RECIPROCITY OF IRRADIANCE AND DURATION OF THE EXPOSURE TO ULTRAVIOLET RADIATION ON *ERYSIPHE NECATOR*

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Tyler Brent McCann

December 2018

ABSTRACT

Nighttime application of ultraviolet radiation (UV), particularly wavelengths below ~280nm, has been shown to reduce populations of species causing powdery mildew at relatively low doses, while avoiding harm to the plants being exposed. The Bunsen-Roscoe principle, or dose reciprocity, describes a photochemical reaction where the outcome is determined solely by the dose level used, not how quickly or intensely that dose is supplied. Several microorganisms, however, have shown sensitivity to the intensity of UV used at a given dose. No study has investigated whether the method in which UV-C (100-280nm) is applied impacts the level of control achieved, or the consequences for the implementation of field application. *Erysiphe necator,* the causal agent of grape powdery mildew, was examined to determine if dose reciprocity held within the dose range (4 - 210 J/m^2) for germination, colony expansion rates, and latency. Initial experiments comparing all doses at UV exposures over 4 and 400 seconds found dose reciprocity held, with increasing dose leading to decreased odds of germination success ($P<0.05$). Experiments to confirm this finding using larger sample sizes and irradiance of 0.3 and 30 W/m^2 found conidia exposed to higher irradiance trended toward increased detrimental effect on *E. necator* at a dose of 120 J/m^2 ($P=0.2$); at 210 J/m^2 mean germination was significantly different ($P<0.05$) between the higher irradiance (4%) and the lower irradiance (9%). Colony expansion and latency for colonies exposed to 210 J/m^2 were affected to the same degree irrespective of irradiance of duration of exposure. This study is the first to examine dose reciprocity at irradiance levels closer to those shown to be effective in field applications. These results

suggest that UV dose may be lowered, if irradiance is increased, to achieve powdery mildew disease suppression.

BIOGRAPHICAL SKETCH

Tyler McCann attended the University of Florida in Gainesville, FL, and obtained his Bachelor of Science degree in Botany with a minor in Geography in 2013. As an undergraduate he worked in the laboratory of Drs. Doug and Pamela Soltis assisting in several research projects focusing on plant evolution and phylogenetics. In the summer of 2012 he participated in Cornell University's Summer Scholar program at the New York Agricultural Experiment Station in Geneva, NY under the guidance of Dr. David Gadoury. He participated in the NSF-REU program at the Donald Danforth Plant Science Center in St. Louis, MO under the guidance of Dr. Sona Pandey during the summer of 2013. Through these experiences his interest in science communication for public audiences grew. Then in the summer of 2015, Tyler began his graduate studies at Cornell University's School for Integrative Plant Science in the Section of Plant Pathology and Plant-Microbe Biology.

ACKNOWLEDGEMENTS

I would like to thank each of my committee members for the assistance they provided me in the completion of this thesis. Dr. Lance Cadle-Davison never failed to lend time for our conversations and illuminate new questions in the work. Dr. Marvin Pritts as a professor and advisor helped me identify and develop my skills as a scientist and leader. Dr. Mark Rea offered me the invaluable perspective of a physicist looking into the world of plant and microbe biology, which led me to consider my language and thinking more rigorously. Finally, I would like to thank my committee chair, Dr. David Gadoury, for his ever-present curiosity and skepticism in this work, and encouraging me to pursue professional development in and outside of the laboratory.

I would also like to thank Breanne Kisselstein, Daniel Martinez Lacasa, Surya Sapkota, William Weldon, and Mary Jean Welser for their assistance, encouragement, and support in the Gadoury Lab. Hans Ragnar Gislerød, Arne Stensvand, Aruppillai Suthaparan, and Belachew Asalf Tadesse, along with numerous other scientists at the Norwegian University of Life Sciences (NMBU) provided me with critical lessons in powdery mildew studies during the first summer of my graduate work, and helped me feel welcome in their community. Andrew Bierman at the Lighting Research Center in Troy, NY also helped my understanding of lighting, energy and measurement. Additionally, I'm grateful to countless other members of the community at the New York Agricultural Experiment Station who assisted my efforts to complete this work.

Finally, I would like to thank my friends, teachers, and family who consistently reminded me of the big picture throughout this work.

TABLE OF CONTENTS

CHAPTER 1 INTRODUCTION

SECTION 1: *ERYSIPHE NECATOR* AND GRAPEVINE POWDERY MILDEW

Powdery mildew diseases are caused by fungal pathogens in the order *Erysiphales*. The group is globally distributed infecting nearly all agronomic and horticultural crops with as many as 10,000 different plant species reported as hosts (Glawe, 2008). As obligate biotrophs, powdery mildew pathogens require living host tissue for their survival. Consequences of infection include reduced photosynthetic activity, reduced yields due to the impacts on photosynthesis, and/or an unmarketable quality and appearance of infected fruit.

Erysiphe necator is the causal agent of powdery mildew in grapevines. Each year *E. necator* appears in vineyards shortly after new shoots and leaves appear in the spring, and can increase exponentially as the growing season progresses. Primary inoculum sources include overwintered sexual fruiting bodies, called chasmothecia (syn. cleistothecia), which adhere to dead leaves or the trunk's bark, and, in some climates with warmer winters, mycelia within infected dormant buds. Both ascospore-derived colonies and colonies on flag shoots can produce large number of asexual conidia, which are then dispersed aerially and can spread the disease (Gadoury *et al.*, 2012).

Erysiphe necator can actively grow and infect all green tissues of a grapevine (Gadoury *et al.*, 2012). When a conidium lands on susceptible tissue germination will typically occur within a few hours at temperatures near 25C. More time is required at higher or lower temperatures, and virtually no germination occurs below 6C or above 35C (Delp

et al., 1954). The pathogen then needs to penetrate the epidermis to absorb nutrients from the host. This is accomplished with both cell wall-degrading enzymes and a lobed structure called the appressorium, which is produced from the conidium's hyphae. The appressorium provides pressure to mechanically puncture the plant cuticle and cell wall, and enter the subtending plant epidermal cell. Upon penetration the pathogen develops a haustorium inside the plant cell to absorb nutrients. This is the only component of the fungal body that is inside the host cell – all other tissues of the pathogen are exterior to the host.

Upon reaching a certain vegetative threshold *E. necator* will produce conidiophores, the organs responsible for producing conidia. In *E. necator* the subsequent production of conidia requires light exposure on the colony, followed by approximately 5 hours until mature spores can be detached (Gadoury *et al.*, 2011; McCann *et al.*, 2013). Prior work suggested *E. necator* initiation of sporulation is more sensitive to blue light (peak ~447.5nm, FWHM=21nm, average irradiance=38.65 W/m^2) than red light (peak ~660 nm, Full Width Half Maximum=19nm, average irradiance=62.25 W/m^2). Though intensity differed between these two treatments, colonies in blue light required a smaller amount of energy to produce mature conidia than colonies in red light after 5 hours (McCann *et al.*, 2017). The supply of conidia is the primary determinant of the rate of epidemic progress throughout the growing season.

SECTION 2: CURRENT MANAGEMENT OF GRAPEVINE POWDERY MILDEW

Wherever powdery mildew occurs it poses a perennial problem for growers. For example, left untreated *E. necator* reduced yields of 'Cabernet sauvignon' and 'Sauvignon blanc' by ~24% primarily through decreased berry size and weight (Calonnec *et al.*, 2004). Untreated 'Concord' grapevines infected with powdery mildew exhibited significantly reduced soluble solids at harvest, in some cases below 15%, the minimum level required for grape processing (Gadoury *et al.*, 2001). Growers are encouraged to manage the disease with fungicides early in the disease cycle (Smith *et al.*, 2016) to markedly reduce the rate of disease progress (via a reduction in the number of established colonies and subsequent inoculum), especially during the period when fruit are at the greatest risk due to ontogenic susceptibility (Gadoury *et al.*, 2003).

Fungicides are a critical input of agriculture, and whether they are synthetic or organic in origin, they are a major expense for growers. Accounting for the cost of purchasing and applying materials, Sambucci *et al.* (2014) calculated that California growers spent $189 million on the control of *E. necator* in 2011. The cost of using fungicides also increases as fungicide resistance develops within pathogen populations because higher rates of products may be needed to maintain sufficient disease control. Powdery mildew pathogens have been shown to rapidly develop resistance to multiple classes of fungicide (Erickson & Wilcox, 1997; Holloman & Wheeler, 2002; McGrath, 2001; Miles *et al.*, 2012; Vincelli, 2002). Powdery mildew isolates have shown resistance to benzimidazoles, demethylation inhibitors (DMI), and strobilurins (QoI) (Colcol *et al.*, 2012; Colcol *et al.*, 2016), along with many other materials (FRAC, 2018). In addition to

cost and fungicide resistance there are risks associated with spraying fungicides to the environment (Jacobson *et al.*, 2005; Komárek *et al.*, 2010; Sambucci *et al.*, 2014; Biondi *et al.*, 2012) and farmworkers (Calvert *et al.*, 2008; Damalas & Koutroubas, 2016; Hyland & Laribi, 2017).

Given the history of powdery mildew pathogens, it is almost guaranteed that resistance will develop for any new single-site fungicide groups registered for use. With an emphasis on conservation and integrating multiple tools to complement chemical fungicides, the effective use of many of these fungicides could be prolonged. There are several existing tactics to reduce farmer reliance on fungicides while maintaining control of powdery mildew epidemics. These include: (1) developing new pathogen-resistant grapevines, though this process requires many of years before commercialization can be attempted, it has no guarantee of durability, and in grapes can be limited by adoption (Fuller *et al.*, 2014); (2) canopy pruning and training grapevine canopies to maximize sunlight exposure (Austin & Wilcox, 2011), but labor-intensive cultural practices can be impractical for established vineyards; and (3) biological controls, such as *Trichoderma* species (Woo *et al.*, 2014; Pascale *et al.*, 2017) *Bacillus sp.* (Ockey *et al.*, 2016) and mycophagous mites (English-Loeb *et al.*, 1999; English-Loeb *et al.*, 2007) have shown promise but need greater research and product development to increase adoption (Knudsen & Dandurand, 2014; Verma *et al.*, 2007; Woo *et al.*, 2014;). In order to maintain the ability to control pathogens impacting food crops and conserve fungicides, it is critical to continue developing current and new non-chemical tools that both

suppress pathogen populations below economic thresholds and reduce selection pressure for fungicide resistance.

SECTION 3: ULTRAVIOLET RADIATION

One non-chemical tool that farmers could utilize to manage powdery mildew is ultraviolet (UV) radiation. UV radiation is defined by the wavelength range from 100 to 400 nm. The UV spectrum can be broken down into the following intervals: Vacuum UV (100-200 nm), UV-C (200-280nm), UV-B (280-315 nm), and UV-A (315-400 nm). The majority of UV radiation reaching the Earth's surface is UV-A, with UV-B largely attenuated by the planet's atmosphere. UV-C and vacuum UV are completely blocked by the Earth's atmosphere (Urban *et al.*, 2016).

Unlike wavelengths in the visible range that are most often measured in terms of photon flux (moles of photons / m^2 / sec), the ultraviolet range is commonly reported in terms of irradiance (Watts / m^2 / sec) and energy transfer or dose (Joules / m^2), where 1 watt is equal to 1 joule / sec. Converting between the two is not simple, and requires measurements for all of the wavelengths of interest to be known and integrated. This distinction can be explained in part by the history of spectrophotometry and its emphasis on light as perceived by the human eye, and radiometry with its focus on energy and detecting wavelengths invisible to the human eye (Johnston, 2001).

UV damages cells through various means, including the interference of DNA replication and transcription through photoproduct formation (e.g. cyclobutane pyrimidine dimers),

and the induction of peroxides in the host that degrade cell membranes and other components of the cell (Urban *et al.*, 2016).

Most organisms can avoid or repair the damage caused by daily UV exposure, however. Melanization is a common strategy taken by a wide range of organisms, where pigments, including melanin, help the organism avoid damage from UV by screening the incoming radiation; powdery mildew species (with the exception of one species [Glawe, 2008]), however, produce protective compounds in the walls of sexual fruiting bodies only (Suthaparan *et al.,* 2012). Phenolic compounds in plants also play an important role in protection from UV (Schmitz-Hoerner & Gottfried Weissenbock, 2003). Many organisms also utilize repair enzymes to fix the damage to DNA by UV exposure (Lucas-Lledo & Lynch, 2009). Recently, Pathak *et al.* (2017) reported transcriptomic evidence of the presence and expression of genes similar to known photo-responsive genes, including photolyase, in a powdery mildew species.

SECTION 4 UNDERSTANDING UV USE IN AGRICULTURAL SETTINGS

Growers looking to implement UV-based control systems should be aware of the risks of this radiation. Similar to the application of chemical fungicides, personal protective equipment should be used with UV radiation; glasses or goggles to shield the user's eyes, and long sleeves and pants to minimize exposed skin (Urban *et al.,* 2016). In the absence of widespread use of UV in agriculture to manage plant pathogens, there are no studies on the occupational risks of use in the US; however, studies have documented the risks of acute and chronic UV exposure generally (Sklar *et al.*, 2013) A

UV system could decrease some human safety risks through the elimination of spray drift, a major cause of injuries by chemical pesticides (Calvert *et al.,* 2008), and potentially reduce accidental exposure due to the high visibility of lamps from large distances away and the absence of lingering toxic compounds in areas where it is supplied.

There are many elements that need to be considered and investigated before UV can be used confidently in the field to suppress plant pathogens (Gadoury *et al.,* 2017). Firstly, the UV dose needed to reach a desirable level of pathogen control needs to be identified. Secondly, any UV dose that results in disease suppression should be used to expose plants susceptible to that pathogen to identify any phytotoxic effects that occur. Following this should be a consideration of the ground speeds that a mobile UV unit can travel and the amount of time any individual plant will be exposed under this unit. Tractor-drawn units may become impractical relative to other methods below a certain speed, but as speed increases the amount of UV plants receive will also decrease.

Any dose of UV radiation is a product of the duration of exposure and the intensity, or irradiance, of the radiation. For any given dose, it can be supplied more quickly if the irradiance is increased, or supplied more slowly if irradiance is decreased. This means that the speed of mobile UV units could increase only if the number of lamps also increased or if more powerful UV bulbs were used (i.e., irradiance increased). Some organisms have shown enhanced sensitivity to UV when the irradiance used to supply a dose is increased or decreased beyond some point (Sommer *et al.*, 1996; Sommer *et*

al., 1998; Suthaparan *et al.,* 2012; Taylor-Edmonds *et al.,* 2015). Evidence of a pathogen showing greater sensitivity to higher irradiance treatments would mean that ultimately lower doses could be used via a higher irradiance to achieve sufficient pathogen control, and the speed of field units could be increased without risking a crop epidemic.

An understanding of whether *E. necator* shows enhanced sensitivity to different irradiance levels in the application of UV is currently lacking. The identification of where dose reciprocity does not hold for a UV dose that suppresses *E. necator* would reveal whether lower doses, and commensurately faster ground speeds, could be used to achieve adequate suppression of grape powdery mildew in field application.

The objectives of this thesis were:

- to identify the UV-C dose range where *E. necator* growth is suppressed, while minimizing host phytotoxicity; and

- to evaluate the effect of irradiance on pathogen growth (germination, colony expansion, latency) at doses found to effectively suppress pathogen growth; and

- to examine whether *E. necator* behaves in accordance with dose reciprocity within the experimental range of dose and irradiance.

CHAPTER 2 LITERATURE REVIEW

SECTION 1 HOST RESPONSE TO UV EXPOSURE

One of the principal questions related to the use of UV to control powdery mildew is the response of the host plant to the UV exposure. However, many of the agricultural studies of UV application have focused on post-harvest effects in plants, and have shown success in delaying senescence or rot in fruits and vegetables (Maharaj *et al.*, 2010; Obande *et al.*, 2011; Pombo *et al.*, 2009; Pombo *et al.*, 2011; Urban *et al.*, 2016). A smaller number of studies have examined the effects of UV exposure on a number of plant species before harvest (Table 1).

Early testing with UV-C by Gadoury *et al.* (1992) in grapevine was effective in reducing the severity of grapevine powdery mildew, but season-long use resulted in some defoliation, possibly indicative of a phytotoxic UV dose level, (estimated near ~1000 J/m^2)(D.M. Gadoury, personal communication). In contrast, Obande *et al.* (2011) examined two key market features, color and firmness, in tomato plants using preharvest UV-C levels of either 3 kJ/m^2 over 150 sec or 8 kJ/m^2 over 400 sec. Fruits were placed 70 cm from the lamps, but irradiance was not explicitly reported by the authors (calculated: 20 W/m^2). Preharvest exposure on red fruits prolonged firmness at either UV dose compared to the non-exposed control, and the higher UV dose prolonged fruit firmness in the presence of *Penicillium digitatum*. Preharvest treatments exhibited delayed color development in green fruits compared to the non-exposed fruit.

