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Original Research Paper

Effectiveness of the field application of UV-C for cucumber downy mildew control

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ABSTRACT

There is growing interest in the application of ultraviolet (UV-C) energy to control crop pathogens. In the present study, the efficacies of UV-C treatments for controlling cucumber downy mildew (*Pseudoperonospora cubensis*) were investigated on a commercial farm in eastern Massachusetts, USA. Controlled doses of UV-C, delivered by a tractor-mounted array of sources, between 120 and 480 $J \cdot m^{-2}$ were applied and compared to conventional fungicide treatments as well as to untreated controls, for each of two consecutive years (2020 and 2021). Visual assessments of foliar disease severity in the trial plots were made several times from planting through the end of productive life. In contrast to the successful control of powdery mildew, the UV-C treatments for controlling cucumber downy mildew were not as successful as conventional fungicides. None of the UV-C treatments affected the overall progression rate of downy mildew once the disease became apparent, although disease onset was delayed slightly compared to untreated controls. This delay may have been due to UV-C induced resistance to infection by the host. Unlike powdery mildews, downy mildew spores from *P. cubensis* are darkly pigmented, possibly decreasing the efficacy of the UV-C treatments for controlling the disease. DM spores may also be only susceptible to UV exposure prior to encysting in the leaves of the host, thereby perhaps limiting the window of opportunity when UV-C treatments can be effective. Although not the primary focus of this study, the use of reflective mulch appeared to delay disease onset relative to black mulch in fields with significant sunlight exposure, perhaps due to lowering plant stress by maintaining a lower soil temperature.

Keywords : Crop pathogens, cucurbits, downy mildew and ultraviolet energy

INTRODUCTION

Pseudoperonospora cubensis is the oomycete pathogen responsible for cucurbit downy mildew It can infect many cucurbits such as cantaloupe, pumpkin, watermelon, and squash, but cucumber is particularly susceptible. In the United States, use of resistant cucurbit varieties was an effective means of control of downy mildew until 2004. Since then, effective control has depended upon chemical fungicides in addition to planting resistant varieties (Savory *et al.*, 2011).

P. cubensis is spread by means of aerially dispersed sporangia. When viable sporangia land on a host leaf, they germinate in moisture on the leaf's surface producing biflagellate zoospores that encyst in stoma where a germ tube is formed that penetrates the leaf's surface through the stoma. Hyphae form in the mesophyll layer and produce clavate-branched haustoria in the host's cells. When sporulation is triggered, sporangiophores emerge through stomates bearing sporangia at their tips. When released, these sporangia are carried by the wind and the life cycle repeats at the next site.

Both visible light and ultraviolet (UV) energy have been reported to reduce the viability of fungal spores (Rotem *et al.*, 1985; Kanetis *et al.*, 2010). UV has been particularly effective for controlling powdery mildew (Patel *et al.*, 2020; Skinner *et al.*, 2020; Onofre *et al.*, 2021). Unlike powdery mildew however, downy mildew spores are darkly pigmented with melanin (Lee *et al.*, 2021), a strong absorber of

UV (Meredith and Sarna, 2006), which reduces the potential for damage from natural UV solar energy (Cordero and Casadevall, 2017).

UV energy can be classified into three bands, UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (100-280 nm). Sunlight reaching the surface of the earth is limited to the longer UV wavelengths, and the amount and spectrum depends upon weather, latitude, and season. UV energy in all three bands can be produced by electric sources. Today, low pressure discharge sources are the most common; however, LED sources will undoubtedly displace them in the next few years.

UV energy can inactivate viruses, bacteria, and fungi through several different mechanisms (Nelson *et al.,* 2018). UV-C in the range of 250 to 270 nm acts directly on nucleic acids (RNA and DNA) within the cell (Nelson *et al.,* 2018). When a UV photon is absorbed, the molecular strands become broken and dimers (molecular lesions) are formed. These dimers, typically thymine dimers, prevent cellular replication unless repaired (Kneuttinger *et al.,* 2013). In fact, every organism has nucleic acid repair mechanisms. UV-C can also induce secondary reactions within the cell when a photon is absorbed by an endogenous chromophore and subsequently produces free radicals which, in turn, breakdown one or more vital cellular functions. UV-B and UV-A can also induce these secondary reactions. Generally, the longer the UV wavelength, the higher the dose needed to induce secondary endogenous photocatalytic inactivation. Secondary inactivation can also occur through exogenous photocatalysis. Photocatalysts like TiO_2, with moisture, will also produce free radicals that can kill bacteria and fungi by damaging cell walls, thus disrupting their permeability (Ramesh *et al.,* 2016).

Most plants and their obligate parasites have evolved mechanisms that limit cellular damage from natural UV (Sancar, 1994; Cordero and Casadevall, 2017). Pigmentation that absorbs UV energy and then dissipates it as heat is one protective mechanism, minimizing damage to DNA. Pigmentation can also reduce the number of photons available for absorption by chromophores within the cell that might initiate lethal, secondary reactions. Melanin is the most widely recognized putative protective pigment in fungi (Cordero and Casadevall, 2017), with high absorption throughout the UV spectrum (Meredith and Sarna, 2006). Unlike powdery mildew fungi that are devoid of melanin (Suthaparan *et al.,* 2012), some downy mildew sporangia such as *P. cubensis* (Lee *et al.,* 2021) have high concentrations of protective melanin. PM incorporates a different protective mechanism that entails upregulating a photoactivated (short visible wavelength) repair mechanisms for UV-induced cellular damage (Sancar, 1994).

