Received: 5 March 2024 | Revised: 10 September 2024 | Accepted: 12 September 2024

DOI: 10.1002/ppp3.10586

Plants People Planet PPP Open Access

RESEARCH ARTICLE

Exposure dose, light distribution and wavelength affect the fate of introduced bacterial biological control agents in the phyllosphere of greenhouse grown tomato

Maria Hellström | Maria E. Karlsson | Isabella Kleman | Anna Karin Rosberg | Julia Darlison | Emina Mulaosmanovic | Lena Will | Beatrix W. Alsanius

Microbial Horticulture Group, Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Lomma, Sweden

Correspondence
Maria Hellström, Microbial Horticulture Group, Department of Biosystems and Technology, Swedish University of Agricultural Sciences, PO Box 190, SE-23422 Lomma, Sweden.
Email: maria.hellstrom@slu.se

Funding information
Kungl. Skogs- och Lantbruksakademien, Grant/Award Number: HORT2022-0010; Svenska Forskningsrådet Formas, Grant/Award Number: R-18-25-006; Stiftelsen Lantbruksforskning

Societal Impact Statement

The use of chemical plant protection products must be reduced to promote sustainability in food production. One possible alternative is biological control agents (BCAs), but their efficacy under commercial conditions does not always reach the standard of chemical control agents. Previously, light has been found to induce mechanisms in bacterial BCAs that can affect their distribution and establishment. This could promote BCA efficacy. We looked into how monochromatic and polychromatic (which is what growers use) light treatments affected the occurrence of three BCAs post-application. By combining two non-chemical methods: a biological (BCA) and a cultural (light) control method, this offers a new integrated pest control strategy.

Summary

- The dynamics and functionality of beneficial and non-beneficial, non-phototrophic bacteria can be influenced by light quality. We investigated if light could aid the survival of three bacterial biological control agents (BCAs; *Bacillus amyloliquefaciens* DSM7, *Pseudomonas chlororaphis* 50083 and *Streptomyces griseoviridis* CBS904.68) in the canopy of greenhouse-grown tomatoes at four light treatments.
- Tomato plants were exposed to 50 µmol m^{-2} s^{-1} of either polychromatic light (white) or monochromatic light (blue: 420 nm, green: 530 nm and red: 660 nm) using DYNA LED lamps for a total of 48 h post foliar application of the BCAs. Leaves were harvested from two levels in the canopy at the top and middle of each plant at 0, 2, 4, 8, 12, 24 and 48 h post inoculation. The occurrences of the BCAs were quantified by plate count and droplet digital PCR (ddPCR).
- *S. griseoviridis* persisted under most treatments, whereas *P. chlororaphis* and *B. amyloliquefaciens* preferred the polychromatic and green light treatments as depicted by the viable count analyses. Significant differences between the DNA and cDNA concentrations were only noted for *P. chlororaphis*, with prominent wavelength effects.

- Light exposure dose, placement in the canopy and wavelength were found to be decisive factors for BCA re-isolation, indicating that they have different optimal light environments.

KEYWORDS
Bacillus amyloliquefaciens, ddPCR, greenhouse production, light emitting diode (LED), *Pseudomonas chlororaphis*, *Streptomyces griseoviridis*, viable count

1 | INTRODUCTION

Due to an increased demand for both in-season and off-season products (Baskins et al., 2019), a higher proportion of the food production has shifted to controlled environment agriculture (CEA) as higher yields are possible. The shift has led to, among others, the need for alternative plant protection measures, as growers in Northern Europe, for example, have fewer chemical alternatives to cope with foliar diseases in greenhouse crops with continuous harvest. Microbial biological control agents (BCAs) could be a key alternative being more sustainable and could aid in decreasing the development of resistant pathogens, given that challenges regarding consistent establishment and expression of biocontrol mechanisms can be resolved. One of the greatest hurdles a microorganism needs to overcome within the phyllosphere of a crop is the topography of the leaf itself (Andrews, 1992). The leaf is not as homogenous as one might expect (Remus-Emsermann & Vorholt, 2014).

An array of microhabitats occur within one leaf (Leveau & Lindow, 2001), which in turn affect the survival of introduced BCAs. After a life in a fermenter and a storage box, the BCA must adapt to the fluctuations in cyclic and noncyclic environmental variables such as temperature, irradiation and relative humidity once introduced to the phyllosphere (Andrews, 1992; Remus-Emsermann & Vorholt, 2014). The introduced microorganisms need to successfully compete for space and resources within the already established microbial aggregates on the leaf surface (Schlechter et al., 2023). This in turn affects not only their survival but also their metabolic activity. An in vitro study by Carlström et al. (2019) indicated that the removal or late addition of certain strains did affect the community structure to varying degrees with prominent priority effects. This conveyed the complexity of intra- and inter-kingdom relationships of leaf microbiota in a synthetic microbiota study in the *Arabidopsis* phyllosphere.