Lead Author	Year	Crop	Single Dose (J/m^2)	Duration	Irradiance (W/m^2)	Cumulative Dose (kJ/m^2)	Phytotoxic Effects
Gadoury	1992	grape	~1000	ND	ND	ND	Defoliation
Obande	2011	tomato	3000 or 8000	150 or 400 sec	(20)	3000 or 8000	None
Darras	2012	geranium 'Victor', 'Glacis'	500 - 1000	ND	ND	(3.5 - 80.0)	10kJ/m^2: height, number of inflorescence reduced
Suthaparan	2012	rose	(144 – 1440)	1 – 2 hr	0.1, 0.2	ND	1.44 kJ/m^2: Leaf area, dry weight, height reduced
Suthaparan	2014	cucumber	(300 - 900)	5-10 min	1.0	(3.3 – 9.9)	600 J/m^2: chlorophyll efficiency reduced
Darras	2015	geranium 'Victor', 'Glacis'	2500	ND	ND	(20)	Temporary reduction in photosynthetic activity
Janisiewicz	2016a	strawberry 'Albion', 'Monterey'	6.18 or 12.36	30 or 60 sec	0.206	(0.087 or 0.198)	None
			(1483 - 4450)	2 – 6 hr	0.206	(1.48 – 4.45)	All: Chlorophyll degradation
Janisiewicz	2016b	strawberry 'Monterey'	12.36	60 sec	0.206	(0.074, 0.371)	None
Oliveira	2016	strawberry 'Camarosa'	500	120 sec	(4.17)	(14.0)	Reduced photosynthesis efficiency, yield
Xu	2017	strawberry 'Albion'	600	60 sec	(10.0)	9.6 – 29.4	All: Increase flower abortion 15 kJ/m^2: deformed fruit

Table 1 Summary of selected studies examining the impacts of UV on plants. Numbers in parentheses are based on calculations from the available information included in each publication. "ND", no data.

Two recent studies of UV-C irradiated strawberry plants with relatively short UV exposures reported no significant host damage compared to non-exposed control plants. Janisiewicz *et al.* (2016a) found no negative impact on weight, shape, or total count of fruit for strawberry plants over 8 weeks in high tunnels with twice weekly UV-C exposures for 30 or 60 sec at 0.206 W/m^2 (single exposure doses of 6.18 and 12.36 J/m^2, respectively). Janisiewicz *et al.* (2016b) reported strawberry plants irradiated twice weekly for 60 sec at 0.206 W/m^2 (single exposure dose of 12.36 J/m^2) produced larger fruits compared to non-exposed controls. In both studies plants were irradiated at a reported distance of 30cm from the UV lamp.

Xu *et al.* (2017) also investigated the impact on fruit yield and quality in UV-C treated strawberry plants, but with a significantly larger dose range and maximum than Janisiewicz *et al.* (2016ab). Plants received cumulative doses of 9.6, 15, or 29.4 kJ/m^2 from single 60-second exposure events of 0.6 kJ/m^2 every 3, 2, or 1 day, respectively, for 7 weeks. Samples were placed 70cm from the lamp, but irradiance was not reported in the study (calculated: 10 W/m^2). UV-C treatment within the tested dose range was associated with a greater number of aborted flowers and misshapen fruit (increasing as the cumulative dose increased), but the maturation time, yield of marketable fruit, and the mean size of fruit was unaffected by UV-C exposure (Table 2).

Treatment	Total flowers	Aborted flowers	% Aborted	Mature time (day)	Total fruits	Deformed fruits	% Deformed	Marketable yield (g)
Control	48.00 ± 7.55	8.33 ± 3.79b	17.4	25.92 ± 0.50	40.00 ± 4.00	7.46 ± 2.29c	18.7	408.64 ± 47.35
Low	58.67 ±11.85	19.33 ± 2.89a	32.9	26.15 ± 0.23	39.67 ± 8.37	8.60 ± 4.57bc	21.7	432.50 ± 65.80
Middle	58.00 ± 3.61	20.67 ± 2.31a	35.6	26.17 ± 1.05	36.33 ± 5.77	18.86 ± 5.93a	51.9	355.39 ± 28.54
High	56.00 ± 12.29	18.00 ± 6.08a	32.1	26.44 ± 0.19	38.67 ± 8.33	18.06 ± 6.36ab	46.7	399.85 ± 45.40

Table 2 Mean values with standard deviation for irradiated strawberry plants. Control, no UV-C irradiation; Low, UV-C irradiation every third day; Middle, UV-C irradiation every second day; High, UV-C irradiation every day. Each UV-C exposure provided 0.6 kJ/m^2. Different letters within a column indicate significant differences. Adapted from Xu *et al.* (2017).

These studies provide evidence that plant physiological changes (e.g., firmness and maturation rate) may occur in response to UV-C exposure though yield and marketable fruit characteristics were unaffected for the doses tested. Oliveira *et al.* (2016), however, contradicted this finding, reporting that UV-C exposure decreased fruit yield of strawberry by 20% with single UV-C doses of 500 J/m^2 over 2min (irradiance was unreported, but calculated as ~4.17 W/m^2).

This difference does not appear related to dose or irradiance level among these four studies, however. In comparison with Oliveira *et al.* (2016), Janisiewicz *et al.* (2016ab) found a positive effect on yield with a dose ~40X and irradiance ~25X smaller, and Xu *et al.* (2017) despite finding more aborted flowers and deformed fruits than controls, found no significant change in yield with larger single and cumulative doses, and larger irradiance. This suggests that among strawberry plants single exposure doses up to 600 J/m^2 supplied with an irradiance equal to 10 W/m^2 may be used without significant damage to fruit, although UV sensitivity is expected to vary for different plant species.

Darras *et al.* (2012) examined plant growth for two cultivars of geranium ('Victor', 'Glacis') irradiated with UV-C. Potted plants were placed ~20-30cm from the lamps and irradiated weekly with UV-C doses ranging from 0.5 - 10 kJ/m^2 (duration of exposure and irradiance were unreported). The impact of UV-C on growth was affected by both environmental conditions across the years of the study, and cultivar. In the first year, 'Victor' produced fewer inflorescences under the highest UV-C dose, but this effect disappeared in the second year. Irradiated 'Glacis' plants produced a greater number of

inflorescences at any level of UV-C dose compared to the control plants in both years. Under higher temperatures and lower PAR (photosynthetically active radiation, 400-700nm), 'Glacis' produced almost triple the number of inflorescences than the control.

The amount of visible light plants are exposed to has previously been shown to play a role in the phytotoxicity of UV exposure in plants. For example, Cen & Bornman (1990) reported *Phaseolus vulgaris* plants under 700 μmol/m^2/s photosynthetic photon flux density and supplemental UV-B (6.17 kJ/m^2/day) showed virtually no growth differences with non-exposed plants, while plants grown under 500 and 230 μmol/m^2/s exhibited smaller mean leaf area (~47% decrease relative to control) and decreased photosynthetic efficiency. Leaves under 700 or 500 μmol/m^2/s were also thicker than non-exposed plants by ~18% and ~20%, respectively, which the authors suggest could have provided some protection against UV damage. Suthaparan *et al.* (2017) also reported similar findings for specific visible wavelengths, lengths of exposure, and time of application in combination with nightly UV treatments (3 W/m^2 for 3 min, calculated dose = 540 J/m^2). Chlorophyll content, leaf area, and shoot dry weight were lowest among plants grown under shorter photoperiods (8 hrs), but while longer days (16hrs) produced better plant growth, it also increased pathogen severity (from 0% up to 6%).

Any inhibition of photosynthesis may hold consequences for fruit yield, thus it's essential to identify at what dose and irradiance levels photosynthesis is inhibited. Darras *et al.* (2015) found geranium plants ('Victor', 'Glacis') irradiated at a single UV-C dose, 2.5 kJ/m^2 (duration of exposure and irradiance unreported), exhibited decreased

photosynthetic efficiency, but within 48hrs after irradiation returned to control levels. Suthaparan *et al.* (2012) found no negative impact in irradiated rose plants on chlorophyll content and photosynthetic efficiency with an irradiance of 0.1 or 0.2 W/m^2 supplied over 1 or 2 hours (calculated dose 144 - 1440 J/m^2). Janisiewicz *et al.* (2016a; 2016b) similarly found no negative impact on photosynthetic efficiency in UV-C irradiated strawberry plants at a dose of 12.36 J/m^2 supplied with an irradiance of 0.206 W/m^2 with twice weekly applications for up to 8 weeks; a single 2hr dose (1.48 kJ/m^2) at the same irradiance degraded chlorophyll in strawberry plants ('Albion', 'Monterey') via Imaging-PAM Chlorophyll Fluorometer System. Oliveira *et al.* (2016) also found a significant, but small decrease in photosynthetic efficiency in 'Camarosa' strawberry plants after irradiated four times at 500 J/m^2 (calculated irradiance 4.17 J/m^2). Taken together these studies do not show enduring inhibition of photosynthesis or damage to chlorophyll for UV doses up to 2500 J/m^2 in some crop species; however, the range of irradiance values used was small, with a maximum of 4.17 W/m^2. While grapevine was not tested for inhibition of photosynthesis in these studies, the expectation is that there is a set of UV doses, which are useful for managing pathogens and do not affect plant processes.

The wavelengths comprising UV-C are generally regarded as more damaging to all organisms than wavelengths comprising UV-B and UV-A. Santos *et al.* (2013) examined 9 different bacterial species under equivalent doses of UV-A, UV-B, and UV-C, and found LD_{50} maximum values 297.5 kJ/m^2 for UV-A, 50.1 kJ/m^1 for UV-B, and 45.0 kJ/m^2. The action spectra of UV in plants has also been studied, but none to date have

included wavelengths below the UV-B range (<280nm), presumably because no UV-C reaches the Earth's surface. Suthaparan *et al.* (2016a) suggested longer wavelengths closer to the UV-B range were superior for plant pathogen suppression due to their smaller phytotoxic effect; however, this group provided no evidence to support this claim. The available data show a trend in plants where greater photosynthetic inhibition occurs as wavelengths grow shorter approaching 280nm (Coohill *et al.,* 1989). Despite the absence of a clear comparison of the phytotoxic effects between UV-B or UV-C wavelengths in plants, there is continued interest in the use of UV-C to enhance plant growth (Urban *et al.*, 2016). Additionally, UV-C lamps currently produce more radiant energy than UV-B lamps due to the mechanism used to produce the spectral distribution. UV-C wavelengths are generated via the process of ionizing mercury in a clear glass tube, whereas UV-B wavelengths are generated by converting these UV energy with phosphors coating the inside of the glass tube, a process which is only ~2% efficient (David Gadoury, *personal communication*). Thus the use of UV-C lamps allows for greater UV irradiance needed to apply UV over short durations.

In summary, we can see a wide variety of methodologies were used to supply UV to plants, with irradiance ranging from 0.206 up to 20 W/m^2 and durations of exposure from seconds to hours long (Table 1). With these three measurements (i.e., dose, irradiance and duration of exposure) it may be possible to reproduce a study testing the effect of UV on an organism, and specifically to vary irradiance for any specific dose to determine whether the outcome changes. Additionally, Teranishi *et al.* (2004) reported plant sensitivity to UV varying over cultivars. At present there are no in-depth studies

evaluating the sensitivity of different grape cultivars to UV exposure, specifically looking at supplemental UV exposure and its impact on yield over the course of a season. This should become a research priority if UV is found to be a viable tool against powdery mildew on a commercial scale. Until then efforts should be made to use the lowest possible doses found to be effective is disease management.

While there is some variability in reports for negative consequences on different plant species that are exposed to UV, the studies discussed above using UV-C and UV-B show doses up to 8000 J/m^2 and irradiance values up to 20 W/m^2 can be safe to use on some crops. For studies in grapevine only single exposure doses below 1000 J/m^2, however, should be considered due to demonstrated phytotoxicity (Gadoury *et al.,* 1992). There is also no consensus on the upper limit of irradiance that is associated with plant damage, and the majority of studies discussed used irradiance <5 W/m^2. Thus future experiments concerned with the time required to supply a given dose (e.g., field application of UV) to grapevines should continue to explore irradiance treatments above 5 W/m^2 to allow for shorter durations of exposure, while only considering the range of doses found to be effective for suppression of *E. necator.*

SECTION 2 PATHOGEN RESPONSE UNDER NATURALLY OCCURRING UV RADIATION

Studies of powdery mildew under solar irradiation provide a foundation when considering how to develop UV management strategies. Willocquet *et al. (*1996) reported the effect of single and multiple day exposures of sunlight on *E. necator*

germination rates and mycelial growth on leaf disks. Treatments were provided from 10AM – 2PM in the Bordeaux region during summer with global radiation values (300-3000nm) averaging 721-758 W/m^2 over an hour; however, measurements of the two remaining UV treatments (acrylic and shade cloth) were not reported. Warmer temperatures increased the differences among treatments. When leaf temperatures were 5 – 10C higher in the first experiment germination rates relative to the control were ~25% for full sun, ~38% for attenuated, and 75% for shade. With lower temperatures germination rates relative to the control for both full sun and attenuated treatments corresponded to ~68%, while the shade treatment corresponded to ~75%. The authors also observed a general trend where germination decreased more with multiple consecutive days of sun exposure than a single exposure. The largest decrease in germination from sunlight corresponded to an exposure immediately following inoculation, while the greatest decrease on mycelial length occurred with an exposure 24hr after inoculation.

Austin & Wilcox (2012) improved upon this 1996 study with the use of full size plants in a research vineyard. After two weeks powdery mildew foliar severity was highest in the interior of grape canopies of grapevines in the shade of adjacent trees (mean severity ~64%, mean daily solar irradiation [400-1100nm] ~12% of full sun treatment), and lowest on leaves fully exposed to the sun (mean severity ~11%). In a follow-up experiment artificial shading was used to evaluate the impact of solar irradiance on fruit cluster severity; double shade cloth reduced 88% of all measured UV radiation with an 80% reduction in solar irradiation between 400-1100nm, and a UV-attenuating acrylic

barrier reduced all measured radiation by 87% with a 9% reduction in solar irradiation between 400-1100nm. Over 5 years in 'Chancellor' vines they reported a mean cluster severity of 17.4% in full sun, 45% under UV-attenuating acrylic, and 53.5% under double shade cloth. This 2012 study complemented their earlier work that showed powdery mildew severity decreased with leaf pruning 2 weeks after postbloom (7-43% reduction relative to control) and with a training system (24% reduction under VSP system relative to Umbrella-Kniffen system) to maximize sunlight exposure (Austin & Wilcox, 2011).

While some plant pathogens have been shown to be negatively impacted when susceptible plants are irradiated with UV prior to inoculation (Buxton *et al.,* 1957; Kunz *et al.*, 2008; Patel *et al.*, 2017), and others have been reported to be able to infect more easily (Norell, 1954), powdery mildews appear to be strongly affected by direct damage from UV. Austin and Wilcox (2012) investigated how solar irradiance affected the severity of powdery mildew on grapevine when reduced (via an acrylic barrier) or provided fully for 7 days prior to inoculation or 14 days after inoculation. The authors found that the highest severity among treatments was found on plants that did not receive full sunlight after inoculation. Interestingly the authors also reported a further decrease in severity for plants receiving full sun after inoculation when sunlight was reduced before inoculation (Table 3). This phenomenon, however, was not discussed by the authors. Suthaparan *et al.* (2012) also reported powdery mildew severity on rose plants irradiated for 1 week prior to inoculation with supplemental UV-B were equivalent

to infected control plants that were never exposed to UV, thus indicating there was no

added benefit of pre-inoculation irradiation in the control of powdery mildew.

Pre-inoculation exposure	Post-inoculation exposure			
	Abaxial severity		Adaxial severity	
	UV-	UV+	UV-	UV+
UV-	38.1 ± 6.0	16.9 ± 4.0	31.0 ± 3.1	1.0 ± 0.4
UV+	38.9 ± 3.7	26.4 ± 0.6	31.5 ± 2.1	2.7 ± 0.7

Table 3 Mean foliar powdery mildew severity +/- standard error on grapevines exposed to UV treatments before and after inoculation. UV-, vines covered with UV-attenuating acrylic; UV+, vines exposed to full sunlight. Pre-inoculation exposures began 1 week prior to inoculation. Post-inoculation exposures began immediately after inoculation and remained for 2 weeks. The post-inoculation treatment was significant ($P < 0.001$). Adapted from Austin & Wilcox (2012).

Relying on sunlight for a management strategy, however, can be limited by the repair mechanisms of pathogens. UV exposure can damage microorganisms in several ways, including the production of cyclobutane pyrimidine dimers and other photoproducts that alter DNA structure and impede subsequent replication and transcription. Microorganisms mitigate this damage with a system of repair enzymes, including photolyases that enable repair of damaged DNA from UV exposure. These photolyases are activated by "blue" wavelengths and UV-A (300-500 nm), in a process called photoreactivation (Thompson & Sancar, 2002). Thus in darkness, this repair system is inactive, and pathogen suppression by UV is ultimately greater (Suthaparan *et al.*, 2012; Suthaparan *et al.,* 2014; Suthaparan *et al.,* 2016; Janisiewicz *et al.* 2016a; 2016b; Taylor-Edmonds *et al.*, 2015).

The severity of powdery mildew, in particular, has been shown to also be affected by visible light. Suthaparan *et al.* (2010) first reported an inhibiting effect of select visible wavebands on *P. pannosa*, the causal agent of rose powdery mildew. Germination rates decreased by ~10% under blue illumination (420 to 520 nm, peak 465 nm), while airborne conidia decreased by 67% under 1-hr of red illumination (620 to 720 nm, peak 675 nm) provided during the night, each in comparison to full-spectrum white light illumination. This was followed by an investigation of the efficacy of nighttime UV-B exposure with short visible wavelengths in *P. xanthii*, the causal agent of cucumber powdery mildew (Suthaparan *et al.*, 2014). UV-B nighttime treatments alone resulted in no fungal colonies forming, and an area under the disease progress curve (AUDPC) <50. Treatments, which included UV-A or blue illumination along with UV-B, showed an increase in the number of successful colonies (to 5 and 7, respectively) and AUDPC (~125 and ~175, respectively). Recently, Suthaparan *et al.* (2017) reported cucumber plants infected with *P. xanthii* and treated with nighttime UV-B followed by 4hr red illumination, exhibited less than 5% severity while also eliminating losses in shoot dry weight, leaf area, and relative chlorophyll content seen when treating infected plants with longer days and nighttime UV-B. Higher amounts of blue light enrichment during the day was associated with small but significant increases in powdery mildew severity under UV-B treatments (2% increase), necessitating larger amounts of UV to maintain disease suppression, while blue light supplied immediately after UV treatments at night increased severity up from ~5% to ~65%. In the context of day light integrals, nighttime UV treatments were less effective on longer days (severity increase from 0-1% to 4-6%).