UV treatments have been particularly successful for mitigating obligate powdery mildew in a variety of crops (rose, strawberry, cucumber, etc.). Of particular note, nighttime applications of UV have been shown to be more efficient than daytime applications at similar doses (Suthaparan *et al.,* 2012). As noted above, powdery mildew has a short-wavelength sensitive repair mechanism which works quite well for pathogen survival because visible light is always in the same solar spectrum as UV. By providing only nighttime UV-C exposure, the powdery mildew pathogen has no access to its short-wavelength repair mechanisms. Through field trials, it has been shown that UV-C doses at night between 100 $J{\cdot}m^{-2}$ and 200 $J{\cdot}m^{-2}$ can be more effective and less expensive than conventional fungicides for limiting the proliferation of powdery mildew (Onofre *et al.,* 2021).

In the present study we wanted to learn more about the effects of UV-C on mitigating cucurbit downy mildew. Rotem *et al.* (1985) exposed three different types of spores with increasing pigment density to short wavelength UV energy. Not surprisingly, they found that the spores with higher pigment density were less sensitive to the effects of UV exposure. Based on these findings, we expected that UV-C doses higher than those applied to control powdery mildew would likely be needed to be effective because of high concentrations of protective pigment in downy mildew spores. Of some concern, we wanted to determine whether UV-C doses higher than those previously used might reduce yield from the host plant. To further our understanding of the potential for UV-C to control downy mildew, we added a UV-reflecting mulch to the study. The reflective mulch would not only increase the effective dose but would also redistribute the UV-C to surfaces otherwise in shadow. In addition to better understanding the direct effects of UV-C on controlling downy mildew, we also wanted to see if there was evidence for UV-induced resistance to infection by the host (Kunz *et al.,* 2008; Paul *et al.,* 2012), the idea being UV-C exposure prior to the presence of downy mildew in the field might reduce either the severity or delay the onset time of infection. Additionally, since *P. cubensis* sporangia may only be susceptible to UV treatment prior to encysting in host leaves, both once- and twice-weekly treatments using different UV doses were investigated.

MATERIALS AND METHODS

UV-C Treatment Device

A tractor three-point hitch mounted UV-C treatment attachment (aka the "Dragon") was designed to treat one crop row at a time. The unit consisted of an array of six 300 W UV-C fixtures [four UV-C lamps (TUV75W/HO, Philips) per fixture, each fixture powered by 2 two-lamp ballasts (Pure VOLT IUV-2S60-M4-LD, Philips/Advance)], which were arranged in a hemi-cylindrical manner arching over the row of plants (Fig. 1). The power to operate the light fixtures was provided by an on-board gasoline powered inverter generator (iGen 2600, Westinghouse Outdoor Power Equipment). Vinyl curtains with a UV reflective foil tape (76145A62, McMaster-Carr) applied to the inner side were installed on both ends of the enclosure to help contain the UV-C within the treatment attachment and to redirect it back into the unit.

Since the output of the UV-C array is fixed, prescribed dose levels were obtained by varying the speed of the tractor over the crop. The speeds required to achieve the specific doses were verified by driving the UV-C attachment over an integrating UV-C logger. A ground speed of approximately 4.0 $km \cdot hr^{-1}$ (2.5 MPH) was required to achieve 120 $J \cdot m^{-2}$ and 2.0 $km \cdot hr^{-1}$ (1.25 MPH) to achieve 240 $J \cdot m^{-2}$. The 480 $J \cdot m^{-2}$ dose level was achieved by making two passes at the 240 $J \cdot m^{-2}$ ground speed. The UV-C logger used was designed and built by the research team. It consisted of a UV-C detector and a microcomputer housed in a weather resistant housing with the detector located under a UV transparent window. The microcomputer recorded the output of the UV-C detector to a memory chip once every 10 ms. The duration and amount of UV-C irradiance incident on the detector was used to compute dose.

Plastic Mulch

Black (BioTelo, Heartnut Grove Inc.) and reflective mulch (Brookdale Farm Supplies) were used to make the beds in the study plots. The reflectance of both mulch types was measured at 254 nm and 436 nm to determine their reflectance of UV-C and a short visible wavelength (Table 1).

Table 1 : Reflectance at two wavelengths of the black and the reflective mulches

Mulch Type	Reflectance	
	254 nm	436 nm
Reflective	66%	75%
Black	5%	1%

Year 1 Field Trials

The focus of the first-year field trial was to identify a combination of UV dose and frequency of application that was effective for controlling cucurbit downy mildew without negatively affecting yield. In addition, we wished to evaluate the efficacy of UV dosing for mitigating downy mildew with UV-reflective mulch relative to black mulch.

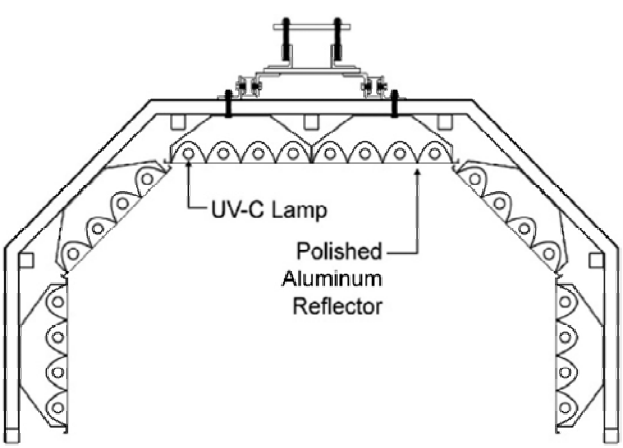

Fig. 1 : (Left) Cutaway end view of the UV-C enclosure showing placement of the fixtures. (Right) UV-C treatment attachment mounted to a tractor and shown positioned over an unplanted row.

