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Effects of Nighttime Applications of Germicidal Ultraviolet Light Upon Powdery Mildew (*Erysiphe necator*), Downy Mildew (*Plasmopara viticola*), and Sour Rot of Grapevine

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Abstract

Nighttime applications of germicidal ultraviolet were evaluated as a means to suppress three diseases of grapevine. In laboratory studies, UV-C light (peak 254 nm, FWHM 5 nm) applied during darkness strongly inhibited the germination of conidia of *Erysiphe necator*, and at a dose of 200 J/m^2, germination was zero. Reciprocity of irradiance and duration of exposure with respect to conidial germination was confirmed for UV-C doses between 0 and 200 J/m^2 applied at 4 or 400 s. When detached grapevine leaves were exposed during darkness to UV-C at 100 J/m^2 up to 7 days before they were inoculated with zoospores of *Plasmopara viticola*, infection and subsequent sporulation was reduced by over 70% compared to untreated control leaves, indicating an indirect suppression of the pathogen exerted through the host. A hemicylindrical array of low-pressure discharge UV-C lamps configured for trellised grapevines was designed and fitted to both a tractor-drawn carriage and a fully autonomous robotic carriage for vineyard applications. In 2019, in a Chardonnay research vineyard with a history of high inoculum and severe disease, weekly nighttime applications of UV-C suppressed *E. necator* on leaves and fruit at doses of 100 and 200 J/m^2. In the same vineyard in 2020, UV-C was applied once or twice weekly at doses of 70, 100, or 200 J/m^2, and severity of *E. necator* on both leaves and fruit was significantly reduced compared to untreated controls; twice-weekly applications at 200 J/m^2 provided suppression equivalent to a standard fungicide program. None of the foregoing UV-C treatments significantly reduced the severity of *P. viticola* on Chardonnay vines compared to the untreated control in 2020. However, twice-weekly applications of UV-C at 200 J/m^2 to the more downy mildew-resistant *Vitis* interspecific hybrid cultivar Vignoles in 2021 significantly suppressed foliar disease severity. In commercial Chardonnay vineyards with histories of excellent disease control in Dresden, NY, *E. necator* remained at trace levels on foliage and was zero on fruit following weekly nighttime applications of UV-C at 200 J/m^2 in 2020 and after weekly or twice-weekly application of UV-C at 100 or 200 J/m^2 in 2021. In 2019, weekly nighttime applications of UV-C at 200 J/m^2 also significantly reduced the severity of sour rot, a decay syndrome of complex etiology, on fruit of 'Vignoles' but not the severity of bunch rot caused by *Botrytis cinerea*. A similar level of suppression of sour rot was observed on 'Vignoles' vines treated twice-weekly with UV-C at 200 J/m^2 in 2021. Nighttime UV-C applications did not produce detectable indications of metabolic abnormalities, phytotoxicity, growth reduction, or reductions of fruit yield or quality parameters, even at the highest doses and most frequent intervals employed.

Keywords: disease management, fruit, fungi, small fruits

With the exception of the haustoria inserted into epidermal cells, powdery mildew pathogens are wholly external to the host and occupy a niche exposed to sunlight throughout the disease process. Only the chasmothecial wall cells of their ascigerous state possess pigments that offer physical protection from biocidal wavelengths of the solar spectrum: wavelengths of UV-B between 280 and 290 nm (Suthaparan et al. 2016a). Although powdery mildews are favored by shade and repressed to some degree by direct sunlight exposure (Austin and Wilcox 2011, 2012; Willocquet et al. 1996), in part they persist in the above niche due to their ability to repair UV-B-inflicted damage to their DNA through a robust photolyase mechanism driven by blue light and UV-A (Suthaparan et al. 2014; Thompson and Sancar 2002). This link between sunlight and the ability to withstand exposure to UV-B and UV-C has recently been exploited by exposing plants to fungicidal doses of UV-B or equivalent doses of UV-C during darkness. When damage to fungal DNA was not repaired within 4 h, it was generally lethal to a large percentage of exposed conidia and mycelia (Janisiewicz et al. 2016a, b; Suthaparan et al. 2012a, 2014, 2016a, 2017). The UV spectrum used in such studies has ranged from a UV-B waveband between 280 and 290 nm to near-monochromatic UV-C produced by low pressure discharge lamps yielding a peak output near 254 nm (Onofre et al. 2021). Reduction of the severity of several powdery mildews has been attributed to direct damage to the pathogen by UV exposure (Austin and Wilcox 2012; Gadoury et al. 2012a; Onofre et al. 2021; Suthaparan et al. 2012a, 2014, 2016a). UV-B and UV-C have been reported to be directly inhibitory to *Botrytis cinerea* on strawberry (Janisiewicz et al. 2016a) and geranium (Darras et al. 2015) and to phytophageous mites on strawberry (Osakabe 2021). In contrast, pathogens other than powdery mildews have been suppressed by exposure of their hosts to UV-C prior to inoculation (Buxton et al. 1957; Kunz et al. 2008; Patel et al. 2017), possibly due to enhancement of host resistance, and Ledermann et al. (2021) recently reported that preinfection exposure of grapevines to UV-C increased host resistance to infection by *Erysiphe necator*.

The adaptation of nighttime UV-C treatments to commercial field plantings has necessitated the development of UV lamp arrays powerful enough to apply effective doses at speeds that allow the equipment to complete treatments during the available night interval, often in late spring and early summer, during some of the shortest nights of the year. A tractor-drawn UV-C apparatus was designed and constructed in an earlier

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study (Onofre et al. 2021) to suppress strawberry powdery mildew (*Podosphaera aphanis*). This apparatus contained two hemicylindrical arrays of UV-C lamps and was the basis of a later array design fitted to an autonomous robotic carriage (Onofre et al. 2021). UV-C treatments applied once or twice weekly at doses from 70 to 200 J/m^2 effectively suppressed strawberry powdery mildew to a degree that equaled or exceeded that of some of the best available fungicides (Onofre et al. 2021). A long-term goal of our research is to develop light-based disease suppression technology for other crops. We chose grapevine and *E. necator* as the next pathosystem for investigation.

Global winegrape production is largely based upon the production of *Vitis vinifera*, a host species comprised of cultivars that are all highly susceptible to infection by *E. necator* (Gadoury et al. 2015), as well as several other fungal and oomycete pathogens. In particular, fungicidal suppression of grapevine powdery mildew is problematic. Resistance to many Fungicide Resistance Action Committee classes, including sterol demethylation inhibitors (DMI), strolbilurins, bezimidazoles, and succinate dehydrogenase inhibitor (SDHI) fungicides is sufficiently widespread that the forgoing classes are no longer effective in some viticultural regions. Additionally, many viticultural regions exist in Mediterranean climates, with little rainfall during the crop production season. This creates an environment where powdery mildew predominates as the principal threat to healthy fruit and foliage. If nighttime UV-C treatments could obviate the threat posed by *E. necator* in these regions, the need for fungicide applications might be substantially reduced. In regions with higher rainfall and multiple fungal pathogens, the potential for nighttime UV-C treatments to remove the threat of powdery mildew would improve options for the remaining members of the pathogen complex. For example, the differential spectrum of activity of various fungicide classes and different infection requirements for grapevine powdery mildew versus downy mildew (*Plasmopara viticola*) makes the optimization of fungicide activity and the integration of fungicide timing exceedingly difficult, which is a problem that has increased as fungicide options are reduced by resistance. Availability of a nonfungicidal option for suppression of powdery mildew to both reduce the selection for resistance and allow optimization of fungicidal suppression of the remaining members of the pathogen complex is therefore highly desired by the grape and wine industry. For all of the foregoing reasons, our objectives in the present study were to (i) determine the potential of nighttime UV-C applications to suppress grapevine powdery mildew; (ii) determine if UV-C at disease-suppressive doses and frequency of application has any deleterious effects on vine growth, yield, or crop quality; and (iii) determine if nighttime UV-C applications targeting powdery mildew have effects on other selected pests or diseases of grapevine. Preliminary reports of this work have been published (Gadoury 2019, 2021).

Materials and Methods

Effects of UV-C on conidial germination in *E. necator*

To establish a range of UV-C doses for field testing, conidia were first exposed to a range of UV-C dose in the lab. Inoculum of *E. necator* for assays was grown on detached *V. vinifera* 'Chardonnay' leaves on 1% water agar in Petri plates as previously described by Gadoury et al. (2012b). Conidia from 10-day-old colonies on these leaves were transferred using a fine artist's brush to glass microscope slides. The slides were transferred within 5 min after collection to a light-tight metal cabinet. The cabinet contained two low-pressure mercury discharge lamps (Osram HNS G13 55W UV-C, peak 253.7 nm, FWHM < 5 nm) mounted at a height of 220 cm above the cabinet base behind a pneumatically operated sliding shutter mechanism. The remotely operated shutter allowed the lamps to be powered during sample manipulations without exposing the samples to UV-C, as well as precise (±0.5 s) times of exposure to UV-C. Irradiance at various distances from the lamps was calibrated after allowing 5 min for the lamp output to stabilize using a spectroradiometer (Giga-Hertz Optik UV-C BTS2048-UV-C). Irradiance at 15, 25, 45, 85, 165, and 220 cm from the lamps measured 50, 25, 10, 4, 1.5, and 1.0 W/m^2, respectively. Conidia were exposed to UV-C doses of 0, 50, 100, and 200 J/m^2, corresponding to exposure times of 0, 5, 10, and 20 s at 45 cm from the lamps. Following UV-C exposure, slides were transferred in darkness to foil-wrapped glass Petri plates containing a 9-cm disk of moist filter paper and were incubated in darkness at 20°C for 24 h. A drop of sterile distilled water and glass coverslip was applied to each slide, and germination among a sample of 100 conidia on each slide was then assessed at a magnification of 100×. Conidia were considered germinated if possessing a germ tube exceeding the length of a conidium (Gadoury et al. 2015). Immediately after germination was assessed, a drop of sterile distilled water containing a 0.5% (wt/vol) aqueous solution of the fluorescent vital stain fluorescein diacetate was placed at the edge of the coverglass to be drawn under through capillary action to contact the condia. The conidia were allowed to absorb the stain for 5 min, and then one 100× microscopic field was observed under fluorescence microscopy (325- to 500-nm excitation filter and transmission filter > 530 nm) and immediately afterward under bright-field microscopy. The number of germinated conidia exhibiting bright green fluorescence under illumination at 325 to 500 nm and the total number of germinated and ungerminated conidia observed under brightfield illumination were recorded. Treatments were replicated on three glass sides, and the experiment was repeated once.

