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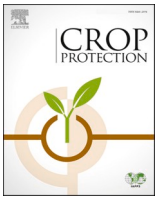

Effects of germicidal ultraviolet-C light on grape mealybug (*Pseudococcus maritimus*)

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A B S T R A C T

Germicidal ultraviolet-C light (UV–C) has been used to effectively suppress grapevine powdery mildew (*Erysiphe necator*). However, effects on arthropod pests that would be simultaneously exposed to UV-C during applications directed at *E. necator,* are poorly understood. The grape mealybug (*Pseudococcus maritimus*) is one such grapevine pest. We exposed mated *P. maritimus* adult females in a lab study to UV-C light doses of 100, 200, 500 and 1000 J/m^2. UV-C was applied during darkness and was followed by an additional 4 h dark period post-treatment to enhance possible suppressive effects of UV-C. Positive (2% horticultural oil) and negative (untreated) controls were included. Adult female *P. maritimus* oviposition was observed two weeks after treatment, and hatching of eggs was enumerated four weeks after treatment. UV-C treatment of mated adult females, regardless of dose, did not prevent oviposition or reduce ovisac viability. In a separate lab experiment, first instar *P. maritimus* nymphs were similarly exposed to UV-C doses of 200 and 1000 J/m^2 with and without a 4hr dark period. UV-C treatments were compared to the same positive and negative controls. Nymph mortality was assessed at 24 and 48 h after UV-C treatment. Increasing doses of UV-C treatments, followed by a dark period, had a small, but significant impact on nymph mortality relative to the untreated control. Further studies on the potential sublethal effects (e.g., fecundity or longevity) of single or multiple exposures or increased UV-C doses upon juvenile stages of *P. maritimus* are warranted, as longer-term and multigenerational effects of UV-C have been observed in other arthropod systems.

1. Introduction

Grape mealybug, *Pseudococcus maritimus* (Ehrhorn) is an important pest in grape (*Vitis* sp.) production, as it is a primary vector of *Grapevine leafroll-associated virus 3*. This virus is the predominate virus of grapevine leafroll disease in many regions around the world, including the Pacific Northwest, USA (Rayapati 2011; Bahder et al., 2013a; Donda et al., 2023). Grape mealybug is typically managed at the nymph stage with contact insecticides applied in the grape phenological period of delayed dormant (wooly bud, BBCH 01 to 03). In addition, management also occurs mid-season after the first male flight with a systemic insecticide which typically aligns around the bloom period (BBCH 57–69) for grapevine development in the Pacific Northwest (Hoheisel et al., 2023). However, these management approaches have not always resulted in desired control outcomes, as appropriate spray coverage targeting moving nymphs can be difficult (Geiger and Daane 2001). Nymphs emerge from protected areas of the bark during a short period where they are at maximum exposure to insecticides (Geiger and Daane 2001; Daane et al., 2012). In addition, insecticide resistance in *P. maritimus* can also result in a lack of pest control (Venkatesan et al., 2016). These challenges associated with spray coverage and product resistance, coupled with an increase in the adoption of sustainability certification programs in western USA wine grape production (for example: Low Input Viticulture and Enology, https://livecertified.org/about; Sustainable Washington, https://sustainablewa.com/; and Lodi RULES, https://www.lodirules.org/), has warranted exploration into alternative pest management technologies.

Germicidal ultraviolet-C light (UV–C) offers a potential alternative for *P. maritimus* management. UV-C is a highly energic wavelength (254 nm) that has been used effectively to suppress powdery mildews such as

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Erysiphe necator in grapes (Gadoury et al., 2023), *Podosphaera aphanis* in strawberry (Onofre et al., 2021), and *Podoshaera xanthii* in cantaloupe (Lopes et al., 2023). Application of UV-C during the night aids in bypassing the photolyase DNA repair mechanism in these powdery mildews, which allows for the use of lower UV-C doses without subsequent phytotoxicity to the plant (Suthaparan et al., 2014; Janisiewicz et al., 2016). Similar photo-enzymatic repair of UV-B damage and enhanced efficacy of nighttime application of UV-C has been reported in *Tetranychus urticae* Koch (two-spotted spider mite). Short et al. (2018) was able to reduce *T. urticae* populations on potted strawberry plants by exposing them to 60 s of UV-C light (14 J/m^2) followed by a dark period. Studies regarding the efficacy of UV-C on arthropods such as mealybugs are lacking, but necessary to evaluate and create management programs for this impactful pest.

