphal elongation both 48 and 96 h after inoculation (Table 4).

*Colony maturity.* At each of the three temperatures, the latent period increased linearly ( $P < 0.0001$ ) in response to UV-B dosage and in a similar manner whether applied acutely or chronically (Figure 1.5). At 25°C, the latent period was increased by 40% relative to the no UV-B check treatment (i.e., from 5 to 7 days) following the maximum total dosage of 86.4 kJ/m<sup>2</sup>, under both the acute and chronic exposure

Figure 1.4. Mean maximum hyphal length for each temperature x UVB combination for *Erysiphe necator* conidial inoculations treated with a six-hour daily dose of 3.0 W/m<sup>2</sup> UVB for 0, 1, 2, or 4 days. For each of three replicate leaves 10 hyphae were measured at 48h and 96h post inoculation. Experiments were repeated twice. Error bars represent one standard error of the mean.

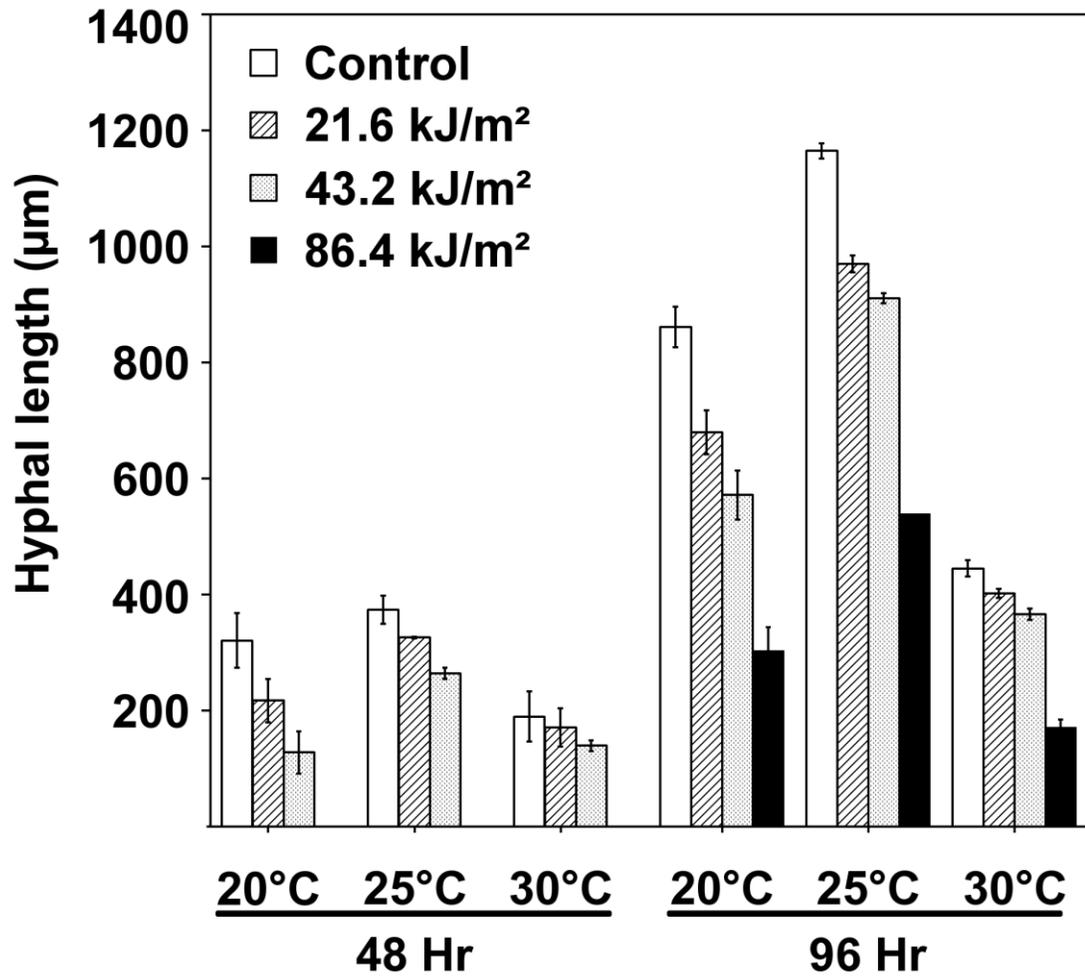
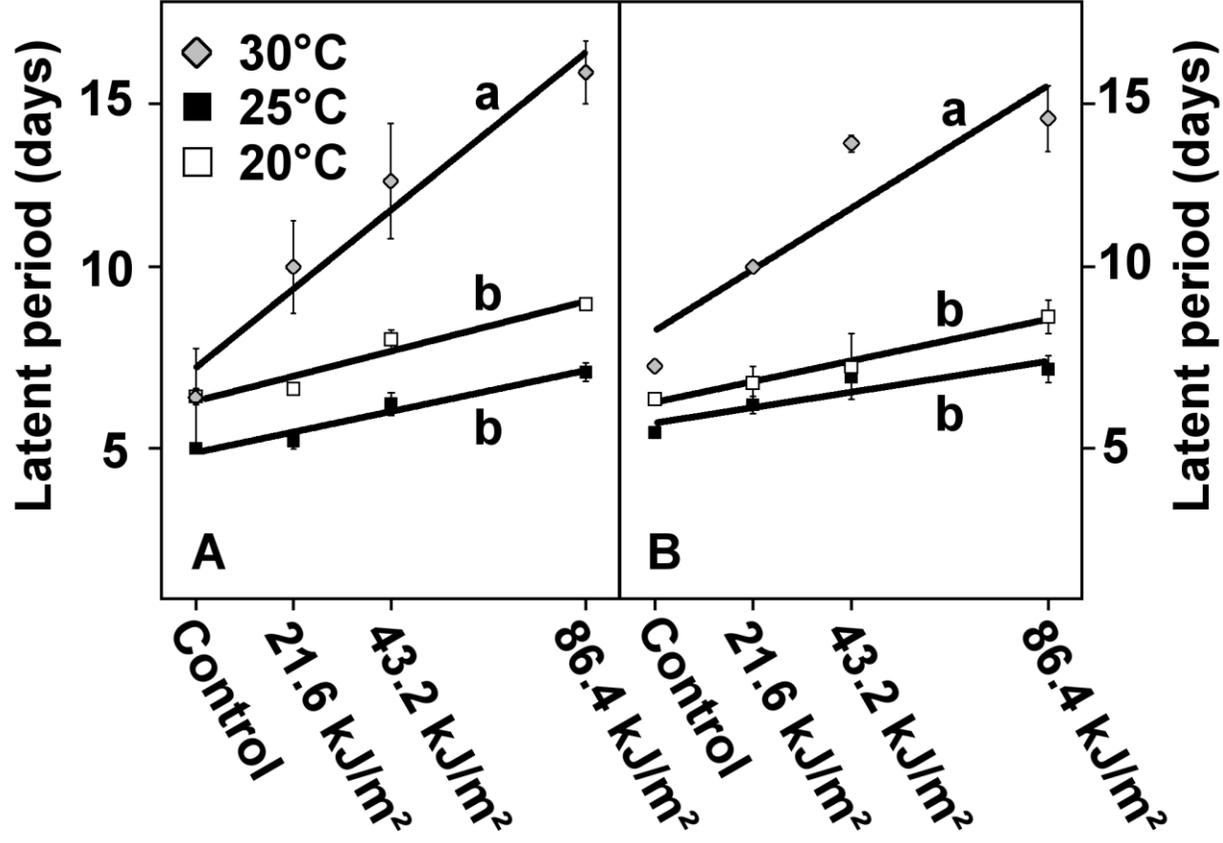


Figure 1.5. The latent period of *Erysiphe necator* as a function of temperature and UV-B exposure, under controlled conditions. Conidia on excised 'Chardonnay' leaves were exposed to four different doses of UV-B radiation at 20°C, 25°C, or 30°C, under acute and chronic exposure regimes. (A) Conidia and leaves received acute exposures of UV-B, supplied at an intensity of 3.0 W/m<sup>2</sup>, for 0, 6, 12, or 24 h, immediately after inoculation, for a total UV-B dose of 0, 21.6, 43.2 or 86.4 kJ/m<sup>2</sup>, respectively. (B) Conidia and leaves received 6-h exposures to UV-B supplied at this same intensity for each of 0, 1, 2, or 4 consecutive days beginning immediately after inoculation, providing the same respective cumulative doses as in the acute treatments. Each of three replicate leaves for all temperature x UV-B combinations was monitored conidiation from the developing powdery mildew colonies starting 4 days post-inoculation. Acute and chronic experiments were repeated three times for each temperature and data was pooled for analysis. Within panels A and B, lines not labeled with a common letter have significantly different slopes ( $P < 0.001$ ). Error bars represent one standard error of the mean.



regimes. Similarly, the same dosage increased latent periods by approximately 1 to 2 days at the suboptimal temperature of 20°C, under both regimes. In contrast, the latent period more than doubled following exposure to this highest UV-B dosage at 30°C, from 7 days with no UV-B exposure to 16 and 15 days following acute and chronic exposures, respectively. A highly significant interaction ( $P < 0.0001$ ) between temperature and UV-B exposure level was found for both the acute and chronic exposure regimes (Table 4). Comparison of regression line slopes for each temperature indicated a significantly ( $P < 0.001$ ) different rate of increase in latent period in response to UV-B radiation at 30°C versus both 20 and 25°C, for both acute and chronic exposure.

## DISCUSSION

Our results conclusively demonstrate that sunlight exposure of grapevine tissue significantly inhibits growth of *Erysiphe necator*, the grapevine powdery mildew pathogen, and development of the disease that it causes. By quantifying the effect of variable levels of natural shade in the Dresden vineyard, and through the use of various artificial shading materials to isolate two specific components of sunlight (i.e., UV radiation and the heating of exposed tissues by longer wavelengths), we observed a consistent increase in disease severity associated with increased levels of shade and showed that both components can be responsible. Although the association between light exposure and disease development has been noted in previous studies (Chellemi and Marois 1992, Zahavi *et al.* 2001,

Willoquet *et al.* 1996), our results are the first to demonstrate the quantitative relationship between disease severity and various forms and levels of shading across multiple vineyards. Furthermore, by using controlled environment experiments we were able to establish the role of UV-B in reducing disease development through its negative effects on various initial stages of pathogenesis and its interaction with elevated temperatures.

Reductions in UV radiation on leaves by natural shading or on fruit via shade cloth or UV-filtering Plexiglas® increased powdery mildew severity in each experiment, sometimes by one or more orders of magnitude. UV-B radiation has been shown to alter fungal populations on plant leaves (Moody *et al.* 2001) and be detrimental to fungal survival (Bjorn 2007, Isard *et al.* 2006, Arabi and Jawhar 2003, Ulevicius *et al.* 2004, Rotem *et al.* 1985). Powdery mildew fungi appear to be particularly vulnerable to UV radiation, due to their generally superficial growth habit and lack of pigmentation. The specific host/pathogen interactions reviewed by (Roberts and Paul 2006, Manning and von Tiedermann 1995) indicate that UV-B exposure after inoculation can often hinder infection, and consistent with our findings, Willocquet *et al.* (1996) demonstrated reduced conidial germination and weaker mycelial growth thereafter when grapevine leaf discs inoculated with *E. necator* were exposed to UV-B radiation.

In our study, differential disease severities associated with varying UV-B doses, observed in the vineyard 2 weeks (leaves) to 2 months (clusters) after inoculation, were similarly observed under

controlled environment conditions within 48 to 96 h of UV-B exposures that immediately followed inoculation. Increasing exposure of conidia to UV-B radiation (acute and chronic) reduced both germination frequency and the proportion of germinated spores that established infection and produced a hypha. Relative to the optimum growing temperature of 25°C, the negative effect of acute UV-B exposure on the proportion of spores able to establish infections and produce hyphae appeared to be somewhat buffered at the lower suboptimal temperature of 20°C. Conversely, the higher suboptimal temperature of 30°C exacerbated the damaging effect of UV-B with respect to conidial ability to produce hyphae 96 h after inoculation. The impact of increased acute doses of UV-B radiation was greater on initial conidial development than the same doses applied chronically. Indeed, for the chronic exposure treatments, which for the higher UV-B doses are more representative of exposure patterns likely to occur in the field, UV-B effects on germination and appressorium formation were inconsistent and insignificant ( $P = 0.81$  to  $0.91$ ), but were highly significant ( $P = 0.001$  to  $0.0001$ ) for measures of subsequent colony development after infections had been established (i.e., hyphal length and latent period). Perhaps most interesting, we found that temperature and UV-B exposure were highly interactive on both the early stages of colony development (hyphal formation and elongation) and their maturity (latent period duration). However, there was no significant interaction on conidial germination or appressorium formation. The synergistic effects of UV-B and high temperatures in limiting disease development are of particular practical importance

since in the field, those tissues receiving the most UV-B exposure from direct sunlight are also those most likely to have their temperatures elevated into a range where this interaction will occur.

