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Effects of Ultraviolet-C Light on Grapevine Powdery Mildew and Fruit Quality in *Vitis vinifera* Chardonnay

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Abstract

Background and goals

Germicidal ultraviolet light (UV-C) has suppressed grapevine powdery mildew in multiple cropping systems. Our goals were to further characterize the curative effects of UV-C light against grapevine powdery mildew (*Erysiphe necator)* in laboratory experiments, to understand how UV-C can be integrated into vineyard disease management programs, and to determine the effects of season-long UV-C exposure on basic fruit chemistry.

Methods and key findings

UV-C doses of 100 and 200 J/m^2 were applied to developing *E. necator* colonies at 24 to 144 hrs after inoculation in the lab. Both doses reduced colony development relative to the untreated control, and the effect of the 200 J/m^2 dose was equivalent to that of the control (horticultural oil) treatment. UV-C was also applied in *Vitis vinifera* Chardonnay vineyards at 200 J/m^2 once or twice weekly, under different application regimens (early-season only or season-long). In the most disease-favorable years, both regimens reduced foliar and cluster disease severity relative to the untreated control. Basic fruit chemistry was not affected by UV-C treatments in either the season-long UV-C trials or in an additional UV-C trial in Dresden, New York.

Conclusions and significance

The ability of UV-C to reduce powdery mildew on leaves and fruit in both low and high disease-pressure years, with no adverse effects on yield or fruit quality, suggests that it could be an additional, non-chemical tool to manage grapevine powdery mildew.

Key words: disease management, integrated pest management, phenolics, tannins, UV-C light

Introduction

Germicidal ultraviolet-C light (UV-C) encompasses wavelengths between 200 and 280 nm. UV-C at appropriate doses has the capacity to damage microbial DNA, which can kill or reduce the viability of the target microbe. UV-C has been used for nearly 80 years in hospitals, microbiology labs, and in food handling and processing facilities to suppress harmful microbes (Beggs 2002). A better understanding of how UV-C interacts with plant pathogens has led to successful field-level control of several plant pathogens, notably, powdery mildews of strawberry (*Podosphaera aphanis*) (Onofre et al. 2021), cantaloupe (*Podosphaera xanthii*) (Lopes et al. 2023), and grapevine (*Erysiphe necator*) (Ledermann et al. 2021, Gadoury et al. 2023). Enhanced efficacy has been achieved by applying UV-C (254 nm) during a dark period that continues for at least four hours after UV-C application (Janisiewicz et al. 2016a, Suthaparan et al. 2016a, Onofre et al. 2021). This dark period allows the UV-C damage to the fungus' DNA to be incorporated permanently, as the damage cannot be repaired by the fungal photolyase repair mechanism that is driven by the blue and UV-A components of sunlight (Beggs 2002). By applying UV-C with a subsequent dark period, lower, non-phytotoxic doses of UV-C can be used to suppress pathogens effectively (Suthaparan et al. 2014, 2016b, Janisiewicz et al. 2016b, Onofre et al. 2021).

These findings are the foundation for the use of UV-C light to manage grapevine powdery mildew (*E. necator*) (Ledermann et al. 2021, Gadoury et al. 2023), a globally important pathogen that can infect *Vitis* spp. vineyards, rendering fruit unusable and reducing the health of the vine (Lakso et al. 1982, Gadoury et al. 2001). Growers have traditionally managed

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this disease through fungicides (Varela et al. 2019, Hoheisel et al. 2023, Oliver et al. 2023, Skinkis et al. 2023), but there is an overall desire to reduce synthetic fungicide applications and use softer pest management practices, as seen in an increase in the adoption of sustainability certification programs in western United States winegrape production (e.g., Sustainable Washington, https://sustainablewa.com/; Lodi Rules, https://www.lodirules.org/).

In laboratory experiments, UV-C was shown to inhibit conidial germination of multiple fungal species causing powdery mildew, including *E. necator* (Gadoury et al. 2023), *Blumeria graminis* (Zhu et al. 2019), and *Pseudoidium neolycopersici* (Pathak and Suthaparan 2021). Doses as low as 50 J/m^2 inhibited conidial germination of *E. necator* conidia, with near complete suppression at 200 J/m^2 (Gadoury et al. 2023). The UV-C dose acted independently of the duration of exposure needed to achieve that dose, meaning that cumulative doses had equivalent effects within a broad range of irradiances and exposure durations (Gadoury et al. 2023). UV-C effectively reduced *B. graminis* infestation by halting pathogen development at the stage of appressorium formation and penetration (Zhu et al. 2019). Pathak and Suthaparan (2021) confirmed that independent of *P. neolycopersici* isolate type, UV-C treatments followed by a dark period significantly reduced germination of conidia compared to the untreated control. Effective control of field-level grape and strawberry powdery mildews has been achieved using twice-weekly UV-C (Onofre et al. 2021, Gadoury et al. 2023), achieving control equivalent to a fungicide standard. These studies have provided a framework for dose and frequency of UV-C application required for successful field management of powdery mildews.

The potential curative effects – the ability to eradicate the pathogen before visual disease symptoms occur – of UV-C were not investigated, although results obtained by Gadoury et al. (2023) indicated that UV-C applications to *E. necator* were largely curative rather than protective, and thus more frequent applications might be expected to yield more effective field-level disease suppression. Earlier studies on grapevine (Ledermann et al. 2021, Gadoury et al. 2023) compared season-long use of UV-C to fungicides, but did not examine UV-C application regimens modified to target key periods of epidemic development and ontogenic susceptibility of grape berries.

Most winegrape (*Vitis vinifera*) production in Washington State occurs in a semiarid steppe environment, averaging 1600 growing degree days (GDD; 1 April to 31 Oct, base 10°C), in-season average daily maximum temperature ranging from 12 to 35°C (April to October) with an average of 15 days greater than 33°C, and average annual precipitation of 164 mm, with 76 mm falling between April and October. These climatic conditions are associated with relatively low severity of grapevine powdery mildew, though fungicides are applied routinely to ensure disease remains at commercially acceptable levels. For example, vineyards for winegrape production typically apply fungicides every seven to 14 days from 8 to 16 cm shoot growth until four weeks postbloom (Hoheisel et al. 2023, Oliver et al. 2023). There is an emphasis on more intensive use of systemic fungicides during the critical window of flower and berry susceptibility to powdery mildew, which in Washington State lasts from rachis elongation to four weeks post 100% bloom (Gadoury et al. 2003, Moyer et al. 2010, 2016a). By approximately four weeks post 100% bloom, fruit is nearly immune to infection by *E. necator*. Furthermore, decreasing relative humidity and increasing temperatures in eastern Washington State following bloom are generally unfavorable for expansion or spread of extant powdery mildew colonies on fruit or foliage, and such conditions will cause powdery mildew epidemics to stall in their development (Delp 1954). While information on pathogen biology has generally improved the efficiency and efficacy of fungicide use, the winegrape industry remains interested in further reducing synthetic chemical inputs (Oliver et al. 2023). Commercial interest in UV-C technology in specialty crops such as grapes and strawberries has significantly increased in recent years (Petrovic 2017, Claver 2021, Penn 2021, Clark 2022).

At doses and application intervals that effectively suppressed powdery mildew, there was no effect of UV-C on multiple components of fruit quality and yield in *V. vinifera* in New York (Gadoury et al. 2023). UV-B exposure to developing grape berries can increase phenolic compounds in grape skin such as flavonols, anthocyanins, tannins, stilbenes, and hydroxycinnamic and hydroxybenzoic acids (Del-Castillo-Alonso et al. 2020). UV-C has been used for postharvest disease control of grapes and to increase health-related phenolic compounds, namely stilbene derivatives (Fernandez-Marin et al. 2013, Freitas et al. 2015). As preharvest UV-C holds promise to suppress multiple plant pests and pathogens in grapevine, there is a critical need to further explore its effects on final fruit chemistry in a broad range of viticultural climates. This is particularly true for grape skin phenolics that can affect sensory properties of wine.

Our objective was to expand our knowledge of how UV-C can be used as a non-pesticidal alternative to suppress grapevine powdery mildew in *V. vinifera* vineyards and its effects on basic fruit chemistry, through both laboratory and field experiments in a semiarid steppe environment. Additional data were collected by collaborators in the Finger Lakes viticultural region of NY for purposes of comparison, using common measures of fruit quality.