Our objective was to better understand possible suppressive effects of UV-C light on adult female and nymph stages of the grape mealybug (*P. maritimus)* in a controlled environment. This information could be used to further explore the use of UV-C as a direct alternative, or a complementary approach, to integrated management of grape mealybugs in commercial vineyards.

2. Materials and methods

Laboratory-scale UV-C light array system. An enclosed UV-C lamp array was constructed for use in laboratory experiments. The frame of the apparatus was clad in galvanized steel with 3.0 mm thick PVC curtains at each end to contain the UV-C light within the apparatus. The apparatus contained three 90 cm length lamp fixtures, each bearing two low pressure discharge UV-C lamps (Osram germicidal T8 55W UVC Medium Bi Pin Base model G55T8/OF, Wilmington, MA) powered by dual-lamp ballasts (IUV–2S36-M2-LD PureVOLT™, Philips ADVANCE, Rosemont, IL). Lamps were positioned above a motorized conveyor belt at the base of the unit to move samples through the apparatus (60W, 30–120 rotations/min, 1.5 × 0.4 m belt, Vevor®, Rancho Cucamonga, CA). Magnitude and uniformity of UV-C irradiance at the sample plane, approximately 20 cm below the bottom lamps, was measured using a UV spectroradiometer (model BTS2048-UV-S, Gigahertz-Optik GmbH) as described by Onofre et al. (2021). Target doses of UV-C (254 nm, FWHM 5 nm) 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, 200, 500, and 1000 J/m^2, the conveyor belt was set to 0.5, 0.25, 0.09, and 0.03 m/s, respectively.

Laboratory evaluation of ultraviolet-C light on Pseudococcus maritimus *adult female oviposition*. The potential lethal effects of UV-C on mated adult female *P. maritimus* was evaluated using UV-C doses of 100, 200, 500, and 1000 J/m^2, with a 4 h dark period after treatment then returned to a darkened incubator. Additional treatments included a positive control: 2% v/v horticultural oil (PureSpray Green, Intelligro, Ontario Canada), and a negative control: untreated. The horticultural oil was applied to bioassay until filter paper saturation (3 s) using a handheld pump sprayer (CHAPIN 16100 Home and Garden one-gallon sprayer; adjustable cone nozzle, 0.3–0.4 MPa), with the delivery nozzle placed 18 cm from the adaxial leaf surface; bioassay set up is described below.

Mated adult *P. maritimus* females were collected from a vineyard in Benton City, WA. In eastern Washington vineyards, *P. maritimus* is the only confirmed mealybug species (Daane et al., 2011, Bahder et al., 2013b); the vineyard that the adult females were collected from is isolated from other cropping systems. For further confirmation, the adult females were gently prodded at the time of collection, and excreted an orange fluid, a common diagnostic feature of grape mealybug (see: https://ipm.ucanr.edu/pmg/c302/mt302bpmealybug.html). The timing of collection to ensure that females were mated was done by monitoring delta traps (Pherocon VI Delta Trap, Trece, Incorporated, Adair, OK, USA) with pheromone lures for *P. maritimus* males (Pherocon 3245–25, Trece Incorporated, Adair, OK, USA) by vineyard managers. Scouting and collection of mated adult females occurred once traps had countable adult male *P. maritimus*. Collection dates were 31 August 2022 (first experiment) and 5 September 2022 (second experiment). Once collected, ten females were transferred with a fine tip paintbrush to each of the five 90 mm petri dishes. These petri plates were lined with a dry Whatman filter paper. The bioassays were kept in darkened conditions in a plant growth incubator (3765 model 504L Thermo Fisher, Waltham, MA) at 22 °C for 24 h. After this 24 h holding period, the adult females were treated as described above. Following treatment, the petri plates containing the adult females were incubated in dark conditions until oviposition (approximately 2 weeks). Once oviposition had occurred, the number of ovisacs were visually counted to determine effects to the female's ability to oviposition. Ovisacs were allowed to continue development to hatching under a 16:8 h light regimen in the same incubator for an additional 2 weeks. The number of ovisacs (not individual eggs) with at least one moving first instar nymph was counted as a successful oviposition. This delayed count was done to determine potential longer-term effects that may result from treatment or UV-C light exposure of the adult female on the next generation.