The concept that surface temperature elevated by sunlight exposure can play a role in disease development appears to be novel. Nevertheless, sunlight exposure increasing surface temperature on grapes (Kliewer *et al.* 1968, Smart and Sinclair 1976, Bergqvist *et al.* 2001, Millar 1972, Downey *et al.* 2006) and other fruit (Chen *et al.* 2009, Ferguson *et al.* 1998, Schroeder 1965), and the effects this has on berry physiology have been documented extensively. From our own measurements, supported by the aforementioned literature, it was evident that leaves and fruit in the sun were often at temperatures above optimum for *E. necator* development and in some cases approached or exceeded those detrimental to the fungus, while the temperatures of leaves and fruit in the shade remained closer to ambient and in a range ideal for *E. necator* growth. Fruit disease severity in our cv. Chancellor vineyard in 2006, where vines beneath shade cloth were more severely diseased than those beneath UV filters that admitted longer, heating wavelengths, indicated the significant impact surface temperature can play on powdery mildew development. This effect was not observed on cluster infections in all seasons in our trials; however, infections of leaves (which are often better exposed to the sun than fruit), or on vines in regions where ambient temperatures are regularly near the upper threshold for powdery mildew development, may experience a more consistent effect of elevated

surface temperatures inhibiting the disease. It should be noted that Delp's (1954) seminal work on powdery mildew responses to temperature was conducted in controlled environment chambers in the absence of sunlight, and thus did not distinguish the potential differences between ambient and host surface temperatures, nor the interactive effects between higher temperatures and UV radiation likely to occur under field conditions.

Previous studies on grapevine powdery mildew have used thermocouples to measure surface temperatures and have reported variable responses in temperature associated with sunlight exposure (Willoquet *et al.* 1996, Keller *et al.* 2003, Jones 1999); indeed, Tarnopolsky and Seginer (1998) discussed errors inherent with using thermocouples to estimate leaf temperatures. In unreported preliminary vineyard experiments utilizing thermocouples, we recorded no difference in surface temperatures between shaded and exposed leaves. However, perceptible differences could be detected simply by touching these same leaves with one's hands. We then investigated measuring surface temperatures with a non-contact infrared (IR) radiometer, an instrument that has effectively measured surface temperatures of fruit (Helyes *et al.* 2007) and grapevine leaves (Loveys 2005). IR thermometry indicated that exposed leaves were on average 4-8°C higher than shaded leaves.

In an obligate parasite-host interaction such as *E. necator-Vitis* sp., separating the effects of environmental variables such as temperature and UV-B exposure on one participant from those on the

other is difficult. Keller *et al.* (2001) provided data suggesting that UV-B exposure can affect the grapevine's susceptibility to infection by this pathogen. In the literature, pre-exposure of host plants to UV-B radiation has had variable effects on disease development, with severity increasing in some systems and decreasing in others (Roberts and Paul 2006, Manning and von Tiedermann 1995). Jordan (2002) has reviewed how UV-B can alter reactive oxygen species generation, signal transduction, and changes in gene expression, including pathogenicity related (PR) genes in grapes. Nevertheless, due to the well-documented negative effects of UV radiation on general cellular structure, and the vulnerability of *E. necator* discussed above, there seem good reasons to believe that UV-B will negatively affect this pathogen directly, regardless of any host influence that might be involved as well.

Management of powdery mildew is an essential component of any vineyard program. All commercial grape varieties are susceptible to this disease to some degree, in particular those of high value *Vitis vinifera* cultivars. Our results suggest that reducing internal shading and promoting optimal sunlight exposure into the center of vines is an important component of an integrated-crop-management approach to contend with the disease. Indeed, viticultural practices promoting this occurrence have been shown to limit disease development under field conditions (Austin *et al.* 2009). Powdery mildew growth models have been developed (Calonnec *et al.* 2008, Sall 1979, Gadoury *et al.* 1995) as have forecasting models (Gubler *et al.* 1999, Kast 1997) that help

growers assess disease risk and guide them with respect to fungicide application timing. Understanding the similarities and differences in powdery mildew epidemiology within different viticultural climates could be important to extending the applicability of such management guides.

Existing models are heavily reliant upon measurement of ambient air temperatures; however, our results show that not only is the relationship between air temperature and host tissue temperature affected by its sunlight exposure status, but that the quantitative effect of temperature on pathogen development is influenced, sometimes pronouncedly, by its degree of UV-B exposure. Thus, seasons or microclimatic situations in which UV-B exposure levels are particularly deviant from the mean in a particular region may be more or less favorable for disease development than would otherwise be predicted. This is of particular importance when interpreting existing models or devising new ones. Our study was limited to the *E. necator*/grapevine pathosystem, but the general biological principles involved suggest that the importance of UV-B exposure, surface temperature, and their interaction on pathogen development may also have applicability to others, especially those of various unpigmented powdery mildews whose thallus growth is predominantly on its host surface.

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## CHAPTER TWO

### QUANTIFICATION OF POWDERY MILDLEW SEVERITY AS A FUNCTION OF CANOPY VARIABILITY AND ASSOCIATED IMPACTS ON SUNLIGHT PENETRATION AND SPRAY COVERAGE WITHIN THE FRUIT ZONE

#### ABSTRACT

The quantification of grapevine canopy variability, and the associated effect on sunlight distribution in to the fruit zone, was correlated with the spatial distribution of powdery mildew, caused by the fungus *Erysiphe necator*, severity within a vineyard. Using the enhanced point quadrat analysis technique (EPQA), the number of canopy shading layers and the photon flux within the fruitzone of individual vines were shown to have a significant correlation with mean fruit disease severities for those vines, i.e., less disease developed on clusters with more exposure to sunlight. When all clusters were categorized as heavily shaded ( $\leq 10\%$  photosynthetic photon flux), moderately exposed, or well exposed ( $\geq 51\%$  photosynthetic photon flux), vines with the least disease were also shown to have a significantly greater proportion of clusters in the well-exposed category relative to vines with the highest PM ratings; similarly, these latter vines had significantly more heavily-shaded clusters. The correlation remained strong and the relationship linear even with biweekly applications of 2 or 9kg/ha of sulfur during the growing season. Additionally, through use of a fluorescent tracer and EPQA assessments, deposition of spray materials upon clusters was

shown to be linearly related to their degree of exposure. Thus, canopy management practices designed to optimize sunlight exposure of grape clusters for fruit quality purposes should significantly assist in the management of powdery mildew as well.

## INTRODUCTION

Many of the complex and interacting chemical and physiological processes in grapevines are affected by sunlight. Although numerous studies have examined the effects of sunlight on various vine functions, they have focused almost exclusively on basic components of physiology and/or issues pertaining to crop yield and quality. Interactions with vine-associated microbes, including those that are known pathogens of this host, have received relatively little attention despite their economic importance.

The ultraviolet (UV) component of solar radiation, in particular the UV-B spectrum (wavelength = 290 to 320 nm), has been studied for its many biological effects on a wide range of living organisms. Furthermore, much of the recent literature has stressed the need for increased study of the effects of UV-B radiation in agricultural systems, due to current decreases in its stratospheric filtering (Björn 2007, Manning and Tiedemann 1995, Paul 2000, Keller 2010). UV-B has been shown to alter fungal populations on plant leaves (Moody *et al.* 2001); alter grapevine physiology (Kolb *et al.* 2001, Keller and Torres-Martinez 2004); and generally increase fungal mortality (Bjorn 2007, Isard *et al.* 2006, Arabi and Jawhar 2003, Ulevicius *et al.* 2004,

Rotem *et al.* 1985). Although the response to UV-B radiation is specific for individual organisms and their interactions (Roberts and Paul 2006), the vast majority of studies involving plant pathogens have shown a negative effect of UV-B exposure on their growth.

An additional factor associated with sunlight that can alter microclimates relevant to plant pathogen-host interactions is an increase in the surface temperature of exposed tissues. Sunlight exposure increasing surface temperature--often in the range of 5-15°C above air temperature--has been widely documented on grapes (Kliewer *et al.* 1968, Smart and Sinclair 1976, Bergqvist *et al.* 2001, Millar 1972, Downey *et al.* 2006) and other fruit crops (Chen *et al.* 2009, Ferguson *et al.* 1998, Schroeder 1965). These studies, however, have focused on the physiological effects on the fruit, not the consequence that such temperature elevations may have on microorganisms growing on the sunlight-exposed surfaces.

Powdery mildew, caused by the fungus *Erysiphe necator* (Schw.) Burr., is a disease common in grape growing regions worldwide. Virtually all cultivars of *Vitis vinifera*, a species of European origin that evolved in isolation of this native North American pathogen, are highly susceptible to it, and left uncontrolled the disease can destroy infected leaves and fruit. Although *E. necator* grows well at temperatures as high as 28°C, it ceases growth at 32°C and can begin to die above 35°C, depending on the duration of exposure (Delp 1954). Fungal development, which occurs primarily on the surface of infected tissues, is also hindered by exposure to UV-B radiation (Chapter 1).

Several studies have shown that sunlight exposure can impact the development of grapevine powdery mildew. Chellemi and Marois (1992) reported that basal leaf removal around fruit clusters, intended to benefit fruit quality, resulted in reduced powdery mildew severity on these berries. They hypothesized that this effect was due to improved pesticide coverage and inferred that leaf removal produced a berry microclimate less conducive for disease development. Calonnec et al (2009) found that the spatial spread of powdery mildew was strongly related to the leaf density of individual vines, and hypothesized that this was due to quantitative differences in susceptible tissue and secondary inoculum production among vines of different vigor. Zahavi et al (2001) demonstrated that a training system and row spacing that improved sunlight exposure on clusters was associated with reduced powdery mildew severity on fruit, and concluded that this disease reduction was a direct effect of increased light intensity. Keller et al. (2003) showed that vines grown under sunlight from which UV radiation was filtered had significantly more PM than those exposed to unfiltered sunlight, which they attributed to differences in vine disease susceptibility.

In Chapter 1, it was established that differences in sunlight exposure among adjacent vines can be responsible for pronounced differences in powdery mildew development upon them, and that this was due to both the individual and synergistic effects of UV-B radiation on the fungus and elevated temperatures of the exposed grapevine tissues. Our objective for this present study was to

determine to what extent such effects might be operative in vineyard systems and climates as varied as those in the northeastern and northwestern regions of the United States and two viticultural districts of South Australia, and how these effects might relate to fungicide management practices.

## MATERIALS AND METHODS

*Canopy and disease assessments.* In all vineyards, canopy assessment was performed according to an enhanced version of the Point Quadrat Analysis (PQA) technique developed by Smart and Robinson (1991), as recently proposed by Meyers and Vanden Heuvel (2008). This enhanced point quadrat analysis (EPQA) system provides improved acuity of spatial estimates of sunlight distribution on fruit. We particularly focused on two metrics calculated with EPQA, i.e., (i) cluster exposure layer (CEL), defined as the number of shading layers between clusters and the nearest canopy boundary; and (ii) cluster exposure flux availability (CEFA), defined as the proportion of above-canopy photosynthetic photon flux (PPF) that reaches clusters.

To calculate CEL and CEFA, PQA measurements were collected using a thin stiff rod inserted into the fruiting zone of the canopy parallel to the ground and perpendicular to the row, every 20 cm along the length of a vine. As the rod was being inserted, each leaf or cluster contact with the leading tip of the rod was noted. Sunlight measurements were taken with an AccuPAR LP-80 Ceptometer (Decagon Devices, Pullman, WA) for each vine between 10 am and 2

pm on the same day of PQA assessment. The ceptometer incorporated simultaneous handheld measurements within the vine fruitzone parallel to the row using the ceptometer probe (90cm long with 80 photosensors), as well as above the canopy using a PAR sensor connected to the integrated controller. For each vine, 10 measurements were made over ~10 s and mean values for above- and within-canopy photosynthetic photon flux (PPF) were recorded. PQA measurements and the sunlight readings taken above and within the canopy were then used to calculate CEL and CEFA values via EPQA as described by Meyers and Vanden Heuvel (2008). Unless otherwise noted, CEL and CEFA values are reported for individual vines from the relevant measurements made on them, and analyzed with respect to disease severity ratings made from the same individual vine.

Disease severity was assessed in each vineyard by visually rating individual clusters on a 0-100% scale, based on the proportion of cluster tissue covered with the pathogen. Unless otherwise noted, disease severities reported are vine averages calculated from 10 clusters per vine arbitrarily selected to provide a representative sample of all clusters, with five clusters from each side of the vine.

*Individual vineyards. Prosser, WA (Yakima Valley).* Trials were conducted during the 2008 and 2009 seasons at the Irrigated Agriculture Research and Experiment Center in an own-rooted *V. vinifera* 'Chardonnay' vineyard, within which vine size was highly variable. Twelve rows of 30 vines (five, six-vine panels) each, trained to a midwire cordon with a single catch wire, were selected and

natural powdery mildew infections were allowed to occur. The 12 rows were grouped into four replicate blocks of three adjacent rows each, and each row was randomly assigned a fungicide application regime at the beginning of each season. Fungicide treatments consisted of sulfur (Microthiol Disperss; 80% active ingredient) applied biweekly with a hooded boom sprayer at a rate of (i) 2.24 kg/ha; (ii) 8.96 kg/ha; or (iii) none. On 4 to 8 August 2009, EPQA measurements and disease assessments for 10 clusters were conducted on the second and fifth vine in each of the five panels per row, as described above. On 10 to 13 September 2008, the same techniques were used, but canopy structure was assessed on four vines in each of five panels per row and disease severity was assessed on 10 arbitrarily-chosen clusters across the same four vines. Data are reported as mean values from each of the 15 panels per treatment thus assessed. Data were analyzed by regression analysis using GLM/ANOVA. Comparison of means for the effect of sulfur in WA was made using the Tukey-Kramer HSD test. These and all other statistical analyses were made using JMP statistical software (SAS Institute, Cary, NC).