In summary, there are several studies that have shown a reduction of powdery mildew growth by differential levels of natural solar irradiation. Relying on sunlight alone, however, limits the control mechanism to a set number of daylight hours, natural solar irradiance, and wavelengths that pass through the atmosphere. Additionally, sunlight contains UV-A and short visible wavelengths shown to activate pathogen repair systems and decrease the damage to powdery mildew pathogens. Thus future work in *E. necator* should pursue supplemental UV treatments to avoid the activation of photolyases, thorough an identification of the wavelengths and irradiance values shown to be the most inhibiting for this pathogen.

SECTION 3 POWDERY MILDEW DEVELOPMENT UNDER SUPPLEMENTAL UV

Many of the papers described in Section 1 investigated both plant physiology and pathogen success under supplemental UV exposure. For example, *Botrytis cinerea* germination and hyphal expansion was highly inhibited on strawberry fruit under UV-C exposure at a dose of 12.36 J/m^2 (Janisiewicz *et al.*, 2016a), and the same pathogen on geranium produced smaller lesions at a dose of 500 J/m^2 (Darras *et al.*, 2015). Rose, cucumber, strawberry, tomato and grape powdery mildew severity on susceptible hosts was lowered with UV exposure (Suthaparan *et al.*, 2012; Suthaparan *et al.*, 2014; Suthaparan *et al.*, 2016a; 2016b; Janisiewicz *et al.*, 2016b; Austin & Wilcox 2012).

Supplemental lighting allows for greater control of the wavelengths used in exposure, along with the duration and irradiance of that exposure. Suthaparan *et al.* (2012) first

reported greater efficacy when wavelengths below 290nm were included in UV exposures in the control of rose powdery mildew, caused by *P. pannosa*. Suthaparan *et al.* (2016a) also investigated the action spectra for *Oidium neolycopersici*, the causal agent of tomato powdery mildew. An action spectrum is the biological effect as a function of individual wavelengths (Coohill, 1991). These authors reported that wavelengths between 250 and 280nm, which includes the UV-C range and is often present in the spectral range of UV-B lamps, produced similar negative outcomes in irradiated powdery mildew pathogen tissue for germination, germ tube length, penetration attempt, and secondary hyphal branching, though wavelengths up to 300 nm showed partial inhibition of *O. neolycopersici* at the highest dose tested (615.6 J/m^2/day at 280nm). Studies using UV-B lamps that include a spectral range overlapping in this region of wavelengths may also be considered alongside studies using UV-C lamps for their use in pathogen control, however they emit less energy (as described in Section 1), which limits their use for short duration UV exposure.

Supplemental UV exposure applied at night while there is no visible light present, will avoid the initiation of photoreactivation, and has been shown to control powdery mildew on several hosts. UV-B doses supplied over 2 hours at ~0.2 W/m^2 on rose plants reduced powdery mildew foliar severity from 34% in control plants down to <1% on greenhouse grown roses, or down by a factor of 2.6 with as little as 2 minutes at 1.2 W/m^2 (Suthaparan *et al.*, 2012). Dose was not reported by the authors, but can be calculated in each of these two studies as 1440 J/m^2 and 144 J/m^2. Suthaparan *et al.*, (2014) reported 1 W/m^2 supplied to infected cucumber plants for 10min (calculated dose

600 J/m^2), reduced diseased area of the leaf to 0.57% compared to ~80% diseased leaf area in control plants. The addition of 2 hrs white light illumination increased diseased leaf area to 20-30%. However pathogen suppression is still affected by naturally occurring sunlight. Suthaparan *et al.* (2017) showed how increasing the day length from 8 to 16hr increased powdery mildew severity from ~0% up to 6% (Suthaparan *et al.*, 2017). Thus indicating that plants in greenhouse settings or northern latitudes with longer days, will require larger doses of UV to maintain effective disease suppression.

Additional studies have examined UV-C for its potential in powdery mildew control (Gadoury *et al.,* 2017; Bierman *et al.,* 2017). Buxton *et al.* (1957) studied *E. graminis (syn. Blumeria graminis f. sp. hordeii)* on barley, and reported a UV-C dose of 22 J/m^2 decreased germination to 50%; irradiated spores immediately incubated in 7 hours of daylight recovered to 100% germination for UV doses up to ~65 J/m^2, possibly due to photoreactivation. Janisiewicz *et al.* (2016b) described the lowest effective dose of 12.36 J/m^2 supplied over 60 seconds as effective in controlling powdery mildew infected strawberry plants, without any damage occurring to the plant over 15 weeks of exposure. Suthaparan *et al.* (2016b) in tests of mobile application in greenhouse settings found *P. aphanis* severity was limited to almost nil when a dose as low as 228.3 J/m^2 was supplied nightly. Recently Onofre *et al.* (2018) reported field trials of nighttime UV-C application supplying between ~90 and ~170 J/m^2 once or twice a week resulted in powdery mildew suppression to levels equivalent with commonly used fertilizers without visible signs of plant damage or changes in yield. All of the preceding studies,

whether with UV-B or UV-C, found greater efficacy from the use of UV when accompanied by an immediate dark period.

There is a challenge in comparing and reproducing studies with UV-B and UV-C irradiation due to the level of detail described in experiments. The lack of methodological details leaves open the question of how to apply doses from other studies. UV-B lamps produce variable spectral distributions, with wide bands around their peak (Fig. 1), which can prove challenging in the measurement of dose; an accurate measurement of total energy requires calculating the light integral for the relevant wavelength range and excluding others outside of this range. In comparison, energy from UV-C lamps peaks around 254nm, and the peak is limited to a ~1nm band (Mark Rea, *personal communication*). With the recent knowledge of action spectra for many developmental stages in powdery mildews (Suthaparan *et al.,* 2016a) the inclusion of the spectral distribution of lights used in these studies is critical for replication. Furthermore, though descriptions of lamps used in contemporary studies are more detailed, especially in terms of spectral distribution, than some of the earlier UV work (Buxton *et al.,* 1957), publication works continue to rely on lighting manufacturers to archive and make available this information as older models are phased out, rather than incorporate these lamp specifications into publications directly.
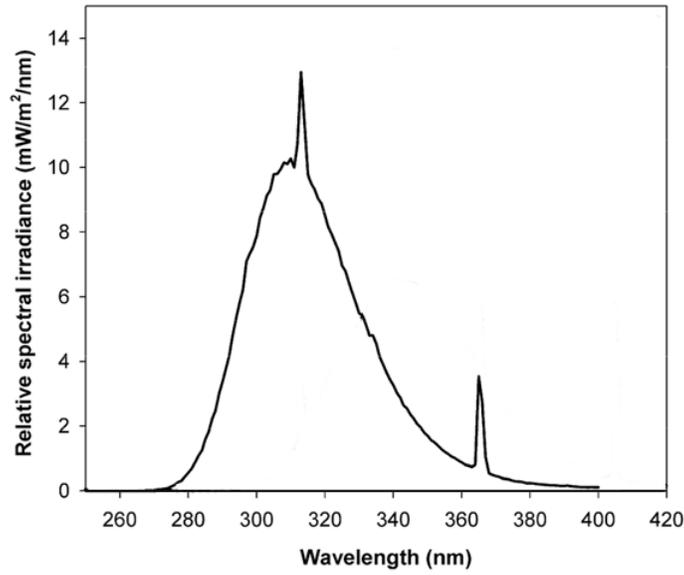
Figure 1 Spectral distribution of UV-B fluorescent tubes (Model UVB-313EL; Q-PANEL Lab Products). Adapted from Suthaparan *et al.* (2014).

Often the challenge of including these methodological details stems from the instrumentation needed for these kinds of studies. The spectroradiometry instruments required for accurate measurements of UV can be prohibitively expensive (e.g., approximately $20,000 in 2018), require careful calibration, and operators should be trained in their use (Bierman *et al.*, 2017). For example, Suthaparan *et al.* (2012; 2014; 2016b) described data on the lighting system used in their studies extensively, but did not report a dose based on irradiance and duration that could inform others trying to reproduce their studies or vary their reported irradiance and times of exposure. Unspecified distances of samples from lamp sources can also prevent the repetition of a study, and inaccurate irradiance measurements may explain reports of aberrant low or high doses needed for disease suppression (Janisiewicz *et al.* 2016a; 2016b). Currently not all published studies of UV in pathogen control include the essential details (e.g.,

spectral distribution, irradiance, duration of exposure) needed to reproduce these studies (Suthaparan *et al.,* 2012; 2014; 2016b; Janisiewicz *et al.* 2016a; 2016b; Austin & Wilcox 2012; Willocquet *et al.,* 1996).

In summary, prior work reporting the wavelengths of UV most inhibiting on powdery mildew pathogens' developmental stages means future work should focus on the range between 250 and 280nm, which includes the spectral range of UV-C lamps. Reported doses not associated with phytotoxic effects, but effective in controlling powdery mildew species ranged from 12.36 J/m^2 up to 600 J/m^2. Thus to avoid any concerns of phytotoxicity, future work with *E. necator* should work well below this upper dose limit.

SECTION 4 THE BUNSEN-ROSCOE PRINCIPLE IN MICROORGANISMS: RECIPROCITY OF IRRADIANCE AND DURATION IN THE APPLICATION OF UV DOSE

A dose of UV radiation is composed of two reciprocal components, irradiance and duration of exposure. The Bunsen-Roscoe principle (hereafter referred to as "dose reciprocity") in the biological context describes how a specific dose of light or UV radiation will produce the same biological response in an exposed organism regardless of how intensely or slowly that dose is given (Sommer *et al.*, 1996). Specifically, if UV dose reciprocity is said to hold at a given dose for powdery mildews, then an irradiated powdery mildew pathogen will respond equivalently to some low irradiance, long duration or some high irradiance, short duration at that dose level. However in reality many organisms show dose reciprocity up to a certain level of irradiance and duration of

exposure. Thus we should determine the UV-C dose where powdery mildew suppression is observed, and investigate whether dose reciprocity holds by manipulating irradiance and durations of exposure in the application of that dose.

Water purification studies offer a foundation to explore where dose reciprocity has broken down among microbes. Sommer *et al.* (1996) found survival rates of the bacteria and virus, *B. subtilis* spores, *E. coli* ATCC 25922, and *S. aureus* phage, were unaffected by the irradiance used (0.02, 0.2, and 2.0 W/m^2) to supply a dose within the range of 20-500 J/m^2. The survival of *S. cerevisiae* yeast strains, however, were more negatively impacted by lower irradiances than higher irradiances at a given dose. This phenomenon was only present in vegetative yeast cells (i.e., not in yeast spores), which increased in magnitude as larger doses of UV-C were supplied. However, a follow-up study, however, contradicted the *E. coli* ATCC 25922 findings, with survival rates decreasing with higher irradiances between 80 and 100 J/m^2 (Sommer *et al.*, 1998). Despite an identical methodology, no explanation was provided by the authors to explain this discrepancy. Zahl *et al.* (1939) reported that dose reciprocity held for *A. niger* fungal spores on cellophane strips for an equivalent UV-C dose supplied with an irradiance ranging from 0.88 W/m^2 up to 14.1 W/m^2 (one meter away from the light source irradiance was reported as ~0.25 W/m^2), whereas Taylor-Edmonds *et al.* (2015) reported greater inactivation in this species under higher irradiance treatments (1.1 vs. 0.22 W/m^2) beginning at 1.0 kJ/m^2 UV-C (Fig 2).
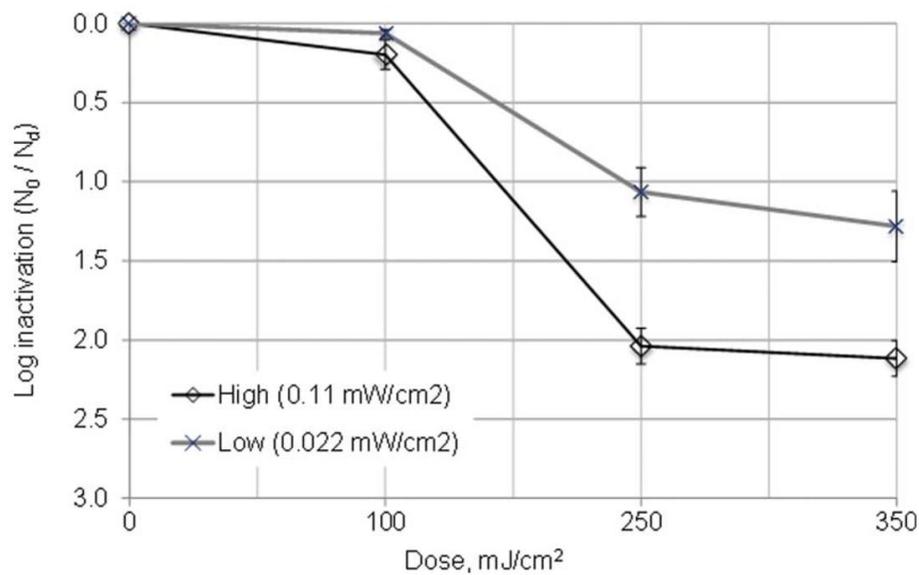
Figure 2 Taylor-Edmonds *et al.* (2015). *A. niger* spores did not behave in agreement with dose reciprocity above 1 kJ/m^2, where the higher irradiance (1.1 W/m^2) treatment resulted in a lower survival rate than the lower irradiance (0.22 W/m^2) treatment.

In powdery mildew studies the question of dose reciprocity has been raised infrequently. Willocquet *et al.* (1996) performed experiments with *E. necator* on leaf disks under simulated natural conditions and found no difference in germination or mycelial length between two irradiance treatments (280 – 320nm, 1.3 W/m^{2}·over 4 hours vs 0.7 W/m^2 over 8 hours) for a similar dose (dose was unreported, but calculated as 18.72 kJ/m^2 and 20.16 kJ/m^2, respectively)(Fig 3). However this study used filters that blocked all wavelengths shorter than 295nm, thus filtering out the majority of the key wavelengths based on the reported action spectra for powdery mildew pathogens.
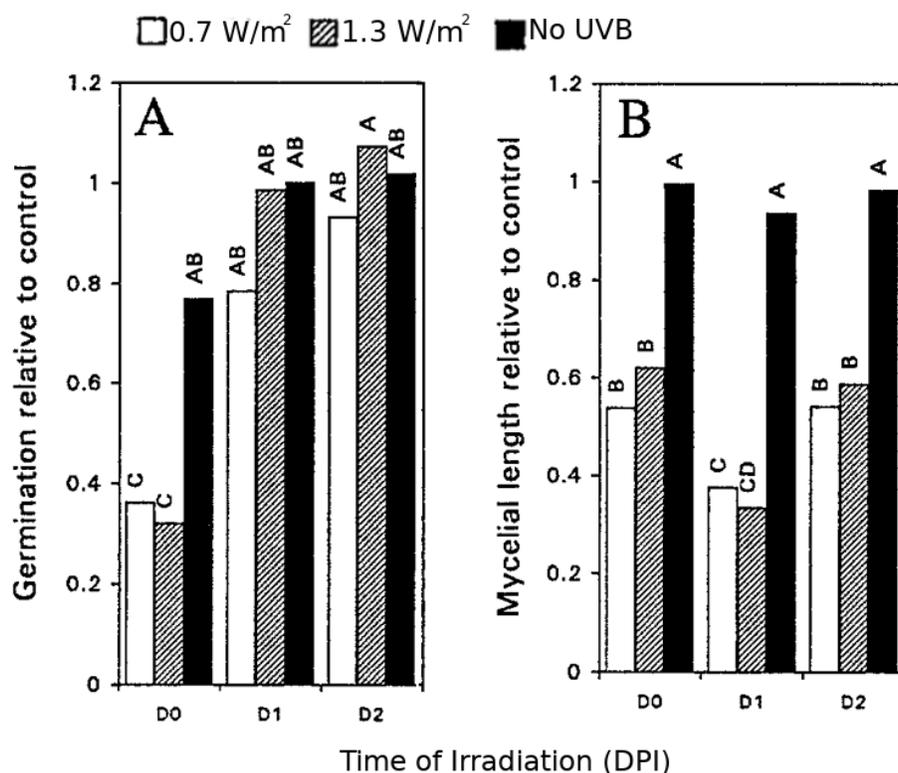
Figure 3 Effects of UV dose (~18-20 kJ/m^2) on *E. necator* at two irradiance levels. Treatments were grouped by the number of days after inoculation that irradiation treatments occurred (i.e., immediate, 24hrs, 48hrs post inoculation). Different letters above bars within a single irradiation time indicate significant difference in the mean values (P = 0.05). The effect of irradiance on germination (A) and maximum mycelial length (B) were examined. Adapted from Willocquet *et al.* (1996).

Suthaparan *et al.* (2012) found a small, significant difference in germination of *P. pannosa* on rose at a calculated dose of 720 J/m^2. Germination rate was higher when using a higher irradiance (0.2 W/m^2 for 1 hour; mean 3.5%) than a lower irradiance UV- (0.1 W/m^2 for 2 hours; mean 11.3%), but there was no difference in hyphal branching (Table 4). Suthaparan *et al.* (2016b) expanded on this previous experiment by looking at

how breaking up UV-B treatments across multiple days or hours within a single night affected the efficacy of powdery mildew control, but found dose reciprocity held in powdery mildew foliar severity for irradiance treatments of 1.6 and 0.8 W/m^2 (calculated cumulative dose/week 2016 J/m^2, single exposure minimum 96 J/m^2, maximum 864 J/m^2); efficacy of UV-B treatments was not clearly affected by breaking up the dose into various treatments (Fig 4).