Materials and Methods

Laboratory-scale UV-C light array system

To evaluate the effects of UV-C light against grapevine powdery mildew, an enclosed UV lamp array was constructed. The apparatus consisted of three lamp fixtures, each fixture holding two low-pressure discharge UV-C lamps (Osram germicidal T8 55W UVC Medium Bi Pin Base model G55T8/OF). Lamps in each fixture were 90 cm long and powered by dual-lamp ballasts (IUV-2S36-M2-LD PureVOLT; Philips ADVANCE). The lamps were

positioned above a motorized conveyor belt (60W, 30 to 120 rotations/min, 1.5 × 0.4 m belt; Vevor). The frame of the apparatus was clad in galvanized steel with 3.0 mm-thick PVC curtains at each end of the conveyor belt to contain the UV-C light within the apparatus. Magnitude and uniformity of UV-C irradiance at the sample plane (~20 cm below the bottom-most lamps) was measured using a UV spectroradiometer (model BTS2048-UV-S; Gigahertz-Optik GmbH) as described (Onofre et al. 2021). Target doses of UV-C were achieved by adjusting conveyor belt speed based upon the length of the array and a mean irradiance at the center line of the belt surface (Gadoury et al. 2023). To deliver a dose of 100 or 200 J/m^2, the conveyor belt was set to 0.5 or 0.25 m/sec, respectively.

Effect of UV-C light on nascent *E. necator* colonies under laboratory conditions

The curative effect of UV-C light was evaluated using a UV-C dosage of 100 or 200 J/m^2 delivered by the laboratory UV-C system described above. These were compared to two controls: an untreated control and a 2% v/v horticultural oil (PureSpray Green; Intelligro) sprayed control. Horticultural oil was applied at a volume equivalent to just-before-runoff using a hand-held pump sprayer (CHAPIN 16100 Home and Garden one-gallon sprayer; adjustable cone nozzle, 0.3 to 0.4 MPa), with the delivery nozzle also placed 18 cm from the adaxial leaf surface. These four treatments were applied to one-, three-, or six-day old *E. necator* colonies (24, 72, and 144 hrs postinoculation [hpi], respectively) grown on detached *V. vinifera* Chardonnay leaves. In total, there were 12 experimental treatments (four treatment options × three *E. necator* developmental stages).

E. necator inoculum for the laboratory experiments was cultured on detached leaves using a method modified from Moyer et al. (2010) and the pathogen was re-sourced regularly from field isolates when cultured isolates began to decline. To create cultures, field-sourced *E. necator* colonies from one vineyard were transferred to detached Chardonnay leaves by tapping infected leaves onto new, uninfected leaves every seven days. The inoculum used in the experiment described above came from seven- to 14-day-old cultures.

Leaves used in the experiment were collected from greenhouse-grown Chardonnay cuttings. Young leaves were collected from the leaf position three nodes down from the growing shoot tip and were ~50 to 75% expanded, with a noticeably shinier cuticle than older leaves (Doster and Schnathorst 1985, Merry et al. 2013). After collection, leaves were surface-disinfested with a 0.5% $NaOCl_2$ solution for 90 sec and rinsed twice in distilled water. Each leaf was then placed in a double petri dish, where the petiole was submerged into deionized water and incubated overnight at 22°C (protocol adapted from Moyer et al. 2010). The leaves were inoculated with 10 µL of a conidial suspension (10^4 to 10^6 conidia/mL), made from vigorously shaking infected leaves in 10 mL of an 0.05% 80 Tween solution; additional Tween solution, or additional infected leaves, were added to reach the desired suspension concentration. Each leaf was inoculated 10 times (five droplets on each side of the midvein). Droplets dried at room temperature for one hour, then the double petri dish was re-covered and placed in a plant growth incubator (3765 model 504L; Thermo Fisher) with 20 watt fluorescent bulbs (F20T12/CW/ALTO; Philips Lighting) at 22°C with day/night periods of 16:8 hr. The germination potential of each inoculation solution was assessed immediately before and after inoculation by transferring a 10 µL sample of the conidial suspension to a glass microscope slide. The slide was incubated in a closed petri dish with moist filter paper for 24 hrs at 22°C, after which the percent germination (i.e., presence of a germ tube at least one-half the length of the conidium) was assessed microscopically on all spores present; postinoculation germination rate was between 40 and 50%.

The number and size of colonies formed on each leaf was recorded at eight and 13 days postinoculation. Conidiophore development of each colony was also observed eight days postinoculation. The experiment was repeated a total of three times; eight replicate leaves per treatment were used in the first trial, and 10 replicate leaves per treatment were used in the second and third trials.

Field-scale UV-C light array system

A field-scale UV-C light array system was configured in an over-the-row triangular arch supported by a metal tower with 12 ballasts, as described above, to power 24 UV-C lamps. The light fixtures were backed by polished aluminum reflectors (XRFP230; Lamar LED). The over-the-row arch was suspended on horizontal arms by a metal tower support that attached to the three-point hitch system of a tractor. A curtain of 3.0 mm-thick PVC strips described above were mounted on each end of the array to contain UV-C within the apparatus. The framework was built by VineTech Equipment. UV-C irradiance was measured as described above. A dose of 200 J/m^2 was achieved by adjusting ground speed based upon the array length and mean irradiance at the center line of the array 1 m aboveground: the approximate height of the fruiting zone within the vineyard trellis (Gadoury et al. 2023). To achieve a UV-C dose of 200 J/m^2, ground speed in 2020 was set to 1.6 km/hr; in 2021 and 2022, the ground speed was set to 1.1 km/hr. The change in speed was due to a change in the field unit used to apply UV-C between the two years – the length of the field array was 1.8 m in 2021 and was shortened to 1.5 m in 2022.

Field performance of UV-C for *E. necator* management

UV-C efficacy trials for management of *E. necator* occurred in a vineyard planted to Chardonnay on its own roots at the Washington State University Irrigated Agricultural Research and Extension Center in Prosser, WA for three growing seasons (2020, 2021, and 2022). The vineyard was planted in 2009 to 1.8 × 2.7 m (vine × row) spacing with north-south row orientation. Vines were trellised on a modified vertical shoot-positioning system and trained to a dual-trunk bilateral cordon with spur pruning. The vineyard was drip-irrigated

with natural vegetation under the vines and between rows; the vineyard floor was maintained through routine in-season mowing. Minimal canopy management was used all three years, with hedging occurring at BBCH 71 (Lorenz et al. 1995) on 17 June 2020 and 10 June 2021, and shoot thinning occurring during BBCH 68 on 22 to 24 June 2022. Vineyard weather data were collected using Washington State University's AgWeatherNet (https://weather.wsu.edu), pulling from the 'Prosser.NE' station. This station is located 1.6 km from the experimental vineyard.

UV-C treatments were applied under different regimens that focused on frequency and seasonal duration of applications. UV-C was applied weekly in 2020 and weekly or twice-weekly in 2021 and 2022, during the early-season only (Table 1) or season-long (Table 2). Early-season applications were made between 15 cm shoot growth (BBCH 13) and prebloom (BBCH 55 to 57), then a fungicide spray program was used postbloom. Early-season UV-C treatments were compared against three controls (season-long unsprayed, fungicide spray program, and unsprayed until prebloom [BBCH 55 to 57]) and the fungicide spray program postbloom (Table 1 and Supplemental Table 1). Early-season treatment plots in 2020 and 2021 consisted of nine consecutive vines in a row and increased to 30 vines in 2022 for ease of application. Data were collected from the center four vines. Season-long treatments were applied between 15 cm shoot growth (BBCH 13) and four weeks post-fruit set (BBCH 73). Season-long UV-C treatments were compared against two control treatments: season-long unsprayed and fungicide spray program (Table 2 and Supplemental Table 1). Season-long treatment plots consisted of 30 consecutive vines in a row, with data collected from four consecutive vines within. For both experiments, treatments were replicated four times within a randomized block design. To reduce potential drift between treatment plots, tarps were used to cover individual treatment replicates during treatment application in 2020. In 2021 and 2022, there were six vines serving as buffers between treatments within a row, and an entire non-experimental row between treated rows.