Three levels of UV dose (120 J·m^{-2}, 240 J·m^{-2}, and 480 J·m^{-2}) were selected and each of these doses were applied once or twice weekly (i.e., on Mondays and Thursdays). All UV treatments and untreated control plots were duplicated on standard black plastic mulch and UV reflective plastic mulch, according to the field study layout in Fig. S1 (supplimentary data). Additionally, plots treated with the farm's conventional fungicide program were included in the study to benchmark the performance of UV-C only treatments. The conventional fungicide treatments for downy mildew applied during the year 1 trial are summarized in Table 2. The various dose/frequency combinations were distributed throughout the black and reflective mulch blocks such that no combination was replicated in adjacent rows. The cucumber variety Raider F1 (Harris Seed) was used for the first year's field trial, since it is not a downy mildew resistant variety and was the choice of the producer. Raider F1 does have resistance to scab, and intermediate resistance to angular leaf spot and cucumber mosaic virus.

A cooperative extension agent performed the downy mildew severity ratings by visually assessing the percentage of leaf area covered in downy mildew lesions (evidenced by yellowing, necrosis, sporulation) within the whole plot. Individual leaf inspections were then conducted on 10 leaves per plot by inspecting both the upper and lower leaf surfaces, to ensure the extension agent was looking carefully at leaf symptoms and not attributing leaf yellowing to downy mildew without evidence of sporulation.

Year 2 Field Trials

The focus of the second-year trial was to compare the downy mildew control efficacy of the best UV-C only condition from the year 1 field study, the grower's conventional fungicide program, and a combination of both treatment types. The treatments used were: (1) UV-C only (480 J·m^{-2}) twice weekly, (2) weekly conventional fungicide, (3) fungicide weekly plus UV-C twice weekly and (4) fungicide every other week plus UV-C twice weekly. The third treatment was included to determine if the addition of UV-C to the conventional weekly fungicide program would offer additional control beyond the conventional fungicide program alone. The fourth condition, added at the suggestion of the participating extension agent, was included to determine if downy mildew control could be maintained by adding UV-C treatments while reducing the amount of conventional fungicide applications.The conventional fungicide treatments for downy mildew applied during year 2 are summarized in Table 3. Each condition was replicated in two rows on both black and UV reflective mulch, for a total of four replications for each treatment. The last 4.5 m (15 feet) of one row was devoted to a control condition with no fungicide or UV-C treatment. The layout for this field study is shown in Fig. S2 (supplimentary data) The cucumber variety Raider F1 (Harris Seeds) was used again for the second year of the trial.

Table 2 : Summary of conventional fungicide applications for DM during the year 1 trial; products listed as DM / PM are labeled for treatment of both downy and powdery mildew.

Date	Product	Rate		Purpose
07/13/2020	Rampart	3.27	L·Ha^{-1}	DM
	Initiate	2.34	L·Ha^{-1}	DM/PM
07/22/2020	Curzate	0.51	L·Ha^{-1}	DM
07/29/2020	Rampart	3.27	L·Ha^{-1}	DM
08/04/2020	Omega 500F	1.32	L·Ha^{-1}	DM
08/13/2020	Rampart	3.27	L·Ha^{-1}	DM
	OxiDate 5.0	2.34	L·Ha^{-1}	DM/PM
08/09/2020	Ranman	0.18	L·Ha^{-1}	DM
08/06/2020	Omega 500F	1.32	L·Ha^{-1}	DM
09/01/2020	Rampart	3.27	L·Ha^{-1}	DM
	OxiDate 5.0	2.34	L·Ha^{-1}	DM/PM
09/08/2020	Orondis Ultra	0.58	L·Ha^{-1}	DM
	OxiDate 5.0	1.40	L·Ha^{-1}	DM/PM
09/16/2020	Ranman	0.18	L·Ha^{-1}	DM
	Rampart	3.27	L·Ha^{-1}	DM
	OxiDate 5.0	1.40	L·Ha^{-1}	DM/PM

Each row was divided into ten sections (Fig. S2) and assessments of percentage foliar downy mildew severity were made within each of the 10 sections to increase the sample size within each row. Assessments were performed visually within a square quadrat with 61 cm (24 inch) sides, placed randomly within each of the ten row sections using the same methodology used in the first year. The top and bottom sides of leaves within the quadrat were inspected to verify that the symptoms were consistent with downy mildew in the same manner as year 1. Assessments were performed by a farm staff member trained to scout

Table 3 : Summary of conventional fungicide applications made during the year 2 trial; dates marked with an asterisk (*) indicate the products listed were not applied to the plots that received conventional fungicide every other week (Products listed as DM / PM are labeled for treatment of both downy and powdery mildew)

Date	Product	Rate		Purpose
07/21/2021	Ranman	0.18	L·Ha^{-1}	DM
	Initiate 720	2.34	L·Ha^{-1}	DM/PM
07/26/2021*	Microthiol	6.73	kg·Ha^{-1}	DM/PM
	Kocide 3000	1.12	kg·Ha^{-1}	DM/PM
08/02/2021	Previcur Flex	1.40	L·Ha^{-1}	DM
08/10/2021*	Omega 500F	1.17	L·Ha^{-1}	DM
08/17/2021	Ranman	0.18	L·Ha^{-1}	DM
08/23/2021*	Previcur Flex	1.40	L·Ha^{-1}	DM
08/31/2021*	Nordox 75WG	1.23	kg·Ha^{-1}	DM/PM
09/06/2021	Tanos	0.73	L·Ha^{-1}	DM
09/13/2021*	Rampart	2.34	L·Ha^{-1}	DM
	OxiDate 5.0	2.34	L·Ha^{-1}	DM/PM

cucurbit downy mildew by cooperative extension agents.

