UV-C Light as a Tool to Manage Grape Powdery Mildew

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1 Introduction

Ultraviolet spectrum C (UV-C) light within a waveband from 254 to 283 nanometers has been effectively used to suppress several plant pathogens. The discovery that UV-C efficacy is greatly increased when UV is applied during darkness has significantly improved efficacy of treatments (Janisiewicz *et al.,* 2016; Suthaparan *et al.,* 2016; Onofre *et al.,* 2019; Zhu *et al.,* 2019). Microbial pathogens possess a photolyase repair mechanism that can reverse the harmful effects of UV on DNA. This repair mechanism requires blue light or UV-A to operate, and therefore, is nonfunctional during darkness (Beggs, 2002). Using UV-C as an additional tool in the integrative management of powdery mildew could help reduce the quantity of fungicides applied which saves growers on input costs, thereby increasing profitability and minimizing fungicides introduced into the environment. Our objective was to determine the effects of nighttime applications of UV-C upon the grapevine powdery mildew pathogen *Erysiphe necator*.

2 Inhibition of *E. necator* conidia by UV-C *in vitro*

Two 41.4cm, 36-Watt UV-C low-pressure mercury lamps were suspended above a conveyor belt system whose speed could be varied to apply control doses of UV-C to conidia of *E. necator*. Mean irradiance at the sample plane was 45 watts per square meter (W/m^2), and the conveyor belt speed was adjusted such that conidia were exposed to five UV-C doses 0, 100, 150, 275, 500, and 800 joules per square meter (J/m^2).

Nineteen isolates of *E. necator* were grown on detached grape leaves in a growth chamber. Conidia of each isolate were dispersed onto Gelzan medium filled 24-well microtiter plates in a settling tower one hour after simulated sunset, and were exposed to UV-C doses in the absence of blue light followed by incubation in darkness for an additional 7 hours, before they were returned to a 16-hour photoperiod at 21°C. Plates were imaged before and 48 hours after UV-C exposure with an Echo Revolve digital microscope (Fig. 1), image backgrounds were removed with a rolling ball radius of 10 pixels, converted to binary images, and germ tube growth estimated by subtracting the biomass area imaged before UV exposure from that imaged 48 hr. after UV-C exposure in ImageJ. The half-maximal UV-C dose to inhibit powdery mildew conidia germ tube growth (ED_{50}) was calculated by `drc` package in R using a three-parameter log-logistic function.

ED_{50} values varied across isolates from 98 to 245 J/m^2 with an average of 183 J/m^2 (Table 1). Conidia with stunted germination tubes may not be infective and therefore the ED_{50} to inhibit conidia infection viability may be lower than the ED_{50} values reported here. Regardless, the relative variability in sensitivity may suggests that there may be cellular mechanisms that influence the tolerance or sensitivity of powdery mildew to UV-C.

Isolate	ED_{50} (J/m^2)	±Std. err
HO1	98	34
RMT2A	114	23
E101	122	11
DY4-2	140	15
SE7A	146	14
S402UTC	155	13
Evpop553	164	29
RC2-2	164	11
STPN667-1	178	18
DDOFS2	187	19
STPN777-1	196	20
RMT1A	205	20
HO3	205	36
CL9-3	212	16
THB	213	19
STPN777-2	219	28
PR7-67	227	13
HO2	233	32
R527ST115-1	234	74
CAT1D1	245	38

Table 1: UV-C ED_{50} estimates to inhibit *E.necator* germination.

3 Vineyard UVC application on the management of *E. necator*

Field trials were conducted using twenty vine rows of a 0.4-hectare (ha) vineyard of *Vitis vinifera* 'Pinot Noir" trained to a bilateral Guyot system. In 2020, weekly UV-C treatments were applied using a tractor-drawn array of UV-C lamps arranged as an over-the-trellis arch approximately 182 cm in height, 91 cm in width, and 106 cm in length (Fig. 2). On the interior of the arch were 24 evenly spaced 55 W UV-C low-pressure mercury lamps (Osram G55T8 OF) backed by polished aluminum reflectors (Fig. 2). The device was suspended from a cantilevered arm attached to the 3-point hitch of a tractor. The theoretical irradiance under the array was 100 W/m^2 and the tractor was operated at speeds of 3.2 or 4.8 kilometers per hour (kph) yielding a UV-C dose of 120 and 80 J/m^2, respectively. Five vine sub-plots within the UV-C treatment main plots received different fungicide programs: 5.6 kilograms/hectare (kg/ha) micronized sulfur applied at 7-day or 14-day intervals, 0.74 L/ha azoxystrobin (Abound; QoI) on a 14-day interval, an alternation of sulfur