Reciprocity of irradiance and duration of exposure in UV-C dose/response effects upon conidial germination in *E. necator*

To assess if dose reciprocity (Sommer et al. 1996) held for the range of irradiance and times of exposure anticipated for vineyard applications of UV-C, conidia of *E. necator* raised as described above were transferred as above to the surface of 1-cm disks of *V. vinifera* 'Chardonnay' leaves on 1% water agar. UV-C doses of 4, 6, 16, 40, 100, and 200 J/m^2 were applied to *E. necator* conidia on leaf disks by placing samples at predetermined distances from the UV-C lamps over a period of either 4 or 400 s. Following UV-C exposure, the Petri plates containing the disks were transferred in darkness to incubate in darkness at 22°C for 24 h. Following incubation, disks were cleared (3:1 95% ETOH:glacial acetic acid), stained with 0.5% Chlorazol Black E in 80% lactic acid, and mounted on glass slides in 50% glycerol for observation at 200×. Germination among 30 to 100 conidia on each disk was assessed. Germination was defined as any spore that exhibited (i) a germ tube greater than or equal to the length of a conidium or (ii) a lobed appressorium irrespective of germ tube length. Treatments were replicated on 20 disks, and the experiment was repeated three times.

Laboratory studies of the activity of UV-C against *P. viticola*

To prepare and maintain inoculum for inoculations, the first translucent and fully expanded leaves (generally three to four nodes from the shoot apex) were harvested from disease-free, greenhouse-grown *V. vinifera* 'Chardonnay' grapevines. Leaves were sterilized for 2 min in 0.6% sodium hypochlorite, rinsed several times in sterile distilled water, and placed with the abaxial surface uppermost on 1% water agar in 9-cm polystyrene Petri plates. Sporangial inoculum of *P. viticola* downy mildew was cultured on these leaves and rinsed from them with sterile distilled water when required for experiments.

To evaluate effects of preinoculation exposure of leaves to UV-C, freshly harvested leaves were collected as described above, surface sterilized, and placed, abaxial surface uppermost, on 1% water agar in 9-cm Petri plates. The leaves were then transferred to the above cabinet in darkness, the lids of the plates were removed, and the leaves were exposed to 0, 47, 91, 131, or 204 J/m^2 of UV-C. After UV-C exposure, the leaves were incubated in darkness at 22°C for 12 h and then inoculated using an aerosol suspension of 1×10^5 sporangia of *P. viticola*. Following inoculation, the Petri plates containing the leaves were placed in a growth chamber at 23°C for a 12-h photoperiod. At 7 days after inoculation, 1-cm disks were cut from each leaf, sporangia were rinsed from their surface, the rinsate was adjusted to a volume of 5 ml, the number of sporangia per ml was determined at 100× using a hemocytometer, and the number of sporangia produced per cm^2 of leaf tissue was calculated.

Preinfection and postinfection activity of UV-C against *P. viticola* were compared as follows. Leaf disks 1 cm in diameter were cut from expanding leaves of *V. vinifera* 'Chardonnay' as described above and were randomly placed, adaxial surface uppermost, on 1% water agar in 9-cm Petri plates. Plates containing 10 leaf disks were (i) exposed to 200 J/m^2 UV-C and then inoculated 24 or 48 h later with a suspension containing 1×10^5 sporangia/ml as described above; (ii) inoculated with a suspension containing 1×10^5 sporangia/ml and then exposed to 200 J/m^2 UV-C 24 or 48 h later; or (iii) inoculated with a suspension containing 1×10^5 sporangia/ml and not exposed to UV-C as an inoculated but untreated control. At 7 days after inoculation, sporangia were rinsed from the surface of each disk, the rinsate was adjusted to a volume of 5 ml, the number of sporangia per ml was determined at 100× using a hemocytometer, and the number of sporangia produced per cm^2 of leaf tissue was calculated. Treatments were replicated four times, and the experiment was repeated. Duration of protection from infection by preinoculation exposure to UV-C was similarly evaluated, but the time between exposure to UV-C and subsequent inoculation was varied in eight temporal steps from 0 to 168 h after exposure to UV-C.

Vineyard trials for the suppression of grapevine diseases by UV-C

Trials for the suppression of powdery mildew during 2019 were conducted in a 1-ha vineyard that had been planted to *V. vinifera* 'Chardonnay' in 2004 at the New York State Agricultural Experiment Station in Geneva, NY. All vines were midwire cordon-trained and spur-pruned with vertical shoot positioning, resulting in a wall of foliage maintained at a maximum width of approximately 60 cm through use of catch wires and summer pruning of the canopy, yielding a defined fruiting zone approximately 60 to 80 cm above the vineyard floor. Treatments were arranged in a randomized complete block design of nine-vine plots replicated four times and consisted of the following treatments: (i) an untreated control; (ii) weekly nighttime applications of UV-C at 100 J/m^2; (iii) weekly nighttime applications of UV-C at 200 J/m^2; and (iv) a commercial fungicide standard.

The commercial fungicide standard (D. Combs, unpublished) was applied on 29 May at 2 weeks prebloom (Revus Top [Syngenta US], 0.491 kg product/ha), 3 June (Phostrol [Nufarm Inc] 2.9 liters product/ha), 11 June (Vivando 300 SC [BASF GmbH], 0.701 kg product/ha), 17 June (Zampro 4.4 SC [BASF GmbH] 0.981 kg product/ha), 25 June (Luna Experience [Bayer Crop Science US] 0.421 kg product/ha), 1 July (Phostrol 2.9 liters product/ha), 8 July (Gatten EC [Gatten America], 0.421 kg product/ha), 12 July (Zampro 4.4 SC, 0.981 kg product/ha), 23 July (Microthiol [United Phosphorus Inc], 5.61 kg product/ha), 24 July (Ranman 400 SC [Summit Agro], 0.193 kg product/ha), 5 August (Microthiol, 5.61 kg product/ha), 6 August (Presidio [Valent U.S.A.], 0.259 kg product/ha), and 20 August (Microthiol 5.61 kg product/ha).

Nighttime UV-C applications began 30 min after sunset and were completed within 2 h. Disease incidence (percentage of leaves or fruit clusters infected) and severity (percentage of leaf or fruit cluster infected) was assessed at 2-week intervals on the center five vines of each nine-vine plot from 1 June to 1 August 2019 on at least 10 shoots and 10 fruit clusters per vine. The BBCH stage of growth of foliage and fruit was recorded during disease assessments (Wilcox et al. 2015).

A trial for the suppression of grape sour rot, a complex disease syndrome involving wild yeast species, acetic acid bacteria, and fruit-feeding insects (Hall et al. 2018), was conducted in 2019 in a vineyard planted to the *Vitis* interspecific hybrid cultivar Vignoles in 1999 at the New York State Agricultural Experiment Station in Geneva, NY. The 'Vignoles' vines were pruned, trained, and maintained as in the previously described 'Chardonnay' vineyard. Treatments were arranged in nine-vine plots replicated four times and consisted of the following: (i) an untreated control; (ii) weekly nighttime applications of UV-C at 200 J/m^2; and (iii) applications of the fungicide Oxidate 2.0 (27% hydrogen peroxide plus 2% peroxyacetic acid, BioSafe Systems LLC) in a 1:200 dilution at 935 liters/ha at 7- to 14-day intervals. Incidence and severity of sour rot was assessed on 25 clusters per replicated plot on 25 August 2019.

In 2020, trials at the previously described *V. vinifera* 'Chardonnay' at the New York State Agricultural Experiment Station in Geneva, NY, were expanded to include both powdery and downy mildew. Treatments were arranged in a randomized complete block design of 15-vine plots replicated four times and consisted of the following treatments: (i) an untreated control; (ii) weekly nigttime applications of UV-C at 200 J/m^2; (iii) twice-weekly nighttime applications of UV-C at 200 J/m^2; (iv) weekly nighttime applications of UV-C at 100 J/m^2; (v) twice-weekly nighttime applications of UV-C at 100 J/m^2; and (vi) a commercial fungicide standard.

The commercial fungicide standard (Gold and Combs 2022) was applied on 1 June at 2 weeks prebloom (Quintec 2SC [Gowan Inc], 277 ml product/ha), 4 June (Zampro 4.4SC, 968 ml product/ha), 15 June (Rally 40WSP [Corteva Agriscience], 0.351 kg product/ha plus Ranman 400 SC 190 ml product/ha), 29 June (Quintec 2SC, 277 ml product/ha plus Revus 0.561 kg product/ha), 13 July (Rally 40WSP, 0.351 kg product/ha), 24 July (Ridomil Gold SL [Syngenta US] 1.613 liters product/A), 27 July (Quintec 2SC, 277 ml product/ha plus ProPhyt 4.43 liters product/ha), 10 August (Rally 40WSP, 0.351 kg product/ha plus Ranman 400 SC 190 ml product/ha), and 21 August (Microthiol 5.61 kg product/ha plus Orondis Ultra [Syngenta U.S.A.] 0.473 kg product/ha).