RESULTS AND DISCUSSION

The sets of data from the year 1 and year 2 field trials were analyzed in two ways. First, the area under the disease progress stairs (AUDPS) method (Simko and Piepho, 2012) was used to provide a composite index of the relative impact of each treatment and control condition on disease progression throughout the assessment period in each year. Second, the instantaneous foliar disease severity values (in percent) from each assessment interval were compared among the treatment and control conditions and fitted with mathematical power functions to model disease progression under each condition.

The time reference for the disease progress modeling used in this study is based on an assumed date of initial infection of the cucumber plants in the test plots, based on the average duration of 4 to 12 days between the initial infection and the first observed symptoms in *P. cubensis* (Salcedo *et al.,* 2020). In the year 1 field trials, the first observations of disease occurred on August 18th, when the foliar disease severity for untreated crops ranged from 5% to 12.5%. In the year 2 trials, the initial observations of disease occurred earlier in the year, on August 3rd, when disease severity values ranged from 0.1% to 1%. During the next set of observations on August 11th, disease severity values ranged from 1.5% to 12%; similar to the initial disease observations in year 1. Since these two observations in year 2 occurred 8 days apart and since Salcedo *et al.* (2020) reported a range of 8 days during which initial disease observations could be made following infection, August 18th in year 1 and August 11th in year 2 were defined as 12 days after infection, and August 3rd in year 2 was defined as 4 days after infection.

Year 1 Field Data

Fig. 2 shows the observed foliar disease severity values for each treatment and control condition, when black mulch was used, and Fig. 3 shows the corresponding data for reflective mulch. Each point in Figs. 2 and 3 is a single observation for the once-weekly doses or the average of two observations for the twice-weekly doses. The conventional fungicide program in year 1 was only applied with the black mulch, so that condition is omitted from Fig. 3.

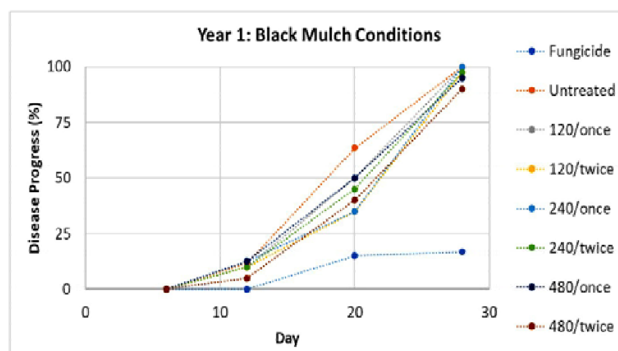

Fig. 2 : Disease progress curves for year 1 under each condition using black mulch.

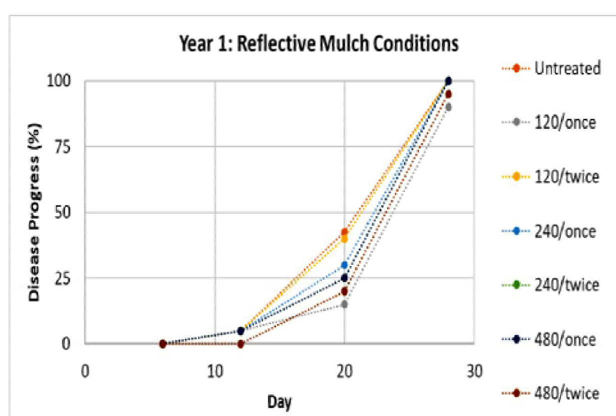

Fig. 3 : Disease progress curves for year 1 under each condition using reflective mulch.

A four-way analysis of variance (ANOVA) was performed on the foliar disease severity data comprising a balanced experimental design with the type of mulch, the UV-C dose, the dosing frequency, and the date of assessment as independent factors. The mulch type had a statistically significant effect ($F_{1,45}$=15.3, $p<0.05$) on disease severity, as did the date of assessment ($F_{5,45}$=1184, $p<0.05$). There was also a statistically significant interaction ($F_{5,45}$=8.89, $p<0.05$) between the mulch type and the date of assessment on disease severity. This can be observed from the fact that the disease severity values for the two mulch types were similar for the earliest and latest assessment dates but differed around day 20.

Qualitatively, the curves in Fig. 2 also illustrate the large difference found in year 1 between the conventional fungicide treatment conditions and the control and UV-C treatment conditions. Disease severity remained under 20% under the fungicide condition for all observation periods, whereas it approached 90%-100% for all other conditions by the last observation period. Generally, the differences among the control and UV-C treatment conditions were small, although the untreated control condition tended to have greater disease severity values than the UV-C conditions.

AUDPS values (Simko and Piepho, 2012) were calculated for each condition representing each treatment type (or control), the frequency of application (for the UV-C treatment conditions) and type of mulch. These values are shown in Fig. 4. Qualitatively, Fig. 4 shows the much lower AUDPS value for the conventional fungicide condition than for all other conditions. It can also be seen that the AUDPS values are usually (with one exception for 120 J·m^{-2} applied twice weekly) lower for the reflective than for the black mulch.