The use of complementary lighting in CEA in Northern climates is essential as available natural light is highly dependent on the season (Modarelli et al., 2022). Different wavelengths can steer plant architecture, such as plant length, leaf size and thickness (Fan et al., 2013; Zheng & Van Labeke, 2017). Plant canopies found in greenhouse settings tend to be denser and more compact, resulting in sharper angles of light infiltration (Slattery et al., 2018). This leads to extreme differences in irradiance intensities between the upper canopy layers when compared with the lower canopy foliage, due to a shading effect (Cutolo et al., 2023).

Light energy can modulate major aspects of the physiology of an organism (Canessa et al., 2013). The metabolism of non-phototrophic bacteria can be affected by light quality, as they are equipped with photosensory proteins (Alsanius et al., 2019; Beattie et al., 2018; Gharaie et al., 2017; Losi & Gärtner, 2021). Several distinct types of photosensory proteins, such as cryptochromes, phototropins, microbial rhodopsins and bacterial phytochromes, have been described within plant-associated bacteria and bacterial pathogens (Alsanius et al., 2021; Hatfield et al., 2023; Imada et al., 2014; Wilde & Mullineaux, 2017; Yu & Lee, 2013). Their photosensory proteins have been found to regulate the shift between a sessile and planktonic lifestyle in microbial biofilm formation due to phototaxis (Gomelsky & Hoff, 2011; Hoff et al., 2009; Purcell & Crosson, 2008). Light quality has also been shown to cause both positive and negative changes in cell motility (Wu et al., 2013). Foliar pathogens such as powdery mildew and grey mould have exhibited phenotypic responses by being suppressed by different light treatments (Canessa et al., 2013; Suthaparan et al., 2014).

The objective of this study was to investigate the integration of BCAs in the phyllosphere of greenhouse tomatoes, aiming to enhance their effectiveness. We hypothesise that the selection of light quality (wavelength), exposure dose and placement within the canopy significantly influences the occurrence and viability of BCAs. By investigating using a novel in vivo greenhouse experiment and sampling methods, we studied how light quality and intensity, in combination with the exposure length, affect the introduction of BCAs in the phyllosphere of greenhouse-grown tomatoes.

2 | MATERIALS AND METHODS

2.1 | Experimental design

We employed a four-factorial experimental design, with factor 1: BCAs, factor 2: light quality, factor 3: exposure length and factor 4: position in the plant canopy (Figure 1).

Three biological control agents were used, namely, *B. amyloliquefaciens* DSM7, *P. chlororaphis* 50083 purchased from DSMZ (Leibniz Institute, Braunschweig, Germany) and *S. griseoviridis* CBS904.68 purchased from Centraalbuureau voor Schimmelcultures, Utrecht, Netherlands. All strains were equipped with spontaneous antibiotic resistances to allow for specific re-isolation (*B. amyloliquefaciens*: streptomycin 100 μg mL^{-1}, *P. chlororaphis* and *S. griseoviridis*: ampicillin 100 μg mL^{-1}). The spontaneous antibiotic