Nighttime UV-C applications began 30 min after sunset and were completed within 2 h. Disease incidence (percentage of leaves or fruit clusters infected) and severity (percentage of leaf or fruit cluster infected) was assessed at 2-week intervals on the center five vines of each nine-vine plot from 1 June to 1 August 2020 on at least 10 shoots and 10 fruit clusters per vine. The BBCH stage of growth of foliage and fruit was recorded during disease assessments.

An additional trial for suppression of powdery mildew was conducted in a 10-ha commercial vineyard of *V. vinifera* 'Chardonnay' at Anthony Road Wine Company in Dresden, NY. The vines were pruned, trained, and managed similarly to those in the previously described Chardonnay vineyard in Geneva. Treatments were arranged in single-row plots of 315 vines/row replicated three times and consisted of the following treatments: (i) weekly nighttime applications of UV-C at 200 J/m^2 and (ii) a commercial fungicide standard. Vines in the UV-C-treated plots were treated with fungicides with selective suppressive activity against downy mildew at 10- to 14-day intervals from 1 July to 15 August. Host phenology and the incidence and severity of powdery mildew on leaves and fruit was assessed as above. The above trial was repeated and expanded in 2021 to include applications of UV-C at 100 or 200 J/m^2 applied weekly and twice-weekly.

In 2021, a trial for the suppression of powdery mildew, downy mildew, and sour rot was conducted in a 0.5-ha planting of the *Vitis* interspecific hybrid cultivar 'Vignoles' at a USDA research vineyard in Geneva, NY. This cultivar is only moderately susceptible to powdery mildew and downy mildew but is highly susceptible to sour rot. Vines were pruned, trained, and maintained similarly to those in the previously described 'Chardonnay' vineyard. Treatments were arranged in a randomized complete block design of four-vine plots replicated five times and consisted of the following treatments: (i) an untreated control; (ii) twice-weekly nighttime applications of UV-C at 200 J/m^2, and (iii) applications of the fungicide Oxidate 2.0 (27% hydrogen peroxide plus 2% peroxyacetic acid, BioSafe Systems LLC) in a 1:200 dilution at 100 gal/A at 7- to 14-day intervals. Disease incidence and severity was assessed as described above.

The UV-C lamp arrays used for field studies were similar in configuration to those described by Onofre et al. (2021) for strawberries but with dimensions adapted for trellised grapevine canopies. Each array consisted of a hemicylindrical or arched arrangement (Fig. 1) of low-pressure discharge UV-C lamps (Osram germicidal T8 55W UV-C Medium Bi Pin Base model G55T8/OF) backed by polished aluminum reflectors (Lamar Lighting Lamar XRFP230) and driven at 700 mA (100% output) using a Philips Advance model UV-2S60-M4-LD ballast. Arrays (Fig. 1) were moved through vineyards either as a tractor-drawn arch suspended from the side of a utility

trailer or using a fully autonomous robotic carriage (model Thorvald, SAGA Robotics, Ås, Norway). Uniformity and intensity of irradiance was confirmed as described by Onofre et al. (2021) using a UV spectroradiometer (model BTS2048-UV-S, Gigahertz-Optik GmbH). Dosing was established by adjusting ground speed to apply the desired dose in Joules/m^2 based upon the array length and mean irradiance at the center line of the array at a height of 60 cm above ground: the approximate height of the fruiting zone within the vineyard trellis. For example, given a mean irradiance of 75 W/m^2 under an array, an array length of 1.5 m, and a target dose of 200 J/m^2, ground speed would be adjusted such that the array would require 2.666 s to pass over the target, yielding a speed of 1.777 m/s, or 6.397 km/h (75 W/m^2 × 2.666 s = 200 J/m^2).

Effects of UV-C applications on grapevine growth and physiology

At the 'Chardonnay' vineyard in Geneva, NY, photosynthetic parameters were measured at midday on the day after UV-C applications using a MultispeQ v2.0 device (PhotosynQ, East Lansing, MI) on the following treatments: (i) untreated control vines and (ii) vines receiving weekly UV-C applications at 200 J/m^2 in 2019 and 2020. Measurements were conducted on the youngest fully expanded leaf of three shoots per vine on two vines in each replicated block. The 'Photosynthesis RIDES' protocol was used to assess relative chlorophyll, chlorophyll fluorescence parameters, ATP synthase conductivity, saturation pulse estimation of Photosystem I parameters, absorbance at eight wavelengths (450, 535, 605, 650, 730, 850, 880, and 940 nm), and rate of turnover of cytochrome b6f complex. Chlorophyll fluorescence parameters include quantum yield of Photosystem II (PSII; Φ_{II}), nonregulatory light dissipation (Φ_{NO}) nonphotochemical exciton quenching (Φ_{NPQ}), and linear electron flow (LEF). ATP synthase conductivity parameters include proton conductivity (gH^+), steady-state proton flux (vH^+), and magnitude of electrochromic shift (ECS_t). Leaf thickness was also measured using the MultispeQ device. In addition to the foregoing, leaf length and width were measured manually on the same leaves, and the number of leaves per shoot was recorded.

Seasonal treatment effects upon sugar accumulation and fruit cluster weights were determined at harvest in 2019 at Geneva, NY, and in 2020 and 2021 at the commercial 'Chardonnay' vineyards in Dresden, NY, by weighing 10 fruit clusters randomly collected from each replicate block of all treatments. Three berries were also harvested from each of the 10 clusters per replicate, the berries were crushed, and the soluble solids level of a 1-ml sample of the juice was measured using a refractometer (Bausch and Lomb model 10, Rochester, NY). Effects of repeated UV-C treatments on foliar development was assessed in the commercial 'Chardonnay' vineyards in 2020 and 2021 by measuring the length and width of the youngest fully expanded leaf of five shoots per vine on five vines in each replicated block, as well as the total number of leaves per shoot approximately 1 week postblooom in each year of the study.

On 14 July 2020, on the morning after UV-C treatment, foliar reflectance spanning the visible (VIS) to shortwave infrared (SWIR) was measured using a high-spectral-resolution SVC HR-1024i (350 to 2,500 nm; collectively VSWIR) portable spectroradiometer (Spectra Vista Corporation, Schnectady, NY) on the third fully emerged and visibly healthy leaf of five to six shoots per vine from four vines within one replicated block of the untreated control and vines exposed weekly to 70, 100, and 200 J/m^2 within the trial at Crittenden Farm in Geneva, NY. All measurements were taken from the leaf adaxial surface using a leaf-clip assembly attached to a plant probe with an internal halogen light source and 99% spectralon used as white reference (Labsphere, North Sutton, NH). Following standard practice (Couture et al. 2018; Gold et al. 2019), bad measurements, such as those with low reflectance or abnormalities due to measurement error, were removed. Data was prepared for analysis in R by removing noisy bands at the shortest and longest wavelengths (resulting in a spectral range of 400 to 2,400 nm) and interpolated to 1-nm resolution from the native 3- to 8-nm resolution of the instrument.

Statistical analyses

The response variables measuring pathogen suppression or host growth were analyzed in a generalized linear mixed model with treatments as fixed effects and blocks as random variables. Significance of treatment effects was confirmed by two-way analysis of variance (ANOVA). Student's *t* test was used for simple comparisons of two sample means, and Fisher's Protected Least Significant Difference test ($\alpha = 0.05$) was used for multiple comparisons. Linear or nonlinear regression were also used to analyze relationships across gradients of dependent and independent variables. Where results for repeated experiments were combined, normality and homogeneity of variance between repeats were first confirmed.

For host responses to UV measured using PhotosynQ, all observations flagged "issue" by the MultispeQ device were removed from the dataset. Measurements with values of greater or less than three times the interquartile range were deemed outliers and removed. Data

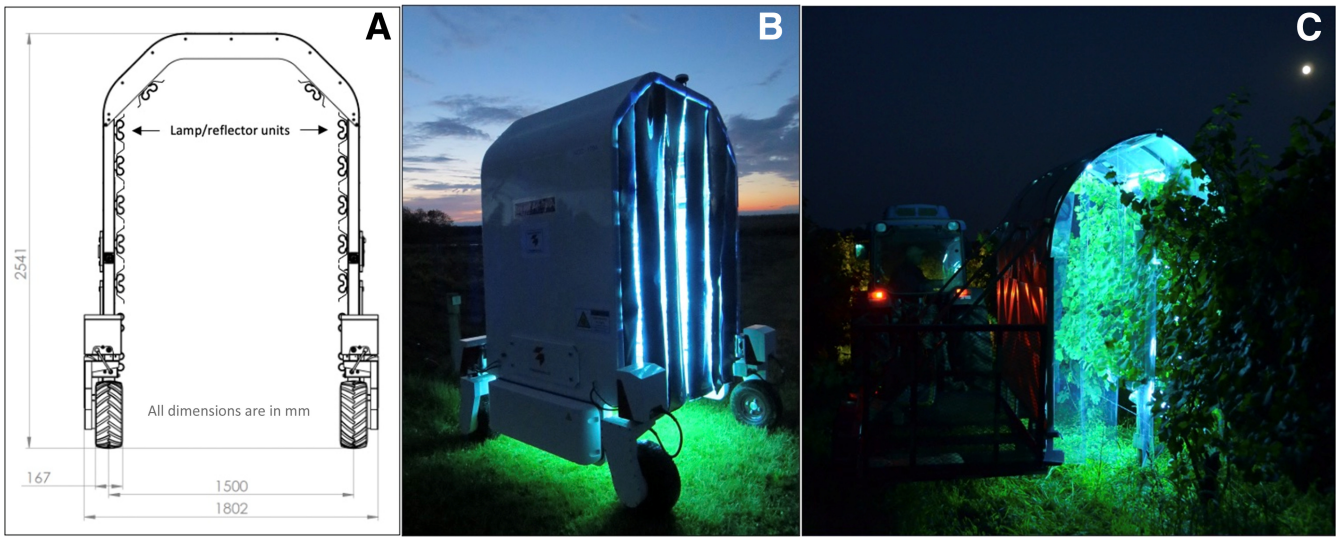

Fig. 1. Robotic and tractor-drawn UV lamp arrays. **A,** Diagrammatic end view of SAGA Robotics "Thorvald" autonomous robotic carriage showing dimensions in mm and positions of lamp/reflector units. **B,** Quartering view of "Thorvald" with reflective end curtains in place at the beginning of nighttime UV applications at the vineyard site. **C,** Tractor-drawn lamp array during nighttime UV application.

was evaluated at each time point and observation independently. Normality for each observation-time combination was determined using the Anderson-Darling test, with data returning P values of >0.05 determined to be normally distributed. When P values fell into the (0.01, 0.05) range, Q-Q plots were visually evaluated, and data was determined to be approximately normal if the Q-Q plot laid along a y = x line without notable skew or kurtosis. To summarize the combined effects and assess the cumulative and long-term effects of the broad variety of physiological responses measured by PhotosynQ on untreated control vines (UTC) compared to vines treated with UV (UV-C), responses were converted to ratios of UV-C/UTC, averaged within years, and then pooled for both years after confirmation of homogeneity of variance. The 95% confidence limits for the ratio for each response were calculated to determine if a confidence limit overlapped 1.0, indicating no significant difference between means for the UV-C treatment compared to the untreated control.