In 2020, the standard fungicide spray program (Tables 1 and 2) was applied using an all-terrain vehicle (ATV)-mounted tank sprayer (ATV2507 ATV Sprayer; WorkHorse Sprayers) at 468 L/ha until 21 May. The applications were then made with a Rears Manufacturing Powerblast Pul-Tank airblast sprayer at 702 L/ha with D5DC25 TeeJet nozzles, without air-assist on 4 June and with air-assist for the last two sprays. In 2021 and 2022, the standard fungicide spray program was applied with a Rears custom-built over-the-row multi-tank with air shear nozzles (Tables 1 and 2). Sprays up to 19 May 2021 and 7 June 2022 were applied at 468 L/ha optimized with three nozzles per side, then 702 L/ha optimized with four nozzles per side for the remainder of the applications. All fungicides applications were made at seven- or 14-day intervals.

Visual disease incidence and severity ratings of *E. necator* were recorded starting from bloom (BBCH 60 to 71; 10 June 2020, 8 June 2021, and 14 June 2022) and continued until harvest each year. Ratings were performed by trained raters and occurred every seven to 14 days. Harvest occurred when fruit was at least 22 Brix. Ratings consisted of visually evaluating the upper and lower surfaces of 40 random leaves and 20 random clusters per treatment replicate in 2020, and 40 random leaves and 40 random clusters per treatment replicate in 2021 and 2022.

Table 1 Treatment application dates and rates for the early-season field evaluation of ultraviolet-C (UV-C; 200 J/m^2) on *Erysiphe necator* management in a *Vitis vinifera* Chardonnay vineyard in Prosser, WA. UV-C treatments and prebloom unsprayed control treatments were applied up to bloom (BBCH 55[a]), then converted to the fungicide spray program for the remaining season. Fungicides were applied at maximum label rates unless listed. All products were registered for use in Washington State at the time of the study. See Supplemental Table 1 for a list of the active ingredients associated with the fungicide trade names presented here.

Treatments		2020	2021	2022
Fungicide program	Prebloom	8 May (BBCH 15) – Microthiol Disperss (5.6 kg/ha) 14 May (BBCH 55) – Microthiol Disperss (4.4 kg/ha) 21 May (BBCH 60) – Vivando + Microthiol Disperss (2.2 kg/ha)	5 May (BBCH 15) – Microthiol Disperss (4.4 kg/ha) + Cinnerate + Complex 12 May (BBCH 19) – Microthiol Disperss (4.4 kg/ha) + Cinnerate + Complex 19 May (BBCH 55) – Vivando + Complex	19 May (BBCH 15) – Microthiol Disperss (4.4 kg/ha) + Complex 25 May (BBCH 19) – Microthiol Disperss (4.4 kg/ha) + Complex 1 June (BBCH 55) – Vivando + Complex
	Postbloom	4 June (BBCH 68) – Quintec + Cinnerate 18 June (BBCH 71) – Torino + Cinnerate 2 July (BBCH 75) – Gatten + Cinnerate	1 June (BBCH 68) – Quintec + PureSpray Green (0.25%) 15 June (BBCH 71) – Torino + PureSpray Green (0.25%)	15 June (BBCH 60) – Quintec + Microthiol Disperss (2.2 kg/ha) + Complex 29 June (BBCH 71) – Torino + PureSpray Green (0.25%) 13 July (BBCH 75) – Aprovia + Complex
Early twice-weekly UV-C	Prebloom		Twice per week from 6 to 27 May	Twice per week from 19 May to 9 June
Early weekly UV-C	Prebloom	Once per week from 7 to 28 May	Once weekly from 6 to 27 May	Once per week from 19 May to 13 June
Early unsprayed	Prebloom	Unsprayed		
Season-long unsprayed				

[a]BBCH Scale (Lorenz et al. 1995).

Yield components and fruit composition of UV-C treated, field-grown fruit in Prosser, WA

Early-season and season-long treatments, as described above, were harvested on 4 Sept 2020, 31 Aug 2021, and 20 Sept 2022. For each treatment replicate, all clusters from four vines were collected, pooled, weighed, and averaged for total yield per vine. Clusters were saved for later processing; two random clusters per vine for a total of eight clusters in 2020 and three random clusters per vine for a total of 12 clusters in 2021 and 2022. Immediately after harvest, clusters were pooled and weighed to capture average cluster weights. To obtain an average berry weight, 48 berries (with pedicel intact) were removed, pooled, and weighed. In the season-long treatments, in 2020 and 2021, those berries were crushed within 24 hrs postharvest for juice analysis. In 2022, whole clusters (12 clusters) were pressed in the collection bag for juice analysis after removing berries for later analysis. Juice from crushed berries was sieved and collected into 50-mL centrifuge tubes and centrifuged three min at 3000 rpm. The supernatant was used to measure soluble solids (TSS), titratable acidity (TA), and pH. TSS, TA, and pH were measured with a temperature-compensating pocket refractometer (ATAGO Co PAL-1), an automatic compact titrator (Mettler Toledo G10S), and an MP225 pH meter (Mettler Toledo), respectively. The pH was converted to molar ion concentration for statistical analysis.

Berry skin tannin and phenolic concentrations were also measured from fruit in the season-long treatments. To conduct these measurements, 30 berries with pedicels attached, per treatment replicate, were selected randomly from clusters saved at harvest and frozen at -20°C. Prior to analysis, frozen berries were pooled, weighed, and the skin of each berry was removed from the flesh by hand. The skins were dried at room temperature for one hour, pooled, then weighed. After drying, berry skins from each treatment replicate were placed into separate 50-mL plastic Falcon vials and stored at -20°C until tannin and phenolic extraction could be completed. To extract skin tannins and phenolics, 30 mL of 70% acetone was added to the vials, then they were shaken for 12 to 18 hrs at 150 rpm (SCILOGEX SK-330-Pro). After agitation, samples were centrifuged at 5000 rpm for four min (Eppendorf 5810R) and decanted into polyvac vials (PB3002-S; Mettler-Toledo). In 2020, samples were evaporated to 9 to 11 mL with a vacuum pump (Buchi Syncore Polyvap), heated to 40°C, and agitated under a vacuum at 300 rpm. In 2021 and 2022, samples were evaporated to 9 to 11 mL with a nitrogen evaporator (N-EVAP 112; Organomation Associates Inc.) and heated to 40°C. Postevaporation, samples were weighed and transferred to plastic vials for storage at -80°C until tannins and phenolics could be measured. Tannins and total iron reactive phenolics were measured as described (Harbertson et al. 2002, 2003).

Composition of UV-C treated, field-grown fruit in Dresden, NY

In a complementary experiment, we evaluated the effects of season-long UV-C treatment on berry skin composition using fruit from a commercial Chardonnay vineyard in Dresden, NY. The vineyard was planted on 3309 rootstocks in 2004 to 1.8 × 2.7 m (vine × row) spacing with north-south row orientation. Vines were trellised on a vertical shoot-positioning system and midwire cordon-trained with spur pruning. The vineyard was nonirrigated, with middle alleys sodded and under-vine treated with herbicide. Shoot thinning and leaf removal was performed by hand four weeks postbloom. Vineyard weather data was collected from the

Table 2 Treatment application dates and rates for the season-long field evaluation of ultraviolet-C (UV-C; 200 J/m^2) on *Erysiphe necator* management in a *Vitis vinifera* Chardonnay vineyard in Prosser, WA. UV-C treatments and controls were applied from 10 cm shoot growth (BBCH 15[a]), until three weeks post-fruit set (BBCH 75). Fungicides were applied at maximum label rates unless listed. All products were registered for use in Washington State at the time of the study. See Supplemental Table 1 for a list of the active ingredients associated with the fungicide trade names presented here.

Treatments	2020	2021	2022
Fungicide program	8 May (BBCH 15) – Microthiol Disperss (5.6 kg/ha) 14 May (BBCH 55) – Microthiol Disperss (4.4 kg/ha) 21 May (BBCH 60) – Vivando + Microthiol Disperss (2.2 kg/ha) 4 June (BBCH 68) – Quintec + Cinnerate 18 June (BBCH 71) – Torino + Cinnerate 2 July (BBCH 75) – Gatten + Cinnerate	5 May (BBCH 15) – Microthiol Disperss (4.4 kg/ha) + Cinnerate + Complex 12 May (BBCH 19) – Microthiol Disperss (4.4 kg/ha) + Cinnerate + Complex 19 May (BBCH 55) – Vivando + Complex 1 June (BBCH 68) – Quintec + PureSpray Green (0.25%) 15 June (BBCH 71) – Torino + PureSpray Green (0.25%)	19 May (BBCH 15) – Microthiol Disperss (4.4 kg/ha) + Complex 25 May (BBCH 19) – Microthiol Disperss (4.4 kg/ha) + Complex 1 June (BBCH 55) – Vivando + Complex 15 June (BBCH 60) – Quintec + Microthiol Disperss (2.2 kg/ha) + Complex 29 June (BBCH 71) – Torino + PureSpray Green (0.25%) 13 July (BBCH 75) – Aprovia + Complex
Twice-weekly UV-C		Twice per week from 6 May to 24 June	Twice per week from 19 May to 29 July
Weekly UV-C	Once per week from 7 May to 9 July	Once per week from 6 May to 24 June	Once per week from 19 May to 29 July
Season-long unsprayed			

[a]BBCH Scale (Lorenz et al. 1995).