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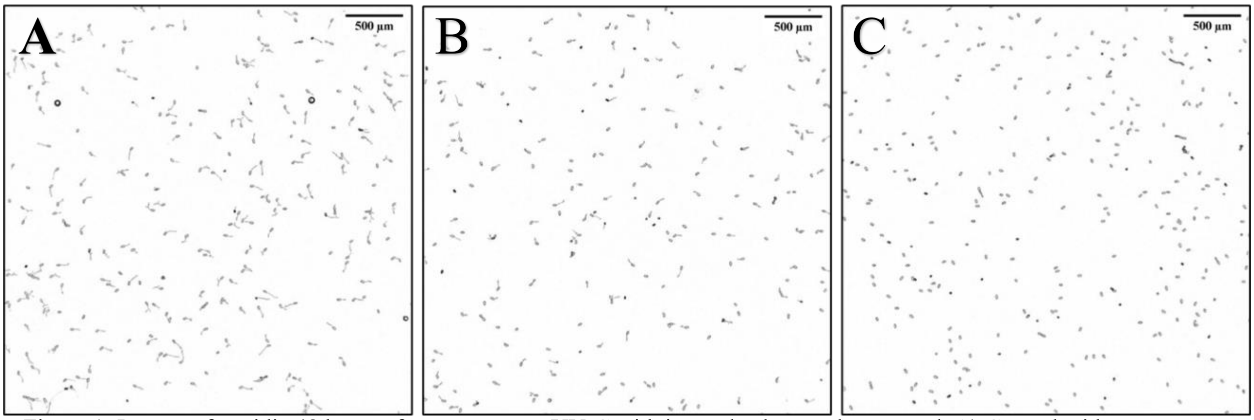

Figure 1: Images of conidia 48 hours after exposure to UV-C with image backgrounds removed. A) Control without exposure to UV-C with strong germination growth. B) Exposure to 150 J/m^2 with weak germination growth, and C) exposure to 800 J/m^2 with no germination.

and azoxystrobin on a 14-day interval, or water treated control. Fungicides were typically applied the morning after the application of UV-C to minimize the exposure of fungicide residues.

In 2021 field trials, UV-C applications were increased to twice per week (every 3-4 days), and the tractor ground speed reduced to 2.2 and 3.2 kph to yield a dose of 200 and 120 J/m^2, respectively. Fungicide applications were adjusted to 4.48 kg/ha micronized sulfur at 14-day or 21-day intervals, 2.24 kg/ha sulfur at 14-day interval, an alternation of 4.48 kg/ha sulfur and 0.74 L/ha azoxystrobin on a 14-day interval, or water treated control. In addition, once weekly applications of 120 and 200 J/m^2 UV-C were also performed in conjunction with a 14-day interval, 4.48 kg/ha micronized sulfur fungicide spray program.

Figure 2: The tractor mounted vineyard UVC application unit in operation at night.

For both seasons, powdery mildew leaf incidence ratings were performed every other week starting in mid-May and ending at the onset of veraison with leaf and cluster mildew severity ratings performed at onset of veraison by rating thirty leaves or clusters per plot. In addition, individuals wore gloves while visually rating plots for disease that were then swabbed with Cap-Shure polystyrene shaft cotton swabs that were then processed for DNA extraction and analyzed by qPCR to estimate the detectable conidia (Thiessen et al., 2016) inoculum in the plot.

Once weekly UV-C application in 2020 led to a significant reduction in the overall leaf incidence epidemic (Area under the disease progress curve; AUDPC) by UV-C application (TukeyHSD, $p < 0.05$) reducing the AUDPC by 28% and 17% for 80 and 120 J/m^2 applications, respectively (Fig. 3A). In 2021, once weekly applications of UV-C in conjunction with 14-day interval, 4.48 kg/ha micronized sulfur fungicide spray program were not significantly different from the twice weekly applications of UV-C with the same fungicide program.