A one-way ANOVA for each treatment condition in Fig. 4 was performed showing that there were statistically significant differences among the treatment conditions ($F_{14,10}$=16.2, $p<0.05$). Tukey's post hoc tests were carried out among each treatment to identify which conditions differed from the others. It was found that the conventional fungicide treatment (with black mulch) was statistically significantly (t=5.07 to 13.2, $p<0.05$) different from all other conditions. No other conditions differed from one another after adjustment of Type I errors for multiple pairwise comparisons.

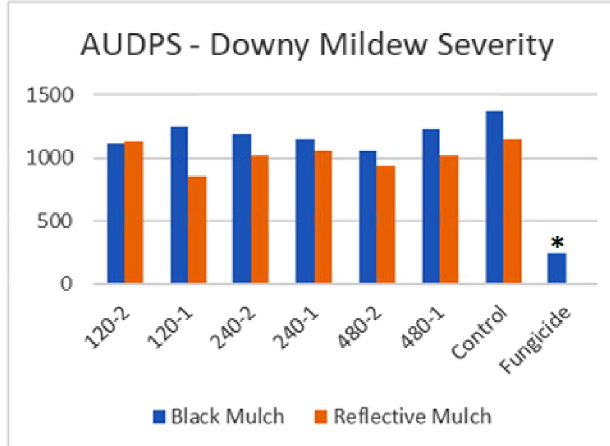

Fig. 4 : AUDPS values (Simko and Peipho, 2012) for each treatment and control condition in year 1. The asterisk (*) for the fungicide condition indicates that this condition was statistically significantly ($p<0.05$) different from all other conditions. Vertical error bars for the conditions are not shown because each value is either a single measurement or the average of two measurement values.

Considering only the UV treatment groups, the AUDPS values could be analyzed using a three-way ANOVA with the UV-C dose, the dosing frequency and the type of mulch as independent variables. This ANOVA revealed a statistically significant main effect of mulch type ($F_{1,7}$=9.80, $p<0.05$), but no other main effects nor interactions among the variables. Because the AUDPS values collapse across the date of assessment, the result of this analysis is consistent with the ANOVA on the disease severity values.

To identify whether and to what extent the treatment types affected the course of disease progression, the data in Fig. 2 and 3 were replotted in Fig.5 and 6, for black and reflective mulch respectively, using logarithmic axes for the abscissa and the ordinate. (Values of zero were omitted as they could not be plotted along a logarithmic axis.) Visual observation suggested that the data for each condition on the log-log plots in Fig. 5 and 6 fell approximately along straight lines, which are represented by power functions of the form $y = ax^b$. The best-fitting power functions to the data (excluding the conventional fungicide condition) had exponent (b) values ranging from 2.42 to 4.63, with an average of 3.19. (The exponent for the best-fitting power function to the fungicide condition was 0.31.) Assuming the disease progression was similar among the UV treatment

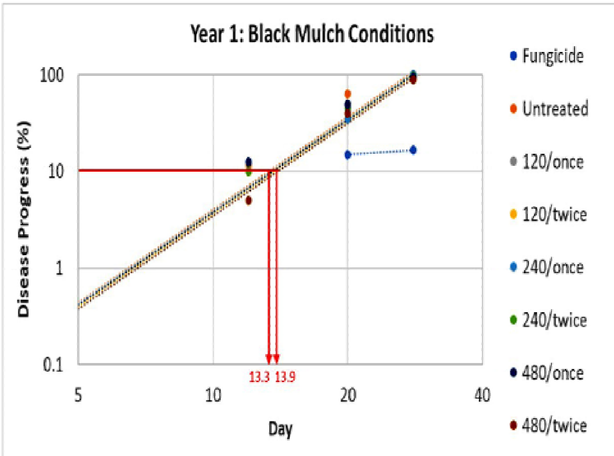

Fig. 5 : Disease progression values (non-zero only) for each condition and using black mulch. Also shown are best-fitting power functions having the form $y = ax^{3.19}$. The range of days at which disease progression reached 10% is also indicated by the red arrows.

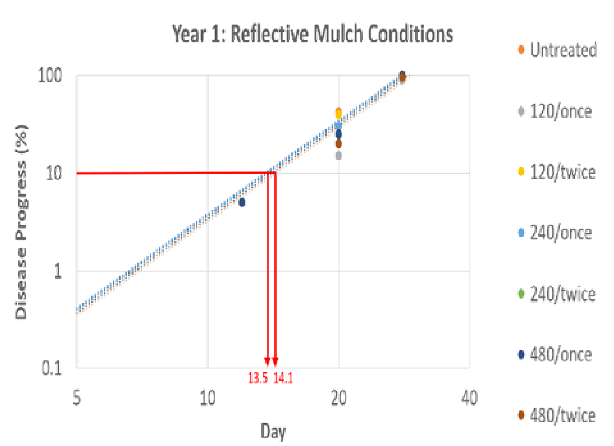

Fig. 6 : Disease progression values (non-zero only) for each condition and using reflective mulch. Also shown are best-fitting power functions having the form $y = ax^{3.19}$. The range of days at which disease progression reached 10% is also indicated by the red arrows.

conditions, a fixed exponent value of 3.19 was used and best-fitting power functions to each set of data were determined having the form: $y = ax^{3.19}$, and these are also shown in Fig. 5 and 6. Goodness of fit (r^2) values for each function ranged from 0.88 to 0.998.

These modeled power functions are nearly coincident with each other, suggesting that disease progressions for the control and for all UV-C treatment conditions were essentially the same. Even the sets of curves for each type of mulch differed very little from each other, despite the statistically significant effect of mulch type in the three-way ANOVA. Indeed, taking an arbitrary disease progression value of 10% to represent a threshold for disease in these conditions, less than a single day separates the time after initial infection at which this observable threshold would be met between the control and all UV-C treatment conditions (Fig. 5 and 6).