Dresden NEWA weather station (https://newa.cornell.edu/). UV-C treatments were applied as described (Gadoury et al. 2023). Briefly, in 2021, weekly and twice-weekly UV-C at 100 or 200 J/m^2 was applied from 10 cm shoot growth to veraison. These were compared with a commercial fungicide treatment, for a total of five treatments arranged in a randomized complete block design of nine-vine plots replicated four times. In each treatment, 20 replicate clusters were harvested on 9 Sept 2021. From each cluster, 10 berries (with pedicel intact) were removed to collect a total of 200 berries per treatment replicate and frozen at -20°C until they were shipped on dry ice to Prosser, WA for analysis. Thirty berries were randomly selected from the 200 berry per treatment replicate, processed, and analyzed for total phenolic and tannin concentrations, as described above.

Statistical analysis

All statistical analyses were performed using the JMP 15 statistical program (JMP 15.0.0; SAS institute, Inc.). For E. *necator* lab analysis, leaves that died from detaching shock or bacterial infection (15, 22, and 15% in experiments 1, 2 and 3, respectively) were removed from analysis and treatment replicates at random were removed to the lowest common denominator to have an equal n. The number of E. *necator* colonies formed (percentage), number of colonies with conidiophores (percentage of colonies with conidiophore / total colonies), and average diameter of colonies present from lab nascent E. *necator* were compared. Foliar and cluster disease severity ratings for field evaluation of UV-C on E. *necator* management were evaluated by calculating the area under the disease progress curve (AUDPC) (Madden et al. 2017). Yield component and fruit composition were averaged by replicate. The restricted maximum likelihood method was used for all analysis, where replicates or blocks were random effects, treatments were fixed effects, and statistical significance was set at $\alpha = 0.05$. Tukey's honest significant difference was used as a post-hoc significance test.

Results

Curative effects of UV-C light on nascent *E. necator* colonies

Data from the three experimental repeats were pooled, as there was no difference among them either at eight ($p = 0.51$, 0.64, and 0.54 at 24, 72, and 144 hrs, respectively) or 13 days ($p = 0.50$, 0.59, and 0.87 at 24, 72, and 144 hrs, respectively). When colonies were treated 24, 72, and 144 hpi (Figure 1A; $p = 0.0001$ at all observation points), both UV-C doses (100 and 200 J/m^2) reduced the total number of colonies formed relative to the untreated control, whether this observation was made at eight- or 13-days posttreatment. Both UV-C doses performed equivalently to the oil treatment's ability to reduce colony formation when applied at 72 hpi and observed at eight- or 13-days posttreatment, and at 144 hpi observed at eight days posttreatment. This effect was not seen with application at 24 hpi, where a UV-C dose of 100 J/m^2 had greater percent colony formation than 200 J/m^2 at both eight- and 13-days posttreatment.

Once colonies were established, UV-C treatments affected the formation of conidiophores (Figure 1B; $p = 0.0001$ at all observation points). UV-C doses of 100 or 200 J/m^2, applied at 24, 72, or 144 hpi, all significantly reduced conidiophore development relative to the untreated control observed at the eight day observation point, and this reduction was sustained to the 13 day observation point. UV-C 200 J/m^2 better suppressed conidiophore development at 24 hpi than UV-C 100 J/m^2. When treatments were applied at 72 hpi, both UV-C doses and the oil control provided equivalent reduction in conidiophore development after both the eight- and 13-day observation points. These results were also seen after eight days when treatments were applied at 144 hpi.

When colonies did form, we measured colony diameter (Figure 1C). Distinct from the above, 72 hpi treatments appeared to have the largest and longest-lasting effects on colony diameter ($p = 0.0001$ after eight and 13 days). At 72 hpi, both UV-C treatments performed equally well to the oil treatment, and there was no extensive continued colony growth between the eight- and 13-day observation points. While UV-C reduced colony diameter relative to the untreated control at 24 ($p = 0.0001$ after both eight and 13 days) and 144 hpi ($p = 0.006$ and 0.006 after eight and 13 days, respectively), we did see continued growth of the treated colonies between the eight- and 13-day observation points.

Field performance of UV-C for *E. necator* management in Prosser, WA

The Washington State weather patterns during 2020 and 2021 resulted in hotter and drier in-season conditions that did not favor E. *necator* development (Figure 2A and 2B). Both 2020 and 2021 had above-average GDD accumulation (Figure 2A; GDD base 10°C, 1 April to 31 Oct) and below-average precipitation (Figure 2B; based on a 10-year average). In 2021, the daytime high temperatures from bloom to veraison exceeded 35°C for 17 consecutive days. The 2022 vintage was starkly different, with below-average temperatures and above-average precipitation early in the season, creating a more favorable climate for E. *necator* development.

Early-season UV-C treatments reduced foliar and cluster disease severity relative to the unsprayed control in 2020 and 2022 (Supplemental Table 2). In 2020, early weekly UV-C reduced foliar and cluster AUDPC by 85% and 86%, respectively, relative to the season-long unsprayed vines (Figure 3A and 3B). In 2021 (Figure 3C), due to extended high temperatures from bloom through veraison, there was little foliar and no cluster disease in the vineyard, resulting in no separation of UV-C treatments, the fungicide program, and untreated vines. In 2022, early weekly or twice-weekly UV-C reduced foliar AUDPC by 77% and 74%, respectively; cluster AUDPC was reduced by 78% and 74%, respectively (Figure 3D and 3E).

Season-long UV-C treatments in WA in 2020 (Figure 4A) and 2021 (Figure 4C) did not significantly reduce foliar AUDPC relative to the unsprayed treatments. Cluster AUDPC in 2020 (Figure 4B) did not differ across treatments. No differences in

foliar AUDPC were seen in 2021 (Figure 4C) and there was no observable disease on clusters regardless of treatment, due to extreme mid-season heat events. Though there was no significant difference, in 2021, foliar AUDPC of vines treated with UV-C weekly was reduced by 50% relative to the unsprayed vines. AUDPC was reduced by 79% when treated with twice-weekly UV-C. In 2022, weekly and twice-weekly UV-C applications significantly reduced foliar AUDPC by 37% and 61%, respectively, relative to the season-long unsprayed control (Figure 4D). Cluster AUDPC in 2022 (Figure 4E) was significantly reduced by 16% and 40% with weekly and twice-weekly UV-C applications, respectively.

Yield components of UV-C treated, field-grown fruit in Prosser, WA

Early-season UV-C did not affect yield, cluster weight, berry weight, or total number of berries per cluster in any year of the study (Table 3 and Supplemental Table 3). The