Treatment	Calculated Dose	Germination (%)	Branched hyphae (%)
No UV-B	0	33.3 ± 2.6 a	13.5 ± 1.8 a
1 hr * 0.1 W/m^2	360	11.3 ± 1.9 b	2.0 ± 1.1 b
1 hr * 0.2 W/m^2	720	3.5 ± 0.9 c	0.3 ± 0.2 b
2 hr * 0.1 W/m^2	720	11.0 ± 1.5 b	1.3 ± 0.5 b
2 hr * 0.2 W/m^2	1440	5.7 ± 0.7 bc	0.2 ± 0.2 b

Table 4 Impact of UV-B exposure on *P. pannosa* on rose plants. Different letters within a column indicate significant difference in mean value (P = 0.05). Adapted from Suthaparan *et al.* (2012).
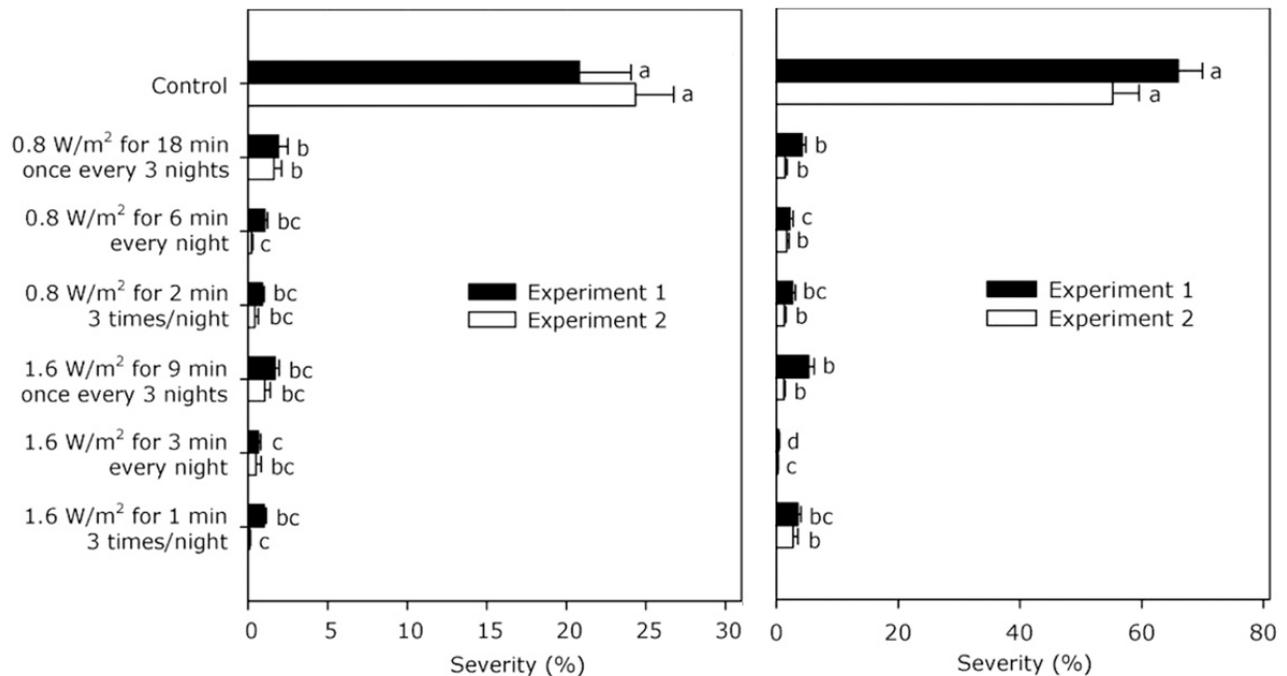
Figure 4 Impact of UV-B exposure across multiple application regimes. Severity was reported for *P. aphanis* on strawberry (left) and *G. biocellatus* on rosemary (right). Calculated UV doses applied were (1) 96 J/m² 3 times per night, (2) 288 J/m² per night, or (3) 864 J/m² applied once every 3 nights. For one pathogen within an experiment, different letters to the right of bars indicate significant differences in mean values (P = 0.05). Adapted from Suthaparan *et al.* (2016b).

It is assumed that powdery mildew pathogens are unable to rapidly adapt to increased radiation. These pathogens have no demonstrated ability to migrate away from UV radiation. Through normal radial growth on susceptible tissue, however, a powdery mildew colony may extend into more shaded areas where pathogen growth is enhanced (Austin & Wilcox, 2012). Additionally, pigmentation has only been documented for powdery mildew species in the sexual fruiting bodies, thus the mycelium and conidia should not be able to readily shield UV radiation.

All of the studies in powdery mildews tested a maximum irradiance range approximating a factor of 2. Among the microorganisms discussed above where dose reciprocity broke within the inhibitory UV dose range, studies tested irradiance treatments varying by a factor of 5 to 100. Due to the constraints of what field application of UV will require (i.e., faster speed of mobile UV units and shorter total application time required), future work in *E. necator* should investigate whether dose reciprocity can be shown to break down using a higher maximum irradiance value to evaluate whether UV doses in the field can be lowered.

CHAPTER 3 METHODOLOGY

SECTION 1 LIGHTING

In all experiments, two low-pressure Mercury discharge lamps (model: OSRAM HNS G13 55W UV-C, peak 253.7 nm, FWHM << 5nm, Fig 5) were used to provide UV-C doses. An enclosed tower was constructed, which consisted of a sample holding area approximately 220 cm tall (Fig 6). Positioned between this holding area and the lamps was a manual shutter mechanism, which could be opened and closed to attenuate UV radiation reaching the samples. Elimination of all UV transmission was achieved with the use of Petri lids over samples in conjunction with the shutter (determined experimentally). Irradiance measurements were conducted by calculating the radiometric value between 240 and 280 nm with a calibrated Giga-Hertz Optik UV spectroradiometer (model: BTS2048-UV). The shutter remained closed during the 2.5 minute warm-up period prior to each experiment, and was manually opened to expose samples.

The level of irradiance was controlled by two actions: (1) changing the sample's distance away from the lamps, and (2) layering non-reflective screening between the lamps and shutter mechanism. In the first experiment across a range of doses, heights were calibrated before the start of the first experiment (Table 5). Irradiance levels were verified to be within 10% of the calibrated level without attenuation at a distance of 25cm from the lamp prior to each day's experiments. In the second experiment examining a dose of 120 or 210 J/m^2, irradiance was measured as ~30 W/m^2 without attenuation and ~0.3 W/m^2 with attenuation at a distance of 21.5cm from the lamp.

Irradiance levels were verified to be within ~10% of the desired level prior to each day's

experiments.

Height from lamps (cm)	Calibrated irradiance (W/m^2)
15	50
25	25
45	10
85	4
165	1.5
220	1.0

Table 5 Calibrated irradiance values for the first experiment of germination.

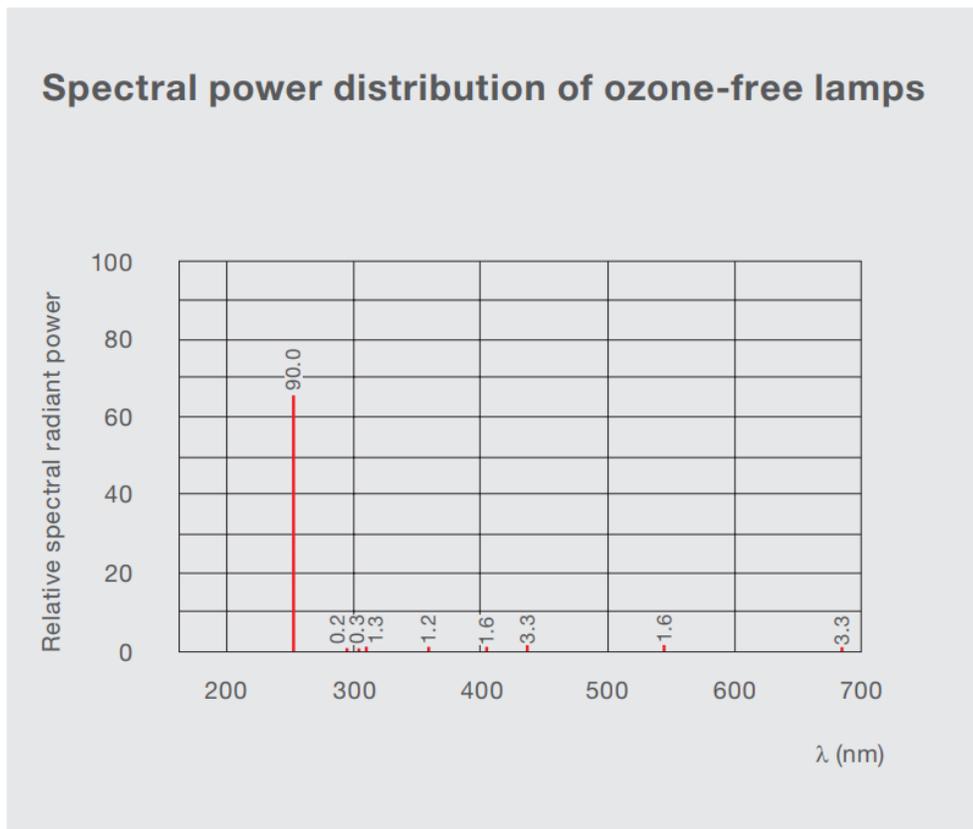Figure 5 Spectral power distribution for OSRAM HNS G13 55W. Data obtained from OSRAM. The lamps produced a near monochromatic peak centered on ~254nm.
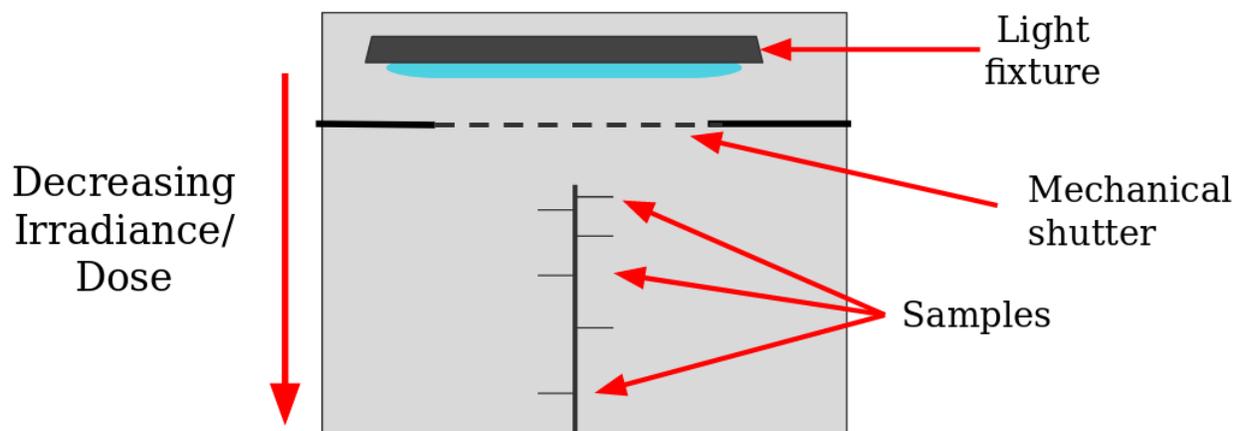
Figure 6 Illustration of UV-C experimental tower. Two UV-C bulbs at the top of the tower were separated from the sample holding area by a mechanical shutter. Samples were placed at multiple distances away from the lamp to provide the UV dose range.

SECTION 2 PLANTS

Leaf material was sourced from disease-free 'Chardonnay' grapevines grown either in a greenhouse or at a nearby research vineyard from unsprayed panels early in the growing season (May-June). Target temperatures for the greenhouse environment were 70F during the day and 65F during the night. Greenhouse vines were also irradiated each night Monday through Friday with 6 minutes of UV-B at a mean irradiance of 1.70 $\pm$ 0.06 W/m^2 at bench level. This was sufficient to reduce issues associated with accidental powdery mildew infections among clean plant stocks, while not producing any visible negative effects in the plants.

SECTION 3 INOCULUM

Clonal isolates of *E. necator* ("NY1-137" and "NY19", described by Feechan *et al.*, 2015) were maintained on susceptible detached leaves of 'Chardonnay' grapevines in growth chambers set to a 15-hr day length beginning at 0800 with 50% relative humidity, and 23C. Inoculum was transferred to new detached leaves once every two weeks in a sterilized transfer hood.

SECTION 4 GERMINATION OF *ERYSIPHE NECATOR* EXPOSED TO UV-C

Six UV-C doses ranging between 4 and 200 J/m^2 were applied to *E. necator* conidia by placing samples at predetermined heights away from the UV lamps and exposing samples for either 4 or 400 seconds (Table 6). These doses were selected based on the lack of phytotoxic effects seen in previous studies described in Chapter 2, and what the experimental apparatus would allow for. Samples were arranged to avoid overlap of

slides holding the samples. In the first experiment, the 4-second treatments used irradiance values of 1 - 50 W/m^2, and the 400-second treatments used irradiance values of 0.01 - 0.50 W/m^2. These values were selected due to the minimum and maximum allowable irradiance in the experimental apparatus. Each UV-C dose within this range was tested on three ~1-cm areas marked on glass slides.

Dose (J/m^2)	Duration of Exposure (sec)
200	4, 400
100	4, 400
40	4, 400
16	4, 400
6	4, 400
4	4, 400

Table 6 Summary of UV-C doses tested for two independent durations of exposure.

Subsequent experiments tested a single dose of either 120 J/m^2 or 210 J/m^2, with irradiance levels of 30 W/m^2 or 0.3 W/m^2 at a single distance away from the UV lamps over 4 and 400 seconds, and 7 and 700 seconds, respectively. In these experiments 40 1-cm leaf disks were held across two 1% agar plates for each experimental treatment and 20 1-cm leaf disks on agar plates for the non-exposed control treatment.

The proportion of germinated conidia was calculated on glass in the first experiments or 1-cm leaf disks in subsequent experiments. Spores were transferred from sporulating colonies by gently touching the fungal colony with a sterilized, dry artist's paintbrush.

For the first experiment covering a range of doses in a single trial, treated samples were placed in darkness for 24hrs before mounting with 50% glycerol or dH_2O and viewing unstained. For subsequent experiments examining a single dose level, treated samples were placed in darkness for 48 hrs before clearing the leaf disks with solution (3:1 95% EtOH:glacial acetic acid), staining with 0.5% Chlorazol Black E in 80% lactic acid and glycerol for 2.5hrs, and mounting with 50% glycerol.

In all experiments, spores were examined at 200X magnification under bright field microscopy. Germination was defined as any spore that exhibited (a) germ tube greater than or equal to the length of the spore, and/or (b) a lobed appressorium irrespective of germ tube length. Counts included the first 30 to 100 spores within an observed inoculation area (either a demarcated area on glass slides or leaf disk). Observations began on the distal edge of an inoculated area. Experiments were repeated 3 times, except experiments at 120 J/m^2, which were repeated twice.

SECTION 5 COLONY EXPANSION IN *ERYSIPHE NECATOR* EXPOSED TO UV-C

The methodology was adapted from Gadoury & Pearson (1991). Inoculation was performed as described in Section 4. Twelve leaves were placed into double Petri containers with a reservoir of water, and randomly assigned to an irradiance or control treatment. Each leaf was inoculated in several discrete areas to account for failed infection caused by non-experimental factors; a maximum of 2 colonies was measured per leaf. To avoid any effect from germination, irradiation was withheld until 48hrs after

inoculation when colonies were irradiated with 210 J/m^2 UV-C once at either 0.3 or 30.0 W/m^2 (700 and 7 seconds, respectively).

Fungal colony diameters were measured as the mean of a single vertical and horizontal measurement (mm) using the Olympus cellSens Entry 1.17 digital measuring tool. Initial measurements took place at 7 dpi, which coincided with colonies visible to the naked eye, and were compared to final measurements at 14 dpi. Any final measurements, which indicated negative growth were assumed to be caused by an error in initial measurements and counted as zero growth. The mean daily growth rate was calculated by dividing the difference in diameter between initial and final mean measurements by 7 days. This experiment was repeated 3 times.

Colonies that failed to germinate (n=2) or exhibited contamination on the final day of measurements (n=1) by other microorganisms were removed from the analysis.

SECTION 6 LATENCY PERIOD OF *ERYSIPHE NECATOR* EXPOSED TO UV-C

Twelve detached leaves were placed into double Petri containers, inoculated as described in Section 4, and assigned to a treatment group and irradiated as described in Section 5.

McCann *et al.* (2013) showed a peak in *E. necator* conidia maturation (i.e., the capacity to separate from the conidiophore) starting approximately 5 hours after light exposure. To reflect the time requirement needed by the fungus to produce mature detachable

conidia, colonies were inspected between 5 and 7 hours after light exposure began in growth chambers once every two days under a dissecting microscope (magnification up to 32X). Due to the mode of data collection every two days, any difference of less than 2 days would not be captured. These observations began at 7dpi based on the appearance of a fungal colony visible with the naked eye.

All fungal colonies which had not produced spores by the final day of observations (13 dpi) were removed from the final colony count. This experiment was repeated 3 times.

SECTION 7 STATISTICAL ANALYSIS

For all experiments that examined germination response, a Wald Chi-Square test was conducted in R to determine the significant parameters for each dataset ($P<0.05$). Latency and colony expansion experiments were analyzed using ANOVA and post-hoc mean comparison was conducted using Tukey's HSD at $P = 0.05$.