*Dresden, NY (Finger Lakes region).* This experiment was conducted in 2008 in a commercial planting of Chardonnay vines on C3309 rootstock, trained to a modified Umbrella-Kniffen system with 3-m row spacing and 2-m vine spacing. Five clusters on 22 vines were arbitrarily selected to provide a representative sample of all clusters and inoculated with a suspension of *E. necator* conidia at 75% capfall; conidia were produced on leaves of potted Chardonnay vines in a

screenhouse, suspended in 0.005% Tween at a concentration of  $10^5$ /ml, and applied with a portable paint sprayer (Chapter 1). Fungal diseases on these vines were managed with minimal sprays of mancozeb and a highly refined petroleum oil (JMS Stylet Oil), chosen for their relative lack of activity against *E. necator* and minimal residual or vapor activity, respectively; inoculated clusters were protected within individual plastic bags during each spray application and the bags were removed immediately after sprays had dried. On 18 August 2008, canopy structure on all 22 vines was assessed via EPQA and all inoculated clusters were evaluated for PM severity, as described above.

*Geneva, NY.* This experiment was conducted on 5-year-old Chardonnay vines on C3309 rootstock trained to a vertical shoot positioning system with 3-m row spacing and 2-m vine spacing. In the early spring of 2009, nine panels of four vines each were selected and cane pruned to leave 20, 40, or 60 buds per vine, to induce canopy variability among these treatments. Each plot consisted of a single panel and treatments were arranged in a randomized complete block design, with three replications. During the subsequent growing season natural PM infection was allowed to occur; vines were sprayed with mefanoxam and mancozeb to limit other fungal diseases, but no chemicals active against *E. necator* were applied. Canopy structure of all vines and disease severity of 10 arbitrarily-chosen clusters per vine were assessed on 9 July 2009, due to rapid degradation of the most heavily-diseased berries and the emergence of botrytis bunch rot.

Data for individual variables are reported as mean values from each of the nine individual panels examined. Data from the Geneva and Dresden experiments were analyzed with simple linear regression/ANOVA.

*Nurioopta, South Australia (Barossa Valley).* This experiment utilized two, 72-vine rows in an own-rooted cv. Chardonnay vineyard planted in 1994 with 3.5-m row spacing and 2.25-m vine spacing. Vines were grown on a three-wire single curtain, non-shoot positioned trellis; pruned to two-bud spurs (~40 buds per vine); and left unsprayed during the 2008/2009 growing season, allowing natural infection to occur. Canopy structure and cluster disease severity were assessed for alternating vines in each row on 10 February 2009, as described above.

*Loxton, South Australia (Riverland).* This 2008/2009 trial was conducted in a vineyard consisting of two rows of 12 mature Chardonnay vines each, with divided canopies. Vines in the first row had parallel bilateral cordons stacked with ~0.5m spacing between cordons, vertically splitting the canopy. Vines in the second row had parallel bilateral cordons ~1m apart, horizontally dividing the canopy. All vines were unsprayed and natural infection was allowed to occur. Canopy structure and disease severity were assessed on 13 February 2009 for every vine in both rows, as described above.

*Weather data:* Weather data for the Prosser, WA site were obtained from AgWeatherNet (The Washington Agricultural Weather Network

V2.0, WSU Prosser, A12 station. <http://weather.wsu.edu>) located ~50 m from the vineyard. Data for the New York vineyards were obtained from the New York State Agriculture Experiment Station's weather station located in Geneva NY, ~15km north of the Dresden vineyard and ~1km west of the Geneva test site. Data for the Barossa Valley, South Australia were obtained from the Nuriootpa Research Center's weather station located ~100m from the vineyard site. Data for the Riverland site were not available. Seasonal weather was defined as the time between 1 April and 31 October for northern hemisphere vineyards, and 1 September to 31 March for Australia. Growing degree days (GDD) were calculated using daily mean temperatures and a base of 10°C. Total solar radiation for the growing season was calculated by summing total photosynthetically active radiation (PAR) values for each day. Total precipitation for the growing season was calculated by summing the daily precipitation measures (Table 2.1).

*Spray coverage efficiency:* Four weeks after bloom in the Geneva Chardonnay vineyard, a highly soluble fluorescent dye (Pyranine 10G, Keystone Aniline Corp., Chicago, IL) was applied at a concentration of 500 mg/L in 468 L/ha of water, using a DP300 FMC airblast sprayer with a modified tower fitted with hollow cone nozzles (TEEJET #TX8004VK); the sprayer operated at a pressure of 70 kPa and traveled at a speed of 5 kph. After the application had dried, one arbitrarily-chosen cluster from each of the four vines per panel was harvested, placed into a plastic bag, and transported in a cooler to the lab for residue analysis. In the lab, dye was washed from individual

clusters in a standard volume of deionized water in a graduated cylinder, and the volume of the cluster was determined based upon the displaced volume of water. These washings were analyzed in a Hidex Chameleon multicell plate reader set to read fluorescence with a 360nm excitation filter and a 515nm emissions filter. The amount of fluorescent dye residue was then standardized for each bunch based on its total volume, and a mean value for the four clusters from each panel was calculated. One week after dye application, canopy structure was assessed via EPQA as described above. Spray coverage data were analyzed with simple linear regression/ANOVA.

*Cluster Exposure Categorical Relationships.* Within the EPQA model, CEFA for every cluster contacted during the PQA assessment is calculated, and as a result every cluster contacted in the vineyard is provided a model-calculated photosynthetic photon flux (%PPF, relative to sunlight availability immediately outside the canopy), i.e., CEFA x 100. This feature was then used to further analyze the relationship between sunlight exposure and powdery mildew development on clusters. First, disease rating data for each vineyard or fungicide-treatment in the case of the WA site (i.e., individual vine or panel averages, as described above) were ranked and separated into terciles, with the one-third of vines (or panels) with the lowest disease levels assigned to the first tercile and the one-third with the highest levels assigned to the third tercile (Table 2.2). Then, the CEFA value for each cluster contacted during the PQA assessment of these same individual vines (or panels) was used to categorize them as (i) heavily

Table 2.1. Weather data for the vineyard locations					
	WA - 2008	WA - 2009	NY - 2008	NY - 2009	Barossa Valley, SA
Seasonal degree days (Base 10°C)	1364	1442	1332	1245	1697
Seasonal precipitation (cm)	4.50	4.62	61.06	55.98	15.21
Total Seasonal Photosynthetically Active Radiation (MJ/m <sup>2</sup> )	4542	4824	3432	3382	4697

shaded (0 to 10% PPF); (ii) moderately shaded/exposed (11 to 50% PPF); or (iii) well exposed (51 to 100% PPF). Finally, the proportion of that unit's clusters in each of the three shading categories was determined and averaged for all units in the first and third terciles. The means of these proportions within each shading category were compared between units in the first versus third tercile using Student's t-test.

## RESULTS

*Washington State:* Increased levels of sunlight exposure linearly reduced disease severity when no sulfur was applied (Figure 2.1). This was particularly so in 2008, when both CEL and CEFA values provided similar strengths of association with measured disease severity (Table 2.3). However, comparisons of the predominant CEL values in 2008 versus 2009--ranging from approximately 0.4 to 1.6 versus 1.0 to 3.0, respectively (Figure 2.1, x-axes)--indicated that canopies were generally denser in the second year. Similarly, comparisons of CEFA values between these two years, with predominant ranges of 0.1 to 0.4 versus 0.05 to 0.2, respectively, indicated that only about one-half as much available sunlight was reaching the clusters in 2009 as in the previous year, with a narrow range of CEFA values across all vines (Figure 2.1). As a result, the relationship between disease severity and CEFA values was weak in 2009, whereas it remained strong for CEL values, which were distributed across a wider range. Furthermore, the greater potential availability of sunlight within canopies in 2008 was associated with a stronger response to incremental increases in

exposure, as indicated by the relative slopes of the regression lines when data sets for a given spray treatment and EPQA measure were compared between the two years (Table 2.3). Biweekly applications of 2kg/ha of sulfur reduced disease severity by approximately two-thirds relative to the unsprayed vines in both seasons (Table 4). Even at this relatively low rate, the strength of the relationship between sunlight exposure and PM severity on fruit was weakened by the effects of sulfur, as indicated by the lower  $R^2$  values compared to those for the unsprayed treatment, as was the impact of incremental changes in exposure, as indicated by differences in the regression line slopes (Table 2.3). Nevertheless, a strong linear relationship remained between disease severity and sunlight exposure in 2008, as reflected by both CEL and CEFA values (Figure 2.1, Table 2.3). However, when canopies were denser and sunlight penetration reduced in 2009, there was no association between CEFA values and disease severity for vines treated with this low sulfur rate and--in stark contrast with the unsprayed vines--the association between disease severity and CEL values was very weak, suggesting that the effect of the sulfur applications outweighed that of the light exposure. Bi-weekly applications of the much heavier sulfur rate of 9kg/ha reduced overall disease severity by approximately an additional two-thirds relative to the 2-kg treatment (Table 2.4). In 2008, this increase in the sulfur rate further weakened the relationship between disease severity and the impact of incremental changes in sunlight exposure, although this remained significantly linear for both of the EPQA measures ( $P = 0.02$

Table 2.2. Tercile disease ranges and cluster counts used for percent photosynthetic photon flux (% PPF) categorical comparisons at each location <sup>a</sup>										
	WSU 08 – 0kg	WSU 08 – 2kg	WSU 08- 9kg	WSU 09 – 0kg	WSU 09 – 2kg	WSU 09 – 9kg	NY 08	NY 09	Barossa Valley	River- land
1 <sup>st</sup> Tercile Range	13.4 - 43	5.6 - 10.1	0.3 - 3.8	35.8 - 70.3	3.1 - 13.2	0.7 - 4.1	17 - 44	40 - 76.4	0.3 - 2.2	0.6 - 2.9
3 <sup>rd</sup> Tercile Range	77 - 97.4	29.8 - 65.3	10.3 - 18.9	88 - 97.7	29.5 - 62.8	10 - 15.6	81.4 - 92.5	92.6 - 94.4	15.8 – 95.5	21.2 - 88.5
Cluster Count	1302	1294	1143	749	664	804	182	252	1009	305

<sup>a</sup>1<sup>st</sup> tercile range represents the lowest one-third of all mean disease severity ratings for individual vines or panels (10 clusters per unit), whereas the 3<sup>rd</sup> tercile range represents the highest one-third of all mean disease severity ratings. The data show the range of disease severity for the tercile in each experiment. Cluster count for each vineyard represents the number of clusters for which a %PPF was calculated by CEFA in the EPQA model.

Figure 2.1. Powdery mildew severity on 'Chardonnay' clusters in a vineyard near Prosser, WA as a function of [A,B,E,F,I,J] Cluster Exposure Layer (CEL), the number of shade layers between clusters and the nearest canopy boundary; and [C,D,G,H,K,L] Cluster Exposure Flux Availability (CEFA), the proportion of above-canopy photon flux that reaches clusters. Vines were subjected to three sulfur application regimes: (i) unsprayed (A-D); (ii) treated biweekly with 2 kg/ha (E-H); or (iii) treated biweekly with 9kg/ha (I-L). Natural infection was allowed to occur and CEL, CEFA and disease severity values were assessed on 10 September 2008 and 4 August 2009. Individual data points in 2008 represent the average disease severity for a total of 10 clusters from four vines in a panel. CEL and CEFA values, are expressed as averages calculated from the middle four vines in that same six-vine panel. Individual data points in 2009 represent average disease severity ratings for 10 clusters from a single vine; CEL and CEFA values were also calculated for that single vine.