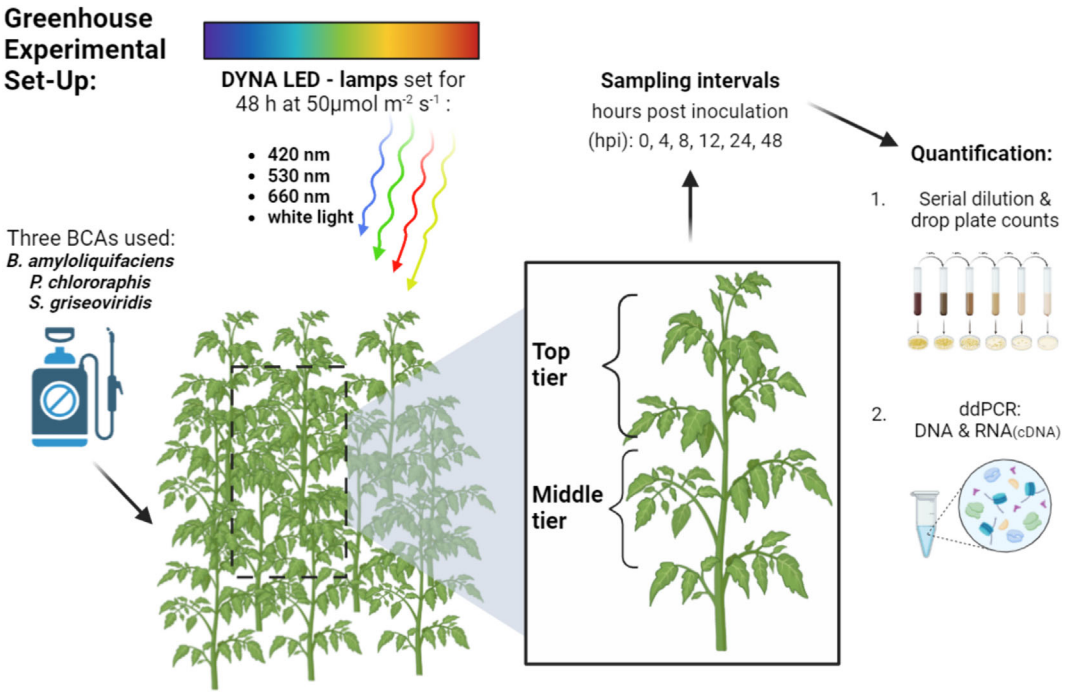

FIGURE 1 A schematic overview of the greenhouse experimental set-up where three biological control agents (BCAs) were sprayed onto tomato plants that were exposed to different light treatments for 48 h. Leaves were harvested from two placements (top and middle tier) in the canopy at 0, 4, 8, 12, 24 and 48 h post inoculation and quantified by plate counts and digital droplet PCR (ddPCR) (illustration: M. Hellström, supported by Biorender.com).

resistance was induced by preculturing the BCAs on agar mixtures containing lower dosages of the respective antibiotics over time.

Four light regimes were chosen using mono- (blue: 420 nm; green: 530 nm; red: 660 nm) and polychromatic (white) LED lamps (DYNA LED, Heliospectra, Gothenburg, Sweden). The spectral distribution is depicted in Figure 2, and lamp specificities can be found in Figure S1 and Table S1.

The total exposure length stretched over 48 h, and samples were taken directly after the onset of the experiment and after 4, 8, 12, 24 and 48 h. The occurrence of the biocontrol strains was monitored in the basal (middle tier) and apical (top tier) parts of the tomato plants.

2.2 | Biological control strain preparation

B. amyloliquefaciens was pre-cultured on full-strength tryptic soy agar (TSA; BD 236950, Becton, Dickinson & Company Sparks, USA) for 48 h before transferring one colony to 6 mL of tryptic soy broth (TSB; BD 211825, Becton, Dickinson & Company Sparks, USA). In parallel, *P. chlororaphis* and *S. griseoviridis* were pre-cultured on full-strength TSA for 24 h prior to transferring one colony to 6 mL of TSB. Cells of *P. chlororaphis* and *S. griseoviridis* were grown for 24 h at 25°C on a rotary shaker (200 rpm). *B. amyloliquefaciens* cells were grown for 48 h at 25°C with no shaker. The cells were washed twice by repeated centrifugation (3200 ×*g*, 10 min, 4°C) and re-suspended in 0.85% NaCl to a density of $OD_{620} = 1$. A tenfold dilution was prepared from the previous re-suspension in 0.085% NaCl for each BCA that was used as the inoculum. A second dilution occurred with the same ratio, and a total of 3 L were prepared for each organism (*P. chlororaphis* and *S. griseoviridis*: average log 7.8 CFU mL^{-1}, *B. amyloliquifaciens*: average log 4.3 CFU mL^{-1}). The average absolute quantification of DNA and cDNA copies of the undiluted and sprayer content can be found in Table S2.

2.3 | Plant propagation

Tomato plants (*Solanum lycopersicum* L.) 'Cappricia RZ F1' (Rijk Zwaan Distribution B.V., Netherlands) were grown under greenhouse conditions. The temperature was set at 22°C ± 2, with ventilation onset at 25°C; relative humidity of 60%; at a density of one plant per pot (3.375 L) growing medium (K-jord, Hasselfors Garden, Sweden). Five grams of fertiliser were added per litre of growing medium (Basacote® Plus 3M 16-8-12(+2+TE), Compo Expert, Germany). The plants were exposed to a photoperiod of 14 h under High Pressure Sodium lamps (HPS-lamps, Philips Greenpower 400 W, Philips, Eindhoven, The Netherlands). The plants were irrigated upon depletion.