Laboratory evaluation of ultraviolet-C light on Pseudococcus maritimus *nymphs*. The potential lethal effects of UV-C on first instar *P. maritimus* nymphs were evaluated using UV-C doses of 200 J/m^2 or 1000 J/m^2, with or without a 4 h dark period following treatment. Additional treatments included a 2% v/v horticultural oil (PureSpray Green, Intelligro, Ontario Canada), and an untreated control. The horticultural oil treatment was also applied as described above until surface saturation of the leaf bioassay. This experiment was repeated a total of three times.

To obtain *P. maritimus* first instar nymphs, rearing methods were adapted from Blaisdell et al. (2016). Mated adult females were collected from a vineyard in Benton City, WA using the methods described previously. Once adult female mealybugs were collected, they were transferred with a fine tip paintbrush to 90 mm petri dishes, with dry 90 cm Whatman filter paper. The mealybugs were kept in darkened conditions at 22 °C in plant growth incubator described above until oviposition. Ovisacs were then allowed to hatch at 22 °C with a day/night regime of 16:8 h. Once hatched, ten first instar nymphs were transferred to leaf disc on moistened circle cotton rounds. Leaves used in the bioassays were from greenhouse-grown *Vitis vinifera* 'Chardonnay' leaves of mature age, fifth leaf from the tip, were collected, surface disinfested in 0.5% $NaOCl_2$ for 90 s, rinsed twice in distilled water and cut using a 4.5 cm diameter core. In total, 5 leave disc bioassays per treatment were used as replicates (n = 5). The leaf bioassays were then left in darkened incubator overnight to be treated the following day, with the treatments described above.

Nymphs were observed under a dissecting microscope (VistaVision VWR, Radnor, PN, USA) at 24 and 48 h post treatment. Nymphs with visible leg or antennae movement in response to gently prodding were considered alive; those lacking movement were considered dead.

Statistical evaluation. All statistical analyses were analyzed using JMP statistical program (v. 6.0.0, SAS Institute Inc., Cary, NC) with the standard least squares model platform. The restricted maximum likelihood (REML) method was used for all analysis, where replicates were random effects, and treatment and experimental repeat were fixed effects. For observations on the impact of UV-C treatment on adult females, there was no statistical difference between the two experiment replications ($p = 1.0$), results were pooled for further analysis. When analyzing the treatment effects on nymph mortality, an additional fixed effect was used: observation timing (24 or 48 h post treatment). Tukey's honest significant difference was used to determine significance ($\alpha = 0.05$). Regression analysis was performed to understand the relationship between UV-C dose against nymph mortality.

3. Results

Laboratory evaluation of ultraviolet-C light on Pseudococcus maritimus *adult female oviposition*. Irrespective of treatment, 100% of the treated adult females were able to oviposit after 2 weeks, including the positive control (2% oil). When 10 ovisacs were selected for further observation at 4 weeks, all contained hatching eggs.