Responses measured through hyperspectral radiometry were subjected to permutation multivariate analysis of variance using distance matrices (PERMANOVA) to generate "pseudo" F and P-values. Treatment significance on spectral reflectance was tested using the R package *vegan* (Oksanen et al. 2013). Foliar nitrogen, total phenolics, sugar, starch, and leaf mass/area (LMA, an indicator of leaf dry-mass investment relating to light interception and leaf longevity) were estimated from spectra using PLS regression (PLSR) calibrations from the UW-ENSPEC profile on EcoSML.org, an open-source repository of spectral mapping functions (https://ecosml.org/package/github/ecosml/Leaf-traits). A two-way ANOVA was fit using a mixed effect model with a maximum likelihood estimation method for each of the metrics, total phenolics concentration, sugar concentration, starch concentration, nitrogen concentration, and LMA, and Tukey's posthoc comparison of means was used to assess significant differences between treatments with the R packages *nmle* and *emmeans* (Lenth 2021; Pinheiro et al. 2020).

Results

Effects of UV-C on conidial germination in *E. necator*

Germination of conidia on glass slides exposed to UV-C during darkness was inversely proportional to the dose applied and was well described by the following equation: $Y = 90.99 - 0.921X + 0.0023X^2$, $R^2 = 0.98$ (Fig. 2A), where Y = the percentage of conidia that will germinate, and X = the dose of UV-C to which conidia were exposed during darkness. Germination declined from approximately 90% in unexposed conidia to near 20% at a UV-C dose of 100 J/m^2 (Fig. 2A). Virtually no conidia germinated at 150 or 200 J/m^2. The rate of germination underestimated the lethality of intermediate doses of UV-C to condia, as only 62.3% (SE = 9.71) of germinated conidia fluoresced in fluorescein diacetate (FDA) after exposure to 50 J/m^2 UV-C, and only 9.2% (SE = 2.02) of germinated spores fluoresced in FDA after exposure to 100 J/m^2. This would indicate that many conidia exposed to either 50 or 100 J/m^2 of UV-C died shortly after germination.

Reciprocity of irradiance and duration of exposure in UV-C dose/response effects in *E. necator*

The rate of germination of condia exposed to increasing UV-C doses between 0 and 200 J/m^2 declined equivalently whether the dose was applied over a period of 4 or 400 s (Fig. 2B). Neither means compared at discrete doses nor coefficients of equations fit to the dose/response differed significantly at $P = 0.05$. In the 4 s exposure, $Y = 43.60^{(-0.03X)}$ and $R^2 = 0.948$, and in the 400 s exposures, $Y = 51.03^{(-0.031X)}$ and $R^2 = 0.971$, where Y = the proportion of germinated conidia, and X = the dose of UV-C to which conidia were exposed during darkness (Fig. 2B).

Laboratory studies of the activity of UV-C against *P. viticola*

When grapevine leaves were exposed to UV-C doses ranging from 47 to 204 J/m^2 12 h before inoculation with *P. viticola*, the number of sporangia produced per unit of infected leaf was inversely proportional to the UV-C dose applied over the range of observations (Fig. 3). A dose of 204 J/m^2 reduced sporangial production by over 90%, from 554,755 to 5,106 spornagia per cm^2 (Fig. 3). Exposure of grapevine leaves to a single UV-C dose of 200 J/m^2 at times between 12 and 168 h before inoculation similarly reduced the subsequent sporulation of *P. viticola*, irrespective of time elapsed between UV-C

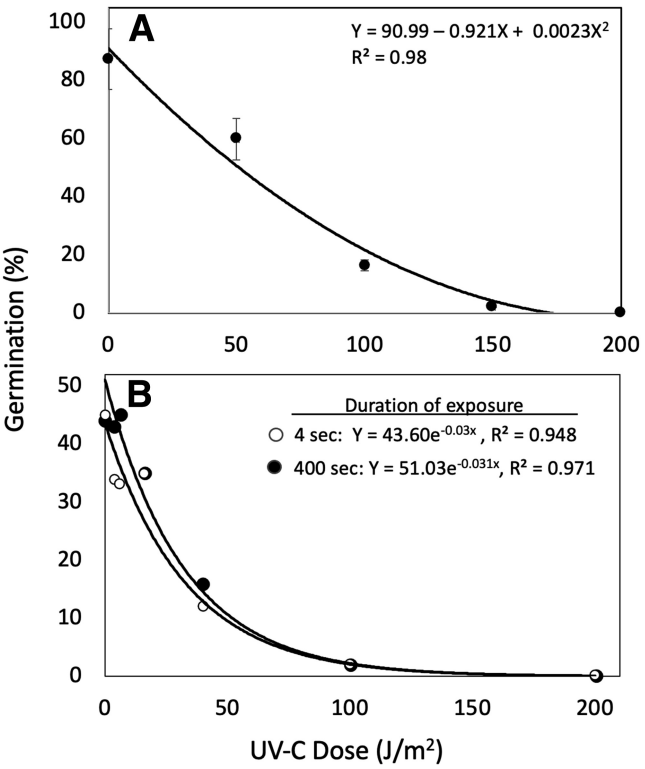

Fig. 2. A, Germination of conidia of *Erysiphe necator* 24 h after nighttime exposure to germicidal UV-C light at various doses. Bars indicate the SE of the mean at each dose. **B,** Effect of UV-C doses applied over periods of 4 or 400 s upon the percentage of germinated conidia of *E. necator*. Neither intercept nor slope coefficients of fitted equations differed significantly ($P = 0.05$), indicating reciprocity of irradiance and time of exposure within the range of UV-C dose applied.

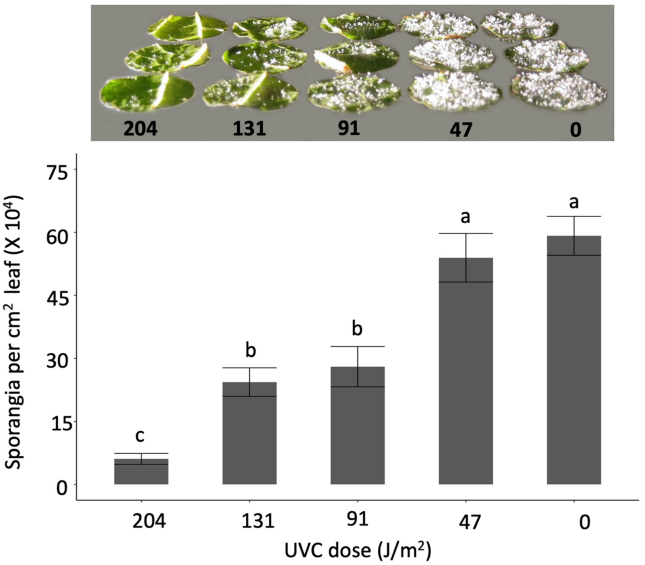

Fig. 3. Effect of a single preinfection application of UV-C to leaves of *Vitis vinifera* 'Chardonnay'. Leaves were exposed to the indicated UV-C doses during darkness and were inoculated with sporangia of *Plasmopara viticola* 12 h later. Leaf samples were then incubated at 23°C for 7 days after inoculation, and the resultant sporangia were harvested and enumerated.

exposure and inoculation (Fig. 4A). Regression of sporangial production at 7 days postinoculation against hours elapsed between UV-C exposure and inoculation yelded the following equation (Fig. 4A): Y = 12.583 + 0.0201X, where Y is sporangial production per cm^2 of leaf tissue 7 days postinoculation, and X is time (h) elapsed between UV-C exposure and subsequent inoculation.

The slope coefficient of the above equation was not significantly different from 0 ($P = 0.01$), indicating that UV-C exposure between 12 and 168 h before inoculation had an equally suppressive effect on sporangial production (Fig. 4A).

Exposure of 'Chardonnay' leaves to UV-C at 200 J/m^2 24 or 48 h before inoculation or 24 h after inoculation substantially but equivalently ($P > 0.05$) suppressed sporangial production (Fig. 4B). When exposure to UV-C was delayed until 48 h after inoculation, sporangial production was still reduced compared to the untreated control (Fig. 4B) but was significantly greater than that resulting from exposure to UV-C at 24 h before or after inoculation, or from exposure to UV-C 48 h before inoculation (Fig. 4B). In a partial repeat of the forgoing experiment, exposure to UV-C at 72 h after inoculation provided no significant ($P > 0.05$) suppression of sporangial production compared to an untreated control.