A limitation of all analyses from year 1 is the small sample size. Only a single observation, or sometimes two observations, were made for the control and treatment conditions in year 1 and this may have limited the ability to achieve statistical significance among those conditions. With or without statistical significance, however, the UV-C applications employed in year 1 were not much of an improvement over the control condition for mitigating DM disease progression.

Year 2 Field Data

As mentioned previously, subsequent field trials in year 2 were carried out to validate the year 1 findings using what would be expected to be the most effective UV-C treatment, 480 $J{\cdot}m^{-2}$ applied twice weekly. Although the 120 $J{\cdot}m^{-2}$ dose applied once weekly (with reflective mulch) was empirically the most effective treatment, the same treatment was not as effective with black mulch, and collapsing across mulch type, 480 $J{\cdot}m^{-2}$ had slightly (albeit not statistically significantly) higher effectiveness than the other doses, and application frequency of twice weekly was slightly more effective than once weekly. Combinations of fungicide (using the producer's usual weekly application schedule or a reduced application frequency of every other week [EOW]) and UV-C treatments were included in year 2 to identify whether UV-C could enhance the effectiveness of fungicide or permit fewer fungicides to be used while providing protection against downy mildew disease. As also stated previously, multiple sections of each treatment row were evaluated for disease to increase sample sizes and statistical power.

Fig. 7 and 8 show the progression of disease for each of the control and/or treatment conditions as a function of time (day after assumed infection as described previously). There are two primary qualitative differences between the data in these figures for year 2 and the corresponding data in Fig. 2 and 3 for year 1. First, there appears to be a greater separation among the conditions in terms of the days that the disease begins to take hold in the plants, especially between the untreated control condition (which

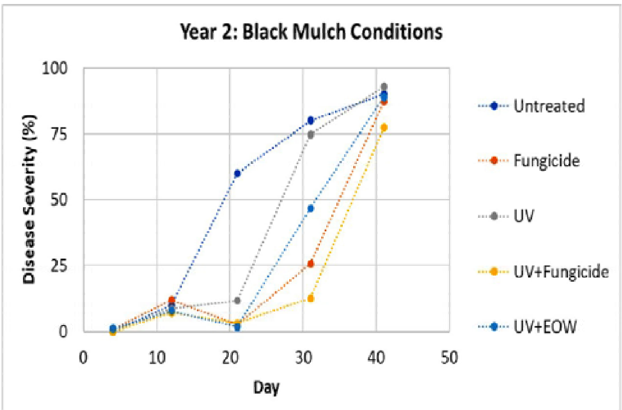

Fig. 7 : Foliar disease progression curves for year 2 under each condition using black mulch.

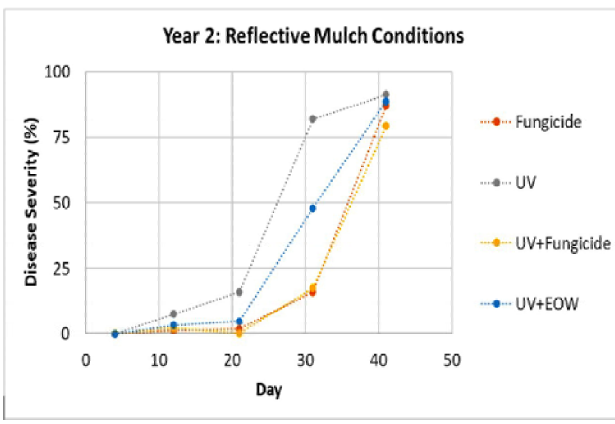

Fig. 8 : Foliar disease progression curves for year 2 under each condition using reflective mulch.

exhibited greater than 50% foliar disease severity by day 21, and the other conditions which exhibited less than 20% disease severity on the same day. Second, the disease severity for the fungicide treatment conditions approached 80% by the end of data collection where as in year 1, disease severity was held to less than 20% with the application of fungicide. (Possibly, disease severity in year 1 for the fungicide treatment condition would have eventually increased to nearly 100%.)

For the four treatment conditions (i.e., fungicide, UV, UV plus fungicide, and UV plus EOW fungicide) for which both types of mulch were used, a three-way ANOVA was performed on the disease severity values, with treatment, mulch type and date of assessment as independent factors. The section number of each row was included in the analysis as a covariate factor to identify whether there were any systematic differences within each row; there were not. The treatment ($F_{3,761}$=118, p<0.05) and the date of assessment ($F_{4,761}$=1958, p<0.05) had statistically significant main effects on disease severity, and there was also a statistically significant interaction between treatment and assessment date ($F_{12,761}$=52.5, p<0.05). This can be observed in Fig. 7 and 8 where the disease severity was similar across all treatments for the first and last treatment dates, with the most variation among treatments for the intermediate dates. Unlike year 1, the type of mulch did not have a statistically significant ($F_{1,761}$=0.98, p>0.05) effect on disease progression.

Mean AUDPS values (Simko and Piepho, 2012) for each treatment and mulch condition were calculated and are shown in Fig. 9. A one-way ANOVA was performed to assess differences among the conditions, which were statistically significant ($F_{8,149}$=45.2, p<0.05), with Tukey's tests to assess pairwise comparisons while controlling for Type I errors (Supplementary data Table S1). In general, there were no significant differences (p>0.05) in AUDPS between mulch types for the same condition. All conditions except for the UV-only conditions differed significantly (p<0.05) from the untreated control condition (which only used black mulch). The combination of fungicide and UV-C treatment with the black mulch was statistically significantly different (p<0.05) from the fungicide-only treatment with the same mulch type, suggesting a small impact of UV-C treatment in conjunction with fungicide.