CHAPTER 4 RESULTS

SECTION 1 GERMINATION

The first experiment examined a range of six UV-C doses across two exposure times, 4 and 400 seconds (Table 6). Within each treatment a large amount of variation was observed across the three experimental repeats for doses under 100 J/m^2 however experimental repeat was not found to be significant ($P > 0.1$). Among the possible factors and interactions of experimental repeat, duration of exposure, and dose, only dose was found to be significant ($P < 0.01$)(Table 7); increasing the UV-C dose increased the odds of reduced germination (Fig 7; 8). This trend became apparent above 16 J/m^2. Spores germinating at higher doses (100 and 200 J/m^2) generally only produced appressoria from extremely short germ tubes (i.e., less than half the length of the spore), whereas spores germinating without any UV exposure and at lower UV doses (<100 J/m^2) were observed with appressoria arising from either shorter germ tubes, as seen under higher doses, or from longer germ tubes that would fit the criteria for germination (Fig 9).

	Wald Chi Square		
Response: Germination	LR Chisq	df	Pr(>Chisq)
Experimental Repeat	4.0016	2	0.14
Duration of Exposure	0.3244	1	0.57
Dose	25.1544	6	<0.01
Experimental Repeat * Duration of Exposure	0.1537	2	0.93
Experimental Repeat * Dose	1.2347	12	1.00
Duration of exposure * Dose	0.4160	6	1.00
Experimental Repeat * Duration of Exposure * Dose	0.5066	12	1.00

Table 7 Analysis of Deviance for germination proportion using GLM in R. Conidia of *E. necator* were irradiated from 4 to 200 J/m^2 of UV-C either over 4 or 400 seconds.

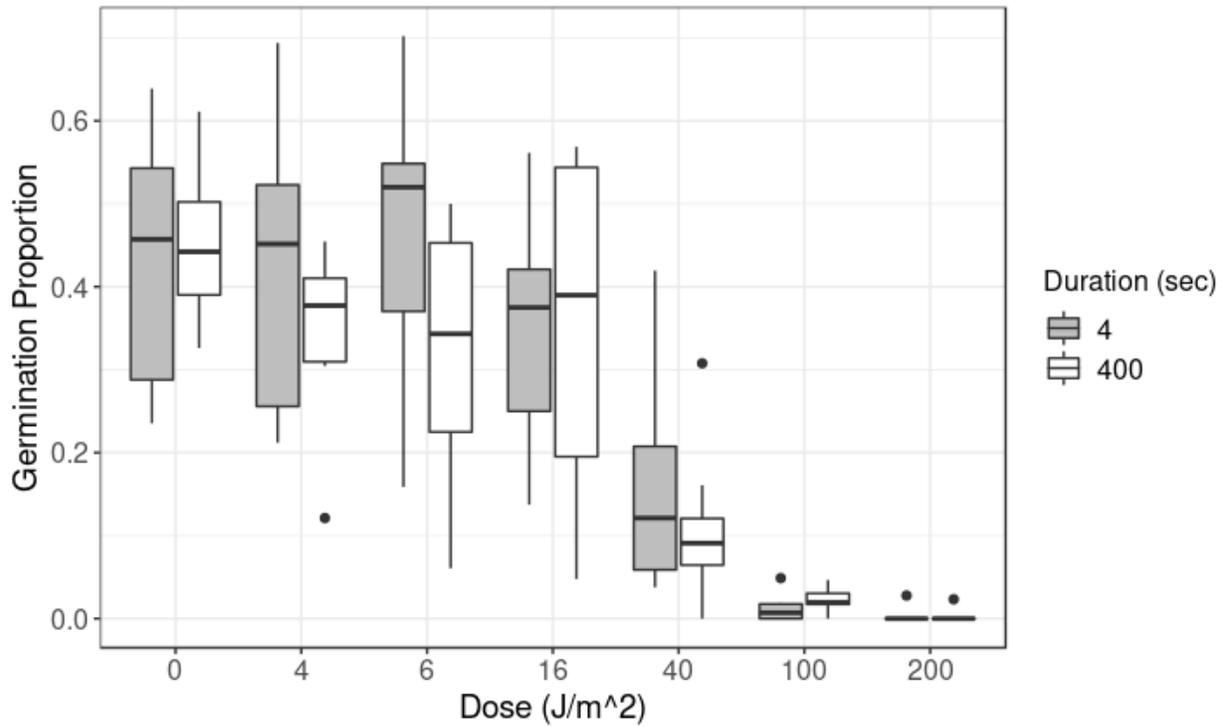Figure 7 Boxplots illustrating the response of *E. necator* spores to UV-C dose at two different durations. The range of doses supplied over 4 seconds had irradiance values ranging from 1 - 50 W/m^2. The range of doses supplied over 400 seconds had irradiance values ranging from 0.01 - 0.50 W/m^2. Dose was found to be the only significant factor ($P < 0.01$). This experiment was repeated 3 times.

Dose	Duration of Exposure (sec)	Standard Deviation	Coefficient of Variance	Duration of Exposure (sec)	Standard Deviation	Coefficient of Variance
0	4	0.16	37.0	400	0.1	21.5
4	4	0.16	37.8	400	0.11	32.7
6	4	0.18	40.8	400	0.15	45.5
16	4	0.13	39.1	400	0.2	56.4
40	4	0.12	77.7	400	0.09	85.3
100	4	0.02	137.2	400	0.01	63.18
200	4	0.01	282.8	400	0.01	300.0

Table 8 Standard deviation and coefficient of variance measurements across the tested
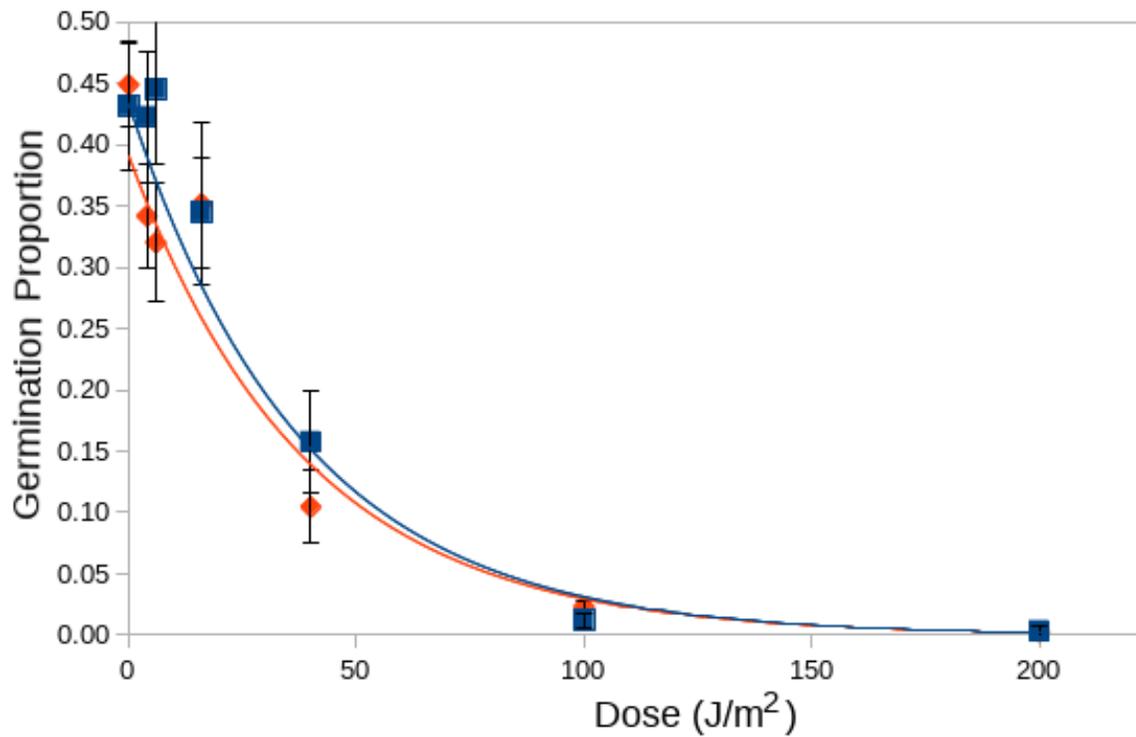
dose range.

Figure 8 Summary of germination rates. Each symbol represents the mean of 3 experimental repeats at each dose within an exposure. Blue squares represent 4 second treatments; fitted curve, $y = 0.39^{(-0.03x)}$. Red rhombuses represent 400 second treatments; fitted curve, $y = 0.43^{(-0.03x)}$. Error bars represent the standard error.
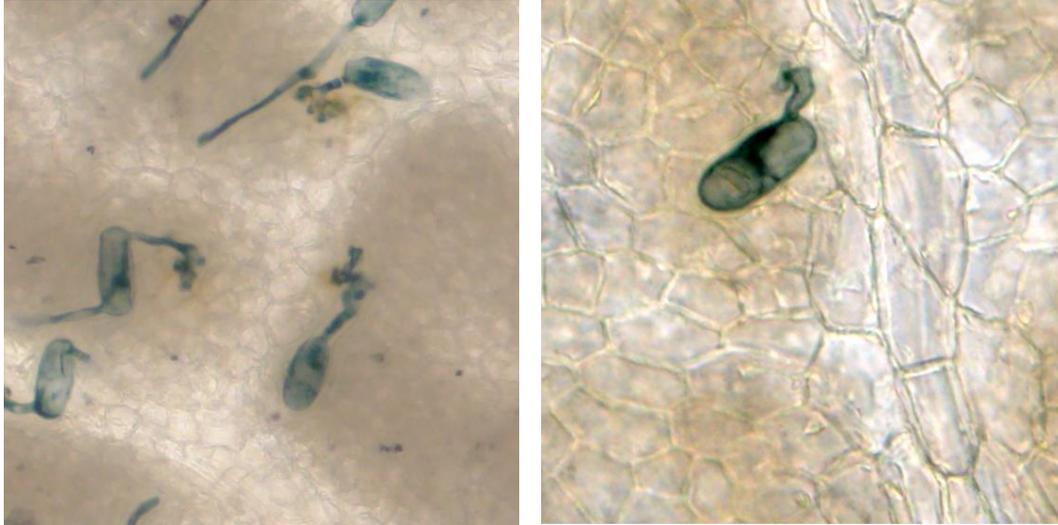
Figure 9 (Left) Conidia not exposed to UV-C at 200X showing both long germination tubes and appressoria. (Right) Conidium irradiated at 210 J/m^2 at 400X showing an appressorium arising from a short germ tube.

Two subsequent experiments investigated the relationship between irradiance and germination at a single dose (120 or 210 J/m^2) using a larger sample size. At 120 J/m^2 no significant factors were identified (Table 9, 10).

Dose (J/m^2)	Duration of Exposure (Sec)	Irradiance (W/m^2)	Mean Germination Proportion (relative to Control)
120	4	30	0.11 ± 0.07
120	400	0.30	0.18 ± 0.09

Table 9 Mean germination proportion (+/- standard deviation) for two pooled experiments at a dose of 120 J/m^2 UV-C supplied via 30 or 0.30 W/m^2 irradiance.

	Wald Chi Square		
Response: Germination	LR Chisq	df	Pr(>Chisq)
Experimental repeat	0.05	1	0.82
Irradiance	1.47	1	0.22
Experimental repeat * Irradiance	0.33	1	0.47

Table 10 Analysis of Deviance for germination proportion using GLM in R. Conidia of *E. necator* were irradiated with 120 J/m^2 of UV-C at 0.3 or 30 W/m^2.

Two isolates were examined at a UV dose of 210 J/m^2. Irradiance was the only significant factor (P<0.05), with experimental repeat, isolate, and all interactions among these factors non-significant (Fig 10)(Table 11).
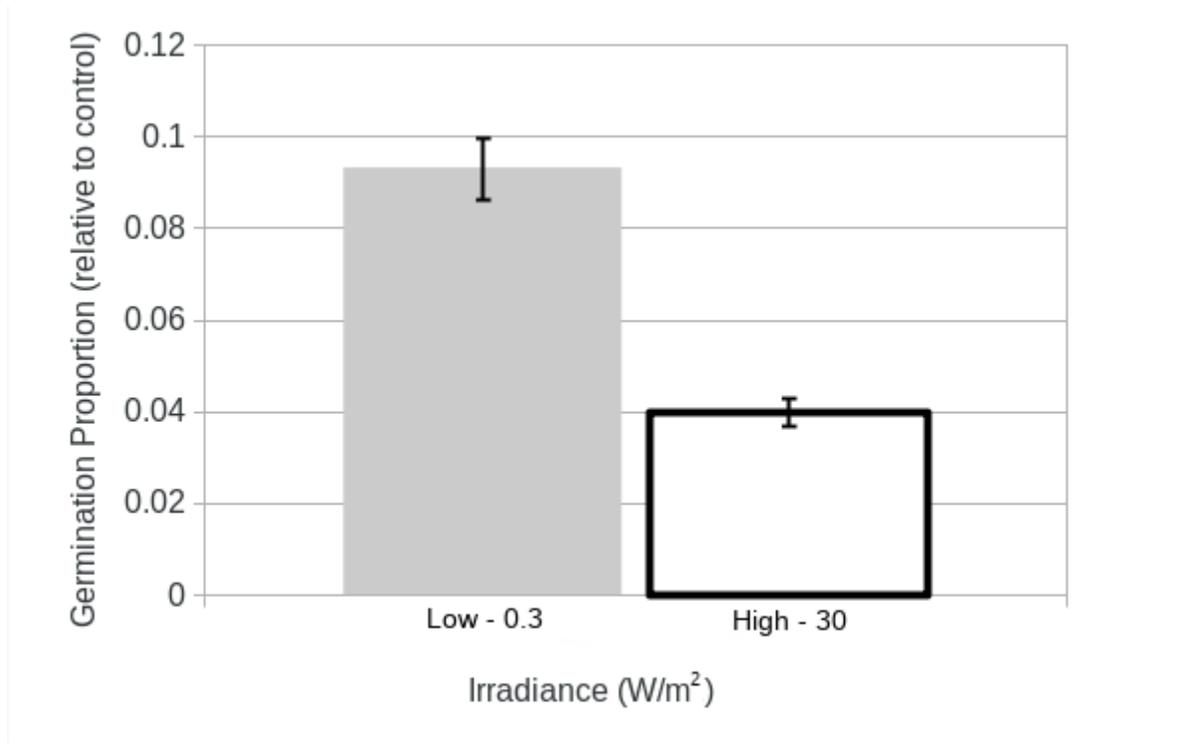
Figure 10 Mean germination success under 210 J/m^2 supplied at 30.0 or 0.30 W/m^2 pooled across two isolates, each with 3 experimental repeats. Irradiance was significant (P = 0.02), indicating the higher irradiance at this dose level inhibits germination more than the lower irradiance.

	Wald Chi-Square		
Response: Germination	LR Chisq	df	Pr(>Chisq)
Isolate	1.52	1	0.22
Experimental repeat	0.17	2	0.92
Irradiance	5.36	1	0.02
Isolate * Experimental repeat	2.71	2	0.26
Isolate * Irradiance	0.11	1	0.74
Experimental repeat * Irradiance	0.09	2	0.96
Isolate * Experimental repeat * Treatment	0.04	2	0.98

Table 11 Analysis of Deviance for germination proportion using GLM in R. Conidia of *E. necator* were irradiated with 210 J/m^2 of UV-C at 0.3 or 30 W/m^2.

SECTION 2 COLONY EXPANSION

Initial colony measurements had a standard deviation of ~19% of the mean. The interaction between experimental repeat and irradiance had a significant effect on the daily mean growth of *E. necator* (P<0.01; Table 12). As a result experimental repeats were analyzed individually. In the first experimental repeat both high and low irradiance treatments were significantly different (P<0.01) from the control, but not significantly different from one another (Table 13); in experimental repeats 2 and 3 the differences among the three treatments was non-significant.

	ANOVA				
Response: Mean Daily Growth	df	Sum Sq	Mean Sq	F value	Pr(>F)
Irradiance	2	0.45	0.23	4.73	0.01
Experimental Repeat	2	0.95	0.48	10.00	<0.01
Irradiance * Experimental Repeat	4	0.93	0.23	4.90	<0.01
Residuals	60	2.86	0.05		

Table 12 ANOVA output for colony expansion rate of *E. necator* colonies exposed to

210 J/m^2 UV-C 48hrs after inoculation.

	Daily Mean Growth (mm)		
Treatment	**Experiment 1**	**Experiment 2**	**Experiment 3**
High	0.29 ± 0.12a	0.50 ± 0.06a	0.67 ± 0.07a
Low	0.34 ± 0.04a	0.36 ± 0.11a	0.63 ± 0.07a
Control	0.80 ± 0.06b	0.34 ± 0.08a	0.75 ± 0.07a

Table 13 Daily mean growth (mm) ± standard error of *E. necator* colony diameters.

Fungal colonies were exposed once 48hrs post inoculation with a dose of 210 J/m^2 at 30

W/m^2 ("High"), 0.30 W/m^2 ("Low"), or not exposed ("Control"). Different letters within an

experiment column indicate statistical difference at P = 0.05.

SECTION 3 LATENCY

Experimental repeat and irradiance were both found to be significant (P<0.05; Table 14). As a result experimental repeats were analyzed individually. Among the first two experimental repeats, no statistical difference was observed between either UV treatment and the control for mean latency. In the third experimental repeat both high and low irradiance treatments significantly differed from the non-exposed control leaves (P<0.01), however the irradiance treatments were not statistically different from one another (P = 0.62; Table 15).

Response: Mean Daily Growth	ANOVA				
	df	Sum Sq	Mean Sq	F value	Pr(>F)
Irradiance	2	24.23	12.11	8.88	<0.01
Experimental Repeat	2	17.59	8.79	6.45	<0.01
Irradiance * Experimental Repeat	4	7.54	1.89	1.38	0.25
Residuals	51	69.57	1.36		

Table 14 ANOVA output for Mean Daily Growth of *E. necator* colonies exposed to 210 J/m^2 UV-C 48hrs after inoculation.