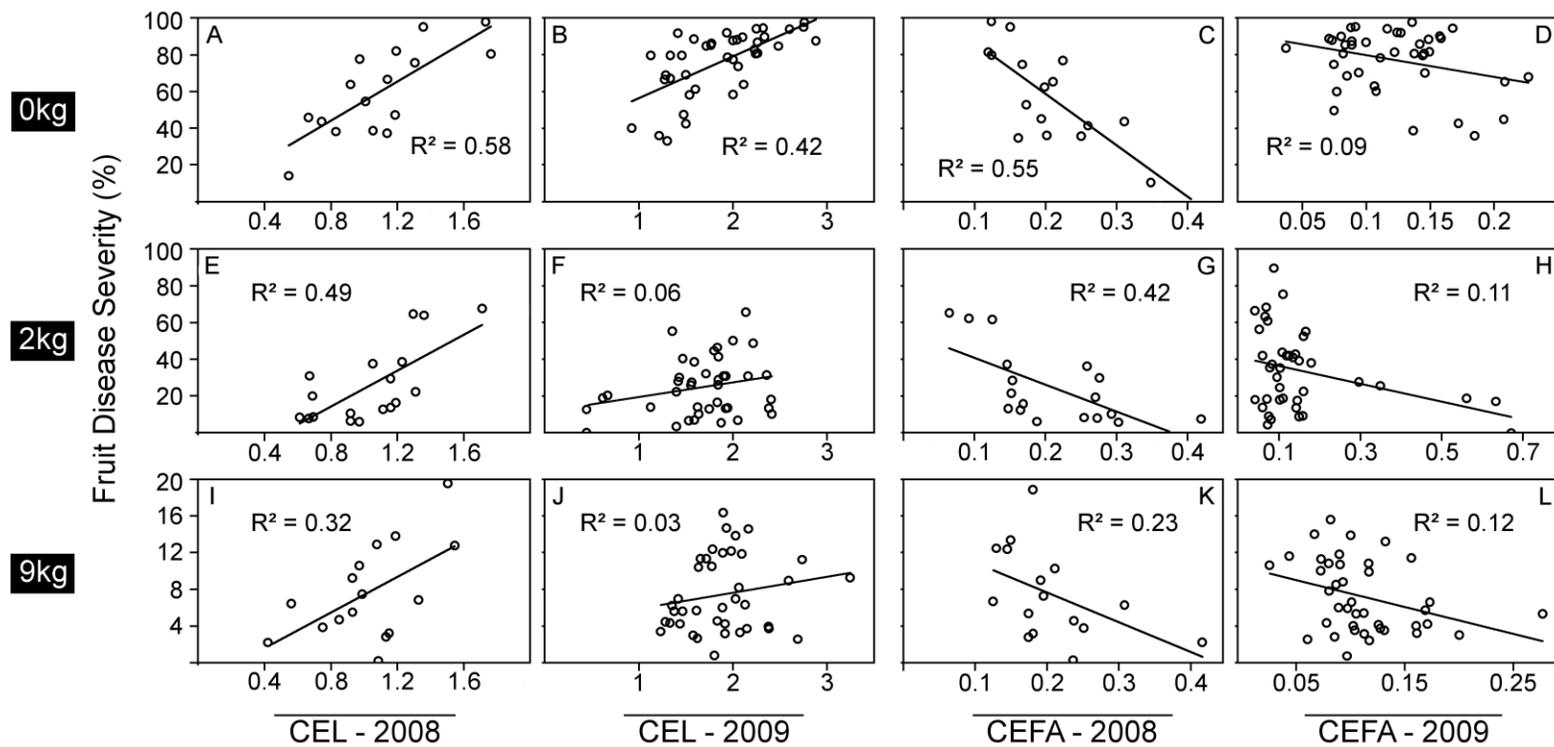


Table 2.3. Statistical parameters for analyses of data from the Prosser, WA vineyard, regressing fruit powdery mildew severity as a function of two different measures of exposure to sunlight

	Measure	Treatment	R <sup>2</sup>	Slope	p
2008	CEL*	0kg	0.58	52.07	0.0006
		2kg	0.49	47.37	0.0012
		9kg	0.32	9.46	0.023
	CEFA**	0kg	0.55	-278.0	0.0009
		2kg	0.42	-148.6	0.0035
		9kg	0.23	-32.68	0.05
2009	CEL*	0kg	0.42	21.84	<0.0001
		2kg	0.03	5.34	0.31
		9kg	0.03	1.70	0.26
	CEFA**	0kg	0.09	-118.4	0.06
		2kg	0.06	-28.45	0.14
		9kg	0.12	-29.37	0.03

Generalized linear model calculations for each WA season and sulfur treatment as function of \*Cluster Exposure Layer (the number of shade layers between clusters and the nearest canopy boundary); and \*\*Cluster Exposure Flux Availability (proportion of above-canopy photon flux that reaches clusters).

and 0.05 for CEL and CEFA, respectively). There was little to no relationship between measures of cluster exposure and disease severity in the denser canopies of 2009 treated with this higher sulfur rate (Figure 2.1, Table 2.3).

*New York:* EPQA measurements in the Dresden vineyard in 2008 were similar to those in the Washington vineyard that year, with CEL values generally below 2 and CEFA values up to ~0.4 (Figure 2.2 A, C). CEL values in the Geneva vineyard in 2009 were similar to those in the Dresden vineyard the previous year; however, CEFA values indicated that a substantially lower proportion of available sunlight was reaching fruit in 2009, with values remaining below 0.25 (Figure 2.1 B, D). For both locations, the relationships between disease severity on clusters and the degree to which they were exposed to sunlight, as defined by CEL and CEFA values, was strongly linear, i.e.  $P = 0.003$  and  $0.0005$ , respectively, at Dresden and  $P = 0.03$  and  $0.004$ , respectively, at Geneva. At both sites, CEFA values provided the stronger association with disease severity (Figure 2.2). The number of buds retained per vine (i.e., 20, 40 or 60) during dormant pruning prior to the 2009 growing season was not a significant factor ( $P = 0.46$ ) in relation to disease severity, as vines with fewer buds compensated with increased growth of the shoots that developed (data not shown). Nevertheless, the strong linear relationship between disease severity and CEFA and CEL values was maintained, regardless of bud number (Figure 2.2).

*Australia:* Measured canopy densities in the Barossa Valley vineyard

Table 2.4. Cluster disease severity on Chardonnay vines receiving variable sulfur application regimes in two seasons in Prosser, WA

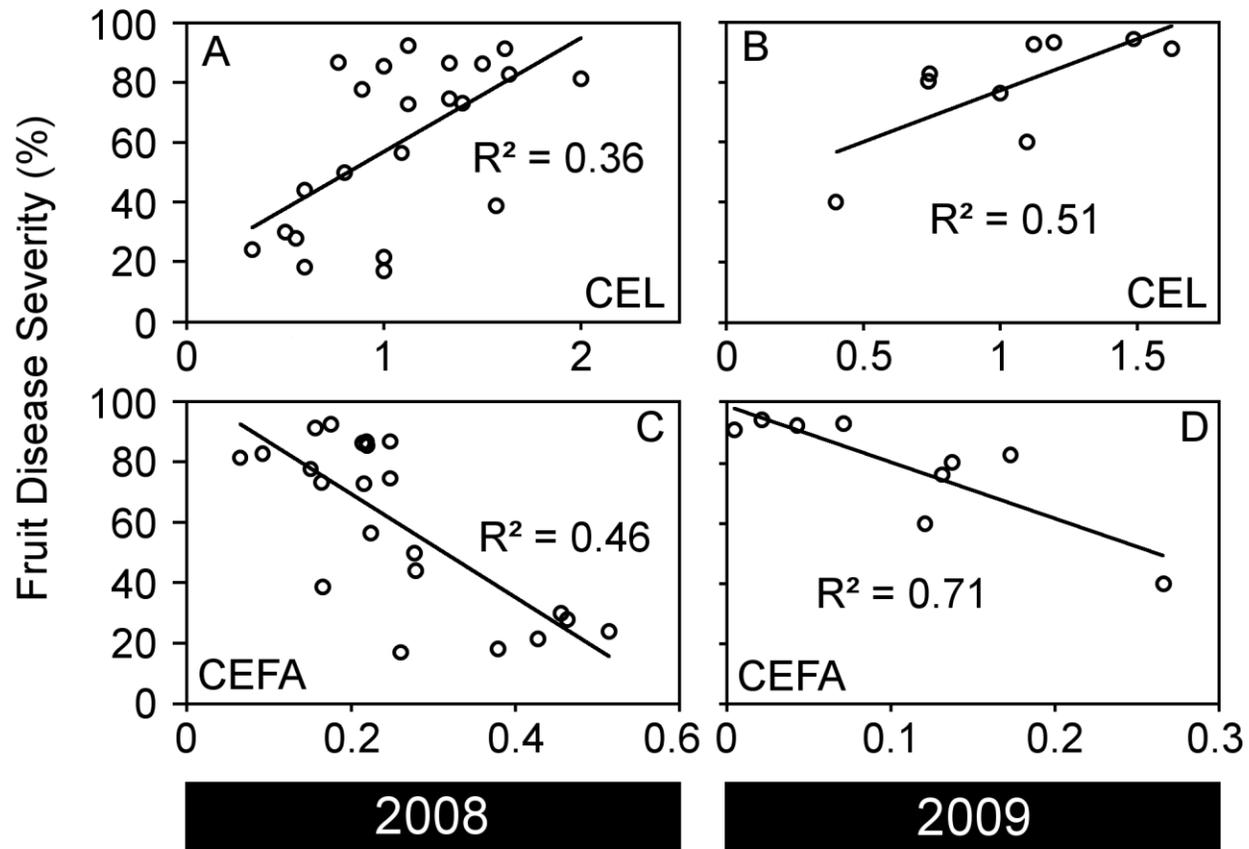
% Cluster area diseased [% control] <sup>a</sup>						
Treatment, rate/ha	2008 <sup>b</sup>			2009 <sup>c</sup>		
None	59.3	a	[0]	77.0	a	[0]
Sulfur, 2kg	20.0	b	[66.2]	24.3	b	[68.4]
Sulfur, 9kg	7.4	c	[87.4]	90.7	c	[90.7]

<sup>a</sup> Means not followed by a common letter are significantly different according to the Tukey-Kramer HSD test ( $P=0.05$ ). Bracketed values denote percent control relative to the untreated check.

<sup>b</sup> Values represent the means from 20 replicate plots per treatment, 10 clusters per plot. Sulfur (Microthiol Disperss) applications: 673L/ha on June 10, June 25, July 9; 841L/ha on July 23, Aug 7, Aug 20, Sept 2.

<sup>c</sup> Values represent the means from 40 replicate vines per treatment, 10 clusters per vine. Application: 580L/ha on May 30; 767 L/ha on June 8; 963 L/ha on June 22; and 1346L/ha on July 6, July 20, Aug 3.

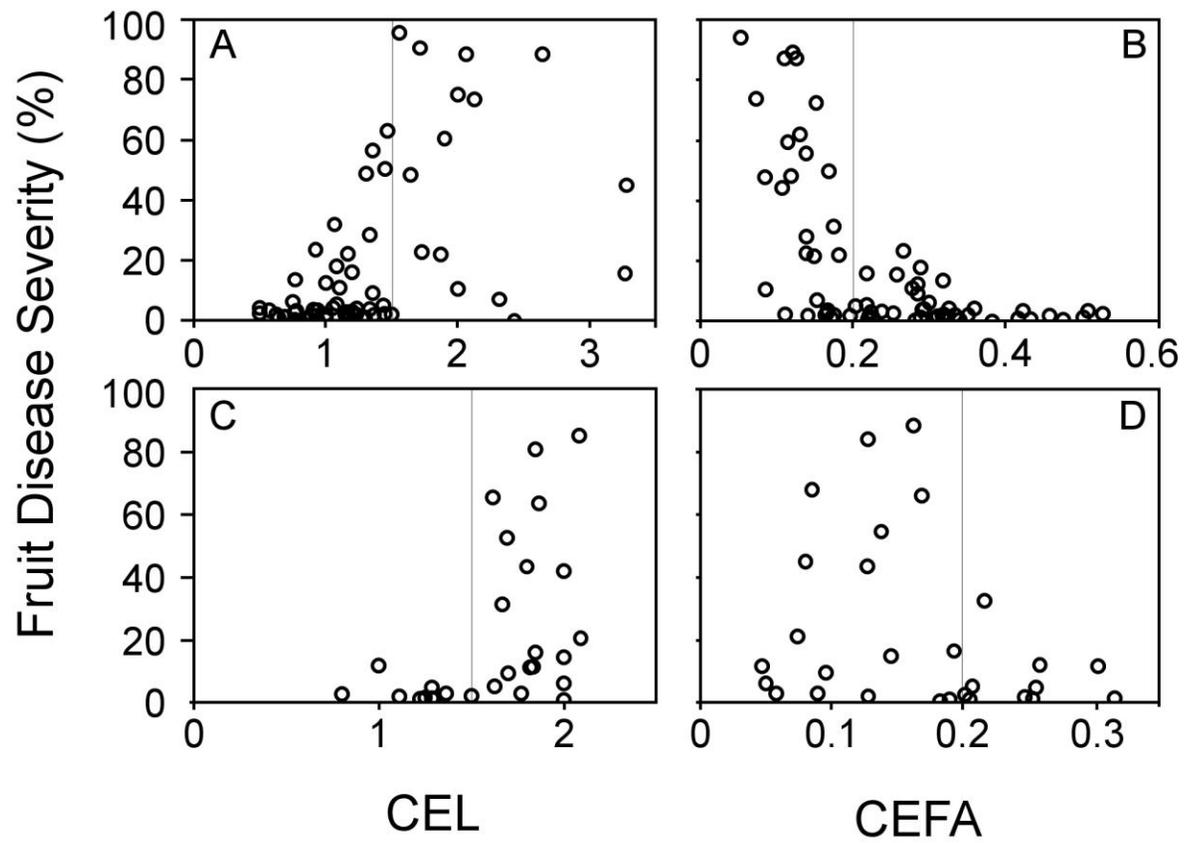
Figure 2.2. Powdery mildew severity in two 'Chardonnay' vineyards in the Finger Lake region of NY as a function of [A,B] Cluster Exposure Layer (CEL), the number of shade layers between clusters and the nearest canopy boundary; and [C,D] Cluster Exposure Flux Availability (CEFA), the proportion of above-canopy photon flux that reaches clusters. Vineyards were located in (A,C) Dresden, NY in 2008 and (B,D) Geneva, NY in 2009. Clusters were inoculated with a suspension of *E. necator* conidia [ $10^5$ /ml] at 75% capfall in 2008, and natural infection was allowed to occur in 2009. CEL, CEFA and disease severity values were assessed on 18 August 2008 and 9 July 2009. Individual data points in 2008 represent the average disease severity of five clusters from single vines, with CEL and CEFA values representing averages calculated for each of the same corresponding vines. Individual data points in 2009 represent the panel average for disease severity assessed on 10 clusters per vine for each of four vines per panel, with corresponding CEL and CEFA values also representing panel averages of four EPQA-assessed vines per panel.



were similar to those in Washington State in 2009, i.e. relatively high maximum CEL values near 3 (Figure 2.3A). However, sunlight exposure on fruit within the canopy also was relatively high, with CEFA values up to ~0.5 (Figure 2.3B). The Chardonnay vineyard in the Riverland had fewer canopy layers than in the Barossa Valley, with maximum CEL values ~2 (Figure 2.3C) although sunlight penetration into the fruitzone was weaker, providing maximum CEFA values of ~0.3 (Figure 2.3D). In both of these vineyards, the distribution of disease severities across CEL and CEFA values was far less even than in their North American counterparts. Therefore, vines were separated into two groups for further statistical comparisons (using Student's t-test), based on visual inspection of the data (Figure 2.3, Table 2.5). In the Barossa Valley, disease severity was significantly lower on vines with a CEL value below 1.5 ( $P < 0.0001$ ) or a CEFA value above 0.2 ( $P < 0.0001$ ) compared to those above and below these respective threshold values (Table 2.5). In the Riverland, the training system did not have a significant effect ( $P = 0.44$ ) on disease severity. Thus, data from these two treatments were pooled, and analysis showed there was significantly less disease for vines with CEL values lower than 1.5 ( $P = 0.004$ ) or CEFA values above 0.2 ( $P = 0.03$ ).