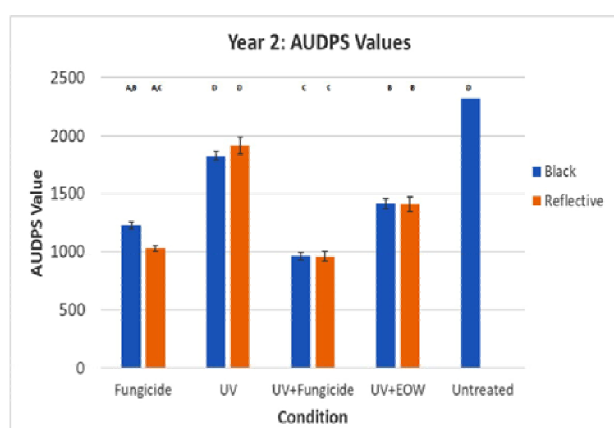

Fig. 9 : AUDPS values (Simko and Piepho, 2012) for each treatment (UV: ultraviolet; EOW: every-other-week fungicide application) and mulch condition in year 2. Letters above each bar indicate non-statistically significant differences among conditions with a common letter.

Excluding the untreated control condition, a two-way ANOVA was performed to assess how the treatment condition and mulch type, and the interaction between them, affected AUDPS. Section from 1 to 10, was included in this analysis as a covariate to identify whether there were any systematic differences across each of the treatment rows; there were not. There was a statistically significant ($F_{3,149}$=110, p<0.05) main effect of treatment, but the mulch type did not exhibit a statistically significant main effect (p>0.05). There was a significant interaction ($F_{3,149}$=2.72, p<0.05) between treatment condition and mulch type on AUDPS; this is seen in Fig. 9 where the black mulch resulted in somewhat higher AUDPS for the fungicide treatment condition, but lower for the UV-only treatment. Aside from the two-way interaction between the treatment and mulch type, this analysis of the AUDPS values was consistent with the ANOVA on the disease severity values in identifying significant differences among the treatments but not between the two types of mulch in year 2.

Using the same analytical procedure as for the year 1 data, the year 2 data for each type of mulch were plotted on log-log axes (excluding zero values) and are shown in Fig. 10 and 11. Similar to data from year 1, these data also seem to fall along straight lines. Using the average exponent value (b=3.19) from the year 1 data, best-fitting power functions of the form $y = ax^{3.19}$ were determined for each condition and these functions are also plotted in Fig. 10 and 11. Goodness of fit (r^2) values for the best-fitting functions ranged from 0.70 to 0.97 with the exception of the untreated (with black mulch) condition, which exhibited a somewhat different shape of its disease progression curve compared to the treatment conditions, as illustrated in Fig. 7 and 8. The goodness of fit value for the untreated data was 0.024.

In general, there are two observations from these figures in comparison to Fig. 5 and 6, which show the corresponding model functions for year 1. First, there was no obvious plateauing effect for the fungicide conditions in year 2 like there seemed to be in year 1. Disease progression for the fungicide conditions in year 2 seemed to follow a similar progression overall as all other conditions, including the untreated control (albeit delayed somewhat). Second, there is somewhat more dispersion among the modeled power functions for year 2 than there was in year 1. For example, using 10% disease severity as a threshold for observable disease (Fig. 10 and 11), the difference in reaching this criterion between the worst (untreated control) and best (UV+fungicide) conditions were 3.8 days for the black mulch, compared to less than 1 day among all non-fungicide conditions in year 1 (Fig. 5 and 6). Depending upon exactly when during the disease progression that the data were collected, the fitted power functions in these figures may have reflected ranges of days closer to the beginning (for year 1) or end (for year 2) of time when disease symptoms were progressing. These differences, as well as the limited sample sizes underlying Fig. 5 and 6, might explain the lack of difference among the fitted curves for year 1.

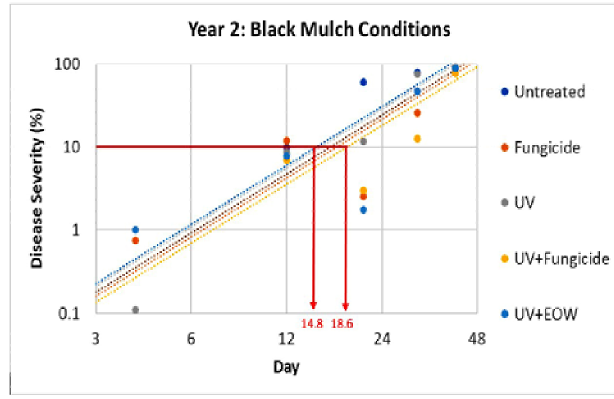

Fig. 10 : Disease severity values (non-zero only) for each condition using black mulch, in year 2. Also shown are best-fitting power functions having the form $y = ax^{3.19}$. The range of days at which disease progression reached 10% is also indicated by the red arrows.