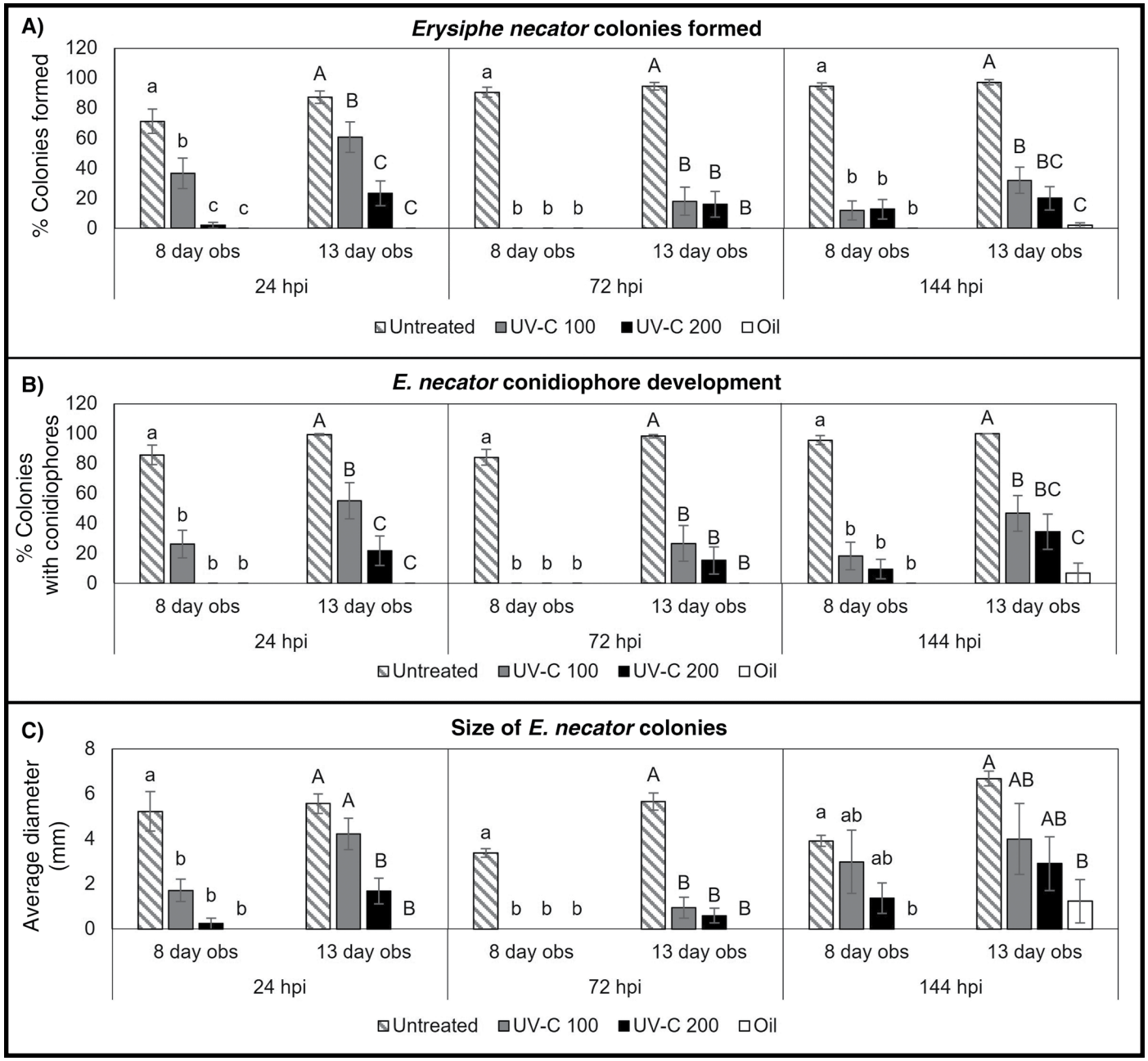

Figure 1 Ultraviolet-C light (UV-C) effects on laboratory-grown *Erysiphe necator* after UV-C and oil treatments at 24, 72, and 144 hours postinoculation (hpi). Untreated, no UV-C or oil; UV-C 100 and UV-C 200, UV-C dose of 100 or 200 J/m^2, respectively; oil, 2% v/v horticultural oil (PureSpray Green, Intelligro). **A**) Percent of colonies (out of 10 inoculated colonies) visible at eight- or 13-day observation (obs) points posttreatment. **B**) Of the colonies that did form after treatment, the percent of colonies that developed conidiophores. **C**) The average colony diameter of those colonies that formed after treatment. Error bars are standard error (n = 10). Different letters denote significant differences among treatment means at $\alpha = 0.05$ using Tukey's honest significant difference test. Same case lettering denotes between treatment differences within an observation timepoint.

only difference was in 2022, under high disease pressure (Table 3), when those metrics were significantly different in vines that remained untreated, either early-season only or season-long, and where the primary driving factor was disease severity on the fruit, not directly related to the use of UV-C.

While similar effects were seen in the season-long UV-C program in 2020 and 2021 under lower disease pressure (Supplemental Table 4), there was stronger separation between treatments in 2022 under greater disease pressure (Table 4). In 2022, the season-long fungicide program and twice-weekly UV-C had higher average yield per vine, cluster weight, and more berries per cluster than the unsprayed treatment. Fruit quality was also starkly different, with the fungicide treatment and twice-weekly UV-C having a lower juice pH and TSS than the unsprayed control.

Fruit composition of UV-C treated, field-grown fruit in Prosser, WA and Dresden, NY

The effects of season-long UV-C treatments on berry skin total phenolics and tannins were inconsistent among the three years in WA (Figure 5A and 5B). In 2020, total phenolics and tannins increased with weekly UV-C treatments over both the fungicide program and the unsprayed control. In 2021, twice-weekly UV-C and the fungicide program resulted in berries with higher phenolic and tannin concentrations than the unsprayed control. In 2022, there were no differences among all treatments for either total phenolics or tannins in WA. In 2021, Dresden, NY was particularly hot with a GDD similar to what was seen in Prosser, WA that same year (Figure 2A). In the Dresden, NY vineyard in 2021, there was no difference in total phenolics and tannins between all UV-C treatments and the fungicide program (Figure 5C and 5D).

Discussion

In this study, we found that UV-C had eradicant activity toward nascent powdery mildew colonies. This effect was strongest at 72 hpi (Figure 1A and 1B), where both UV-C doses were equivalent to the oil treatment at reducing colony development. At 24 and 144 hpi, UV-C at 100 J/m^2 was less effective than at 200 J/m^2, but still reduced