2.4 | Light experiment

Sixty-five-day-old tomato plants, which had reached the phenological stage 6 according to the BBCH scale (Feller et al., 2001), were placed

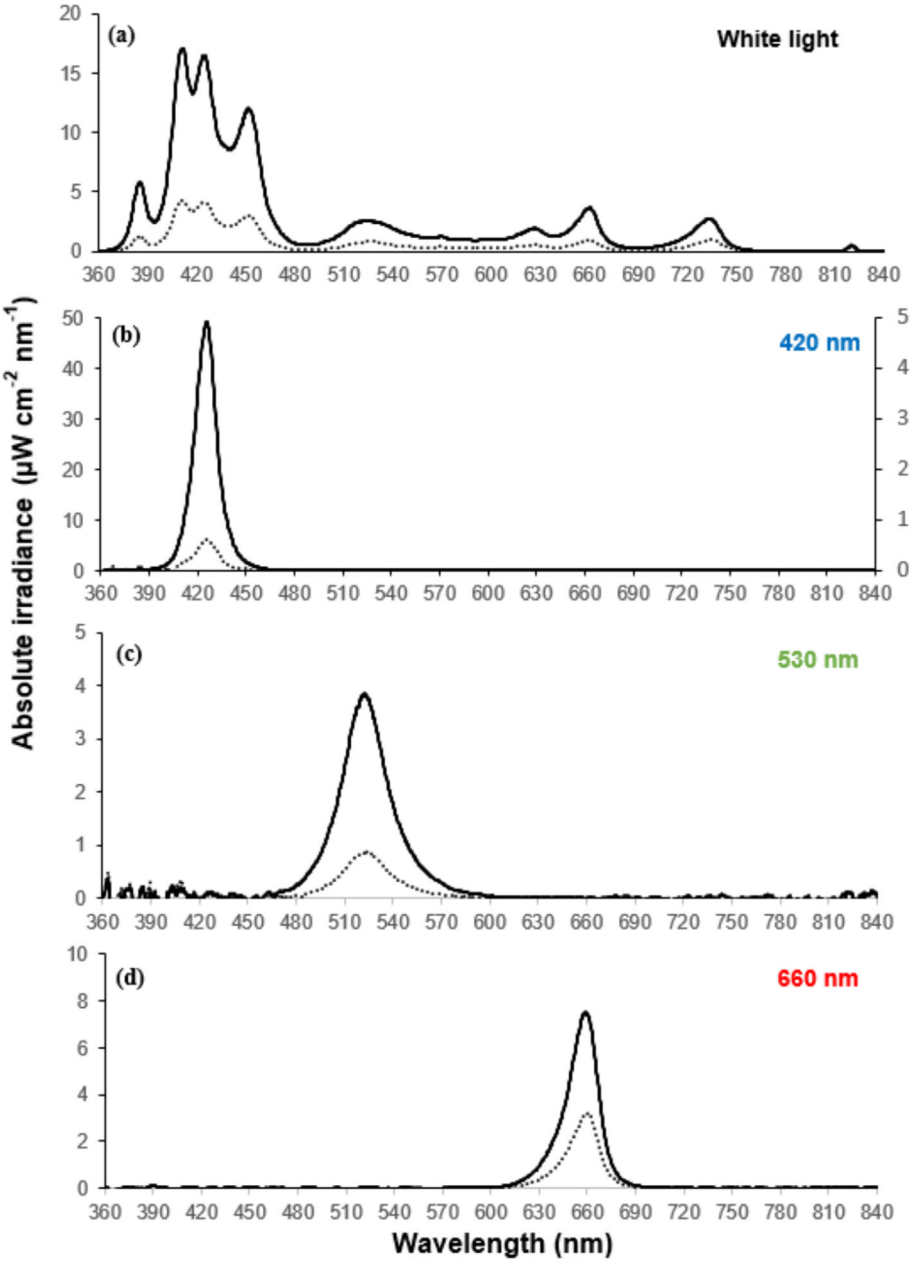

FIGURE 2 Spectral distribution of the four light treatments (DYNA LED lamps) used in the greenhouse experiments. Depicted is the absolute irradiance for both the top tier (solid line) and the middle tier (broken line) of the tomato canopy, (a) white light; (b) blue: 420 nm (note: varying y-axis); (c) green: 530 nm; and (d) red: 660 nm. The secondary y-axis in subfigure b, considers the middle tier, this as the peak was dismal when the primary y-axis was used to initially depict the data.