Vineyard trials for the suppression of grapevine diseases by UV-C

Weekly nighttime applications of UV-C at 100 or 200 J/m^2 significantly but equivalently ($P > 0.05$) reduced the severity of powdery mildew on leaves (Fig. 5A) and fruit (Fig. 5B) of 'Chardonnay' grapevines at Geneva, NY, in 2019 compared to the untreated control but did not suppress disease as effectively as the fungicide standard (Fig. 5A and B). In 2020, severity of powdery mildew on leaves and fruit of the untreated control vines at Geneva (Fig. 6) was approximately twice that observed in the same vineyard in 2019 (Fig. 5). All UV-C treatments reduced the severity of powdery mildew in leaves and fruit compared to the untreated control (Fig. 6). At doses of 70 or 100 J/m^2, the degree of disease suppression on leaves (Fig. 6A) or fruit (Fig. 6B) was not significantly increased ($P > 0.05$) by applying the dose twice weekly compared to once per week. However, at 200 J/m^2, UV-C applied twice weekly provided significantly better suppression of powdery mildew on both leaves and fruit than did once-weekly applications ($P < 0.05$), and the 200 J/m^2 dose applied twice weekly provided suppression of foliar and fruit disease severity that was statistically equivalent ($P > 0.05$) to that provided by the fungicide standard (Fig. 6). When subjected to linear regression analysis within frequencies of UV-C application (weekly and twice weekly), the direct proportionality of dose to efficacy of disease suppression was described (Fig. 7) as follows: Y = 32.380 – 0.1483X (weekly UV-C application, Fig. 7A, $R^2 = 0.8474$), and Y = 36.312 – 0.1826X (twice-weekly application, Fig. 7B, $R^2 = 0.9822$), where Y is cluster surface colonized (%), and X is UV-C dose (J/m^2).

As the frequency of UV-C applications increased from weekly to twice-weekly, the slope coefficient increased significantly ($P < 0.05$), as did the R^2 of the fitted equations (Fig. 7).

In 2020, at the commercial 'Chardonnay' vineyard in Dresden, NY, incidence and severity of powdery mildew was at trace levels in both the grower's fungicide program and weekly applications of

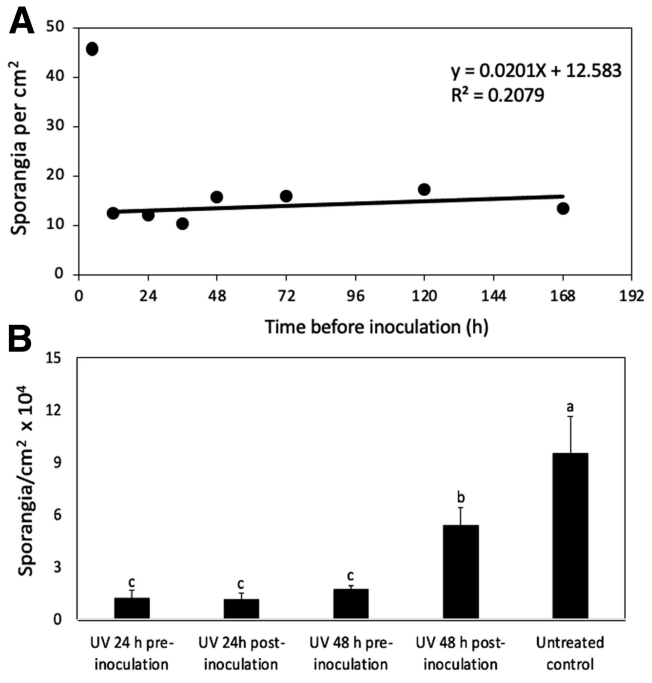

Fig. 4. A, Effect of a single preinoculation exposure of *Vitis vinifera* 'Chardonnay' leaves to a nighttime UV-C dose of 100 J/m^2 at times from 12 to 172 h before inoculation with *Plasmopara viticola*. The plants were returned to the greenhouse until the indicated times after UV-C exposure and then inoculated and incubated, and the sporangia were harvested and enumerated 7 days later. The slope coefficient of the linear equation fit to data from 12 to 172 h was not significantly different from 0 at $P = 0.05$. Data shown for the non-UV-C-treated control (mean = 47.13 sporangia per cm^2 at time = 0) was not included in the regression. **B,** Effects of preinfection and postinfection exposure of 'Chardonnay' grapevine leaves to UV-C upon sporangial production of *P. viticola*. Detached leaves were exposed to 200 J/m^2 of UV-C during darkness at the indicated times before or after inoculation and remained in darkness for 4 h after UV-C exposure. The untreated control was inoculated but not exposed to UV-C. The number of sporangia produced per cm^2 of leaf tissue was determined 7 days after inoculation.

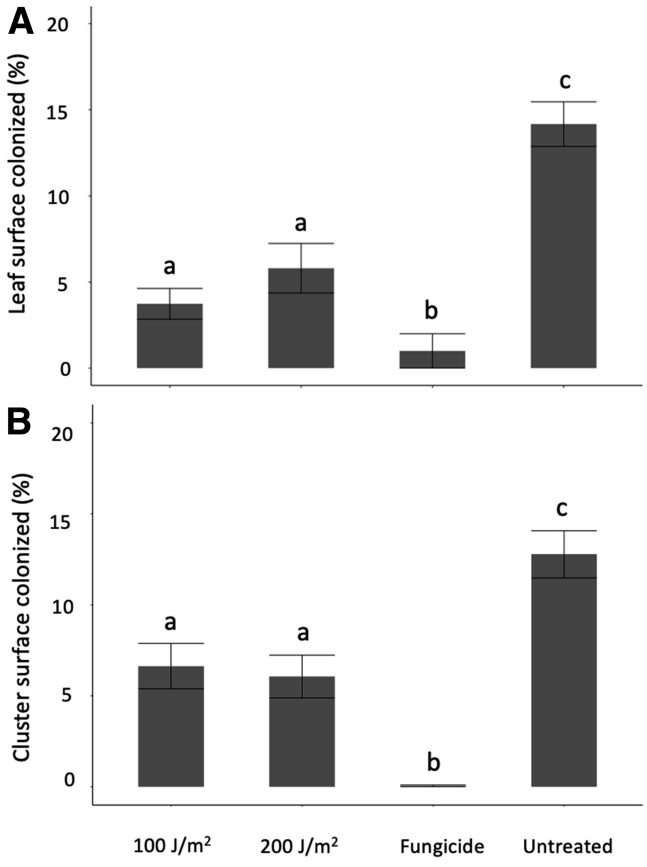

Fig. 5. Severity of powdery mildew on **A,** foliage and **B,** fruit of *Vitis vinifera* 'Chardonnay' at Geneva, NY, in 2019. The percentage of leaf or berry surface colonized by *Erysiphe necator* was assessed at veraison (mid-August 2019). UV-C treatments were applied weekly during nighttime hours at doses of 100 or 200 J/m^2. The fungicide treatment consisted of six fungicide sprays applied at 10- to 14-day intervals. Untreated vines received neither fungicides nor UV-C treatment. Error bars indicate one SE of the mean.

UV-C at 200 J/m^2. At the time of veraison in 2020 (13 August), the mean number of powdery mildew-infected leaves per shoot was 0.07 on fungicide-treated vines compared to 0.05 infected leaves per shoot on the UV-C-treated vines. The mean percentage of the leaf surface colonized on infected leaves was 0.33% on fungicide-treated vines compared to 0.23% on vines treated with UV-C. Similar trace levels of powdery mildew were observed among both levels (100 and 200 J/m^2) and frequency of application (once or twice weekly) on 'Chardonnay' vines in Dresden, NY, in 2021. The number of infected leaves per shoot did not exceed 0.03 among UV-C- or fungicide-treated vines in weekly disease assessments conducted from 11 June (immediate prebloom) to 5 August (veraison). On the interspecific hybrid cultivar Vignoles at the time of veraison (12 August) in 2021, application of UV-C at 200 J/m^2 reduced the mean number of powdery mildew-infected leaves per shoot to 0.62 (SE = 0.112) compared to 3.34 (SE = 0.39) on untreated control vines. The mean percentage of surface colonized on infected leaves was 2.17 (SE = 0.52) on vines treated with UV-C compared to 7.56 (SE = 1.83) on untreated control vines.

In 2020, no rate or interval of UV-C application significantly reduced the incidence or severity of foliar downy mildew on 'Chardonnay' grapevines compared to the untreated control (Fig. 8). The incidence of downy mildew reached commercially unacceptable levels on all UV-C treatments relatively early in vine development, approximately 1 week after the completion of bloom (25 June). The downy mildew part of the trial was discontinued beginning 30 June, and the vines received applications of fungicides with selective activity against oomycete pathogens at 7- to 14-day intervals thereafter to prevent their early defoliation. However, on the more downy mildew-resistant cultivar Vignoles in 2021, foliar downy mildew was reduced from 1.12 infected leaves per shoot (SE = 0.236) on the untreated control to 0.27 infected leaves per shoot (SE = 0.087) on vines treated twice-weekly with UV-C at 200 J/m^2.