Laboratory evaluation of ultraviolet-C light on Pseudococcus maritimus *nymphs*. In our initial analysis, observation time after treatment (24 or 48 h) was not a significant fixed effect ($p = 0.15$). As such, analyses and presentation are from the 48 h observation time point only. Data from the three experimental replicates were not pooled, as experiment was a significant factor for nymph mortality ($p = 0.0003$). When the oil control (2% oil) was included in statistical analyses (Fig. 1) none of the UV-C treatment means differed significantly from the untreated control (Tukey's HSD, $\alpha = 0.05$) with the exception of the 200 J/m^2 with a dark period in Experiment 3. However, when the oil control was excluded, we did see a trend in response to UV-C treatment. When individual pairwise comparisons were performed between the remaining treatments (untreated control, UV-C 200 J/m^2 and 1000 J/m^2), we saw that UV-C at both doses, with a dark period, resulted in increased nymph mortality relative to the untreated control ($p = 0.09$ and 0.08, respectively) in Experiment 1. In Experiment 2, UV-C 1000 J/m^2 with a dark period and UV-C 200 J/m^2 without a dark period increased nymph mortality compared to the untreated ($p = 0.1$ for both). In Experiment 3, the dominant treatment effect was driven by the significant increase of nymph mortality when exposed to UV-C 200 J/m^2 with a dark period compared to the untreated control ($p = 0.02$). These trends were encouraging, given the small sample size that can lead to type II statistical error (false negatives).

Given these subtle treatment effects, the relationship of UV-C dose to nymph mortality was subsequently analyzed using linear regression wherein the untreated control was defined as UV-C at a dose of 0 J/m^2, UV-C at 200 J/m^2 and UV-C at 1000 J/m^2, with or without a dark period following treatment. When UV-C treatment was not followed by a dark period, the dose of UV-C did not influence nymph mortality (Fig. 2, $p = 0.46$). However, when a dark period was included after a UV-C treatment, there was a small, but significant positive relationship between increased UV-C dose and increased nymph mortality (Fig. 2, $p < 0.0001$).

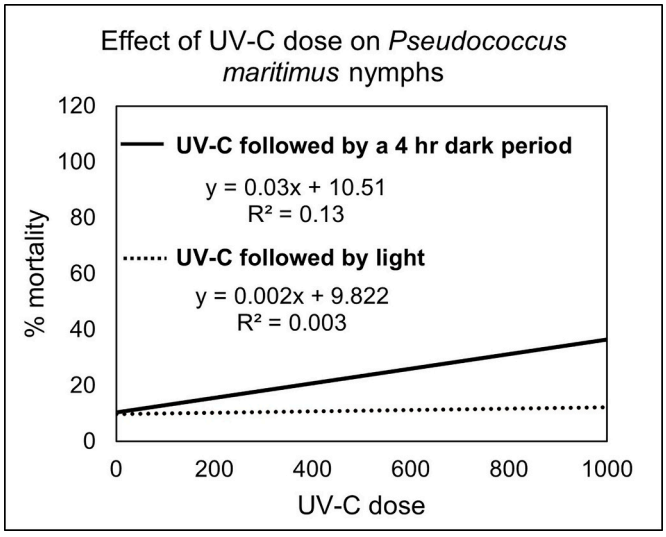

Fig. 2. Relationship between dose of UV-C treatment and nymph mortality in *Pseudococcus maritimus*. UV-C was applied to first instar nymphs in a laboratory setting either with a 4 h dark period following treatment, or without a dark period following treatment.