in rectangular formations on the greenhouse floor at a density of approximately 20 plants m^{-2}. Four rectangular formations occurred per light treatment, as one BCA was sprayed per square. This was done in the form of non-inoculated *P. chlororaphis*, *B. amyloliquifaciens* and *S. griseoviridis*. Edge plants surrounded both around and in between all treated and non-inoculated plants throughout the experiment. The BCAs were individually sprayed using 5 L compression sprayers (GLORIA, Haus- und Gartengeräte GmbH, Witten, Germany), until runoff from leaves was observed as described in Wilson and Lindow (1992). Plants were left to dry for 10 min before any light treatment. For the continuous 48-h light treatments, post-bacterial inoculation, plants were exposed to mono- and polychromatic light (420, 530 and 660 nm; white LED) using DYNA LED lamps (10.5–390 W) (Heliospectra AB, Sweden, intensity: 50 µmol m^{-2} s^{-1} at the top of the plant canopy). Blackout screens omitted all external light throughout the LED experiments, post-inoculation. The relative spectral irradiance distribution for each light treatment was measured as was using a JAZ spectrometer (Ocean Optics, USA) (Figures 2 and S1).

2.5 | Analyses

2.5.1 | Plant analyses

After a 24-h exposure to LED light, six untreated control plants were used to measure: chlorophyll content (PAM-2500 chlorophyll fluorometer, Heinz Walz GmbH, Effeltrich, Germany), leaf angle (Angle

Meter 360, Alexey Kozlov), stomatal conductance and photosystem efficiency (L.MAN-LCpro, ADC BioScientific Ltd., United Kingdom), all recorded using non-destructive methods (Table S3). Destructive methods were used to quantify the leaf surface area (cm^2) of the two tiers within each control plant (LI-3100C, LI-COR Biosciences, Nebraska, USA). The number of leaves, height and width of the plant (cm) were also noted (Table S4). Dry weight (g) was measured after drying the leaves at 80°C for 7 days.

2.5.2 | Viable count of BCAs post light treatment

Leaf samples were harvested after 0, 2, 4, 8, 12, 24 and 48 hpi at two placements within the crop canopy: top (apical) and middle (basal) tier. Six plants were harvested per organism and time point. Leaves were weighed and macerated (Smasher; bioMérieux, Inc., Durham, USA) for 30 s at normal speed in 50 mL of 0.1 M TRIS buffer using sterile plastic bags fitted with a filter (Separator 400, 180 mm*300 mm*70 µm; Grade Products Ltd., Coalville, UK). Samples for DNA and RNA extraction were taken after each maceration by adding 600 µL DNA/RNA Shield™ (R1100-50, Zymo Research, USA) to 200 µL of the sample in a cryotube for later analysis. The remainder of the samples were serially diluted in 0.85% NaCl, drop-plated on full-strength TSA supplemented with the respective antibiotic compound to allow for selective re-isolation. Plates were incubated at 25°C for 24 h, *P. chlororaphis*, *S. griseoviridis*, and 48 h, *B. amyloliquefaciens* before being enumerated as log colony forming units g^{-1} (log CFU + 1 g^{-1}).

2.5.3 | DNA and RNA extraction

Six replicates for each light treatment and position in the canopy collected at 4, 12 and 48 hpi were extracted using ZymoBIOMICS™ DNA and RNA Kit (Zymo Research, USA). The standard protocol provided by the manufacturer was used, only deviating by proceeding to Step 2 in the sample preparation as the samples were placed in a DNA/RNA shield at the time of harvest and processed for 10 min at full speed before continuing with the DNA and RNA purification step. A total of 700 µL per sample was used for Step 1 of the DNA and RNA purification step, and 50 µL of the DNase/RNase-free water was added instead of 100 µL at Step 6.

2.5.4 | Droplet digital PCR (ddPCR)

DNA and cDNA were used to quantify *B. amyloliquefaciens*, *P. chlororaphis* and *S. griseoviridis* using an automated QX200TM Droplet DigitalTM PCR system (Bio-Rad, USA). The cDNA samples were prepared from extracted RNA samples using the iScript cDNA Synthesis Kits, according to the manufacturer's instructions (Bio-Rad, USA). A reaction mixture was prepared composed of 10 µL of QX200 EvaGreen Digital PCR Supermix, 0.5 µL each of forward and reversed species-specific primers (Table 1), 4 µL of DNase/RNase free MilliQ water and 5 µL of DNA or cDNA sample, leading to 20 µL in total. Samples were put into the automated droplet generator (Bio-Rad, USA). The plate containing droplets was sealed with pierceable aluminium foil using a PX1 PCR plate sealer (Bio-Rad, USA) set to 180°C for 5 s. The PCR ran with the following thermal conditions (Touch Thermal Cycler, Bio-Rad, USA): enzyme activation at 95°C for 5 min followed by 40 cycles of denaturation at 95°C for 30 s, annealing and extension for 1 min with the temperature specific for the primer used. The procedure was finalised by signal stabilisation at 4°C for 5 min and 90°C for 5 min and infinite hold at 4°C. After thermal cycling, the plate was added into a QX200™ droplet reader (Bio-Rad, USA) for reading. QuantaSoftTM software was used to analyse the data. Six replicates per tier and light treatment were quantified per organism and time point.