*Categorical relationships.* In the Washington vineyard in 2008, vines in the lowest tercile for cluster disease severity had significantly ( $P = 0.05$ ) more clusters in the well-exposed category (51-100% PPF) than did those in the 3<sup>rd</sup> tercile, for both the unsprayed and 2-kg sulfur treatments. Conversely, the third of the vines with the highest mean

Figure 2.3. Powdery mildew severity in two South Australian 'Chardonnay' vineyards as a function of [A,C] Cluster Exposure Layer (CEL), the number of shade layers between clusters and the nearest canopy boundary; and [B,D] Cluster Exposure Flux Availability (CEFA), the proportion of above-canopy photon flux that reaches clusters. Vineyards were located in the Barossa Valley near Nuriootpa (A,B) and in the Riverland region near Loxton (C,D). Natural infection was allowed to occur and no pesticides were applied in either vineyard. CEL, CEFA and disease severity values were assessed on 10 February 2009 in the Barossa and 13 February 2009 in the Riverland. Individual data points represent mean disease severity ratings for 10 clusters assessed on a single vine, and CEL and CEFA values represent the averages calculated from canopy assessments on the same vine.



disease severities had significantly more heavily-shaded clusters, regardless of fungicide treatment ( $P= 0.04$ ,  $0.02$ , and  $0.02$  for the 0-, 2-, and 9-kg treatments, respectively) (Figs. 4A, 4C, 4E). In the Washington vineyard in 2009, where sunlight penetration was generally lower than 2008, fewer significant differences were observed. However for unsprayed vines, those in the first disease tercile had significantly ( $P= 0.04$ ) more well-exposed clusters than those in the third tercile. Also in 2009, the most-diseased vines in the 9-kg treatment had significantly more moderately- or heavily-shaded clusters than did the least-diseased vines ( $P= 0.05$  and  $0.03$ , respectively). This same pattern continued in all four other vineyards wherein a significantly greater proportion of well-exposed clusters were found on the least-diseased vines and/or a significantly greater proportion of heavily-shaded clusters were found on those third of the vines with the highest disease severity ratings (Fig. 4).

*Spray Coverage:* There was a strong linear relationship between the exposure of clusters to sunlight, particularly as indicated by CEFA values, and the deposition of sprays upon them. Within the range of mean canopy layers in the Geneva Chardonnay vineyard (0 to 2), spray coverage nearly doubled for each shading layer removed. Similarly, spray coverage increased by approximately 50% for each 10% increase in proportional light penetration within the canopy (Fig. 2.5).

## DISCUSSION

Although the impact of sunlight exposure on grape fruit chemistry has

Table 2.5. Categorical comparison of South Australian vineyards for disease severity on clusters separated on the basis of Cluster Exposure Layer (CEL) and Cluster Exposure Flux Availability (CEFA) values.

EQPA value	Barossa Valley			Riverland		
	n	Mean	Std. Error	n	Mean	Std. Error
CEL ≤ 1.5	54	9.3	2.7	10	3.1	7.4
CEL > 1.5	14	53.1	5.3	18	32.4	5.6
CEFA ≤ 0.2	28	37.4	4.0	18	30.0	6.0
CEFA > 0.2	40	5.0	3.6	10	7.4	8.0

Individual vines in each location were separated based on a mean Cluster Exposure Layer threshold value of 1.5 and a mean Cluster Exposure Flux Availability threshold value of 0.2. Data provided refer to the total number of vines in each group and disease severity ratings for them.

been extensively researched (Downey et al 2006, Smart 1985), its impact upon powdery mildew development on berries has received little focused attention. In this study, utilizing a single standard cultivar to eliminate potential genetic sources of variability, we have demonstrated a consistent and quantitative relationship between disease severity and sunlight exposure levels in five different vineyards in regions as diverse as New York, Washington, and South Australia. Furthermore, we have, for the first time that we are aware of,

quantified the heretofore-presumed effect that canopy density exerts on the deposition of spray materials onto developing clusters, and have shown that canopy density can influence powdery mildew development through its interactive effects on both the host-pathogen interaction itself and fungicide coverage of the clusters.

In the relatively cool and humid New York vineyards, there was a strong linear relationship between disease severity on clusters and their degree of exposure to sunlight, as measured both directly (CEFA) and indirectly (CEL). The Washington State vineyard was in a significantly warmer, drier climate that provided substantially more seasonal sunlight than in NY, yet in the absence of sulfur, we observed similar linear relationships between fruit disease severity and sunlight exposure within the fruitzone. Sunlight attenuation within the fruitzone was stronger in 2009 than in 2008, with correspondingly weaker relationships between measures of cluster exposure and disease severity.

In South Australia, where UV radiation levels are particularly high, we also observed a strong impact of sunlight on fruit disease severity; however, these relationships were not linear as in the northern hemisphere vineyards. In the Australian vineyards, little disease developed on vines with a CEFA value above 0.2 or a CEL value below 1.5, whereas all moderately- to heavily-disease clusters occurred on vines with a CEFA value below 0.2 and a CEL value above 1.5. In all vineyards, the third of the vines with the lowest disease ratings consistently had the highest proportion of their

Figure 2.4. Categorical associations of disease severity and shading level for all vineyards. At each location, disease severity ratings for individual units (single vines or panels, depending on the experiment) were ranked, and the first tercile of vines or panels was defined to be those one-third of the total number with the lowest fruit disease severity ratings. Conversely, the one-third of the units with the highest levels of fruit disease severity were grouped into the third tercile. Then, photosynthetic photon flux (PPF) was calculated for every cluster contacted during the PQA assessment of these same units and used to assign them to one of three shading categories, i.e., 0 to 10% PPF; 11 to 50% PPF; or 51 to 100% PPF. Finally, the proportion of that unit's clusters in each of the three shading categories was determined and averaged for all units in the first and third terciles. For each data set, the mean proportions for the three PPF categories were compared between the first and third tercile using Student's t-test ( $P=0.05$ ); columns with a (\*) indicate a significantly greater proportion of clusters in that category relative to its tercile counterpart. Tercile disease ranges and numbers of clusters for which PPF was calculated and categorized are provided for each location in Table 2.

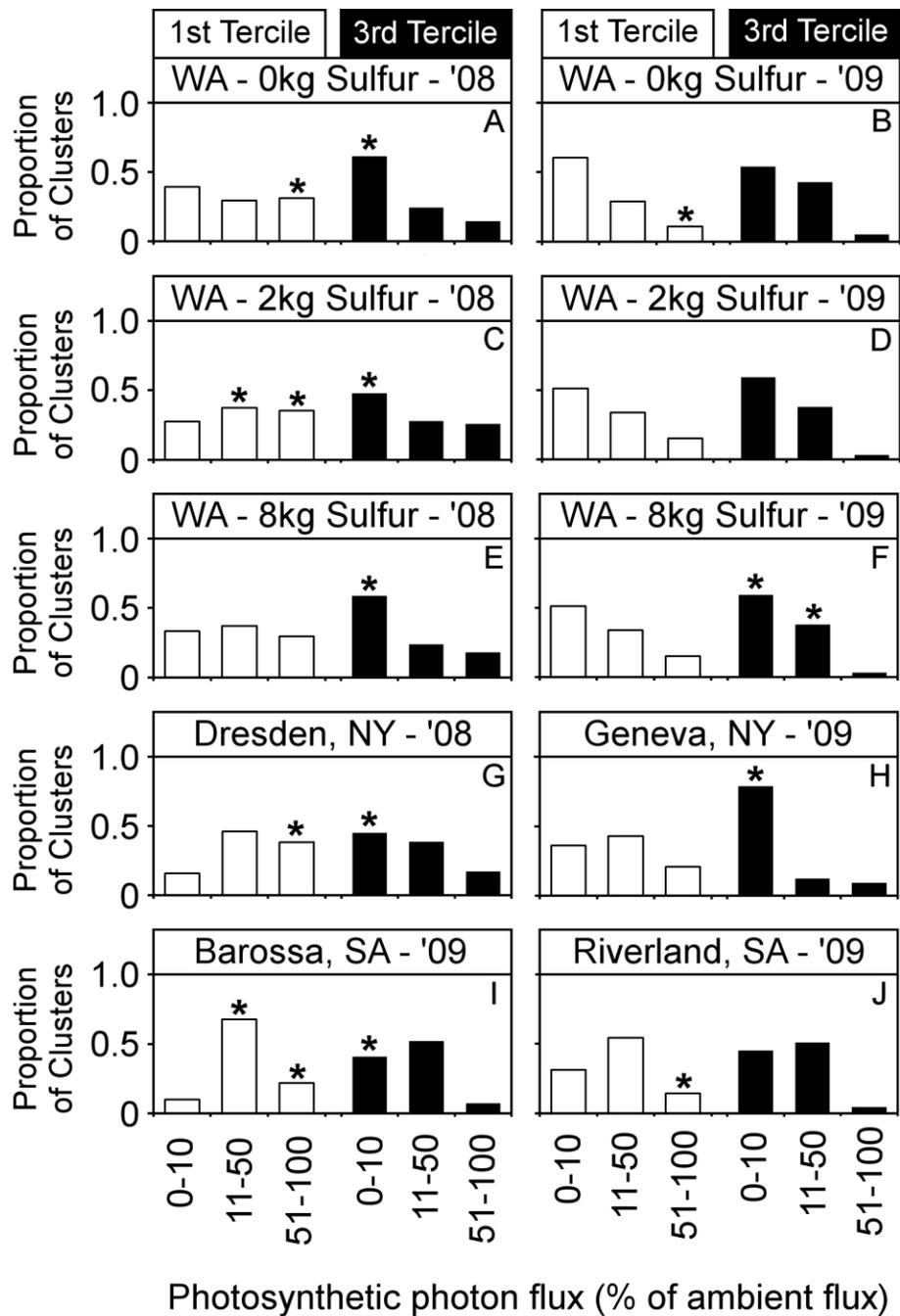
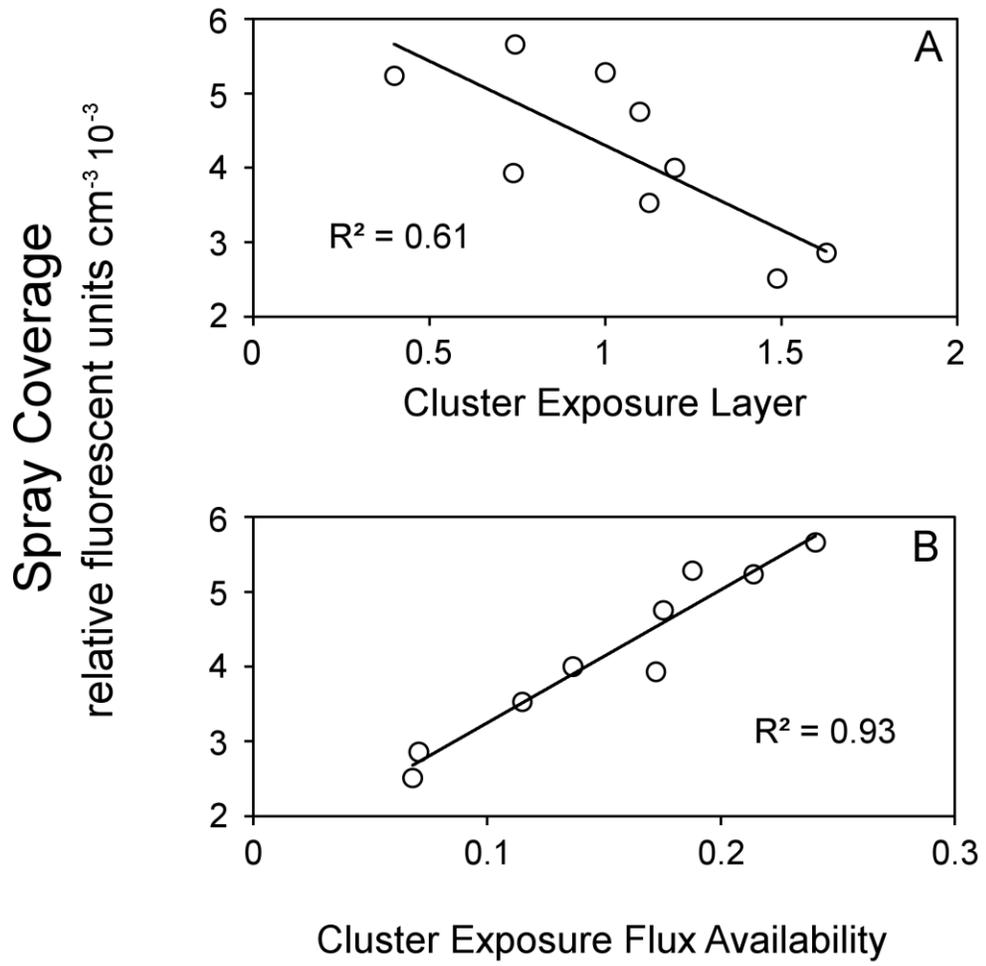