	Latency Period (Days)		
Treatment	Experiment 1	Experiment 2	Experiment 3
High	10a	10a	10a
Low	10a	11a	10a
Control	9a	10a	8b

Table 15 Mean latency period for *E. necator* colonies irradiated with a ~210 J/m^2 dose. Colonies were irradiated with 30 W/m^2 ("High"), 0.3 W/m^2 ("Low"), or were not exposed ("Control"). Any colonies, which had not sporulated by the final day of observations were not counted. The experiment was repeated three times. Different letters within a column indicate statistical significance at P = 0.05

Out of 72 observed colonies among the three experimental repeats, 4 colonies failed to germinate completely, while eight colonies showed only minor hyphal growth on the final observation date (four from 30 W/m^2 treatments, three from 0.3 W/m^2 treatments, and one from control treatments). These 12 colonies were excluded from the analysis.

CHAPTER 5 DISCUSSION

SECTION 1 RECIPROCITY OF UV IRRADIANCE AND DURATION OF EXPOSURE

WITHIN A RANGE OF EFFECTIVE DOSE

The results presented here, while variable across experiments, suggest that dose reciprocity may begin to break down above 100 J/m^2 when supplied at an irradiance of 30 W/m^2. This phenomenon, however, was dependent on the aspect being measured among germination, colony expansion, and latency. In all cases of statistical significance and in most non-significant trends, higher irradiance was more damaging to *E. necator* than lower irradiance at doses greater than 100 J/m^2.

A range of six UV-C doses (4-200 J/m^2) was applied to *E. necator* spores at an irradiance ranging from 0.01 W/m^2 up to 50 W/m^2 to evaluate if UV-C dose reciprocity held in this powdery mildew species for exposure lasting 4 and 400 seconds. This range was selected to avoid phytotoxicity, as discussed in Chapter 2, and provide an adequate level of disease suppression. Subsequent experiments exploring UV dose reciprocity behavior in this fungus sought to further investigate the germination behavior with an increased treatment sample size at the higher range of doses, through testing two specific doses, 120 and 210 J/m^2, at an irradiance of either 0.30 or 30 W/m^2 for germination. Additional experiments examined latency and colony expansion at this higher dose to determine if dose reciprocity held at the same dose and irradiance levels used in germination tests.

The primary experiments testing a range of UV-C doses illustrated the effect of increasing dose on germination rates of *E. necator* conidia. The data revealed a threshold beginning above 16 J/m^2 where germination rates decreased rapidly, although with pronounced variability across the majority of the dose range tested (Fig 7). For germination rates, the data show UV-C dose reciprocity held for doses up to 200 J/m^2 (with 3 leaf disks per treatment; Fig 7; Fig 8; Table 7). The coefficient of variance for each dose increased as the dose level increased, in part due to the rapid decline in germination to almost nil above 40 J/m^2 (Table 8).

During subsequent experiments that tested if dose reciprocity held at the two highest doses (with 40 leaf disks per treatment), however, dose reciprocity broke down at the highest dose tested (210 J/m^2; Fig 10). Specifically, the higher irradiance, shorter duration treatment (30.0 W/m^2 * 4 sec) was more effective (mean = 4%) than the lower irradiance, longer duration treatment (0.30 W/m^2 * 400 sec) (mean = 9%). This trend held for both isolates of powdery mildew tested. It's possible that reciprocity appears to hold at lower doses because of a relationship between accumulated damage and the rate of energy supplied. Similar to the current work, Taylor-Edmonds *et al.* (2015) described a shoulder in their study of *A. niger*, specifically dose reciprocity held for doses less than 1 kJ/m^2.

Colony expansion rate was largely unaffected by UV exposure at 210 J/m^2. The lack of consistent difference between exposed and control plants in colony expansion suggests two possibilities: (1) a higher dose is required to suppress growth occurring after

germination, or (2) colony expansion as a measurement does not adequately capture the whole colony consequences of UV exposure at this dose. Measurements using this method were difficult to record due to leaf topology and the challenge of illuminating hyphal strands on the surface of the leaves. Additionally measuring the diameter of fungal colonies likely does not capture the full impact of UV exposure; within an irradiated colony, even if only a few spores survive and produce hyphae, the diameter will increase overtime. Measurement variables which capture this difference should be used in any future tests.

The consequences of UV exposure on colony expansion is linked to the latent period for a powdery mildew pathogen. Gadoury *et al.* (2011) showed that sporulation occurs in *E. necator* once a threshold of vegetative growth is reached, thus if colony expansion is not affected by the UV dose applied, it is unlikely that latency will be affected either. Austin & Wilcox (2012) reported latency increasing linearly under UV-B irradiation at doses starting at 64.8 kJ/m^2, however, in the current study there was no effect of high or low irradiance on latency at a dose of 210 J/m^2. The pathogen in this developmental stage may exhibit greater resilience to UV exposure, and explain why Austin & Wilcox (2012) observed increasing latency at much higher UV doses than that tested in this work. Additionally, these results should be interpreted cautiously because data collection was limited to every 2 days due to experimental constraints. *E. necator* produces a single conidium per conidiophore per day, thus single day delays as a result of UV treatments, may have occurred, but would have been obscured by the lack of daily measurements.
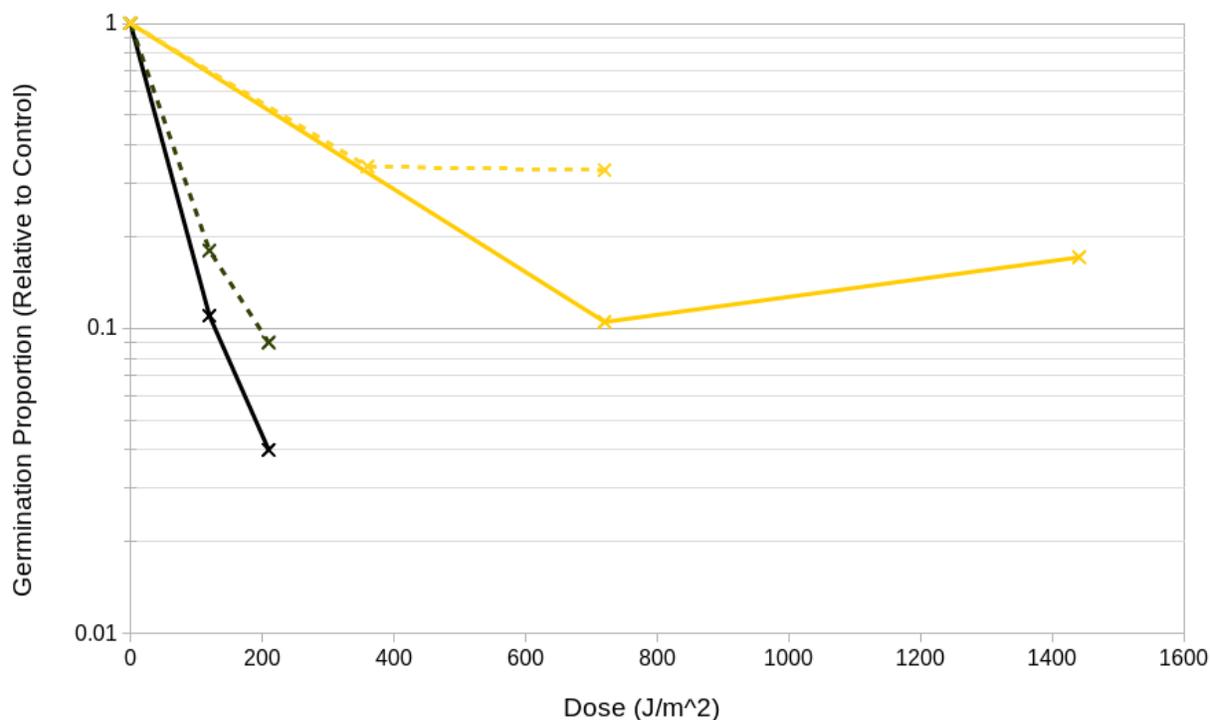
Figure 11 Germination data across two powdery mildew studies testing dual levels of

irradiance. Dashed lines represent the lower irradiance within each study, and solid

lines represent the higher irradiance within each study. Yellow lines describe data from

Suthaparan *et al.* (2012) on rose [0.1 W/m^2 vs 0.2 W/m^2]; black lines describe data in

the current study on grapes [0.3 W/m^2 vs 30 W/m^2].

Among the three previous studies that have examined dose reciprocity in powdery

mildew species, only one reported a small, but significant difference on germination

rates; Suthaparan *et al.* (2012) found for a calculated dose of 720 J/m^2, conidia

irradiated at a rate of 0.2 W/m^2 had a mean germination of 3.5% while treatments

irradiated at 0.1 W/m^2 had a mean germination of 11.3%; this study only examined this

single dose at two irradiance levels. This mirrors the germination results presented in

the current study in that the difference between a more suppressive higher irradiance

treatment and a less suppressive lower irradiance treatment was less than 10%, although our study allowed for much smaller UV doses. A comparison between germination results for the current study and Suthaparan *et al.* (2012) can be seen in Figure 11. Willocquet *et al.* (1996) reported no effect of irradiance on germination rates, however the authors did not report the dose; computing the dose, however, shows it could have varied between ~18 kJ/m^2 in the higher irradiance treatment and ~20kJ/m^2 in the lower irradiance treatment. For measures other than germination, however, all prior studies reported equivalent behavior under the different irradiance treatments at a given dose tested within each experiment (e.g., hyphal branching, spore production, foliar severity) (Willocquet *et al.,* 1996; Suthaparan *et al.,* 2012; Suthaparan *et al.*, 2016b).

The inactivation of repair systems during dormancy has been hypothesized as the cause for why UV dose reciprocity holds only for specific tissues of specific organisms within tested dose ranges (Sommer *et al.*, 1996). This hypothesis likely does not apply to actively growing powdery mildew colonies, however. I speculate that the cause for dose reciprocity breaking down at higher irradiances in *E. necator* spores is due to an overwhelming of the repair system, including but not limited to photolyase, and/or a quantity of photoproducts which cannot be removed before cell death occurs. Prior work in the fungus *Neurospora crassa* found acute UV exposure (i.e., short duration, high irradiance) resulted in more mutations in conidia, and specifically more multigenic gene deletions (compared to point mutations), than chronic UV exposure (i.e., long duration, low irradiance) at the same dose (Stadler & Macleod, 1984; Stadler *et al.*, 1987).

The absence of appressoria originating from long germination tubes in UV-C doses at or above 100 J/m^2 may be demonstrative of sub-lethal damage to the conidium, which enabled these few spores exposed to UV with appressoria to appear viable. Suthaparan *et al.* (2016a) described how germination rates for *O. neolycopersici* spores were higher for the wavelengths between 250nm and 290nm when dark incubation lasted 48hrs instead of 24hrs at a UV dose of 60 µmol/m^2 delivered over 1 minute; Zahl *et al.* (1939) also discussed slowed germination after UV exposure in *A. niger,* however in that study larger amounts of UV were associated with longer germination times. Future experiments should investigate the long-term survival of *E. necator* spores at 100 J/m^2 or higher to determine whether this small number of spores is capable of growth and spreading the disease further. If these spores cannot spread the infection, then the dose required for disease suppression could be lower.

The current study has built upon past work to expand the range of tested dose and irradiance levels for UV treatment in powdery mildews, specifically for germination, colony expansion, and latency. While previous studies provided the first indication of UV dose reciprocity breaking down at higher irradiances in a powdery mildew species (Suthaparan *et al.,* 2012), this study in comparison used much smaller doses (≤210 J/m^2 vs >700 J/m^2) to avoid any possibility of undiscovered phytotoxic effects. Additionally, the irradiance values tested in the current study are an order of magnitude larger than those tested previously due to the necessity of shortened exposure times in field application. With the exception of *S. cerevisiae* (Sommer *et al.,* 1996), *E. necator*

conidia, behave in the same manner as other tested microorganisms where dose reciprocity breaks down at higher irradiance values.

SECTION 2 MANAGEMENT IMPLICATIONS

The question of UV dose reciprocity has special relevance in the application of UV in the field, due to the mobile nature of application. Infected plants can only be under a UV apparatus for the length of time it requires to drive over it. This means higher doses of UV or faster mobile UV unit speeds will require more UV bulbs (i.e., to increase irradiance). If however, higher irradiance exposure is more effective at suppressing a plant pathogen, then ultimately a lower dose is needed and mobile UV unit speeds can be further increased.

Mobile UV units require significant design and engineering expertise, along with calibrated measurements of UV irradiance and calculations of speed needed to achieve an effective dose (Bierman *et al.*, 2017; Gadoury *et al.*, 2017). Three tractor-drawn UV units have been constructed for the purposes of understanding efficacy of UV in the field for several crops, and their use has been shown to produce strong suppression of powdery mildew (Gadoury *et al.*, 2017; Onofre *et al.*, 2017; Onofre *et al.*, 2018). Tractor ground speeds in these field trials are currently in the lower to middle range of possible speeds that could safely be used, though other crops and terrain may influence the range of possible safe speeds that could be used by a grower (David Gadoury, *personal communication*). From the perspective of growers considering UV management of

pathogens, speed is of critical importance for adoption to be considered; a technology must be practical and an improvement for growers over existing control strategies.

The observation that *E. necator* conidia are more sensitive to higher irradiance (at least 30 W/m^2) is beneficial for the improvement of field units. With additional studies it should be possible to determine a lower UV dose needed when supplied via high irradiance to adequately control powdery mildew species. Lowering the dose should be approached cautiously because at the tested UV irradiance in this study only conidia were affected. A field of susceptible plants will likely have a range of developmental stages distributed across the area, so UV treatments need to be designed to control enough of the pathogen population to avoid economic harm. Alternatively, if the dose cannot be lowered, using a higher irradiance at the same dose will provide growers more effective control of the pathogen's germination, while still allowing for some increase in the ground speed of mobile UV units to maintain that same dose level.

Though this strategy is promising, the engineering and design requirements of the unit for this setting are considerably more challenging than that required for controlled agriculture, i.e., greenhouse, plastic tunnel production (Gadoury *et al.*, 2017). Powdery mildew are among the most routinely problematic and severe diseases in glasshouses and plastic tunnel culture (Xiao *et al.*, 2001; Burlakoti *et al.*, 2013; Carisse *et al.*, 2013; Onofre *et al.*, 2016, Onofre *et al.*, 2017). This difference between powdery mildew under protected culture versus the field has been attributed to changes in multiple environmental conditions underneath plastic including the duration of leaf wetness,

decreased light intensity, UV irradiance, changes in temperature, and to the spectral distribution transmitted through these plastics (Jordan & Hunter, 1972; Elad 1997). Manufactured plastics that allow the transmission of UV-B wavelengths have been shown to decrease powdery mildew severity compared to traditional plastics (Onofre *et al.*, 2017; McCann, *unpublished data*). Farmers engaged in greenhouse production should also be familiar with the installation of lamp fixtures for crop productivity, making installation easier and adoption of UV more likely.

UV-based management in greenhouses has been implemented into two research greenhouses at the Cornell University experiment station located in Geneva, NY, for the control of powdery mildew. These systems were highly effective when applied up to 5 times per week along with secondary control measures (e.g., pruning, biocontrols, horticultural oils, sulfur). At least one powdery mildew epidemic occurred in each greenhouse in 2017-2018, but this was determined to be caused by the UV-B lamps not turning on as programmed. Thus, current UV systems in research settings when left as the only control strategy or applied late in the epidemic may fail to adequately manage powdery mildew infection; however farmers are likely already engaged in a multifaceted program to mitigate pathogen and pest damage more generally. Though it was not an issue for these studies, consideration should also be given to the growing plant and its changing distance from UV sources to avoid doses too low to suppress disease, or so high that it causes damage to the plant (Aruppilai Suthaparan, Norwegian University of Life Sciences, *personal communication*).

The question of dose reciprocity may still be relevant in these stationary environments, especially if robotic application units are used or UV lighting is attached to existing mobile sprayers. (Suthaparan *et al.*, 2016b; Stensvand *et al.*, 2017). At present several growers have taken these principles into action (Stensvand, 2018), and are reporting success in pathogen control with UV systems. Preliminary reports also show UV treatments are effective at controlling immature two-spotted spider mites and their eggs (Johansen *et al.*, 2017), leaving open the possibility that future interdisciplinary studies could develop UV application schedules that manage multiple pathogens and pests concurrently.

SECTION 3 UNKNOWN ECOLOGICAL CONSEQUENCES OF UV EXPOSURE

Reactions to this UV related work at annual conferences of the American Phytopathological Society have included a focus on the unintended consequences of UV exposure in agroecosystems, specifically on the infected plants and to the phyllosphere. In Chapter 2 the evidence that UV exposure can cause damage to plants was discussed and found to be minimal for many studies from the perspective of crop productivity and yield. If phytotoxicity does occur, tests to split the dose into multiple test periods may maintain pathogen control without causing further harm (Suthaparan *et al.*, 2016b). The possibility of greater sensitivity to higher irradiance by pathogens, may also allow the total amount of UV needed to decrease.

As for organisms beyond the host and pathogen, UV-based pathogen management could decrease off-target consequences and environmental risk. Unlike chemical

fungicides, there is no lingering toxic compounds after UV exposure so re-entry by farmworkers could occur immediately after exposure occurred. Additionally, the toxic element of UV exposure is limited to the radiation originating from UV-bulbs, which can be directed precisely and addressed the issue of spray drift. The development of an effective UV-based tool could fit into an integrated pest management system that helps to reduce the use of chemical fungicide to only those situations where it was deemed absolutely necessary.

Plant microbiomes, composed of microbial communities in association with roots (rhizosphere) and above ground plant parts (phyllosphere), are an emerging field of plant science. Similar to how the human microbiome is described, some researchers have characterized the plant microbiome as the plant's "second genome" (Berg *et al.*, 2014). Currently the research has not reached the stage of large-scale community manipulation for improved plant health. There are, however, a few examples that show phyllosphere communities, especially fungal endophyte populations, are both highly malleable by environmental stressors, including UV, (Kadivar *et al.*, 2003; Gunasekara & Paul, 2007; Jacobs & Sundin, 2001) and can impact the susceptibility of plants to infection (Arnold *et al.*, 2003; Johnson & Temple, 2013).