Weekly applications of UV-C at 200 J/m^2 provided excellent suppression of sour rot on the *Vitis* 'Vignoles' compared to the fungicide standard used in 2019 (Fig. 9A). However, UV-C did not provide significant suppression of Botrytis bunch rot (Fig. 9B). In

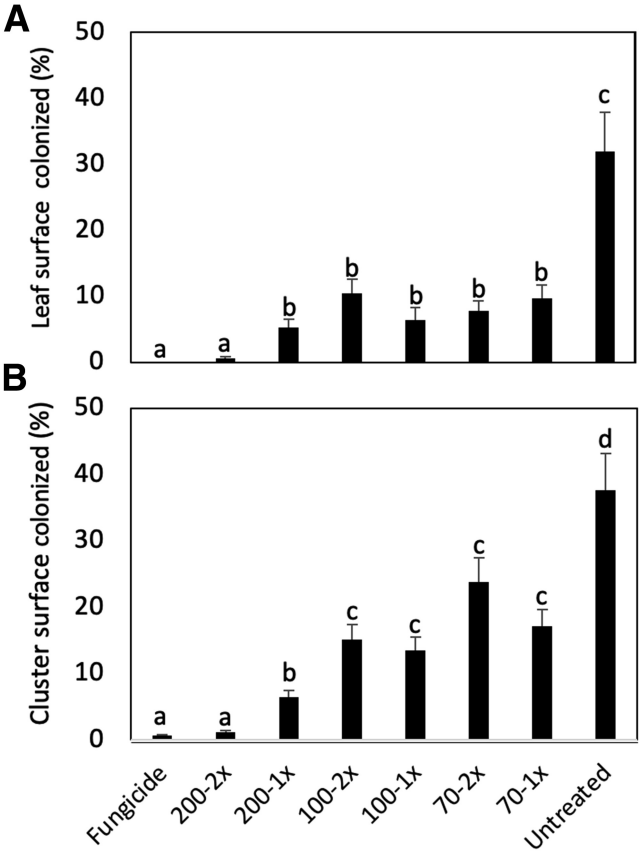

Fig. 6. Means and SEs of severity of powdery mildew on **A,** foliage and **B,** fruit of *Vitis vinifera* 'Chardonnay' at Geneva, NY, in 2020. The percentage of leaf or berry surface colonized by *Erysiphe necator* was assessed at veraison (mid-August 2020). The fungicide treatment consisted of seven fungicide sprays applied at 10- to 14-day intervals, whereas UV-C treatments were applied during nighttime hours at doses of 200, 100, or 70 J/m^2 either weekly (1×) or twice weekly (2×) during the same period. Untreated vines received neither fungicides nor UV-C treatments. Error bars indicate one SE of the mean. Treatment means followed by the same letter are not significantly different at $P = 0.05$.

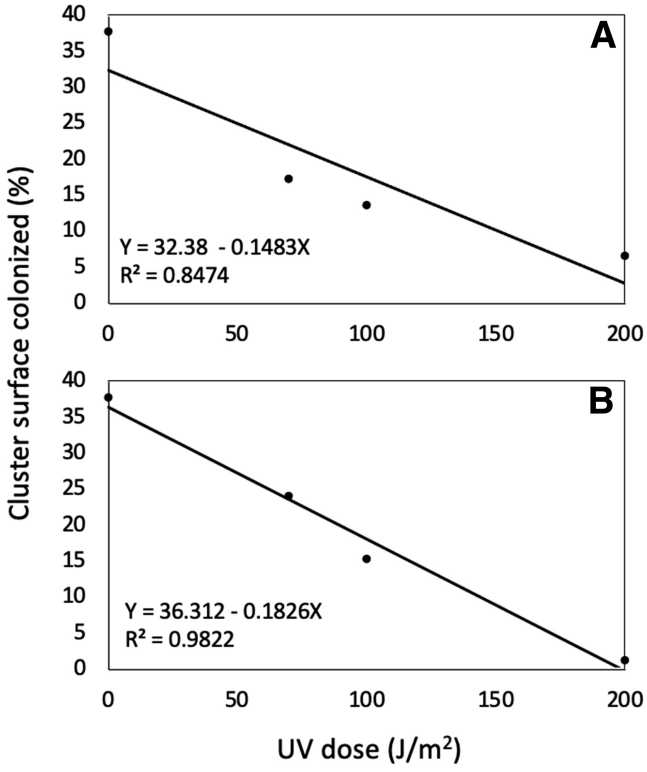

Fig. 7. Relationship between suppression of powdery mildew (*Erysiphe necator*) on 'Chardonnay' grapes and the dose of UV-C applied **A,** weekly or **B,** twice-weekly in 2020. UV-C treatments were applied during nighttime hours at doses of 0 (untreated), 70, 100, and 200 J/m^2. Severity of fruit infection was assessed at veraison (August 2020).

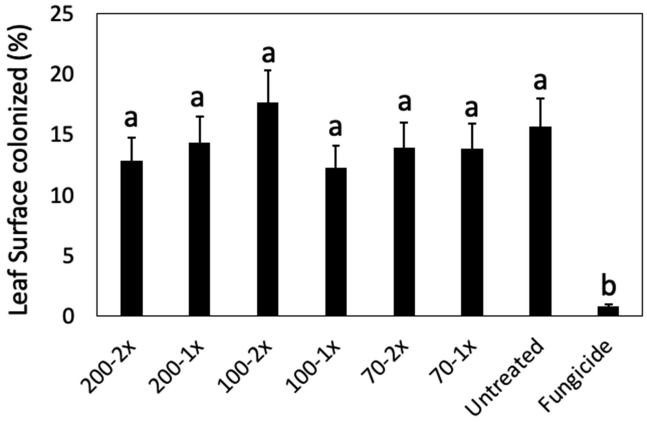

Fig. 8. Means and SEs of the severity of downy mildew (*Plasmopara viticola*) on foliage of *Vitis vinifera* 'Chardonnay' following weekly (1×) or twice-weekly (2×) treatments with UV-C light at 70, 100, or 200 J/m^2 compared to an untreated control and a standard fungicide program in Geneva, NY. Treatment means followed by the same letter are not significantly different at $P = 0.05$. Disease was assessed on 30 June 2020.

2021, twice-weekly application of UV-C at 200 J/m^2 also provided significant suppression of sour rot on 'Vignoles' grapevines compared to untreated vines (Fig. 9C).

Effects of UV-C applications on grapevine growth and physiology

In no year of the study, at no UV-C dose, and upon no cultivar was leaf length, leaf width, or the number of leaves per shoot significantly ($P > 0.05$) affected on UV-C-treated vines compared to untreated control vines, indicating that the rates of UV-C application had no deleterious effects on host physiology and development and suppressed relevant diseases to a level where they likely did not impair host growth and development. Likewise, berry size, berries per cluster, berry cluster weight, and soluble solid levels in fruit did not differ significantly ($P > 0.05$) between UV-treated vines and any vines not exposed to UV treatments. At the highest levels of UV-C treatment (twice-weekly at 200 J/m^2), mean cluster weight of 'Chardonnay' grapes was 129.7 g (SE = 23.2 g) compared to 122.9 g (SE = 19.4) for the untreated uncontrol. Mean soluble solid levels in 'Chardonnay' fruit treated twice weekly with UV-C at 200 J/m^2 ranged from 21.3 to 23.4% at harvest (SE = 0.49%) compared to a range of 21.3 to 23.3% (SE = 0.55%) among 'Chardonnay' fruit from vines not treated with UV-C. Consistent with the lack of impact of UV-C on the above physical expressions of growth of 'Chardonnay' grapevines in 2019 and 2020, of the 20 parameters measured by PhotosynQ analysis, none of the ratios of responses of UV-C/UTC was different at $P = 0.05$ (Fig. 10).

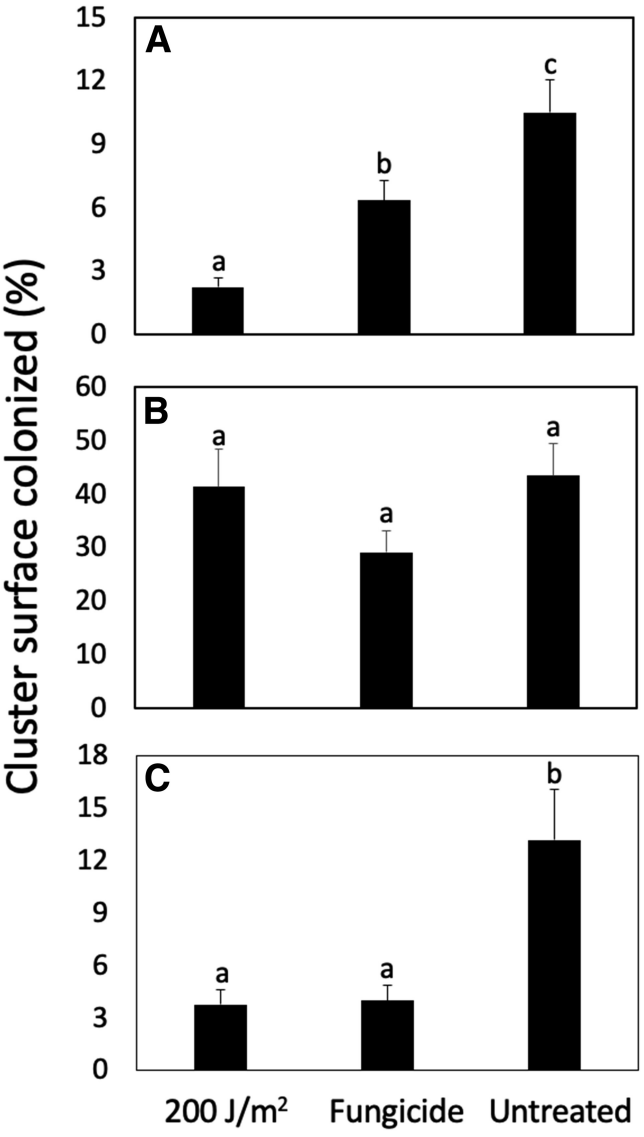

Fig. 9. Severity of **A,** sour rot complex in 2019; **B,** Botrytis (*Botrytis cinerea*) bunch rot in 2019; **C,** sour rot complex in 2021 on 'Vignoles' grapes. Vines were either treated weekly (2019) or twice weekly (2021) with ultraviolet light (UV-C) at 200 J/m^2, treated five to seven times at 7- to 14-day intervals with a fungicide, or were untreated. Error bars indicate one SE of the mean.