2.6 | Calculations and statistical analyses

Viable count results were log transformed prior to statistical analysis. Based on the correlation between leaf area and weight, all values were converted to log CFU + 1 cm^{-2}. Exposure dose calculations were based on formulas described by Alsanius et al. (2024) (Table S5).

All statistical analyses were conducted using RStudio (R Core Team, 2021), and figures were compiled using packages 'circlize' (Gu et al., 2014) and 'ggplot2' (Wickham, 2016). A linear mixed model approach, LMM, 'lmer4' (Bates et al., 2015), was used with a random factor, plant_id, set to compensate for the two measurements that occurred per one plant. A four-way ANOVA was used to analyse the data, with the average log CFU + 1 cm^{-2} leaf area set as a dependent variable and placement, light treatment, BCAs and time of harvesting were set as independent variables. Similarly, linear mixed models and ANOVAs were used to determine if placement had an effect

TABLE 1 Primer sequences used based on 16S rRNA to quantify the selected biological control agents (BCAs).

Primer	Sequence (5′-3′)	Target species	Annealing temp. (°C)	Source
Ba_F	CTGGACGTCGCAAAAGGCATTA	*B. amyloliquefaciens*	56	Current study, modified from Wattiau et al. (2001)
Ba_R	TTCTGCCGCATGCTCCAGA			
PC_F	CCCACCGACAGCCAGCAACG	*P. chlororaphis*	63	Garrido-Sanz et al. (2017)
PC_R	CGGTCTTGTCGCTGATGCCG			
STR_ACT	CGCGGCCTATCAGCTTGTTG	*S. griseoviridis*	61	Al_husnan and Alkahtani (2016)
STR_ACT	CCGTACTCCCCAGGCGG			

irrespective of sampling time. LMMs and ANOVAs were used to analyse ddPCR data for each organism. For the initial analysis, the copies μl^{-1} cm^{-2} of either DNA or cDNA were set as dependent variables, and the independent variables were the organism, placement within the canopy, light treatment and time of harvesting.

3 | RESULTS

3.1 | Re-isolation of the BCAs

The plate counts of the three re-isolated BCAs from the leaves of greenhouse-grown tomatoes differed greatly when exposed to the four light treatments. *S. griseoviridis* had the highest total average log CFU + 1 cm^{-2} tomato leaves from both tiers under all light treatments, contrasting to *B. amyloliquefaciens* total under the 420 and 660 nm treatments, as no colonies were recovered after 4 hpi (Figure 3). For both *P. chlororaphis* and *S. griseoviridis*, a large dip was noted between 0 and 4 hpi under the 660 nm treatment but continued at a steady rate thereafter. In general, samples collected from the top tier had higher viable counts, and white light samples for all three BCAs were highest irrespective of placement.

Statistical differences occurred for all three BCAs when compared solely to time of harvest (hour), placement in the canopy and wavelength exposed, as indicated by their high probabilities (* < .05, ** < .01 and *** < 0.001) (Table 2). Similarly, the interaction between hour and wavelength and BCA's resulted in significant differences (Table S7). Sampling time and placement did have an effect on the average log CFU + 1 cm^{-2} of *P. chlororaphis*. A canopy effect was noted for all three BCA. However, a preference for placement,