4. Discussion

Our laboratory assays presented here demonstrated that treatment of adult female *P. maritimus* with UV-C did not significantly affect their ability to lay viable eggs. In this regard, UV-C treatments share a lack of efficacy with most chemical treatments. Efficacy of existing contact and systemic insecticides are primarily limited to younger life stages in mealybugs (Hoheisel et al., 2023), and do not seem to impede adult female egg laying. Additionally, adult female mealybugs are often concealed under the exfoliating plates of bark on grapevines (Geiger and Daane 2001), reducing the opportunity of both chemical sprays and UV to reach them. Finally, contact products dissolved or suspended in water aerosol sprays encounter difficulty in penetrating the waxy coating of adult mealybugs (Mani and Shivaraju 2016). We had hypothesized that UV-C would exhibit suppressive activity against adult female grape mealybugs. While positive results in treating adult arthropod pests with UV-C have been seen in other studies; notably on *T. urticae* (Short et al., 2018), we did not observe this same positive effect. Sensitivity to UV light, as well as photo-enzymatic repair of UV-B damage has been observed in *T. urticae* (Ohtsuka and Osakabe 2009; Murata and Osakabe 2017). These traits have not been previously reported for *P. maritimus* and a lack of suppression by UV-C treatments has been reported for other arthropod pest species (Hori et al., 2014).

The immature stages of the grape mealybug are mobile, as noted by

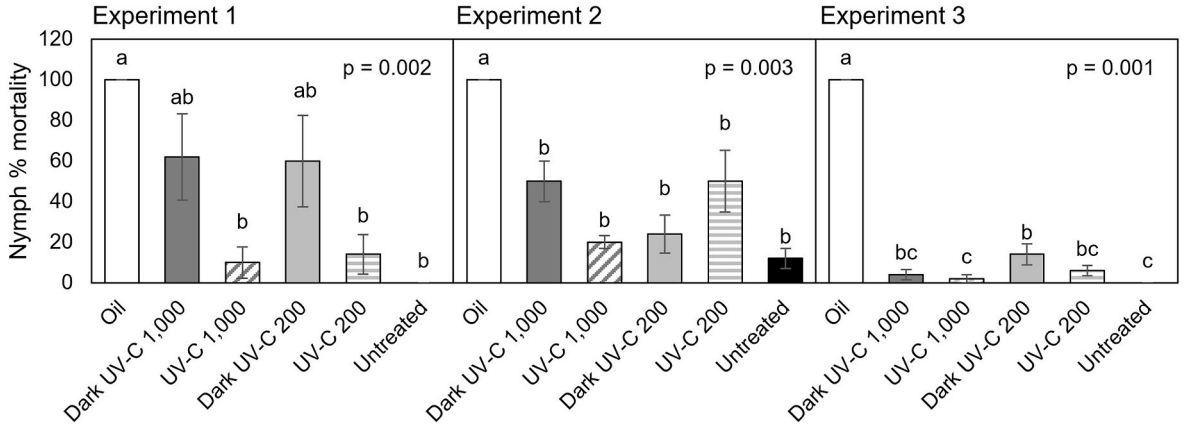

Fig. 1. Effects of ultraviolet-C light on first instar *Pseudococcus maritimus* nymphs observed at 48 h after treatment in laboratory assays. Mortality is defined as lack of nymph movement after gentle prodding. (Left) Experiment 1; (Middle) Experiment 2; and (Right) Experiment 3. 'Dark' in the treatment title names indicate the UV-C exposure was followed by a 4 h dark period. Numbers indicate the UV-C dose in J/m^2. Error bars are standard error ($n = 5$). Different letters denote significant differences among treatment means within an experiment at $\alpha = 0.05$ using Tukey's honest significant difference.

the common name "crawler", and consequently it is a common target of many commercial management programs (Varela et al., 2019; Hoheisel et al., 2023; Skinkis et al., 2023). Nymphs emerge from beneath bark after hatching and are predominately found on stems and leaves (Geiger and Daane 2001), where UV-C would have the greatest potential to reach them. In our laboratory assays, we had mixed results on the efficacy of UV-C light on *P. maritimus* nymphs. When considering the relationship with UV-C dose (when followed with a dark period) as seen in Fig. 2, and the results presented in Fig. 1 coupled with the treatment comparison when the oil control treatment was removed, you can see an emerging trend suggesting small, but potentially impactful, effects of UV-C on *P. maritimus* nymphs. Across all three experiments, when nymphs were exposed to either 200 or 1000 J/m^2 of UV-C followed by a dark period, there was a range of 9–98% probability that UV-C treatment resulted in increased nymph mortality relative the untreated control. While highly variable, our experimental design had limitations, from the number of nymphs that could be reared for experiments and the UV-C dose that could be achieved in the lab. Future studies could focus on experimental methods that allow for increased nymphs in bioassays, along with developing a system that can generate UV-C doses that exceed 1000 J/m^2. The effects of a post-treatment dark period could also be further explored, to understand what aspect of insect physiology is affected by UV-C treatments and why a dark period following treatment may enhance UV-C lethality.