The spectroradiometric analysis indicated a significant difference among the average reflectance of healthy grapevine leaves that were untreated and treated with 70, 100, and 200 J/m^2 of UV-C (PERMANOVA $P = 0.002$). UV treatment impacted leaf chemistry and physiology as measured by concentration of phenolics, starch, and LMA, but nitrogen and sugar concentrations were unaffected (Fig. 11, $P < 0.05$). Nitrogen and sugar concentrations were not significantly different between any UV treatments. Both starch and LMA concentrations were significantly different between untreated and all levels of UV treatment; however, only LMA concentrations showed any variation among treatment levels. The untreated leaves had the lowest LMA (Fig. 11). In general, this would suggest more resource allocation to photosynthesis or defense compounds than structural compounds, while leaves treated with 70 J/m^2 of UV-C had the highest LMA (Fig. 11), suggesting more resource allocation to structural compounds. However, this was not confirmed by the above PhotosynQ measurements of leaf thickness in 2019 and 2020. Spectroradiometric analysis indicated that starch concentration was the lowest in untreated leaves and that there were no significant differences in the starch concentration among leaves treated with different doses of UV-C (Fig. 11). Spectroradiometric analysis indicated that total phenolic concentration was the lowest in leaves treated with 100 and 200 J/m^2 of UV-C and that there were no significant differences among leaves that were untreated and those that received 70 J/m^2 UV-C (Fig. 11). Total leaf phenolics concentration was significantly different between both untreated and 100- and 200-J/m^2 treatments, as well as between 70-J/m^2 treatments and both 100- and 200-J/m^2 treatments.

Discussion

Effects of UV-C on conidial germination in *E. necator*

A significant inhibitory effect of UV-C upon germination of conidia can be observed at 50 J/m^2 (Fig. 2A). Thus, in vitro germination as a response variable can reveal reproducible trends in lethality as the dose of UV-C is increased (Fig. 2A). However, our results also indicate that use of germination as a response variable may significantly underestimate the lethality of UV-C to conidia of *E. necator*. The germination process in *E. necator* is initiated within minutes after conidia land upon and adhere to surfaces (Delp 1954; Ficke et al. 2004). In contrast, the lethality of nighttime exposure to UV-C appears to involve a comparatively slower process, as evidenced by the reversibility of its effects if followed by exposure to blue light or UV-A within 2 to 4 h and the irreversibility of the effect if such exposure is delayed beyond 4 h (Janisiewicz et al. 2016a; Suthaparan et al. 2014). It is notable that a high percentage of conidia that germinated after exposure to UV-C failed to respond positively to a vital stain in the present study. Thus, it appears that a significant proportion of conidia that successfully geminate after exposure to what initially appears to be a sublethal dose of UV-C could ultimately die as a consequence of exposure before successfully infecting the host.

Reciprocity of irradiance and duration of exposure in UV-C dose/response effects in *E. necator*

Within a broad range of UV-C doses that has been considered for efficacy under field conditions (i.e., 50 to 200 J/m^2), our results indicate that equivalent effects upon the pathogen can be expected within application times spanning two orders of magnitude. Effects of UV-C doses witin this range were equivalently effective in suppressing conidial germination of *E. necator* whether they were applied over a period of 4 or 400 s (Fig. 2B). The practical impact of this demonstration of reciprocity is that a given UV-C dose can be

assumed to have equivalent suppressive effects on a pathogen independent of the irradiance of a lamp array and ground speed of application equipment within the broad ranges of irradiance and time used in the above study.

Laboratory studies of the activity of UV-C against *P. viticola*

Both pre- and postinoculation exposure of grapevine leaves to UV-C partially suppressed subsequent production of sporangia under laboratory conditions, and sporulation in the most effective treatments was generally in the range of 20 to 30% of that observed in untreated controls. Suppression of sporangial production was linear between 12 and 168 h before inoculation (Fig. 4A), and equivalent suppression was observed at 24 h preinoculation and 24 h postinoculation (Fig. 4B). However, the degree of suppression decreased if UV-C exposure was delayed until 48 h after inoculation (Fig. 4B), and no suppression occurred if exposure was delayed until 72 h after inoculation. Thus, the principal impact of UV-C exposure would appear to operate through alteration of host susceptibility, similar to that observed for preinoculation exposure to UV-B in suppression of basil downy mildew (Patel et al. 2017). In the case of grapevine and *P. viticola*, the effect was most pronounced when UV-C exposure occurred before inoculation or within 24 h after inoculation; was equivalent for UV-C exposure 1 to 7 days before inoculation, and the suppression of sporangial production was not significant if the infection process was allowed to proceed for 72 h before the UV-C exposure.

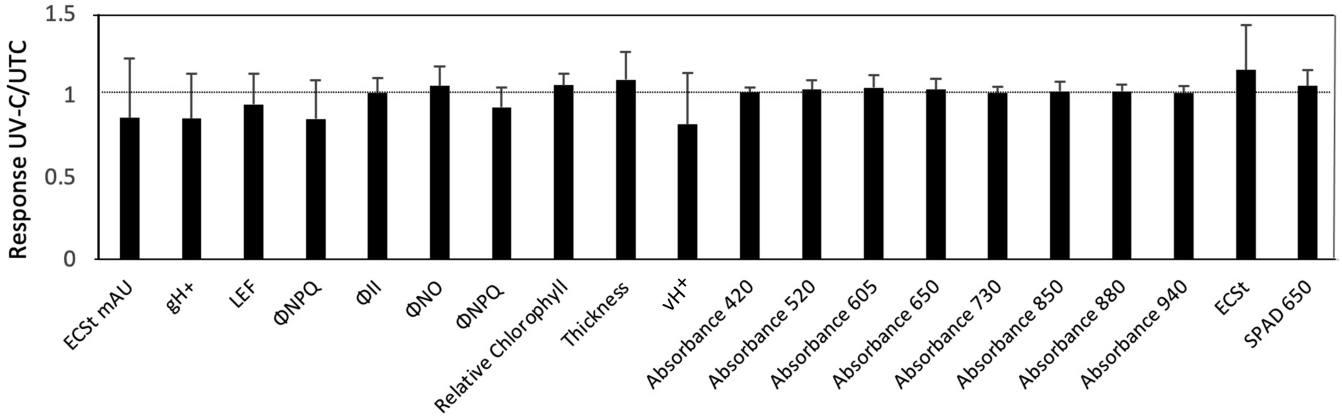

Fig. 10. Response ratios of 'Chardonnay' grapevines treated with UV-C (UV-C) and untreated controls (UTC). A broad variety of physiological responses among 'Chardonnay' grapevines treated weekly at 200 J/m^2 or untreated was measured via PhotosynQ in 2019 and 2020 on four dates approximately 24 h after UV-C applications between bud break and fruit set within each year. Mean responses were converted to ratios of UV-C/UTC, averaged within years, and pooled for both years after confirmation of homogeneity of variance. Error bars indicate the 95% confidence limit for each ratio, and areas that overlap a ratio of 1.0 indicate no significant difference between means for the UV-C treatment compared to the untreated control. ECS_t mAU = total magnitude of electrochromic shift measured as milli-absorbance units during a light-dark transition; gH^+ = ATP synthase proton conductivity, expressed gH^+ s^{-1} proton conductivity of chloroplast ATP synthase; LEF = linear electron flow: total flow of electrons from antennae complexes into Photosystem II, accounting for leaf absorptivity and is expressed in μmol electrons m^{-2} s^{-1}; Φ_{NPQ} = nonphotochemical quenching: a dimensionless parameter of incoming light regulated away from photosynthetic processes; Φ_{II} = quantum yield of Photosystem II: a percentage of incoming light that goes into Photosystem II; Φ_{NO} = percentage of incoming light lost to nonregulated processes. Relative Chlorophyll = a unitless measure of leaf chlorophyll; Thickness = relative thickness of leaf blade expressed in mm; vH^+ = dimensionless steady-state rate of proton flux; Absorbance 420 to 940 = Absorbance at the indicated wavelengths; ECS_t = rate of electrochromic shift (ECS) in proton translocation through chloroplast ATP synthase; and SPAD 650 = a unitless measure of chlorophyll based on red light transmittance at 650 and 940 nm.

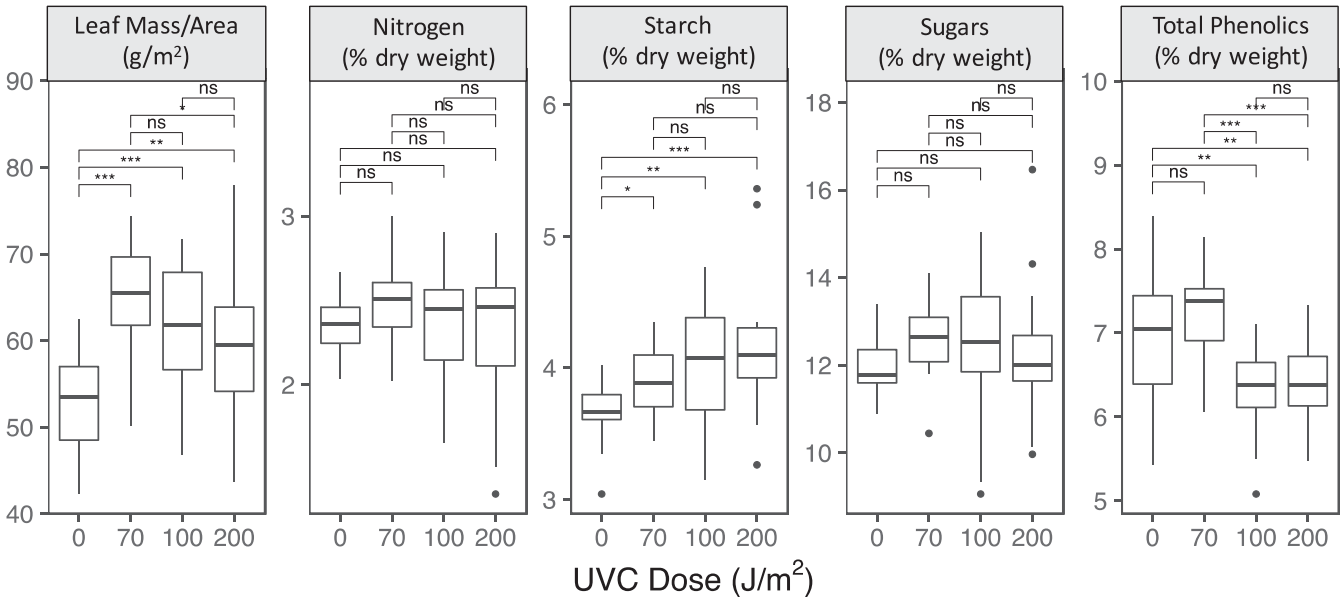

Fig. 11. Leaf mass area and nitrogen, starch, sugar, and total phenolic content in 'Chardonnay' grapevines that were treated weekly at indicated doses of UV-C. Data on trait indices was collected nondestructively through hyperspectral radiometric measurements beginning at 10 AM on 14 July 2020, approximately 3 weeks postbloom and 12 h after UV-C treatments the previous evening.