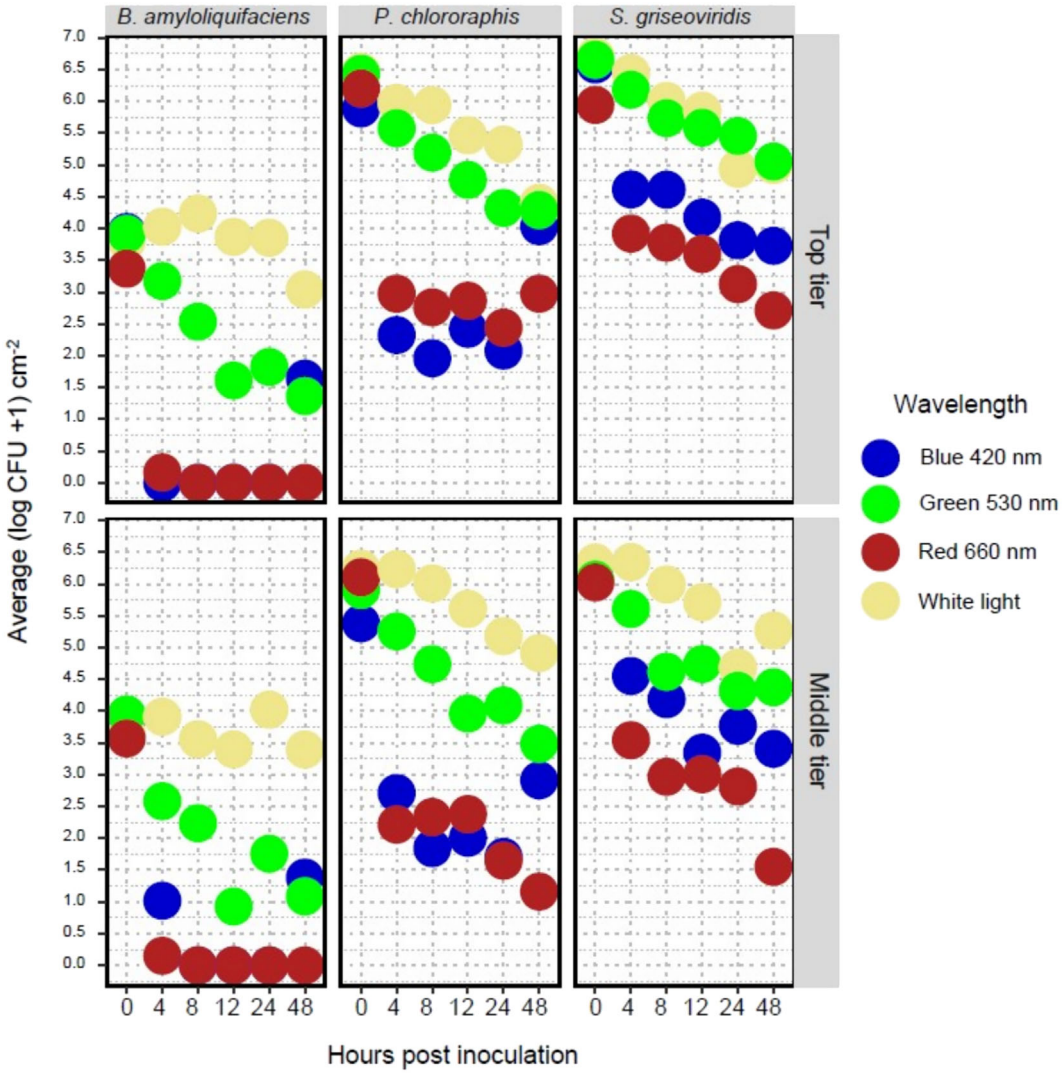

FIGURE 3 Average log colony forming units (CFU + 1) biological control agents cm^{-2} tomato leaves at two placements in the crop canopy; top tier and middle tier over time ($n = 36$, per organism, tier and light treatment, standard deviation, Table S6). The three introduced organisms via foliar spray were: *Bacillus amyloliquefaciens* DSM7, *Pseudomonas chlororaphis* 50083 and *Streptomyces griseoviridis* CBS904.68. They were re-isolated from greenhouse grown tomatoes starting at 0, 4, 8, 12, 24 and 48 h post inoculation of exposure to either white light (polychromatic) or monochromatic (blue: 420 nm, green: 530 nm, red: 660 nm) light.

TABLE 2 Statistical summaries of ANOVAs based on linear mixed models (LMMs), one for each biological control agent (BCA) (*Bacillus amyloliquefaciens* DSM7, *Pseudomonas chlororaphis* 50083 and *Streptomyces griseoviridis* CBS904.68) conducted separately. Hours post inoculation (hour), placement and wavelength were taken into account when compared with the average log colony forming units (CFU + 1) biological control agents cm^{-2} tomato leaves during the entire 48-h period (total $n = 288$). Bold numbers indicate significant effects where $p < .05$. Six replicates were plated for each measurement and organism.