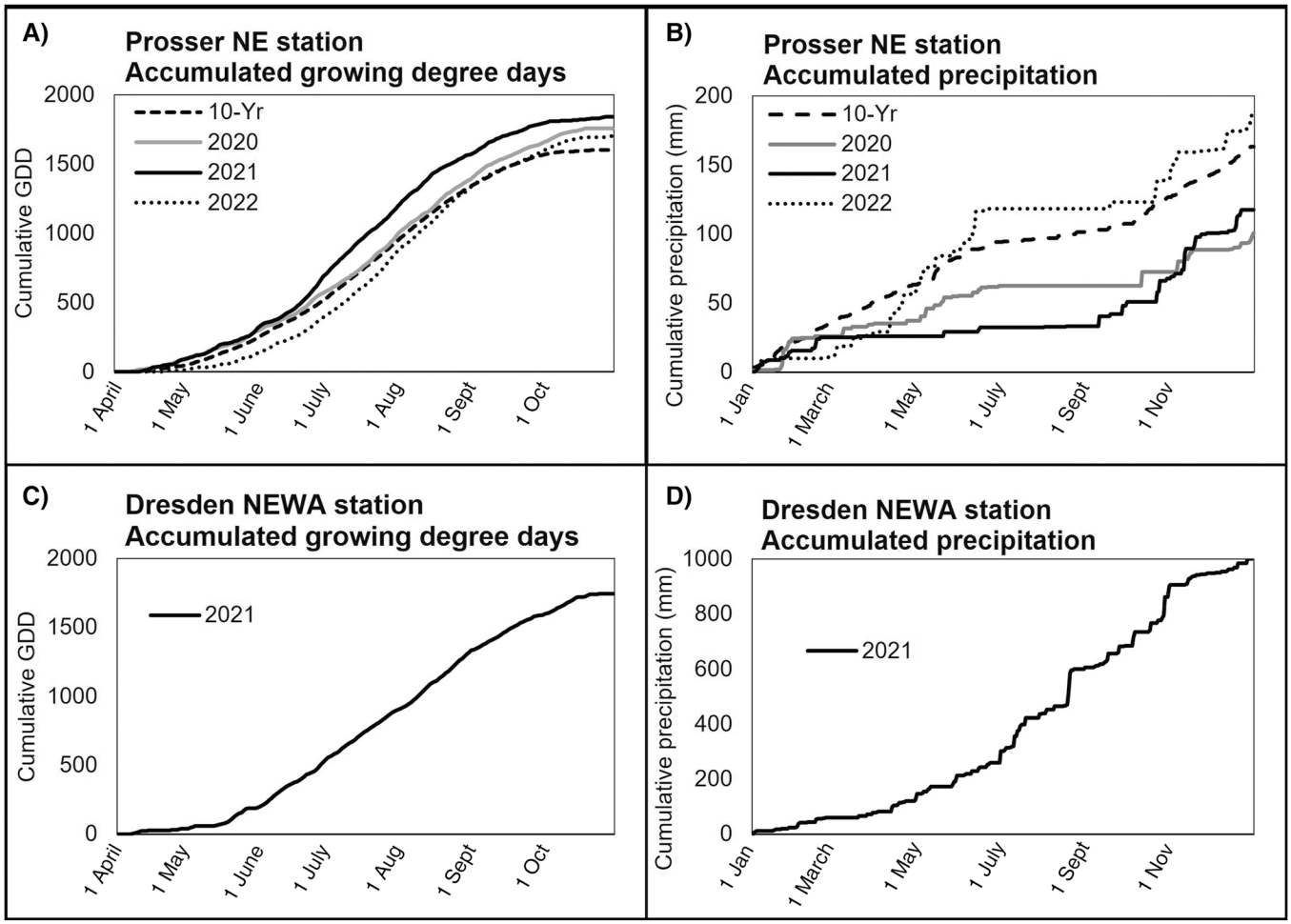

Figure 2 **A**) Accumulated growing degree days (GDD, base 10°C), from 1 April to 31 Oct, for Prosser, WA, with the 10-year average calculated from data from 2009 to 2019. **B**) Accumulated precipitation for Prosser, WA. **C**) Accumulated GDD for Dresden, NY in 2021. **D**) Accumulated precipitation for Dresden, NY in 2021. All Prosser, WA and Dresden, NY weather data were acquired from AgWeatherNet (weather.wsu.edu) 'Prosser.NE' station and from 'Dresden' NEWA station (newa.cornell.edu/), respectively.

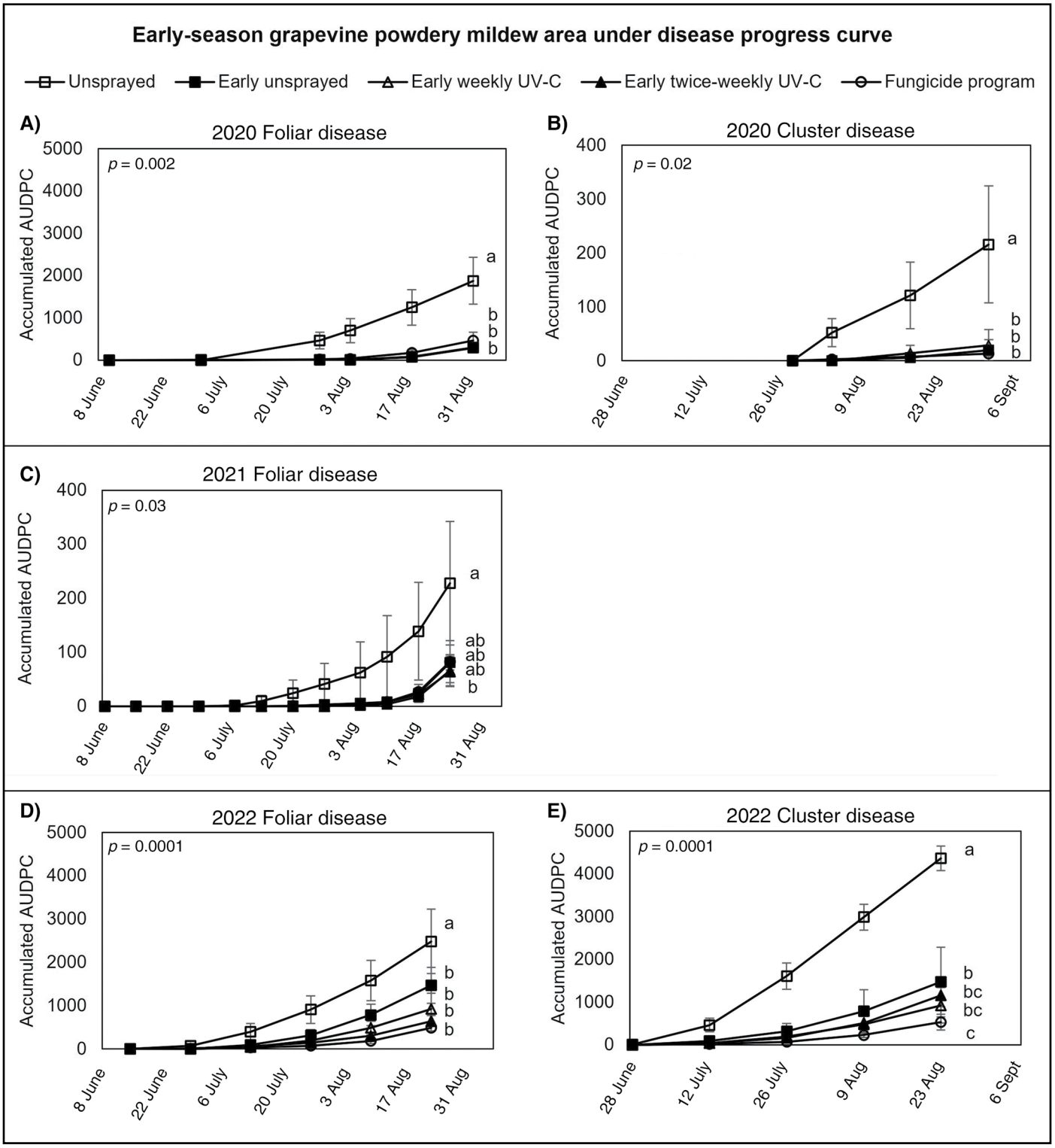

Figure 3 Foliar and cluster disease severity ratings represented as accumulated area under disease progress curve (AUDPC) for early-season disease management treatments, including unsprayed controls (early season and all-season), a full fungicide program, and weekly- or twice-weekly ultraviolet-C light (UV-C) treatments. **A**, **C**, **D**) 2020, 2021, and 2022 foliar disease AUDPC, respectively. **B**, **E**) 2020 and 2022 cluster disease AUDPC, respectively. There is no data for 2021 cluster disease AUDPC, as there was no recorded disease on the fruit in the field. Error bars are standard error (n = 4). Different letters denote significant differences among treatment means at $\alpha = 0.05$ using Tukey's honest significant difference test.