The current study maintains a focus on minimizing damage to the host, maximizing damage to the fungal pathogen with UV exposure, and treating the environment as a static space. Much like the burgeoning field of plant microbiomes, there is still much to observe and understand about the efficacy of UV exposure for the purposes of

pathogen control in crop production. At present concerns about disturbances to the phyllosphere may be addressed by the re-incorporation of biocontrol agents onto plant surfaces (Janisiewicz & Takeda, 2017). Future work should explore the interactions of biological controls and existing phyllosphere community members under supplemental UV exposure.

SECTION 4 EXPERIMENTAL LIMITATIONS

This research was limited by technical barriers, small treatment effects within a given dose, large biological variation and initial experimental designs that did not account for this variation. The initial UV sensor used for calculating UV irradiance became uncalibrated midway through the first experiments, which did not affect the data collection because the heights were kept constant, but it did preclude the testing of additional levels outside this original range until a calibrated spectroradiometer could be obtained. The original experimental apparatus while offering the potential for rapid data collection across a range of doses was limited to small sample sizes per independent experimental repeat (n=3), which proved problematic in the interpretation of data due to the high variability between experimental repeats. This was resolved by testing single doses (120 J/m^2 or 210 J/m^2) with sample sizes of 40 disks per experimental treatment. Additionally, the shutter mechanism in this experimental apparatus limited the minimum exposure duration possible for testing to approximately 4 seconds. A new shutter mechanism has subsequently been built which reduced this time down to <1 second.

Additionally, latency experiments should have reflected the biology of *E. necator* by observing colonies once per day in the evening. *E. necator* sporulates once per conidiophore per day, and while observations in the current work took place at the predicted time of peak maturity of conidia (McCann *et al.*, 2013) a more accurate sense of conidiation could have been captured by waiting several hours after the expected peak had occurred.

The phytotoxicity of the treatments used in this study are unknown in grapevine. This was accommodated by looking to other studies for a lower range of UV doses needed to suppress powdery mildew, and assuming field studies of other crops can be related to grapevine. Future work should include measurements of phytotoxicity to accompany any reports of successful pathogen suppression with UV.

CHAPTER 6 CONCLUSION

SECTION 1 CALIBRATED UV-C DOSE IS A POTENTIAL TOOL AGAINST POWDERY MILDEW

Erysiphe necator is a ubiquitous pathogen of grapevine found consistently across years, varieties of grape, and geographic locations. Currently, grape growers rely heavily on the use of fungicide sprays to minimize damage from this pathogen and maintain high yields, but this places many fungicides at a greater risk of failure due to the evolution of fungicide resistance by pathogens. There is a growing interest in the use of pre-harvest protection with UV against pathogens, particularly wavelengths comprising UV-B and UV-C. Dose reciprocity predicts that the total dose provided determines the photochemical reaction, not the rate of energy or duration used to supply that dose. Using *E. necator* as a case study, I evaluated the impact that a 100x difference in UV-C irradiance would have on germination rates, colony expansion, and latency across a range of doses that should avoid any phytotoxicity. Though dose reciprocity held for colony expansion rates and latency, germination of *E. necator* was lower when UV was supplied with 30 W/m^2 compared to 0.3 W/m^2 at a dose of 210 J/m^2. Greater sensitivity to UV when supplied at higher UV irradiance should inform the implementation of field units and allow for faster ground speeds and less time to apply a treatment.

APPENDIX

FUTURE EXPERIMENTS

Future investigations of the behavior of powdery mildew pathogens under a larger number of irradiance values and a larger range of UV-C doses should attempt to overcome the challenges described in Chapter 5 Section 4. This would require experiments that allow for larger numbers of samples to be observed in a shorter amount of time than required in the current study. Any investigations into latency in *E. necator* should measure samples once at the end of the day, instead of at the expected time for peak spore maturation; observations should occur once every 24 hours, rather than 48hrs, to match the biological process of conidiation in this fungus.

Field trials in vineyards are needed to determine whether the comparisons made in this work to other crops in regards to phytotoxicity and yield effects are appropriate. Based on the finding that ~200 J/m^2 strongly inhibited germination of *E. necator* conidia, this dose could be applied in the field over a growing season. Onofre *et al.* (2018) reported a level of disease suppression equivalent to fungicide treatments by UV-C mobile application at a dose between ~90 and ~170 J/m^2 when applied twice weekly in infected strawberry plants. This could serve as a starting point for connecting laboratory findings of disease suppression to the more complex environment in the field, and allow yield comparisons between chemical fungicide treated, UV treated, and untreated grapevines. Also, the inclusion of at least two irradiance levels derived from the maximum ground speed allowable for a mobile UV unit in a vineyard would provide

information about the biological relevance of dose reciprocity breaking down for the tested dose in regards to disease suppression.

Grapevines may also be affected by different levels of irradiance of supplemental UV, but this was assumed in the current study to be negligible. Using the UV-C experimental apparatus from the current study, potted grapevines could be evaluated for UV sensitivity in terms of photosynthetic efficiency (e.g., Janisiewicz *et al.*, 2016a; 2016b; Suthaparan *et al.*, 2012) across the dose range and irradiance levels used in this study. Multiple cultivars (e.g. American vs European origin) should be used to evaluate whether differences in UV sensitivity exist across cultivars (see: Teranishi *et al.,* 2004). Additionally, measurements should be taken at time points occurring 0, 24, and 48hrs post-irradiation to observe whether short-term inhibition of photosynthesis occurs (see: Darras *et al.*, 2015), as well as over the course of several weeks (approximating the length of tissue susceptibility in the field) to observe whether an inhibition of photosynthesis occurs after chronic exposure (see: Oliveira *et al.,* 2016).

Briefly mentioned above, *E. necator* colonies should be measured in terms other than diameter expansion over time to capture the full effect of UV treatments. One possibility would be to utilize imaging software to analyze how the density of colonies changes over time. This could be accomplished either through the use of automated imaging, such as those currently used in the USDA project "VitisGen2" (Cadle-Davidson, *unpublished data*), and/or through the use of fluorescent dyes (e.g., fluorescein diacetate [Moyer *et al.,* 2010] and propidium iodide [Jones *et al.*, 2016]) to illustrate the

survival of fungal colony hyphae. The benefit of using automated imaging is the potential to reduce the time required to make measurements over many more samples. Alternatively, conducting experiments that count the number of conidia, among those that have formed a germ tube, which exhibit hyphal branching (see: Suthaparan *et al.,* 2016a; 2016b) would be a more time-intensive path to determine whether the small number of conidia that appeared to germinate at doses >40 J/m^2, are able to continue on to reproduce, or are instead representative of delayed lethality. Additionally UV doses at 210 J/m^2 did not show consistent differences between exposed and non-exposed samples for either colony expansion or latency; future studies should test doses greater than those included in this study (e.g. 250, 300, 400, 500 J/m^2) to find where a consistent level of suppression is achieved in terms of colony density (as described above) or latency.

In addition to greater experimentation and optimization of UV mobile application and biological effects, outreach with growers about the possibility of using UV for disease management should be considered. Several companies in Europe have already taken up mobile UV systems in protected environments (see: Stensvand, 2018). In the US, the research group collaborates with a large grower in Florida to test the efficacy of UV treatments on strawberry. Surveys with growers should investigate a number of factors including: (1) the level of understanding of UV as a tool in agriculture, (2) safety concerns regarding its use for farm workers and consumers, (3) barriers to future implementation (e.g., cost of application, physical/topological limitations, efficacy

studies) across different crops and farm size. Ideally this work could assist in the design

of future mobile UV units to ensure the greatest utility possible for farmers.

REFERENCES

Arnold, A. E., Mejia, L. C., Kyllo, D., Rojas, E. I., Maynard, Z., Robbins, N., & Herre, E. A. (2003). Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences*, *100*(26), 15649–15654. https://doi.org/10.1073/pnas.2533483100

Austin, C. N., & Wilcox, W. F. (2011). Effects of Fruit-Zone Leaf Removal, Training Systems, and Irrigation on the Development of Grapevine Powdery Mildew. *American Journal of Enology and Viticulture*, *62*(2), 193–198. https://doi.org/10.5344/ajev.2010.10084

Austin, Craig N., & Wilcox, W. F. (2012). Effects of Sunlight Exposure on Grapevine Powdery Mildew Development. *Phytopathology*, *102*(9), 857–866. https://doi.org/10.1094/PHYTO-07-11-0205

Berg, G., Grube, M., Schloter, M., & Smalla, K. (2014). Unraveling the plant microbiome: looking back and future perspectives. *Frontiers in Microbiology*, *5*. https://doi.org/10.3389/fmicb.2014.00148

Bierman, A., Radetsky, L., Patel, J. S., Figueiro, M., Plummer, T., Rea, M., & Gadoury, D. M. (2017). Practical and accurate measurement of ultraviolet radiation (UV) for research and application in plant pathosystems. (Abstract) *Phytopathology*, *107*(12), 48.

Burlakoti, R. R., Zandstra, J., & Jackson, K. (2013). Comparison of Epidemiology of Gray Mold, Anthracnose Fruit Rot, and Powdery Mildew in Day-Neutral Strawberries in Field and High-Tunnel Conditions in Ontario. *International*

Journal of Fruit Science, *13*(1–2), 19–27. https://doi.org/10.1080/15538362.2012.696956

Buxton, E. W., Last, F. T., & Nour, M. A. (1957). Some Effects of Ultraviolet Radiation on the Pathogenicity of Botrytis fabae, Uromycesfabae and Erysiphe gruminis. *Journal of General Microbiology*, *16*, 764–773.

Calvert, G. M., Karnik, J., Mehler, L., Beckman, J., Morrissey, B., Sievert, J., … Moraga-McHaley, S. (2008). Acute pesticide poisoning among agricultural workers in the United States, 1998-2005. *American Journal of Industrial Medicine*, *51*(12), 883–898. https://doi.org/10.1002/ajim.20623

Carisse, O., Lefebvre, A., Van der Heyden, H., Roberge, L., & Brodeur, L. (2013). Analysis of Incidence–Severity Relationships for Strawberry Powdery Mildew as Influenced by Cultivar, Cultivar Type, and Production Systems. *Plant Disease*, *97*(3), 354–362. https://doi.org/10.1094/PDIS-05-12-0508-RE

Calonnec, A., Cartolaro, P., Poupot, C., Dubourdieu, D., & Darriet, P. (2004). Effects of Uncinula necator on the yield and quality of grapes (Vitis vinifera) and wine. *Plant Pathology*, *53*(4), 434–445. https://doi.org/10.1111/j.0032-0862.2004.01016.x

Cen, Y., & Bornman, J.F. (n.d.). The Response of Bean Plants to UV-B Radiation Under Different Irradiances of Background Visible Light. *Journal of Experimental Botany*, *41*(232), 1489–1495.

Colcol, J. F., Rallos, L. E., & Baudoin, A. B. (2012). Sensitivity of *Erysiphe necator* to Demethylation Inhibitor Fungicides in Virginia. *Plant Disease*, *96*(1), 111–116. https://doi.org/10.1094/PDIS-12-10-0883

Colcol, J. F., & Baudoin, A. B. (2016). Sensitivity of *Erysiphe necator* and *Plasmopara viticola* in Virginia to QoI Fungicides, Boscalid, Quinoxyfen, Thiophanate Methyl, and Mefenoxam. *Plant Disease*, *100*(2), 337–344. https://doi.org/10.1094/PDIS-01-15-0012-RE

Damalas, C., & Koutroubas, S. (2016). Farmers' Exposure to Pesticides: Toxicity Types and Ways of Prevention. *Toxics*, *4*(1), 1. https://doi.org/10.3390/toxics4010001

Darras, A. I., Demopoulos, V., Bali, I., & Tiniakou, C. (2012). Photomorphogenic reactions in geranium (Pelargonium x hortotum) plants stimulated by brief exposures of ultraviolet-C irradiation. *Plant Growth Regulation*, *68*(3), 343–350. https://doi.org/10.1007/s10725-012-9722-2

Darras, A. I., Bali, I., & Argyropoulou, E. (2015). Disease resistance and growth responses in Pelargonium×hortorum plants to brief pulses of UV-C irradiation. *Scientia Horticulturae*, *181*, 95–101. https://doi.org/10.1016/j.scienta.2014.10.039

Delp, C. J. (1954). Effect of temperature and humidity on the grape powdery mildew fungus. *Phytopathology*, *44*(11), 615–626.

Elad, Y. (1997). Effect of filtration of solar light on the production of conidia by field isolates of Botrytis cinerea and on several diseases of greenhouse-grown vegetables. *Crop Protection*, *16*(7), 635–642. https://doi.org/10.1016/S0261-2194(97)00046-X

English-Loeb, G., Norton, A. P., Gadoury, D. M., Seem, R. C., & Wilcox, W. F. (1999). Control of Powdery Mildew in Wild and Cultivated Grapes by a Tydeid Mite. *Biological Control*, *14*(2), 97–103. https://doi.org/10.1006/bcon.1998.0681

English-Loeb, G., Norton, A. P., Gadoury, D., Seem, R., & Wilcox, W. (2007). Biological Control of Grape Powdery Mildew Using Mycophagous Mites. *Plant Disease*, *91*(4), 421–429. https://doi.org/10.1094/PDIS-91-4-0421

Erickson, E. O., & Wilcox, W. F. (1997). Distributions of Sensitivities to Three Sterol Demethylation Inhibitor Fungicides Among Populations of *Uncinula necator* Sensitive and Resistant to Triadimefon. *Phytopathology*, *87*(8), 784–791. https://doi.org/10.1094/PHYTO.1997.87.8.784

Feechan, A., Kocsis, M., Riaz, S., Zhang, W., Gadoury, D. M., Walker, M. A., … Cadle-Davidson, L. (2015). Strategies for *RUN1* Deployment Using *RUN2* and *REN2* to Manage Grapevine Powdery Mildew Informed by Studies of Race Specificity. *Phytopathology*, *105*(8), 1104–1113. https://doi.org/10.1094/PHYTO-09-14-0244-R

FRAC. (2018a). FRAC list of plant pathogenic organisms resistant to disease control agents. http://www.frac.info/docs/default-source/publications/list-of-resistant-plant-pathogens/list-of-resistant-plant-pathogenic-organisms_may-2018.pdf

Fuller, K. B., Alston, J. M., & Sambucci, O. S. (2014). The value of powdery mildew resistance in grapes: Evidence from California. *Wine Economics and Policy*, *3*(2), 90–107. https://doi.org/10.1016/j.wep.2014.09.001

Gadoury, D. M., & Pearson, R. C., (1991). Heterothallism and pathogenic specialization in Uncinula necator. *Phytopathology*, *81*(10), 1287–1293.

Gadoury, D. M., Pearson, R. C., Seem, R. C., Henick-Kling, T., Creasy, L. L., & Michaloski, A. (1992). Control of fungal diseases of grapevine by short-wave ultraviolet light. (Abstract) *Phytopathology*, (82), 243.

Gadoury, D. M., Pearson, R. C., Riegel, D. G., Seem, R. C., Becker, C. M., & Pscheidt, J W. (1994). Reduction of Powdery Mildew and Other Diseases by Over-the-Trellis Applications of Lime Sulfur to Dormant Grapevines. *Plant Disease*, (78), 83–87.

Gadoury, D. M., Seem, R. C., Pearson, R. C., Wilcox, W. F., & Dunst, R. M. (2001). Effects of Powdery Mildew on Vine Growth, Yield, and Quality of Concord Grapes. *Plant Disease*, *85*(2), 137–140. https://doi.org/10.1094/PDIS.2001.85.2.137

Gadoury, D. M., Seem, R. C., Ficke, A., & Wilcox, W. F. (2003). Ontogenic Resistance to Powdery Mildew in Grape Berries. *Phytopathology*, *93*(5), 547–555. https://doi.org/10.1094/PHYTO.2003.93.5.547

Gadoury, D. M., Wakefield, L. M., Cadle-Davidson, L., Dry, I. B., & Seem, R. C. (2011). Effects of Prior Vegetative Growth, Inoculum Density, Light, and Mating on Conidiation of Erysiphe necator. *Phytopathology*, *102*(1), 65–72. https://doi.org/10.1094/PHYTO-03-11-0085

Gadoury, D. M., Cadle-Davidson, L., Wilcox, W. F., Dry, I. B., Seem, R. C., & Milgroom, M. G. (2012). Grapevine powdery mildew (Erysiphe necator): a fascinating system for the study of the biology, ecology and epidemiology of an

obligate biotroph. *Molecular Plant Pathology*, *13*(1), 1–16. https://doi.org/10.1111/j.1364-3703.2011.00728.x

Gadoury, D. M., Bierman, A., Rea, M., Stensvand, A., Suthaparan, A., & Onofre, R. Borba. (2017). Design and operational considerations for use of UV and red light for suppression of plant diseases under field conditions. (Abstract) *Phytopathology*, *107*(12), 5–6.

Glawe, D. A. (2008). The Powdery Mildews: A Review of the World's Most Familiar (Yet Poorly Known) Plant Pathogens. *Annual Review of Phytopathology*, *46*(1), 27–51. https://doi.org/10.1146/annurev.phyto.46.081407.104740

Gunasekera, T. S., & Paul, N. D. (2007). Ecological impact of solar ultraviolet-B (UV-B: 320-290 nm) radiation on Corynebacterium aquaticum and Xanthomonas sp. colonization on tea phyllosphere in relation to blister blight disease incidence in the field. *Letters in Applied Microbiology*, *44*(5), 513–519. https://doi.org/10.1111/j.1472-765X.2006.02102.x

Holloman, D. W., & Wheeler, I. E. (2002). Chapter 16: Controlling Powdery Mildews with Chemistry. In *The Powdery Mildews A Comprehensive Treatise* (pp. 249–255). APS Press.