Figure 2.5. Spray coverage as a function of (A) cluster exposure layer and (B) cluster exposure flux availability in a Chardonnay vineyard in Geneva, NY subjected to different canopy management treatments. A solution containing 500mg/L fluorescent dye was applied 2 weeks post-fruit set at a rate of 468 L/ha using a conventional air blast sprayer. Intact clusters were collected immediately after sprays had dried and assessed in the lab for the quantity of dye per unit volume of the bunch. Each vine was assessed one week after dye application via Enhanced Point Quadrat Analysis (EPQA) to establish fruit exposure levels to sunlight. Individual data points represent mean standardized values for spray coverage assessed on four clusters from each of nine individual panels; corresponding CEL and CEFA values represent averages of four EPQA-assessed vines per panel.



clusters classified as well-exposed (51-100% PPF), whereas conversely, the third with the highest disease ratings had the highest proportion of their clusters in the heavily-shaded (<10% PPF) category. This relationship notwithstanding, it must be remembered that levels of cluster exposure ideal for PM control might also result in sunburning of fruit, hence truly optimal exposure levels must integrate various factors to account for specific considerations unique to each region and producer.

In addition to vineyard and canopy management practices that incrementally influence sunlight intensity in the fruit zone, and thus cluster disease severity, external factors may also affect the intensity of powdery mildew development. Although the association between high disease pressure and structural external shade factors (e.g., adjacent tree lines) have been noted (Chapter 1), the potential impact of transient and irregular shading factors, such as prolonged periods of heavy cloud cover associated with rainy portions of the growing season, is perhaps less appreciated. This applies not only to identifying the relative seasonal risk in regions such as New York, where weather can vary considerably from one year to the next, but also to identifying unanticipated risks in unusually cloudy years or critical portions thereof--such as when fruit are highly susceptible to powdery mildew shortly after bloom (Gadoury et al. 2003)--in regions such as WA and SA that are typically sunny. Existing climate-based models intended to indicate PM risk (Gubler et al 1992, Kast 1997, Sall 1980) might be enhanced by including a component to account for

variable levels of solar radiation, although this has yet to be fully quantified.

In most of these present experiments, as in the study of Zahavi et al. (2001), vines were left unsprayed in order to examine the effect of canopy density on PM development. Whereas this is a useful technique for examining the effect of sunlight on disease *per se*, it does not reflect typical commercial vineyard conditions. Thus, in our WA experiments, we also looked at the interactive effects of canopy density and fungicide applications, utilizing sulfur at both a relatively low and high rate within the range typically employed by U.S. growers using sprayable formulations of this material (sulfur dust is used at much higher rates). Here, the effect of the sulfur itself dampened the influence of sunlight exposure on disease severity, and these exposure effects were reduced even more at the higher of the two application rates. Nevertheless, significant quantitative relationships remained between fruit disease severity and both EPQA measures of cluster exposure. In such an experiment, it is not possible to distinguish the direct effects of sunlight on the host-pathogen interaction resulting from increased cluster exposure versus the indirect effects that increased exposure might have on sulfur activity. Our experiment in the Geneva, NY vineyard clearly demonstrated a twofold difference in spray deposition upon clusters as a function of canopy densities within the relatively narrow range examined, thereby quantifying the well-accepted assumption of higher pesticide residue on well exposed fruit. Furthermore, warming of tissues exposed to sunlight likely can

increase the volatility of sulfur within an open canopy, providing further suppressive effects on PM development. Our results not only demonstrate the rate at which spray coverage increases in response to canopy layers and sunlight exposure, but link the interactive effect that canopy structure has on the disease-suppressive activities of sunlight and fungicide applications. Regardless of the mechanism involved, these results demonstrate that improved powdery mildew control is yet another benefit to be derived from canopy management practices designed to optimize sunlight exposure of the clusters.

Various methods have been developed to assess canopy structure and sunlight distribution on fruit, although they vary in their ease of use and high-throughput applicability (Smart and Robinson 1991, Schultz 1995, Gladstone and Dokoozlian 2003, Meyers and Vanden Heuvel, 2008). There is a balance between ease of use and precision of the resulting output, which should guide the choice or development of the method used for any particular purpose. Meyers and Vanden Heuvel's (2008) EPQA method of canopy mapping provides the ability to quickly assess hundreds of vines in one day, while also providing detailed information on sunlight exposure of fruit. EPQA further provides the ability to calculate PPF proportions for individual clusters within a vine and diagnose vines with high degrees of shaded fruit within the vineyard. For example, based on the results of our study, vines with high levels of heavily shaded clusters (i.e., those receiving < 10% of PPF) could be identified as 'high risk' vines for PM development. Precision viticulture approaches utilizing

technologies such as GIS and GPS have been proposed (Reynolds et al 2007, Hall et al 2002, Bramley 2007) to improve management of blocks or sub-regions within a vineyard for uniformity of flavor, color and yield, and could be adapted to powdery mildew management as well. Software programs (Hall et al 2003, Delenne 2009) that use technologies such as NDVI imaging and aerial photography could be correlated with ground measures of canopy structure, and entire vineyard disease risk assessments could thereby be made on an individual vine basis. This has the potential to incorporate precision disease management into precision viticulture, and to improve proactive management of this near-universally important disease.

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## CHAPTER THREE

### EFFECTS OF FRUIT ZONE LEAF REMOVAL, TRAINING SYSTEM, AND VARIABLE IRRIGATION ON POWDERY MILDEW DEVELOPMENT ON *VITIS VINIFERA* L. CHARDONNAY

#### ABSTRACT

Relative to untreated controls, basal leaf removal applied 2 weeks postbloom reduced cluster disease severity significantly in both years of a study in a New York vineyard, whereas removal of leaves 5 weeks postbloom had no effect. The effect was not significantly different whether one leaf or two above and below each cluster was removed. Shoot density of VSP-trained vines was lower than for Umbrella-Kniffen training; this factor was associated with a significant reduction in disease development in one year of the study, and when combined with early leaf pulling reduced mean disease severity by 32% relative to untreated clusters on Umbrella-Kniffen-trained vines. However, there was no effect of training system in the second year of the study. In South Australia, doubling the irrigation volume supplied to vines receiving a standard reduced deficit irrigation program resulted in two- and sevenfold increases in foliar PM severity in two consecutive seasons. Although these data supported the hypothesis that increased levels of irrigation in a hot, dry climate might increase powdery mildew severity by better regulating leaf temperature through evapotranspirative cooling, data to support this mechanistic explanation were lacking. These results underscore the

fact that viticultural practices targeted primarily at controlling general vine growth and crop quantity/quality issues can also significantly affect the development of powdery mildew.

## INTRODUCTION

Grapevine management to optimize fruit quality and yield is a fundamental tenet of viticulture. Justification for one method over others may depend on a vineyard's geographic location, cultivar grown, desired market for the fruit, economic costs, or microclimate. One method of manipulation is the training system by which the vines are grown. One of the primary roles of a training system is to optimize light interception and/or distribution and subsequent yields and fruit quality (Reynolds and Vanden Heuvel, 2009). Sunlight interception by fruit has been associated with an improvement in fruit quality and is generally desirable to some degree in most vineyards (Lee et al. 2007, Smart 1985). A second practice used to improve sunlight exposure of fruit is basal leaf removal. In addition to increasing direct sunlight exposure, removing these leaves around fruit clusters increases the evaporative potential within the fruitzone, lowering humidity and making the microclimate less conducive for the development of fungal diseases (English et al 1990), particularly botrytis bunch rot (Gubler et al 1987, English et al 1989, Staff 1997, Zoecklein et al 1992). Critical factors such as yield and basic fruit composition (°Brix, pH, acidity) have been shown to be generally unaffected by basal leaf removal (Howell et al. 1994). However, the surface temperature of sunlight-exposed berries can be 5-15°C warmer than ambient air temperature

(Smart and Sinclair 1976), potentially improving berry color in cool climates (Dokoozlian and Kliewer 1996), and altering berry physiology (Downey et al. 2006).

Powdery mildew (PM), caused by the fungus *Erysiphe necator* (Schw.) Burr., is a disease of economic importance in viticultural regions throughout the world. Growth of the causal fungus is almost entirely epiphytic, and as a result it is directly exposed to various environmental components. A training system and row spacing that allowed more sunlight into the fruiting zone was associated with lower powdery mildew incidences in Israel (Zahavi et al 2001), and sunlight exposure within the fruitzone has been thought to create a microclimate unfavorable for this disease (Chellemi and Marois 1992). Furthermore, vines grown under sunlight from which UV radiation was filtered had significantly more PM than those exposed to unfiltered sunlight (Keller 2003). UV has been shown to alter fungal populations on other plant leaves (Moody *et al.* 2001) and generally increases fungal mortality (Bjorn 2007, Rotem *et al.* 1985), including that of *E. necator* (Willoquet et al 1996). Sunlight exposure increasing surface temperature has been well documented on grapes (Kliewer and Lider 1968, Smart and Sinclair 1976, Bergqvist *et al.* 2001, Millar 1972) as well as other fruit (Chen *et al.* 2009, Ferguson *et al.* 1998, Schroeder 1965), although these studies have focused on the physiological effects on the fruit rather than any attendant consequence on disease development. Experiments described in Chapters 1 and 2 firmly established that sunlight exposure inhibits PM development through

at least two interactive and synergistic mechanisms--i.e., exposure of the causal fungus to ultraviolet (UV) radiation and the heating of sun-exposed tissues into a range unfavorable for fungal growth--and that disease development regularly differs within canopies of variable densities in a manner inversely proportional to the degree of admitted sunlight.

Irrigation is a standard viticultural practice in many regions, particularly in arid and semi-arid climates, and has been proposed to be of growing significance in the face of global climate changes (Schultz and Stoll 2010). Use of “deficit irrigation” techniques has been applied as a means to both improve water use efficiency within vineyards and improve grape and wine quality (McCarthy 1997). Water stress has been correlated with increased leaf temperatures (Möller et al. 2006) and hyper spectral reflectance indexes (Rodríguez-Pérez et al 2007), as stressed vines cease to transpire and evaporatively cool, causing their canopies to become warmer than those of unstressed vines. Therefore, an increase in canopy temperature is often used as an indirect estimate of whole vine water status.

Irrigation has been shown to elevate levels of the powdery mildew on tobacco by promoting growth of susceptible tissue (Cole 1966) and on wheat by increasing mesoclimate relative humidity (Sharma et al 2004). However, there are no published reports of irrigation affecting the development of any powdery mildew through an effect on the temperature of host tissues. Nevertheless, increased

ambient temperature (Delp 1954) and surface temperature (Chapter 1) can have significant detrimental effects on the development of grapevine powdery mildew.

Viticultural practices can have pronounced effects on the development of specific diseases and, ideally, these effects should be considered within the broad context of an integrated crop management system. Therefore, the primary objective of this study was to determine whether two standard viticultural practices employed to maximize fruit quality by increasing sunlight exposure within the fruitzone--i.e., use of a relatively open training system and basal leaf pulling around the clusters--might also provide benefits with respect to powdery mildew management. A secondary objective was to determine whether differential irrigation regimes might influence powdery mildew development through differential effects on canopy temperature. Portions of this work have been published previously (Austin et al 2009a, Austin et al 2009b, Austin et al 2008).