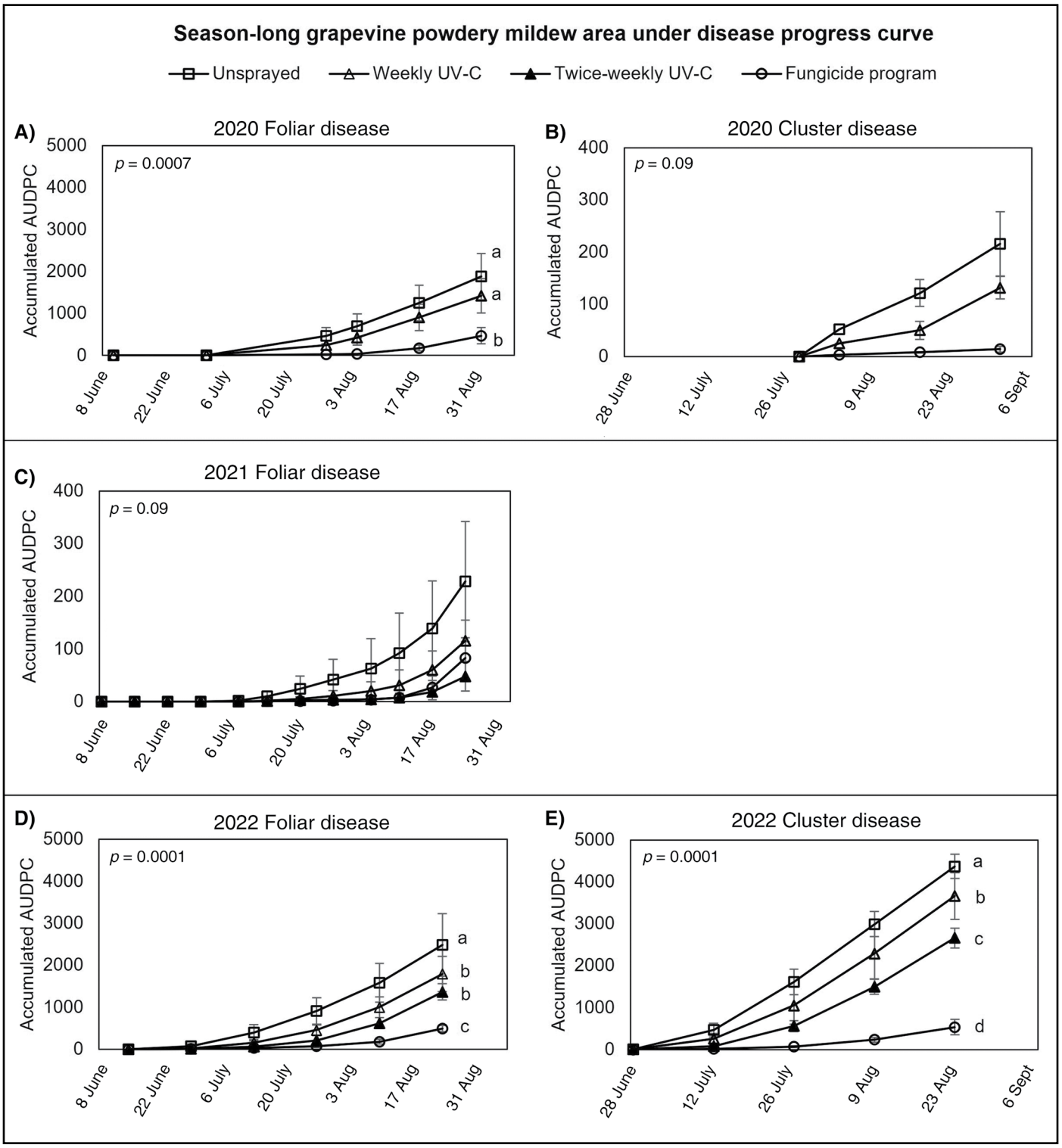

Figure 4 Foliar and cluster disease severity ratings represented as accumulated area under disease progress curve (AUDPC) for season-long treatments, including an unsprayed control, a full fungicide program, or weekly or twice weekly ultraviolet-C light (UV-C) treatments. **A**, **C**, **D**) 2020, 2021, and 2022 foliar disease AUDPC, respectively. **B**, **E**) 2020 and 2022 cluster disease AUDPC, respectively. There is no data for 2021 cluster disease AUDPC, as there was no recorded disease on the fruit in the field. Error bars are standard error (n = 4). Different letters denote significant differences among treatment means for season-long AUDPC (final date) at $\alpha = 0.05$ using Tukey's honest significant difference test.

colony development relative to the untreated control. The reduced efficacy of UV-C applied at 144 hpi may partially explain the increased efficacy of twice-weekly UV-C treatments over weekly treatments in previous studies (Onofre et al. 2021, Gadoury et al. 2023) and in our 2022 season-long field trials (Figure 4E). When UV-C was applied in the early-season only (replacing fungicides until bloom), both weekly and twice-weekly applications provided the same level of disease suppression as a season-long fungicide program (Figure 3). Twice-weekly UV-C all season did not compromise fruit quality in our trials when compared to fungicide programs. The effects of UV-C on fruit quality were inconsistent year-to-year, suggesting that factors other than UV-C were more influential on these measures (Figure 5).

Studies on the ability of UV-C to suppress *in vitro* germination of *E. necator* conidia (Gadoury et al. 2023) and early *B. graminis* establishment *in vivo* (Zhu et al. 2019) both focused on UV-C treatments applied immediately after inoculation. No conidia germinated under *in vitro* conditions with UV-C treatments at 200 J/m^2, while treatments of 100 J/m^2 UV-C had 20 to 27% conidia germination (Zhu et al. 2019, Gadoury et al. 2023). Our results likewise show that nascent colonies treated with 100 J/m^2 UV-C at 24 hpi were less suppressed than colonies treated with 200 J/m^2 UV-C (Figure 1A). When colonies were treated after they were further along in development (72 or 144 hpi), when more mycelium surface area is exposed to UV-C, treatment inhibited disease development much more strongly. Moyer et al. (2010) reported a peak in hyphal mortality when colonies of *E. necator* were exposed to acute cold events at 72 hpi (three days postinoculation), attributing this to a peak in host resistance induced by acute cold temperatures. At 72 hpi, colonies of *E. necator* are in transition from purely mycelial growth to the formation of asexual reproductive structures (Delp 1954). The increased sensitivity of such colonies to UV-C and acute cold-induced host resistance may indicate that colonies at this stage are particularly vulnerable to the germicidal activity of UV-C, as well as to other stress factors. Increasing the frequency of UV-C applications to every three days increases the likelihood that an *E. necator* colony would be at its most susceptible developmental stage.

In our season-long field experiments, disease suppression increased with increased frequency of UV-C application (Figure 4E). Similar results were found in commercially managed plots, where disease pressure may have been less than occurred in our 2022 trials (Onofre et al. 2021, Gadoury et al. 2023). The degree of disease suppression provided by season-long UV-C in our trials was generally less than that reported by Gadoury et al. (2023) and Onofre et al. (2021), and was substantially less than that provided by a common eradicant fungicide treatment (Figure 4). Nonetheless, early-season UV-C treatments in our trials provided commercially acceptable levels of disease suppression that could effectively replace early-season fungicide sprays (Supplemental Table 1 and Figure 3).

The lack of protectant activity following a UV-C treatment can be a positive attribute, as potentially toxic residues that might affect nontarget organisms are not an issue. However, this also means that small-plot experiments assessing disease management strategies could be disproportionately affected by interplot interference if effective treatments border an untreated control or marginally-effective treatment. The two commercial-scale studies discussed above minimized interplot interference by using

Table 3 Yield components and berry metrics of *Vitis vinifera* Chardonnay in Prosser, WA, treated with ultraviolet-C light (UV-C; 200 J/m^2) in the early-season in 2022. Different letters denote significant differences among treatment means at $\alpha = 0.05$ using Tukey's honest significant difference test.

Yield components and harvest metrics	Fungicide program	Early twice weekly UV-C	Early weekly UV-C	Early unsprayed	Season-long unsprayed	*p* value
Average total yield per vine (kg)	3.5 a	3.3 a	2.6 a	2.6 ab	0.9 b	0.003
Cluster weight (g)	79.8 a	74.5 a	72.3 a	64.4 a	23.7 b	0.002
Berry weight (g/berry)	1.3 a	1.2 a	1.2 a	1.1 a	0.6 b	0.0002
Number of berries per cluster	60.8 a	64.8 a	61.1 a	57.4 ab	40.3 b	0.006

Table 4 Yield components and berry metrics of *Vitis vinifera* Chardonnay in Prosser, WA, treated with ultraviolet-C light (UV-C; 200 J/m^2) season-long in 2022. Different letters denote significant differences between treatment means at $\alpha = 0.05$ using Tukey's honest significant difference test.

Yield components and berry metrics	Fungicide program	Twice weekly UV-C	Weekly UV-C	Season-long unsprayed	*p* value
Average total yield per vine (kg)	3.5 a	2.0 b	1.7 bc	0.9 c	0.0002
Cluster weight (g)	79.8 a	51.7 b	42.5 bc	23.7 c	0.0005
Berry weight (g/berry)	1.3 a	0.8 b	0.6 b	0.6 b	0.00006
Number of berries per cluster	60.8 ab	65.4 a	71.7 a	40.3 b	0.007
Titratable acidity (g/L)	8.2	8.6	9.1	8.8	0.40
Total soluble solids (Brix)	21.9 c	24.2 bc	25.1 ab	27.1 a	0.0001
pH	3.4 a	3.5 ab	3.7 bc	3.8 c	0.0007

large experimental plots in commercial fields (Onofre et al. 2021, Gadoury et al. 2023). Our plots were much smaller due to availability of vineyard space. The lower magnitude of treatment effects observed in our study may reflect interplot inference as airborne conidia spread within the experimental area and diluted treatment effects that would be more distinct in larger plots.

Effective access to target surfaces during UV-C applications requires a direct line of sight. This can make access to fruit within dense and complex canopy architecture a challenge, particularly as canopies expand over a growing season. Vineyard canopy management has known effects on spray penetration (Moyer et al. 2016b), and overly-dense canopies can also increase the favorability of disease development (Austin et al. 2011). This might explain improved and more-consistent disease suppression when UV-C was applied only in the early season, when canopies were less dense and allowed greater UV-C penetration. This also suggests that UV-C efficacy may respond favorably to pruning and training systems that open canopies and expose the fruiting zone. In all systems, but particularly in systems with larger, more dense canopies, shoot training, shoot thinning, and leaf removal become increasingly important to ensure that UV-C penetration is not occluded by excessive vegetative growth.