Twice weekly UV-C application on average significantly reduced the overall leaf incidence epidemic AUDPC (TukeyHSD, $p < 0.05$) reducing the AUDPC by 53% and 56% for 120 and 200 J/m^2 applications, respectively (Fig. 3B). UV-C application significantly reduced cluster disease incidence by 22% and 35%, as well as the overall inoculum detected from glove swabs by 24% and 37% with 120 and 200 J/m^2 applications, respectively, compared to non-UV-C treated plots. (TukeyHSD, $p < 0.05$). This indicates that in addition to there being a reduced epidemic seen in visual scouting, powdery mildew inoculum levels in the plots may have also been reduced by UV-C application.

4 UV-C effect on fruit chemistry

Fruit chemistry was measured to examine if UV-C if had effects of fruit quality. The four metrics measured were soluble solids, pH, anthocyanins, and phenols. For each plot, five random clusters were randomly selected, frozen, and three, 100-gram subsamples of berries were randomly sampled. Whole berries were thawed, homogenized, and

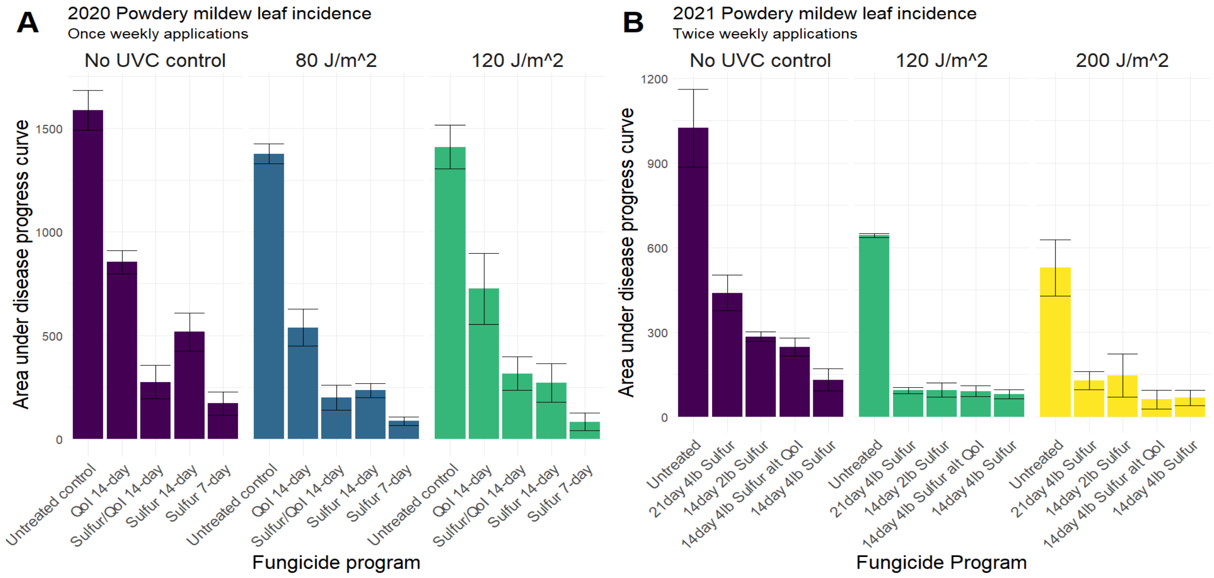

Figure 3: Area under the disease progress values (units arbitrary) for powdery mildew leaf incidence epidemic in 2020 (A) and 2021 (B) with once or twice weekly UV-C applications, respectively, in conjunction with fungicide spray programs. Error bars represent the standard error of the mean.

centrifuged, the supernatant used to calculate the degrees Brix and pH using a refractometer and pH probe, respectively. Anthocyanins and phenolics were estimated by the method described in Bindon *et al.* (2014) and Mercurio *et al.* (2007) by extracting grape berry homogenate in acidified ethanol and reading the color metrics with a spectrometer. For 2020 grape samples, there were no significant differences between the weekly UV-C treatments in any of the four metrics tested.

5 Conclusions and Future Directions

These results suggest that UV-C treatments, in conjunction with fungicide programs and other integrated pest management strategies, may have the potential to improve disease management of grape powdery mildew. Being able to use less fungicides or increase spray intervals between applications with the use of UV-C could reduce the input costs of fungicides and limits the environmental fungicide inputs.

Even though UV-C offers promising improvement of powdery mildew disease control, the application of UV-C must be made more economically feasible for commercial use, thus future work will examine the use autonomous robotic platforms for the application of UV-C. Autonomous robotics can also be recharged using renewable energy such as solar or wind energy thereby minimizing the cost of fuel to operate the system. However, such practices will require investments in renewable energy infrastructure.

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