## MATERIALS AND METHODS

*Training system and leaf removal.* The experiment was conducted in 2008 and 2009 in a vineyard of *Vitis vinifera* 'Chardonnay' on C3309 rootstock with 3-m row spacing and 2-m vine spacing, planted in 2005 at the NY State Agricultural Experiment Station in Geneva. Vines were trained to two different systems: (i) Vertical Shoot Positioning (VSP), or (ii) Umbrella Kniffen, in which arching of the canes allows more buds per linear unit of row than in the VSP system (Table 3.1). Three

panels of four vines each were randomly assigned to be trained to either the VSP or Umbrella Kniffen system beginning in 2006. Vines in both training systems were balanced pruned each year using a 20 + 20 formula (20 buds left on the vine plus 20 more for every 454 g of pruning weight). For all treatments, 10 clusters in each of three replicate four-vine panels were arbitrarily identified and inoculated at 75% capfall, using a portable spray paint apparatus to apply an *E. necator* spore suspension containing  $10^5$  conidia/ml (Chapter 1). Basal leaf removal occurred in both seasons either 2 or 5 weeks post-75% capfall, respectively. At each timing, leaves were removed by hand at the node opposite the inoculated cluster as well as one node above and below ('Light') or two nodes above and below ('Heavy'). Control clusters had no leaves removed. Ambient temperature and relative humidity within the canopy were measured with HOBO dataloggers (Onset, Pocasset, MA) at hourly intervals throughout the growing season. Fungal diseases on vines were managed with minimal sprays of mancozeb and a highly refined petroleum oil (JMS Stylet Oil), chosen for their relative lack of activity against *E. necator* and minimal residual or vapor activity, respectively. Inoculated clusters were protected within individual plastic bags during each spray application and the bags were removed immediately after sprays had dried. Disease progress was monitored bi-weekly throughout the season. Final assessments were made on 5 August 2008 and 25 July 2009 due to the rapid degradation of heavily infected clusters, as well as the emergence of botrytis bunch rot. Disease severity was assessed by visually rating individual clusters on a 0-100% scale, based on the

proportion of cluster tissue covered with the pathogen. Training system and basal leaf removal effects on cluster disease severity were analyzed for significance using GLM. Pairwise comparisons of means for the effects of each leaf removal treatment in the model were compared using Tukey-Kramer HSD. These and all other statistical analyses and calculations were made using JMP statistical software (SAS institute, Cary, NC, USA).

Table 3.1. Pruning weights for UK and VSP vines after the 2009 season <sup>a</sup>				
	Shoots/vine	Pruning wt (kg)	Shoots/m	Pruning wt (kg)/m
VSP	18.4	1.28	11.17	0.78
UK	18.0	0.96	20.74	1.11
Significance <sup>b</sup>	0.75	0.0003	0.0001	0.0003

<sup>a</sup>Vertically shoot positioned (VSP) and Umbrella-Kniffen (UK) trained vines were balance pruned using a 20 + 20 formula each season.

<sup>b</sup>Significance: *P*-values for mean comparisons within each category using Student's t-test

*Irrigation effects.* The potential effects of irrigation on powdery mildew development were investigated in an own-rooted Chardonnay vineyard in Nuriootpa, South Australia. This vineyard was planted in 1994 with 3.5-m row spacing and 2.25-m vine spacing, and the experiment was conducted using two rows of 72 vines each, pruned to two-bud spurs with ~40 buds per vine and grown on a three-wire single curtain, non-shoot positioned trellis. The two rows were divided into three blocks. Each block consisted of six opposing panels from each row (12 in total). Blocks were then subdivided into four individual three-panel

plots, half of which were randomly assigned either (i) the standard reduced deficit irrigation (RDI) regime, provided through a single dripper line that supplied water to all vines; or (ii) twice that water volume, provided through the common dripper line plus an identical second line supplementing only those vines.

Vines were irrigated once per week for 12 hours at a rate of 4L/hr per dripper tube. Irrigations began 6 Dec 2006 and 13 Dec 2007, with a total of 11 and 12 irrigations for the 06/07 and 07/08 seasons, respectively. In both seasons, vines were unsprayed and natural powdery mildew infections were allowed to develop. Foliar disease progress was monitored throughout each season, and final assessments were made on 25 February 2007 and 23 February 2008. Disease severity was assessed by visually rating the adaxial surface of 15 leaves per plot on a 0-100% scale, based on the proportion of leaf tissue covered with the pathogen, and these ratings were averaged for each of the three replicate blocks per treatment. Midday leaf water potentials to estimate vine water status (Chone et al. 2001) were determined using a pressure bomb on days after every third irrigation, for three leaves per plot. Surface temperatures of 10 leaves per plot were measured at these same times using a non-contact a Mikron infrared radiometer MI-N14 (LumaSense Technologies Inc. Santa Clara, CA). Air temperature and relative humidity in the canopy were measured as described above. Foliar disease severity ratings for irrigation treatments were compared using Student's t-test.

## RESULTS

*Training system and basal leaf removal.* In 2008, there was a highly significant ( $P < 0.0001$ ) effect of training system on powdery mildew development on clusters (Table 3.2), with mean disease severity levels for vines trained to the VSP system reduced by 24% relative to those trained to the Umbrella-Kniffen system when averaged across all leaf-pulling treatments (Figure 3.1). Leaf pulling also had a highly significant ( $P < 0.0001$ ) effect, which was a result of the early treatments but not the late timing (Table 3.2). For example, disease severities were reduced by 16 and 18% relative to the control treatment on VSP trained vines subjected to the light and heavy pulling levels, respectively, when these were applied 2 weeks post-bloom, whereas neither level had an effect when applied 5 weeks post-bloom (Figure 3.1). Integrating the effects of training system and early leaf pulling, final disease severities on VSP-trained vines subjected to Light and Heavy leaf pull 2 weeks post-bloom were 31 and 33% lower, respectively, than on vines in the Umbrella-Kniffen system without leaf pulling. There was no significant interaction ( $P = 0.61$ ) between leaf pulling and training system. There were no differences in air temperature or relative humidity measured within the canopies of vines in the two different trainings systems throughout either this or the following season (Table A3.1).

In 2009, when disease pressure was generally higher, training system no longer had an effect ( $P = 0.51$ ). However, the effect of leaf removal was again highly significant ( $P < 0.0001$ ), with an influence of

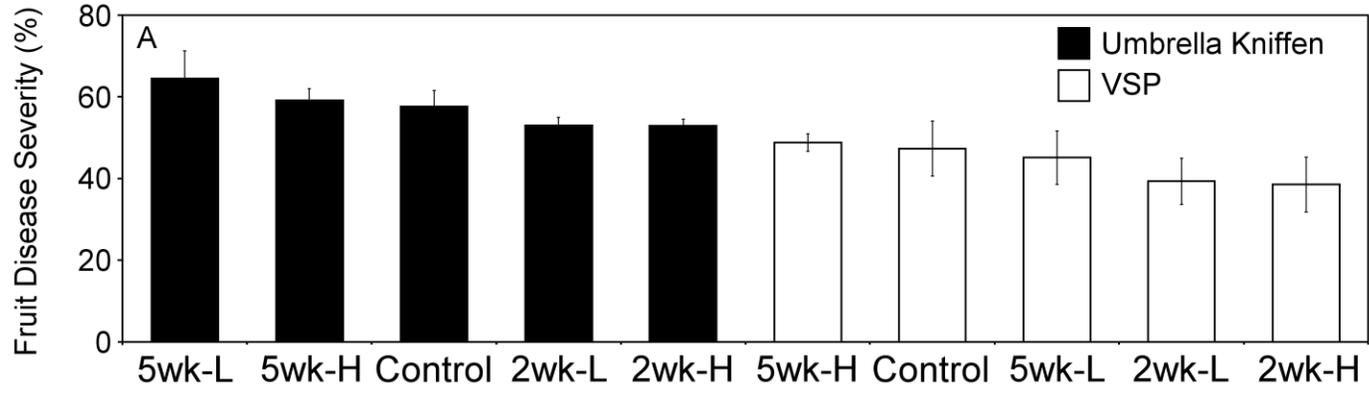
Table 3.2. Statistical analyses for training system and basal leaf removal effects on powdery mildew severity on fruit				
Factor	Effects Tests <sup>a</sup>		Means Comparisons <sup>b</sup>	
	F-ratio	<i>P</i> -value	Level	Statistical group
2008				
Training System	45.22	<0.0001		
Leaf Removal	6.39	<0.0001	Control	AB
			2wk-H	C
			2wk-L	BC
			5wk-H	A
			5wk-L	A
Training x Leaf Removal	0.68	0.61		
2009				
Training System	0.44	0.51		
Leaf Removal	21.18	<0.0001	Control	A
			2wk-H	B
			2wk-L	B
			5wk-H	A
			5wk-L	A
Training x Leaf Removal	0.24	0.91		

<sup>a</sup>Generalized linear model analysis of variance for training system and basal leaf removal effects on fruit disease severity.

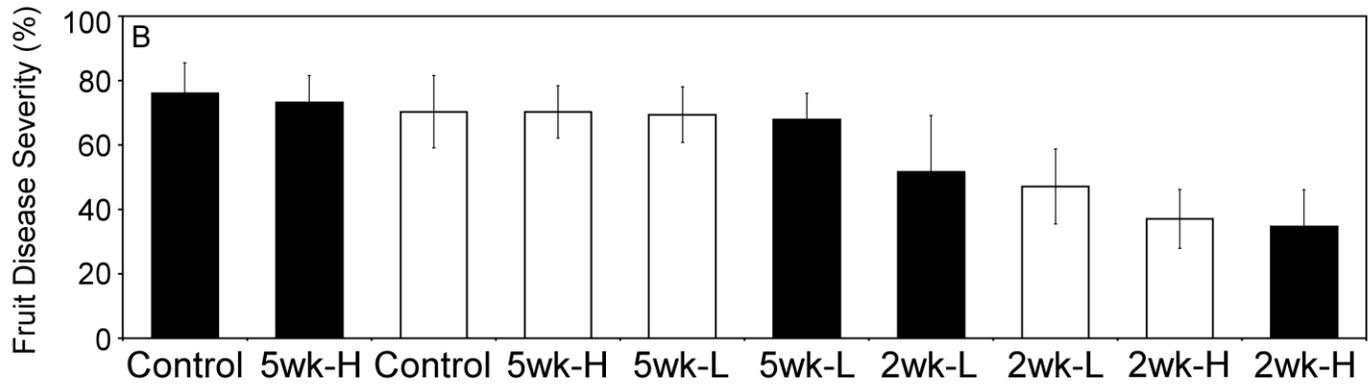
<sup>b</sup>Comparisons of means among leaf removal treatments were made using the Tukey-Kramer HSD test. Means not assigned to a common group are significantly different ( $P = 0.05$ ).

Figure 3.1. Powdery mildew severity on Chardonnay clusters subjected to five different leaf-removal treatments in each of two vine-training systems for the 2008 [A] and 2009 [B] seasons. Clusters were inoculated at 75% capfall and disease severity was visually assessed on a 0-100% scale on 1 August 2008 and 25 July 2009. Leaf-removal was either 2 or 5 wk post-bloom, and for each timing leaf removal was either; H = heavy, or L = light (two leaves or one leaf above and below each cluster, respectively). Each data bar represents the mean for 30 clusters per treatment, and error bars represent one standard error of the mean.

2008



2009



Leaf Removal Treatment

treatment timing and a lack of an interaction between leaf pulling and training system similar to those in 2008 (Table 3.2). As in the previous year, leaf pulling treatments imposed 5 weeks post-bloom had no effect on disease severity, whereas in contrast, the Heavy treatment imposed 2 weeks post-bloom reduced severity by approximately one-half relative to the untreated vines in both training systems. Disease severity levels were somewhat higher in the Light versus Heavy pulling treatment when both were applied early, although these differences were not statistically significant ( $P > 0.05$ ) (Figure 3.1, Table 3.2).

*Irrigation.* Vines that received double the standard RDI volume of water had significantly higher levels of foliar powdery mildew than did those receiving that standard regime, in both seasons (Figure 3.2). In 2007, disease severity on the doubly irrigated vines was twice that found on their RDI counterparts, whereas it was almost sevenfold greater in 2008. Midday measurements of leaf water potential (Table A3.2) and leaf surface temperature (Table A3.3) did not indicate any significant difference between the two irrigation treatments. There were no differences between treatments in canopy temperature or relative humidity during the season (Table A3.3).