What remains a challenge with UV-C light as a field management tool is coverage, or light occlusion, of the target organism. Short et al. (2018) found this to be true in their study on *T. urticae,* where penetration of UV-C light through the strawberry canopy declined with increasing foliage layers. As a result, the greatest reduction in *T. urticae* was on the upper/exposed leaves after UV-C treatment. If UV-C is adopted as a potential integrated pest management (IPM) tool, improved coverage and penetration of the canopy may substantially improve efficacy against arthropod pest, particularly those that are mobile. Given the need for UV-C to reach obscured areas of the vine where mealybug nymphs reside, and the potential need for phytotoxic doses (e.g., >1000 J/m^2), the delayed dormant period of vine development (early spring) may present the most practical timing for its use in mealybug management. Additionally, when serial applications of UV-C are directed at grapevine powdery mildew, their sublethal effects on grapevine mealybug may ultimately prove more consequential in arthropod management as deleterious and accumulating impacts on arthropod longevity, fecundity and fertility has been observed in phytophageous mites.

While the goal in the development and deployment of any new insect management strategy is often complete control, sublethal effects can play an important role in IPM programs. Sublethal effects can affect insect biology, physiological responses, and/or modify insect behavior that can reduce populations overtime but do not cause instant mortality (De França et al., 2017). These effects can be combined with other additional controls such as cultural or biological that could synergistically work together to keep pests below economic thresholds. Unfortunately, in the management of grape mealybug, sublethal effects are not enough. This is because grape mealybug is primarily managed due to its role in the vectoring *Grapevine leafroll-associated virus 3*. Grape mealybugs have a high success rate of transmitting this virus; only one nymph is needed to infect a grapevine under a very short feeding period (O'Hearn and Walsh 2020). As a result, there is a zero-tolerance policy for grape mealybug survival in the vineyard. In our controlled environment studies presented here, UV-C did not induce the desired 100% mortality of *P. maritimus* nymphs. However, if efficacy of UV-C treatments can be improved, either through a better understanding of dose or timing, it could provide a complementary IPM tool for existing control tactics such as insecticides or mating disruption in commercial vineyards.

5. Conclusion

UV-C treatment of adult female *P. maritimus* did not reduce their ability to lay viable eggs. While the study was limited in size, there were potential impacts of UV-C light treatment on nymph mortality, and these results warrant further exploration. The current results suggest that UV-C may not provide the 100% mortality effect that growers are looking for; but it does suggest that with further refinement, this tool could become a complementary component to multi-part IPM approach to grape mealybug management or an additional benefit when treating for grapevine powdery mildew. The studies that are needed to refine this tool include investigating the chronic and potential sublethal effects of UV-C on juvenile stages (e.g., fecundity or longevity), or effects of multiple UV-C exposures at specific instar development stages. Knowledge of how *P. maritimus* perceives light and if it possesses the ability to repair UV damage through photo-enzymatic repair would also be helpful in the exploration and adaptation of this tool for practical mealybug management.

CRediT authorship contribution statement

Alexa L. McDaniel: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **David M. Gadoury:** Methodology, Writing – review & editing. **Michelle M. Moyer:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Michelle M. Moyer reports financial support was provided by Washington State Grape and Wine Research Program. Michelle M. Moyer reports financial support was provided by National Institute of Food and Agriculture. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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