New technologies or changes in viticulture practices must be evaluated for potential adverse effects on yield and basic fruit quality. In 2022, we found differences in yield, but these were not associated with UV-C applications. TSS was lower in the fungicide and twice-weekly UV-C treatments because there was less powdery mildew, less desiccation of fruit, and therefore less concentration of solids in the berry pulp. The more severely infected berries shriveled, so their greater TSS

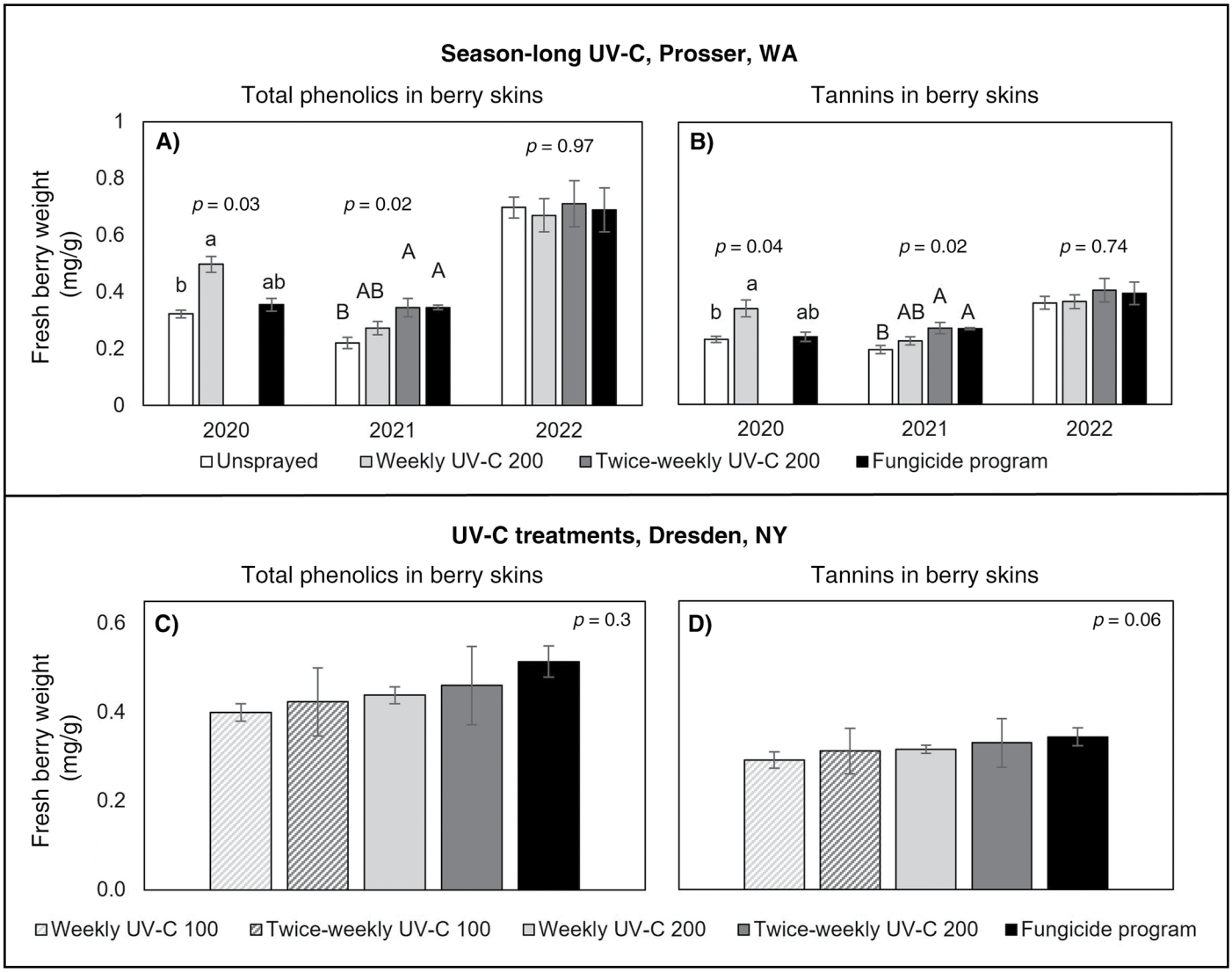

Figure 5 Influence of season-long ultraviolet-C light (UV-C) treatment on fruit quality of *Vitis vinifera* Chardonnay grown in Prosser, WA and in Dresden, NY. **A**, **B**) Total phenolics and tannins, respectively, in berry skins for vines receiving full-season UV-C treatment in 2020, 2021, and 2022 in Prosser, WA. **C**, **D**) Total phenolics and tannins, respectively, in berry skins for vines receiving full-season UV-C treatment in 2021 in Dresden, NY. Error bars are standard error (n = 4). Different letters denote significant differences among treatment means at $\alpha = 0.05$ using Tukey's honest significant difference test. Same case lettering denotes comparisons between treatments within a year. UV-C 100 and UV-C 200, UV-C dose of 100 or 200 J/m^2, respectively.

content reflected water loss more than sugar accumulation. These effects are common when powdery mildew is severe on fruit (Gadoury et al. 2001). Other studies reported no negative effects of UV-C on net photosynthesis (Ledermann et al. 2021, Gadoury et al. 2023), overall vine growth or changes in metabolic measurements in Chardonnay (Gadoury et al. 2023), or fruit quality in Albion, Monterey, or Sensation Florida 127 strawberries (*Fragaria* × *ananassa*) (Janisiewicz et al. 2016a, Onofre et al. 2021). The lack of effect is likely related to the lack of phytotoxicity of doses used (Otake et al. 2021), and the blocking of UV light by the epidermis (Hollósy 2002) before reaching cell layers involved in photosynthesis. This suggests that effects of UV-C might be restricted to the skin of grape berries. Thus far, we have not observed such effects at UV-C doses deemed effective for suppressing E. *necator* (Figure 5). Future work should explore the upper range of UV-C doses that might change berry skin phenolics and tannins, if a change can be made at all.

Conclusion

Optimal deployment of UV-C in commercial viticulture requires integrating knowledge of pathogen biology, disease epidemiology, critical periods of host susceptibility, local climate conditions, and vineyard management practices. UV-C works primarily as an eradicant of young E. *necator* colonies, providing little residual activity against future infections, which necessitates frequent applications. Furthermore, a direct line of sight to access target surfaces is critical for effective treatments. Early-season UV-C applications for grapevine powdery mildew, particularly when canopies are smaller and less dense, could reduce overall chemical inputs while providing effective and commercially-relevant disease suppression. Season-long or exclusive UV-C for grapevine powdery mildew management requires further evaluation under Washington State conditions, specifically in larger plots with disease pressure typical of commercial vineyards, and in combination with canopy management practices to help light penetration.

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Supplemental Data

The following supplemental materials are available for this article at ajevonline.org:

Supplemental Table 1 List of trade name, active ingredient, and Fungicide Resistance Action Committee (FRAC) group of the various fungicides and adjuvents used in both the early-season and season-long experiments described in Tables 1 and 2.

Supplemental Table 2 Early-season and season-long final measurement (BBCH 85 to 89[a]) of cluster disease severity (mean ± SE). Ratings consisted of visually evaluating the upper and lower surfaces of 40 random leaves and 20 random clusters per treatment replicate in 2020, and 40 random leaves and 40 random clusters per treatment replicate in 2022. Data from 2021 was not included, as there was no disease on the grape clusters. UV-C, ultraviolet-C light.

Supplemental Table 3 Yield components and berry metrics of *Vitis vinifera* Chardonnay in Prosser, WA, treated with ultraviolet-C light (UV-C) early in the 2020 and 2021 growing seasons. In 2020, there was no twice-weekly UV-C treatment. Different letters denote significant differences among treatment means at $\alpha = 0.05$ using Tukey's honest significant difference test.

Supplemental Table 4 Season-long field evaluation of ultraviolet-C light (UV-C) effects on yield components and berry metrics of *Vitis vinifera* Chardonnay at Washington State University – Irrigated Agricultural Research and Extension Center, Prosser, WA in 2020 and 2021. In 2020, a twice-weekly UV-C was not included. Different letters denote significant differences between treatment means at $\alpha = 0.05$ using Tukey's honest significant difference test.

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