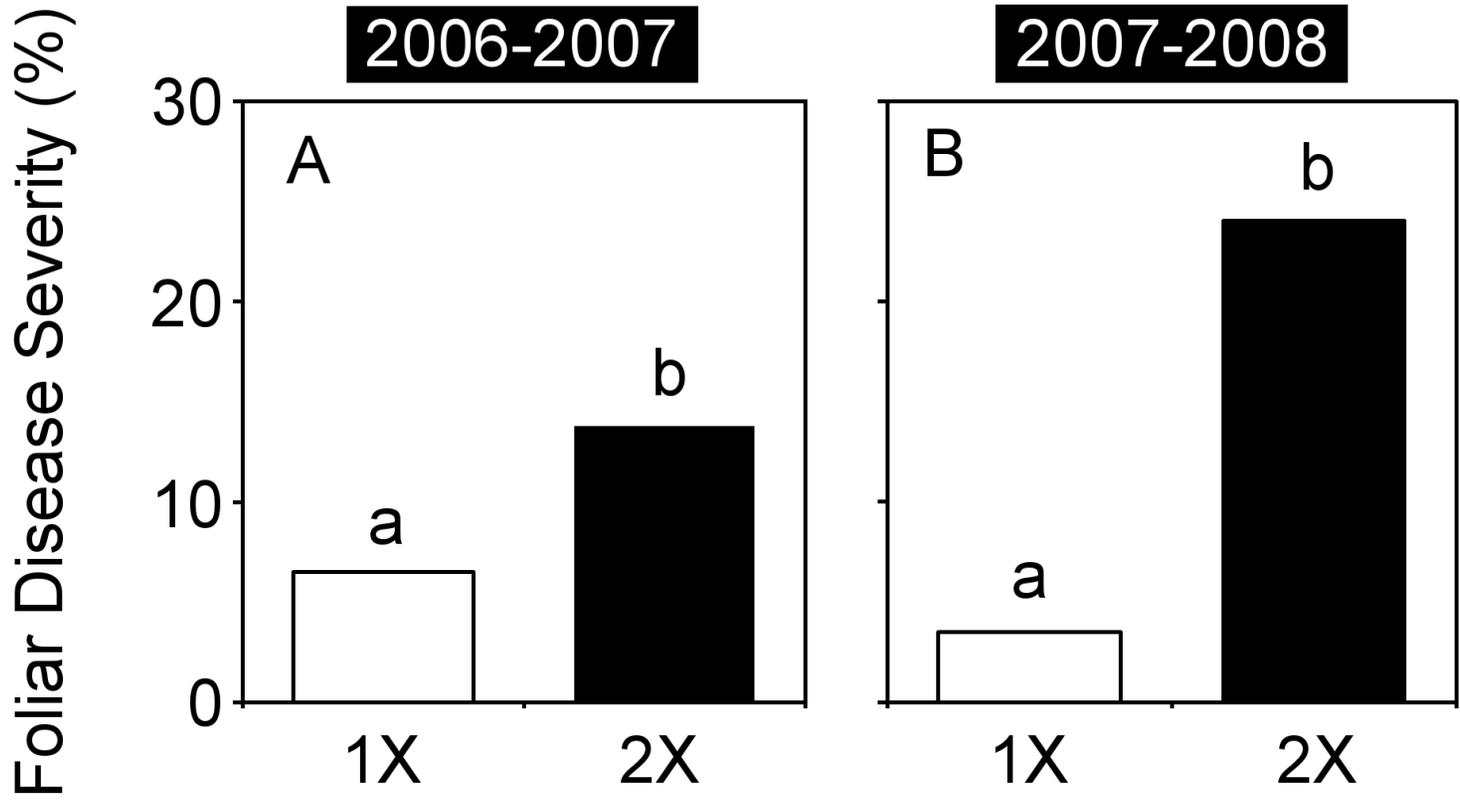
## Discussion

Exposure to sunlight (especially the ultraviolet [UV] component of the spectrum) directly inhibits its development of grapevine powdery mildew (Chapters 1 and 2). Therefore, canopy management practices

designed to reduce shading and promote the penetration of sunlight into the fruiting zone should help limit the development of this disease. Among the most basic decisions with respect to canopy management is the choice of a training system. Although training systems are typically designed or chosen to optimize light interception for reasons of fruit yield and quality (Reynolds and Vanden Heuvel, 2009), I hypothesized that a system chosen for these reasons may also be useful within an integrated approach to managing powdery mildew.

In the Finger Lakes region of New York, older *V. vinifera* plantings are often trained to an Umbrella-Kniffen system, although most newer plantings utilize systems, including VSP, that provide lower density canopies. Thus, I chose these two training systems for comparison in the present study, and consistent with the above hypothesis, disease severity was one-fourth lower in 2008 for the VSP system, in which vegetative growth was also less relative to the Umbrella-Kniffen vines. Zahavi et al (2001) similarly found lower levels of powdery mildew in a sprawl or “free positioned” training system compared to VSP, which in their case was the system that more tightly compressed shoots into a smaller area, thereby providing the denser canopy structure. However, there was no difference in disease severity between the two training systems in our 2009 experiment, a season during which greater severity ratings among the control treatments indicated greater disease pressure than in the previous year. The reason for this seasonal difference is unclear, although there was 54% more precipitation in 2009 than in the

Figure 3.2. Foliar severity of powdery mildew on leaves of Chardonnay vines in Nuriootpa, SA. irrigated with one (1X) or two (2x) dripper irrigation tubes for the 2006-2007 (A) and 2007-2008 (B) seasons. Vines were irrigated once a week for 12h at a rate of 4L/hr per dripper. The vines were unsprayed and natural infection as allowed to occur. Means represent average disease severity from 15 leaves for each of three reps. Disease was assessed on adaxial leaf surfaces four weeks pre-harvest. Within panel A and B, bars not labeled with a common letter are significantly different ( $P < 0.001$ )



previous year during the critical period for cluster infections, i.e., 2 weeks pre-bloom through 2 weeks post-bloom (data not shown). Thus, seasonal effects may override the influence of training system on powdery mildew development.

A common canopy management technique for increasing sunlight exposure within the fruitzone is the removal of basal leaves near the clusters after fruit set. Although this practice was developed primarily to improve fruit quality characteristics (Poni *et al* 2006, Reynolds *et al* 1996, Smart 1985), disease management benefits also have been noted to accrue from it, particularly with respect to botrytis bunch rot (English *et al* 1990, English *et al* 1989, Gubler *et al* 1987, Staff 1997, Zoecklein *et al* 1992) but also, in one report, powdery mildew (Chelemi and Marois 1992). In our study, leaf removal 2 weeks after bloom (i.e., shortly after fruit set) significantly reduced disease severity with respect to the control treatment in both years of the experiment, whereas leaf removal 5 weeks after bloom had no effect in either year. Although grape berries are highly susceptible to infection by *E. necator* 2 weeks after bloom, they become immune to new infection before 5 weeks post-bloom (Gadoury *et al.*, 2003, Gee *et al.* 2008). Thus, the detrimental effects exerted upon the fungus by sunlight via UV irradiation and/or the heating of exposed host tissues (Chapter 1), or through other mechanisms such as increasing host resistance to infection (Keller 2003), may limit disease development more strongly when exposure occurs while berries are still young and highly susceptible. Chelemi and Marois (1992) also noted a significant

reduction in powdery mildew development when basal leaves were pulled 3 weeks after bloom in one year of their study, but unlike our results, they also reported a significant effect when leaves were removed 6 weeks after bloom in a different season. However, it is difficult to determine any potential effect of treatment timing in their study, since (i) they did not directly compare the two timings in a common experiment; and (ii) disease pressure in the experiment examining the 6-week postbloom timing was very low (15% severity on unsprayed controls), potentially confounding the results. It also is possible that the timing effects of basal leaf removal, like the value of the procedure itself, may vary depending on location and weather (Guidoni et al. 2008). Chelemi and Marois (1992) speculated that their observed leaf pulling effects were due to the independent influences of improved fungicide coverage and creation of a microclimate unfavorable for the disease, anecdotally noting that, consistent with data in Chapters 1 and 2, disease was most prevalent on the interior portions of clusters least exposed to the sun. And although the particular vines utilized in this present experiment were not examined for spray coverage, results from another experiment conducted in a different portion of the same vineyard showed that removing one leaf layer between a cluster and the outer edge of the canopy essentially doubled the quantity of spray deposited on that cluster (Chapter 2).

The hypothesis that increased levels of irrigation in a hot, dry climate might increase powdery mildew severity by better regulating leaf temperature through increased evapotranspirative cooling was

supported by disease assessments from the irrigation study in the Barossa Valley of South Australia, where doubling the irrigation volume led to a two- and sevenfold increase in foliar disease severity in two consecutive seasons. However, data to support a hypothesized mechanistic explanation for the phenomenon were lacking, as there were no differences measured in midday leaf water potentials or midday surface temperatures between irrigation treatments. This may be due to the fact that there truly were no differences throughout the season, or because differences were transitory and not present at the time that measurements were made. Vines assessed at midday in this location, independent of irrigation regime, were below the point of critical leaf water potential; thus, complete stomatal closure on all leaves should have resulted in relatively uniform leaf water potentials (Patakas 2005) and increases in leaf temperature as transpiration ceased. However, Loveys (2005) demonstrated that differential canopy surface temperatures can develop for Shiraz vines under variable irrigation regimes in Australia, with those vines receiving more water remaining relatively cooler. The effects that irrigation can have on grapevine physiology are numerous and have been well reviewed (Nagarajah 1989, Williams et al. 1994), and it is quite possible that the increased foliar PM severity in response to increased irrigation resulted from factors in lieu of or in addition to any effects on leaf temperature. Nevertheless, the results presented here underscore the fact that viticultural practices targeted primarily at general vine growth, yield and fruit quality can also significantly affect the development of perhaps the most intensively managed disease of grapevines. This

concept, and these particulars, should be kept in mind when devising integrated pest management and/or integrated crop management programs.

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## APPENDIX ONE

Weather data indicating insignificant differences in vineyard treatments, including; (i) ambient temperature and humidity for artificial shading experiments in New York for the 2005-2009 seasons; (ii) ambient temperature and relative humidity within the canopy of vines trained to either Vertical Shoot Positioning or Umbrella Kniffen near Geneva NY in 2008-2009; (iii) midday leaf water potentials for Chardonnay vines receiving either a standard Reduced Deficit Irrigation regime or twice that amount, and ambient temperature in Nuriootpa SA for the 06/07 and 07/08 seasons; and (iv) leaf surface temperature of sunlight exposed leaves on chardonnay vines receiving either a standard Reduced Deficit Irrigation regime or twice that amount in Nuriootpa SA for the 06/07 and 07/08 seasons.

Table A1.1. Ambient temperature and relative humidity for artificial and natural shading experiments in New York								
	Chardonnay (Dresden) <sup>a</sup>							
	Clearing		Trees		Canopy		Trees & Canopy	
	Temp. °C	RH (%)	Temp. °C	RH (%)	Temp. °C	RH (%)	Temp. °C	RH (%)
2005	21.7	78.6	21.6	78.3	21.7	77.9	21.6	78.5
2006	20.0	77.3	20.0	76.8	20.1	77.0	19.9	77.6
	Chancellor <sup>b</sup>							
	Shade Cloth		Low UV		Full Sun			
	Temp. °C	RH (%)	Temp. °C	RH (%)	Temp. °C	RH (%)		
2005	na	na	21.2	68.9	21.4	69.1		
2006	18.2	76.5	18.4	77.9	18.5	76.0		
2007	20.3	74.3	20.1	72.7	20.4	71.0		
2008	19.4	77.7	19.1	78.0	19.0	80.0		
2009	17.9	78.3	18.0	79.3	18.3	78.5		
	Chardonnay (Geneva) <sup>b</sup>							
	Low UV		Full Sun					
	Temp. °C	RH (%)	Temp. °C	RH (%)				
2007	20.1	80.2	20.4	80.6				
2008	19.1	82.2	19.0	80.7				

<sup>a</sup> Treatments as identified in Fig. 1.1.

<sup>b</sup> Treatments as identified in Fig. 1.2.

Average temperature and relative humidity were calculated from hourly measurements collected from June 1 to August 30 each season.

Table A3.1 Ambient temperature and relative humidity within the canopy of Vertical Shoot Positioning or Umbrella Kniffen trained vines.

	Vertical Shoot Position		Umbrella Kniffen	
	Temp. °C	RH (%)	Temp. °C	RH (%)
2008	19.3	80.1	19.2	80.1
2009	18.8	79.9	18.8	78.5

Average temperature and relative humidity were calculated using hourly measurements collected from 1 June to 30 August each season.

Table A3.2 Midday leaf water potentials for Chardonnay vines receiving either a standard Reduced Deficit Irrigation regime (1X), or twice that water volume (2X)

Date of Assessment	Leaf Water Potential	
	1X <sup>a</sup>	2X <sup>b</sup>
Dec 7, 2006	17.1	17.0
Dec 28, 2006	17.8	17.6
Jan 18, 2007	17.8	18.1
Feb 8, 2007	18.1	18.0
Dec 14, 2007	16.5	16.7
Jan 4, 2008	16.3	16.7
Jan 25, 2008	16.7	16.8
Feb 15, 2008	17.1	16.9

<sup>a</sup> Vines received 4L per hour for 12 hours once a week beginning 6 Dec 2006 and 13 Dec 2007, with a total of 11 and 12 irrigations for the 06/07 and 07/08 seasons, respectively.

<sup>b</sup> Vines received 8L per hour for 12 hours once a week beginning 6 Dec 2006 and 13 Dec 2007, with a total of 11 and 12 irrigations for the 06/07 and 07/08 seasons, respectively.

Table A3.3. Within canopy ambient temperature, relative humidity, and midday surface temperature of sunlight exposed leaves of Chardonnay vines receiving either a standard Reduced Deficit Irrigation regime (1X), or twice that water volume (2X).

	1X <sup>a</sup>	2X <sup>b</sup>
2006/2007		
Canopy Temperature <sup>c</sup>	21.1°C	21.4°C
Canopy RH <sup>c</sup>	49.1%	50.2%
Leaf Temperature <sup>d</sup> above ambient	12.1°C	12.3°C
2007/2008		
Canopy Temperature <sup>c</sup>	21.1°C	21.2°C
Canopy RH <sup>c</sup>	48.2%	48.1%
Leaf Temperature <sup>d</sup> above ambient	10.1°C	10.0°C

<sup>a</sup> 1X irrigation as described in Table A3.2.

<sup>b</sup> 2X irrigation as described in Table A3.2.

<sup>c</sup> Average temperature and relative humidity were calculated from hourly measurements collected within the canopy near the fruit zone from Nov 1 to March 1 each season.

<sup>d</sup> Leaf temperatures averaged from 10 sunlight exposed leaves within each treatment at solar noon on clear sunny days and compared to ambient temperatures at the time of assessment.