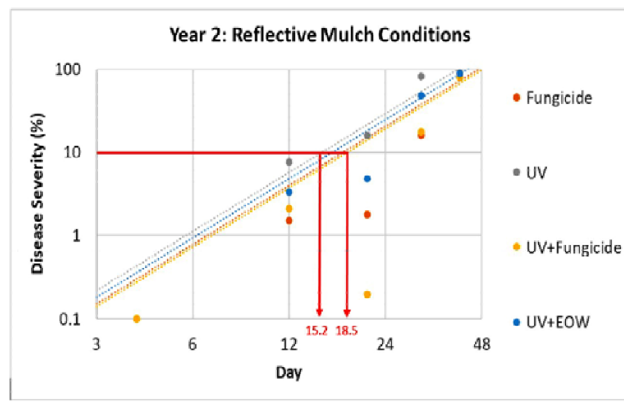

Fig. 11 : Disease severity values (non-zero only) for each condition using reflective mulch, in year 2. Also shown are best-fitting power functions having the form $y = ax^{3.19}$. The range of days at which disease progression reached 10% is also indicated by the red arrows.

The results for the field studies in each of years 1 and 2 exhibited some consistencies and some differences. Overall, the results suggest that, for the doses examined (up to 480 J·m^{-2}), UV by itself is not an effective treatment for downy mildew (*P. cubensis*) control in cucumbers in the field, especially in comparison to the fungicide regimens used during this study. Nor were there any obvious monotonic trends in year 1 for the 120 to 480 J·m^{-2} doses as one might expect if the lowest dose were consistently worse than the highest dose. Possibly, higher UV doses (e.g., 1000 J·m^{-2}) might have been more effective at reducing the severity of disease. However, higher doses than those used in the present study might be damaging to plants, given the reductions in cucumber leaf area observed by Patel *et al.* (2020) in laboratory studies caused by a UV-C dose from an electric light source of only 70 J·m^{-2}. It would also have been challenging to deliver larger doses with the present apparatus without making time-consuming multiple tractor passes over the crops, another practical limitation.

Nonetheless, the application of UV in conjunction with fungicide treatment did show a statistically significantly lower level of disease severity for the black mulch conditions, corresponding to reduced progression of downy mildew (although a significant difference was not observed with reflective mulch). It may be possible to reduce fungicide treatment in conjunction with UV treatment and achieve a level of disease control that is consistent with current conventional practices for fungicide application, but identifying the dosing required to do so is not possible from the present data. In addition, fungicidal products exist that reduce concentrations of melanin pigment in the treated fungi such as tolprocarb (Hamada *et al.,* 2014). If the presence of pigments is a factor in the modest effectiveness of UV found in this study, it is possible to speculate that a combination of melanin-reducing fungicides plus UV might be more effective than the combination of UV and fungicides used in the present study.

Given the parallel disease severity progression curves in Fig. 5, 6, 10 and 11, it would appear that none of the treatments investigated in this study altered the course of disease progression once it was established (shown by the slopes of the curves), but rather that they sometimes delayed it (shown by the horizontal offsets among the curves). This is illustrated by the reasonably good fits of the disease severity progression data to power functions having the same exponent of 3.19. With respect to the UV-C treatments investigated in this study, the observed delays in onset of downy mildew may be attributed to increased resistance to downy mildew induced by the treatments prior to infection (Bonomelli *et al.,* 2004). Even if this could be substantiated in further experiments, however, the observed effect was relatively small.

Yield was not assessed precisely in the first- or second-year trials. However, observations of yield by the grower revealed that none of the treatments (UV at any dose, fungicide, or combination thereof) resulted in an observable reduction in yield relative to untreated crops. The lack of yield reduction suggests that the UV doses applied were below levels would result in significant phytotoxicity in cucumber plants, even though lower doses of 70 J m^{-2} could result in visible leaf damage under laboratory conditions (Patel *et al.,* 2020). It may be possible to increase UV dose to better control *P. cubensis* while still maintaining satisfactory yield, but it seems clear that the thresholds for yield-reducing damage to cucumber plants by UV are not well defined. This is important, however, because as described above, pigmented fungal spores are more resistant to damage from UV than unpigmented spores (Rotem *et al.,* 1985), and *P. cubensis* spores contain melanin pigment as they mature (Lee *et al.,* 2021). Identifying an upper limit for UV doses that do not damage the cucumber plants would be a useful next step in maximizing the potential beneficial impacts of UV-C treatment for downy mildew control. Such investigations should include precise field assessment of crop yields as well as further laboratory studies to identify optimal dosing parameters.

There were two main areas of inconsistency in the field test results between years 1 and 2. First, the disease severity for the fungicide treatment condition in year 1 did not exceed 17% whereas disease severity in year 2 for the fungicide treatment condition exceeded 85%. However, it should be noted that disease assessment was carried out for a greater number of days past the assumed infection date in year 2 (41 days) than in year 1 (28 days). Indeed, on day 31 in year 2, the fungicide treatment conditions had only exhibited 16%-26% disease severity, not much higher than the 15%-17% exhibited on day 28 in year 1.

The second main inconsistency between the results for years 1 and 2 was the impact of mulch types. In year 1, there were larger and more consistent reductions (or delays) in disease progression with the reflective mulch than in year 2. One possible post hoc explanation for this comes from the locations of the fields where the year 1 and 2 trials occurred. In year 1, the test field was in an open area with greater exposure to sunlight, and in year 2, the field was partially shaded by nearby trees. Although the reflective mulch had a much higher UV-C reflectance (66% at 254 nm) than the black mulch (5% at 254 nm), which would be expected to help increase the UV treatment efficacy, this did not seem to be the case in year 2. As one might expect, the reflective mulch also had a substantially higher visible reflectance (75% at 436 nm) than the black mulch (1% at 436 nm), and this could have resulted in soil temperatures being substantially higher with the black mulch because of much higher sunlight absorption compared to the reflective mulch. While not the primary focus of the present study, this suggests that if higher soil temperatures lead to decreased resistance to *P. cubensis*, reflective mulch may have some benefit because of its solar reflectivity.

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