Hyland, C., & Laribi, O. (2017). Review of take-home pesticide exposure pathway in children living in agricultural areas. *Environmental Research*, *156*, 559–570. https://doi.org/10.1016/j.envres.2017.04.017

Jacobs, J. L., & Sundin, G. W. (2001). Effect of Solar UV-B Radiation on a Phyllosphere Bacterial Community. *Applied and Environmental Microbiology*, *67*(12), 5488–5496. https://doi.org/10.1128/AEM.67.12.5488-5496.2001

Jacobson, A. R., Dousset, S., Guichard, N., Baveye, P., & Andreux, F. (2005). Diuron mobility through vineyard soils contaminated with copper. *Environmental Pollution*, *138*(2), 250–259. https://doi.org/10.1016/j.envpol.2005.04.004

Janisiewicz, W. J., Takeda, F., Glenn, D. M., Camp, M. J., & Jurick, W. M. (2016a). Dark Period Following UV-C Treatment Enhances Killing of *Botrytis cinerea* Conidia and Controls Gray Mold of Strawberries. *Phytopathology*, *106*(4), 386–394. https://doi.org/10.1094/PHYTO-09-15-0240-R

Janisiewicz, W. J., Takeda, F., Nichols, B., Glenn, D. M., Jurick II, W. M., & Camp, M. J. (2016b). Use of low-dose UV-C irradiation to control powdery mildew caused by *Podosphaera aphanis* on strawberry plants. *Canadian Journal of Plant Pathology*, *38*(4), 430–439. https://doi.org/10.1080/07060661.2016.1263807

Janisiewicz, W. J., & Takeda, F. (2017). *US2017274106-A1*.

Johansen, N. S., Tadesse, B. A., Suthaparan, A., Stensvand, A., From, P. J., & Gadoury, D. M. (2017). Nighttime treatments of ultraviolet (UV) light targeting powdery mildews also suppress the two-spotted spider mite (Tetranychus urticae). (Abstract) *Phytopathology*, *107*(12), 77.

Johnson, K. B., & Temple, T. N. (2013). Evaluation of Strategies for Fire Blight Control in Organic Pome Fruit Without Antibiotics. *Plant Disease*, *97*(3), 402–409. https://doi.org/10.1094/PDIS-07-12-0638-RE

Johnston, S. (2001). *A history of light and colour measurement: science in the shadows*. Bristol ; Philadelphia: Institute of Physics Pub.

Jones, K., Kim, D. W., Park, J. S., & Khang, C. H. (2016). Live-cell fluorescence imaging to investigate the dynamics of plant cell death during infection by the rice blast fungus Magnaporthe oryzae. *BMC Plant Biology*, *16*(1). https://doi.org/10.1186/s12870-016-0756-x

Jordan, V. W. L., & Hunter, T. (1972). The effects of glass cloche and coloured polyethylene tunnels on microclimate, growth, yield and disease severity of strawberry plants. *Journal of Horticultural Science*, *47*(3), 419–426. https://doi.org/10.1080/00221589.1972.11514485

Kadivar, H., & Stapleton, A. E. (2003). Ultraviolet Radiation Alters Maize Phyllosphere Bacterial Diversity. *Microbial Ecology*, *45*(4), 353–361. https://doi.org/10.1007/s00248-002-1065-5

Knudsen, G. R., & Dandurand, L.-M. C. (2014). Ecological Complexity and the Success of Fungal Biological Control Agents. *Advances in Agriculture*, *2014*, 1–11. https://doi.org/10.1155/2014/542703

Komárek, M., Čadková, E., Chrastný, V., Bordas, F., & Bollinger, J.-C. (2010). Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. *Environment International*, *36*(1), 138–151. https://doi.org/10.1016/j.envint.2009.10.005

Kunz, B. A., Dando, P. K., Grice, D. M., Mohr, P. G., Schenk, P. M., & Cahill, D. M. (2008). UV-Induced DNA Damage Promotes Resistance to the Biotrophic Pathogen Hyaloperonospora parasitica in Arabidopsis. *PLANT PHYSIOLOGY*, *148*(2), 1021–1031. https://doi.org/10.1104/pp.108.125435

Lucas-Lledo, J. I., & Lynch, M. (2009). Evolution of Mutation Rates: Phylogenomic Analysis of the Photolyase/Cryptochrome Family. *Molecular Biology and Evolution*, *26*(5), 1143–1153. https://doi.org/10.1093/molbev/msp029

Maharaj, R., Arul, J., & Nadeau, P. (2010). UV-C Irradiation of Tomato and its Effects on Color and Pigments. *Advances in Environmental Biology*, *4*(2), 308–315.

McCann, T., Grove, G., Mahaffee, W., Gadoury, D. M., Cadle-Davidson, L., & Seem, R. C. (2013). Ontogenesis of conidiation in the grapevine powdery mildew (Erysiphe necator).(Abstract) *Phytopathology*, *103*(6), 93.

McCann, T., Carter, M. D., Gadoury, D. M., & Rea, M. (2017). Radiant energy thresholds and spectral distribution of light as regulatory factors in sporulation of Erysiphe necator. (Abstract) *Phytopathology*, *107*(12), 10.

McGrath, M. T. (2001). Fungicide Resistance in Cucurbit Powdery Mildew: Experiences and Challenges. *Plant Disease*, *85*(3), 236–245. https://doi.org/10.1094/PDIS.2001.85.3.236

Miles, L. A., Miles, T. D., Kirk, W. W., & Schilder, A. M. C. (2012). Strobilurin (QoI) Resistance in Populations of *Erysiphe necator* on Grapes in Michigan. *Plant Disease*, *96*(11), 1621–1628. https://doi.org/10.1094/PDIS-01-12-0041-RE

Moyer, M. M., Gadoury, D. M., Cadle-Davidson, L., Dry, I. B., Magarey, P. A., Wilcox, W. F., & Seem, R. C. (2010). Effects of Acute Low-Temperature Events on Development of *Erysiphe necator* and Susceptibility of *Vitis vinifera*. *Phytopathology*, *100*(11), 1240–1249. https://doi.org/10.1094/PHYTO-01-10-0012

Norell, I. (1954). The Effect of Ultra-violet Light on the Resistance of Potato Tubers to Fusarium Species. *Physiologia Plantarum, 7,* 797-809.

Obande, M. A., Tucker, G. A., & Shama, G. (2011). Effect of preharvest UV-C treatment of tomatoes (Solanum lycopersicon Mill.) on ripening and pathogen resistance. *Postharvest Biology and Technology, 62*(2), 188–192. https://doi.org/10.1016/j.postharvbio.2011.06.001

Ockey, S., Highland, B., Jacobsen, B., & Dimock, M. (n.d.). Bacillus mycoides isolate J (BmJ): Plant protection through Systemic Acquired Resistance. A new option for plant disease management. (Abstract) *Phytopathology, 106*(12), 96.

Oliveira, I. R. de, Crizel, G. R., Severo, J., Renard, C. M. G. C., Chaves, F. C., & Rombaldi, C. V. (2016). Preharvest UV-C radiation influences physiological, biochemical, and transcriptional changes in strawberry cv. Camarosa. *Plant Physiology and Biochemistry, 108*, 391–399. https://doi.org/10.1016/j.plaphy.2016.08.012

Onofre, R. Borba, Gadoury, D., Dufault, N., & Peres, N. (2016). Trapping conidia spores of Podosphaera aphanis in high tunnels and field strawberry plots. (Abstract) *Phytopathology, 106*(12), 21–22.

Onofre, R. Borba, Vieira, R. Luis, & Arupillai, S. (2017). Severity of strawberry powdery mildew in open field vs under plastics that either block or transmit UV light. (Abstract) *Phytopathology, 107*(12), 49.

Onofre, R. Borba, Gatto, J.B, Marin, M, Gadoury, D. M., Stensvand, A., Rea, M., … Peres, N. (2018). Design, Operation, and Efficacy of an Apparatus Using

Ultraviolet Light to Suppress Powdery Mildew of Strawberry in Open Field Production Systems. *Phytopathology*, *108*(10), 165.

Pascale, A., Vinale, F., Manganiello, G., Nigro, M., Lanzuise, S., Ruocco, M., … Lorito, M. (2017). Trichoderma and its secondary metabolites improve yield and quality of grapes. *Crop Protection*, *92*, 176–181. https://doi.org/10.1016/j.cropro.2016.11.010

Patel, J. S., Radetsky, L., Plummer, T., Bierman, A., Gadoury, D. M., & Rea, M. (2017). Pre-inoculation treatment of basil plants with ultraviolet-B radiation induces resistance to downy mildew. (Abstract) *Phytopathology*, *107*(12), 52.

Pathak, R., Sundaram, A., Cadle-Davidson, L. E., Solhaug, K. A., Stensvand, A., Gislerod, H. R., & Suthaparan, A. (2017). Sensing of UV and visible light by powdery mildew pathogens. (Abstract) *Phytopathology*, *107*(12), 47–48.

Pombo, M. A., Dotto, M. C., Martínez, G. A., & Civello, P. M. (2009). UV-C irradiation delays strawberry fruit softening and modifies the expression of genes involved in cell wall degradation. *Postharvest Biology and Technology*, *51*(2), 141–148. https://doi.org/10.1016/j.postharvbio.2008.07.007

Pombo, M. A., Rosli, H. G., Martínez, G. A., & Civello, P. M. (2011). UV-C treatment affects the expression and activity of defense genes in strawberry fruit (Fragaria×ananassa, Duch.). *Postharvest Biology and Technology*, *59*(1), 94–102. https://doi.org/10.1016/j.postharvbio.2010.08.003

Sambucci, Olena S., Alston, Julian M., & Fuller, Kate B. (2014). The Costs of Powdery Mildew Management in Grapes and the Value of Resistant Varieties:

Evidence from California. *Robert Mondavi Institute Center for Wine Economics Publication*, 1–51.

Santos, A. L., Oliveira, V., Baptista, I., Henriques, I., Gomes, N. C. M., Almeida, A., … Cunha, Â. (2013). Wavelength dependence of biological damage induced by UV radiation on bacteria. *Archives of Microbiology*, *195*(1), 63–74. https://doi.org/10.1007/s00203-012-0847-5

Schmitz-Hoerner, R., & Weissenböck, G. (2003). Contribution of phenolic compounds to the UV-B screening capacity of developing barley primary leaves in relation to DNA damage and repair under elevated UV-B levels. *Phytochemistry*, *64*(1), 243–255. https://doi.org/10.1016/S0031-9422(03)00203-6

Sklar, L. R., Almutawa, F., Lim, H. W., & Hamzavi, I. (2013). Effects of ultraviolet radiation, visible light, and infrared radiation on erythema and pigmentation: a review. *Photochem. Photobiol. Sci.*, *12*(1), 54–64. https://doi.org/10.1039/C2PP25152C

Smith, R.J., Bettiga, L.J., & Gubler, W.D. (2016). UC IPM Pest Management Guidelines: Grape Powdery Mildew. *UC ANR Publication 3448*. Retrieved from http://ipm.ucanr.edu/PMG/r302100311.html

Sommer, R., Haider, T., Cabaj, A., Heidenreich, E., & Kundi, M. (1996). Increased inactivation of Saccharomyces cerevisiae by protraction of UV irradiation. *Applied and Environmental Microbiology*, *62*(6), 1977–1983.

Sommer, R., Haider, T., Cabaj, A., Pribil, W., & Lhotsky, M. (1998). Time dose reciprocity in UV disinfection of water. *Water Science and Technology*, *38*(12), 145–150.

Stadler, D., & Macleod, H. (1984). A dose-rate effect in UV mutagenesis in Neurospora. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *127*(1), 39–47. https://doi.org/10.1016/0027-5107(84)90138-6

Stadler, D., Macleod, H., & Loo, M. (1987). Repair-Resistant Mutation in Neurospora. *Genetics*, *116*, 207–214.

Stensvand, A., Suthaparan, A., From, P., Grimstad, L., Bjugstad, N., Solhaug, K., … Rea, M. (2017). Design and operation of static and mobile arrays to suppress powdery mildews in greenhouse and tunnel production systems with UV and visible light. (Abstract). *PHYTOPATHOLOGY*, *107*, 47–47.

Stensvand, A. (2018). Exploiting our knowledge of how fungal plant pathogens use visible and UV light. (Abstract) *Phytopathology*, *108*(10).

Suthaparan, A., Stensvand, A., Solhaug, K. A., Torre, S., Mortensen, L. M., Gadoury, D. M., … Gislerød, H. R. (2012). Suppression of powdery mildew (Podosphaera pannosa) in greenhouse roses by brief exposure to supplemental UV-B radiation. *Plant Disease*, *96*(11), 1653–1660.

Suthaparan, A., Stensvand, A., Solhaug, K. A., Torre, S., Telfer, K. H., Ruud, A. K., … Gislerød, H. R. (2014). Suppression of Cucumber Powdery Mildew by Supplemental UV-B Radiation in Greenhouses Can be Augmented or Reduced by Background Radiation Quality. *Plant Disease*, *98*(10), 1349–1357. https://doi.org/10.1094/PDIS-03-13-0222-RE

Suthaparan, A., Solhaug, K. A., Stensvand, A., & Gislerød, H. R. (2016a). Determination of UV action spectra affecting the infection process of Oidium neolycopersici, the cause of tomato powdery mildew. *Journal of Photochemistry and Photobiology B: Biology*, *156*, 41–49. https://doi.org/10.1016/j.jphotobiol.2016.01.009

Suthaparan, A., Solhaug, K. A., Bjugstad, N., Gislerød, H. R., Gadoury, D. M., & Stensvand, A. (2016b). Suppression of Powdery Mildews by UV-B: Application Frequency and Timing, Dose, Reflectance, and Automation. *Plant Disease*, *100*(8), 1643–1650. https://doi.org/10.1094/PDIS-12-15-1440-RE

Suthaparan, A., Solhaug, K. A., Stensvand, A., & Gislerød, H. R. (2017). Daily light integral and day light quality: Potentials and pitfalls of nighttime UV treatments on cucumber powdery mildew. *Journal of Photochemistry and Photobiology B: Biology*, *175*(Supplement C), 141–148. https://doi.org/10.1016/j.jphotobiol.2017.08.041

Taylor-Edmonds, L., Lichi, T., Rotstein-Mayer, A., & Mamane, H. (2015). The impact of dose irradiance and growth conditions on Aspergillus niger renamed A brasiliensis spores low pressure LP UV inactivation. *Journal of Environmental Science and Health, Part A*, *50*, 341–347.

Teranishi, M., Iwamatsu, Y., Hidema, J., & Kumagai, T. (2004). Ultraviolet-B Sensitivities in Japanese Lowland Rice Cultivars: Cyclobutane Pyrimidine Dimer Photolyase Activity and Gene Mutation. *Plant and Cell Physiology*, *45*(12), 1848–1856. https://doi.org/10.1093/pcp/pch215

Thompson, C. L., & Sancar, A. (2002). Photolyase/cryptochrome blue-light photoreceptors use photon energy to repair DNA and reset the circadian clock. *Oncogene*, *21*(58), 9043–9056. https://doi.org/10.1038/sj.onc.1205958

Urban, L., Charles, F., de Miranda, M. R. A., & Aarrouf, J. (2016). Understanding the physiological effects of UV-C light and exploiting its agronomic potential before and after harvest. *Plant Physiology and Biochemistry*, *105*, 1–11. https://doi.org/10.1016/j.plaphy.2016.04.004

Verma, M., Brar, S. K., Tyagi, R. D., Surampalli, R. Y., & Valéro, J. R. (2007). Antagonistic fungi, Trichoderma spp.: Panoply of biological control. *Biochemical Engineering Journal*, *37*(1), 1–20. https://doi.org/10.1016/j.bej.2007.05.012

Vincelli, P. (2012). QoI (Strobilurin) Fungicides: Benefits and Risks. *The Plant Health Instructor*. https://doi.org/10.1094/PHI-I-2002-0809-02.

Willocquet, L., Colombet, D., Rougier, M., Fargues, J., & Clerjeau, M. (1996). Effects of radiation, especially ultraviolet B, on conidial germination and mycelial growth of grape powdery mildew. *European Journal of Plant Pathology*, *102*(5), 441–449. https://doi.org/10.1007/BF01877138

Woo, S. L., Ruocco, M., Vinale, F., Nigro, M., Marra, R., Lombardi, N., … Lorito, M. (2014). Trichoderma-based Products and their Widespread Use in Agriculture. *The Open Mycology Journal*, *8*(1), 71–126. https://doi.org/10.2174/1874437001408010071

Xiao, C. L., Chandler, C. K., Price, J. F., Duval, J. R., Mertely, J. C., & Legard, D. E. (2001). Comparison of Epidemics of Botrytis Fruit Rot and Powdery Mildew of

Strawberry in Large Plastic Tunnel and Field Production Systems. *Plant Disease*, *85*(8), 901–909. https://doi.org/10.1094/PDIS.2001.85.8.901

Xu, Y., Charles, M. T., Luo, Z., Roussel, D., & Rolland, D. (2017). Potential link between fruit yield, quality parameters and phytohormonal changes in preharvest UV-C treated strawberry. *Plant Physiology and Biochemistry*, *116*, 80–90. https://doi.org/10.1016/j.plaphy.2017.05.010

Zahl, P. A., Koller, L. R., & Haskins, C. P. (1939). The effects of ultraviolet radiation on spores of the fungus Aspergillus niger. *The Journal of General Physiology*, *22*(6), 689–698. https://doi.org/10.1085/jgp.22.6.689