Vineyard trials for the suppression of grapevine diseases by UV-C

Although substantial suppression of sporangial production was observed in our laboratory studies from all pre- and some post-inoculation treatments with UV-C, this was not translated into significant reduction of disease in vineyards of *V. vinifera* 'Chardonnay', which is highly susceptible to downy mildew. Our results indicate that nighttime UV-C treatments against *P. viticola* are unlikely to perform adequately when used as the sole control measure on such cultivars in areas with environmental conditions that favor rapid reproduction by *P. viticola*, although UV-C could be expected to complement the efficacy of fungicides when applied to highly susceptible grapevine species and cultivars. On the more downy mildew-resistant *Vitis* interspecific hybrid cultivar 'Vignoles', twice-weekly applications of UV-C at 200 J/m^2 reduced the incidence of foliar infection by more than 75%. Thus, in instances where more substantial host resistance is present, UV-C treatments alone could conceivably provide adequate suppression of downy mildew.

Within a research vineyard with a history of high levels of overwintering inoculum and severe powdery mildew (Austin and Wilcox 2012, Gadoury et al. 2003; Melidossian et al. 2005; Moyer et al. 2016), nighttime applications of UV-C at doses ranging from 70 to 200 J/m^2 consistently suppressed incidence and severity of powdery mildew of fruit and foliage on 'Chardonnay' grapevines below that observed on untreated control vines (Figs. 5 and 6), and at the highest doses (200 J/m^2) and intervals of application (twice weekly), they provided suppression of foliar and fruit infection that was equivalent to that obtained with a standard season-long fungicide program (Fig. 6). Within the confines of commercial 'Chardonnay' vineyards with a history of excellent disease control and absent untreated control vines, weekly applications of UV-C at 200 J/m^2 maintained powdery mildew at trace levels in both 2020 and 2021, and weekly applications at 100 and 200 J/m^2, whether applied weekly or twice weekly, also maintained powdery mildew at trace levels. Thus, efficacy of UV-C in suppressing grapevine powdery mildew was affected by inoculum dose and could similarly be affected by other key components of epidemic progress: host resistance and environmental favorability.

It is noteworthy that both the research and commercial vineyards used in this research were pruned and trained to a system (cordon-trained with vertical shoot positioning) that resulted in an upright and relatively open canopy, with fruit that were relatively accessible to direct exposure from the UV-C lamps. Such grapevine pruning and training systems are common in many viticultural regions worldwide. The hemicylindrical lamp array used in this study produces multiple reflectance angles that provide an avenue by which UV can reach any surface with a direct line of sight to any part of the lamps or reflective surfaces; angles which change constantly as the mobile array approaches and then recedes from any point in a plant canopy (Suthaparan et al. 2016a). This array design has produced significant reductions of fungal and bacterial diseases in diverse plant canopies in multiple cropping systems (Onofre et al. 2021; Patel et al. 2020; Yannuzzi et al. 2022). Pruning and training systems and edaphic or climatic factors that result in excessively dense canopies could potentially reduce UV penetration and thereby reduce efficacy, as they could for any fungicide application. However, even in crops where very dense canopies eventually preclude effective penetration of UV to the interior, use of UV in the earliest stages of crop development before canopy closure, particularly in pathosystems where ontogenic resistance in fruit may be expressed before canopy closure (Gadoury et al. 2003), could nonetheless present an effective strategy for deployment in such systems.

In the case of a biotrophic pathogen, interpretation of direct effects of UV-C upon the pathogen versus indirect effects mediated through the host can be problematic. Exposure of conidia on glass slides definitively demonstrated direct lethality of UV-C to *E. necator* (Fig. 2). We did not purposely expose grapevine leaves to UV-C before inoculation in our studies, but natural inoculation during the interval between UV applications could undoubtedly occur. Thus, in our season-long vineyard trials, we cannot separate preinfection from postinfection effects. Preinoculation exposure of host plant to UV-B and UV-C had little effect on subsequent disease compared to postinoculation UV exposure at equivalent doses in *Sphaerotheca pannosa* on rose (Suthaparan et al. 2012a, b) and *Podosphaera xanthii* on cucurbit (Suthaparan et al. 2014). Likewise, efficacy of pathogen suppression in our study and many others was heavily dependent upon applying UV-C during darkness and avoidance of daylight-mediated photolyase repair of pathogen DNA (Janisiewicz et al. 2016b; Melis et al. 2019; Onofre et al. 2021; Patel et al. 2020; Pathak and Suthaparan 2021; Suthaparan et al. 2012a, b, 2014, 2016a, b, 2017). No such dependency upon darkness has been demonstrated for UV-mediated stimulation of host resistance. We believe it is therefore reasonable to presume that significant suppression of *E. necator* on grapevine tissues is due to a direct effect of UV-C upon the pathogen. In sharp contrast, the suppression of *P. viticola* in our studies appeared to involve a substantial stimulation of host resistance.

Ledermann et al. (2021) recently reported that exposure of 'Chardonnay' grapevines to 800 J/m^2 at 9- to 13-day intervals from April to July suppressed foliar and fruit infection by *E. necator* by 42 to 65%. In their report, efficacy was attributed to stimulation of host resistance and not to any direct lethality of UV-C to the pathogen. There are three substantial differences between our study and that reported by Ledermann et al. (2021). First, UV-C treatments reported by Ledermann et al. (2021) were applied less frequently than in our study: weekly to twice-weekly in our study compared to every 9 to 13 days in Ledermann et al. (2021). Secondly, the maximum doses applied by Ledermann et al. (2021) were approximately four times higher than used in our studies: 800 versus 200 J/m^2, respectively. Thirdly, applications described in Ledermann et al. (2021) were not reported to be made during darkness. Despite these differences, our results would indicate that an unknown portion of the disease suppression observed by Ledermann et al. (2021) was due to a directly lethal effect of UV-C upon the pathogen. Doses of UV-C substantially lower than those reported by Ledermann et al. (2021) have been shown to be directly lethal to a broad range of powdery mildew pathogens, including *E. necator*, *S. pannosa*, *Podosphaera aphanis*, *Podosphaera xanthii*, and *Oidium neolycopersici* (Gadoury et al. 1992; Janisiewicz et al. 2016b; Onofre et al. 2021; Patel et al. 2020; Suthaparan et al. 2012a, b, 2014; Willocquet et al. 1996). Experiments reported by Suthaparan et al. (2014) for *Podosphaera xanthii* on cucurbits failed to reveal stimulation of host resistance from preinoculation exposure to UV-B. More mechanistic and carefully controlled studies would be needed to definitively resolve the relative contributions of direct lethality of UV-C compared to UV-C mediated stimulation of host resistance with regard to seasonal suppression of grapevine powdery mildew under vineyard conditions.

Effects of UV-C applications on grapevine growth and physiology

Even at the highest doses of UV-C and at the most frequent intervals of application, our results suggest we were operating below a level of UV exposure that was deleterious to grapevines. We measured several indices of foliar growth, including leaf length and width and the number of leaves per shoot. None of these were reduced at the UV-C rates or frequency of application used in our study. This lack of harm to foliar development was reflected in the lack of impact of the UV-C applications upon fruit development as measured by berry size, berry number per cluster, mean berry weight, mean cluster weight, and percentages of soluble solids measured in fruit at harvest. The foregoing lack of any indication of phytotoxicity is consistent with the observed lack of harm to strawberry (Janisiewicz et al. 2016b; Onofre et al. 2021), cucumber (Suthaparan et al. 2012a), and rose (Suthaparan et al. 2012b) plants subjected to similar UV-C and UV-B doses and frequencies of application.

Results from two seasons of MultispeQ measurements did not suggest significant physiological differences between UV-C treated vines and untreated control vines. Although statistically significant differences between vines receiving UV-C treatment and untreated

control vines were occasionally observed, such effects were transient and inconsistent across timepoints and among different parameters. Foliar trait estimation from leaf reflectance data indicated that UV-C treatments raised LMA and starch concentration and lowered total phenolics concentration in UV-C treated leaves, which is an effect that could increase tolerance of environmental stressors. Overall, our findings concur with those of Ledermann et al. (2021), who found that flashes of UV-C light did not have a negative impact on net photosynthesis or maximum photosynthetic rate, water vapor conductance, dark respiration, or PSII and electron flux measurements. The foregoing collectively indicate that when applied at rates of 100 or 200 J/m^2, UV-C does not have an adverse effect on vine physiology and has substantial benefits of suppressing multiple grapevine diseases.

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