		S. griseoviridis			*P. chlororaphis*			*B. amyloliquifaciens*		
Independent variable	**df**	**Chisq**	***p*-value**		**Chisq**	***p*-value**		**Chisq**	***p*-value**	
Hour	5	477.77	**<.001**	***	312.63	**<.001**	***	693.64	**<.001**	***
Placement	1	91.82	**<.001**	***	35.94	**<.001**	***	2.11	.15	
Wavelength	3	538.15	**<.001**	***	508.53	**<.001**	***	1409.7	**<.001**	***
Hour × placement	5	7.59	.18		11.69	**.040**	*	6.33	.28	
Hour × wavelength	15	92.82	**<.001**	***	124.90	**<.001**	***	397.75	**<.001**	***
Placement × wavelength	3	32.54	**<.001**	***	23.50	**<.001**	***	9.30	**.03**	*
Hour × placement × wavelength	15	28.97	**.016**	*	25.92	**.039**	*	124.28	.06	

wavelength and time of sampling was only detected for *P. chlororaphis* and *S. griseoviridis*.

Significant differences were detected between the interaction of placement and the white light treatment impact on the average log CFU + 1 cm^{-2} of *P. chlororaphis* ($p < .01$), whereas no differences were noted for *S. griseoviridis* and *B. amyloliquefaciens* (Figure 4a). For *S. griseoviridis*, statistical significances ($p < .001$) were only observed for its re-isolation under the 530 nm, indicating that placement had an effect in combination with the green light (Figure 4c). A canopy placement effect was detected for *B. amyloliquefaciens* under the 530 nm light treatment (Figure 4c).

3.2 | ddPCR analyses

To study the effect of the four post-inoculation light treatments on the BCAs and to discriminate the proportions of total present (DNA) and alive (cDNA) introduced strains, ddPCR analyses were used. After performing several ddPCR runs with no hits, *B. amyloliquefaciens* was omitted from the analyses.

Under two light treatments, 420 and 530 nm, there was a gradual decrease in the number of DNA copies over time for *P. chlororaphis* in the middle tier (Figure 5a). An increase for all treatments was noted at 12 hpi in the number of DNA copies in the top tier for *P. chlororaphis* (Figure 5a). The highest concentration was evident under the white LED treatment for *P. chlororaphis* at both placements. Notably, an increase in DNA copies occurred at 12 hpi in the top tier for all but the 420 nm treatment. When comparing the number of copies of cDNA for *P. chlororaphis*, a prominent increase occurred at 48 hpi at both placements under the 530 nm treatment (Figure 5b). For *S. griseoviridis*, there were some oscillations between all light treatments over time in the number of DNA copies (Figure 5c). Under the 660 nm, there was a sharp increase at 12 hpi in the top tier and a milder one in the middle tier. An increase was observed during the 12-h mark only in the middle tier for *S. griseoviridis* in the DNA copies concentration. In the case of the absolute quantification of the cDNA, a steady increase occurred under 530 nm for the top tier (Figure 5d). A similar pattern was seen at 420 nm for the middle tier (Figure 5d). In general, no major differences were observed for either the DNA or the cDNA analysis for *S. griseoviridis*, which is concurrent with the ANOVA results based on the LMM (Figure 5c,d, Table S9).

4 | DISCUSSION

When introduced into a novel environment like the phyllosphere, several factors must align for BCA efficacy. In order to succeed, a newly introduced BCA needs to effectively disperse, adhere and demonstrate antagonistic behaviour (Alsanius et al., 2020). Our study found that exposure dose, wavelength and consequently light distribution within the canopy are fundamental factors governing the establishment of introduced BCAs in the phyllosphere. Light quality was found to be a decisive factor in how well BCAs were re-isolated from the phyllosphere of greenhouse-grown tomatoes, both via viable counts and through their absolute quantification using ddPCR. The three BCAs had different light quality preferences as their re-isolation counts varied highly dependent on the light spectra they were exposed to, indicating that the choice of light quality can be crucial in their introduction and establishment.

Several studies have demonstrated that light energy or even the lack of it can control significant aspects of the physiology of non-phototrophic bacteria (Canessa et al., 2013; Fessia et al., 2024; Gharaie et al., 2017; Gomelsky & Hoff, 2011; Karlsson et al., 2023). Some key responses include the induction of antibiotics, biosurfactant and biofilm formation, swarming motility, and virulence caused by different wavelengths and intensities (Alsanius et al., 2019, 2021; Bonomi et al., 2016; Kahl et al., 2022; Wu et al., 2013). We therefore hypothesised that the selection of light quality (wavelength) in combination with the position within the canopy could significantly influence the occurrence and viability of selected BCAs.

The three BCAs used in our study can be found as main constituents in commercial biocontrol products. *B. amyloliquefaciens* is currently

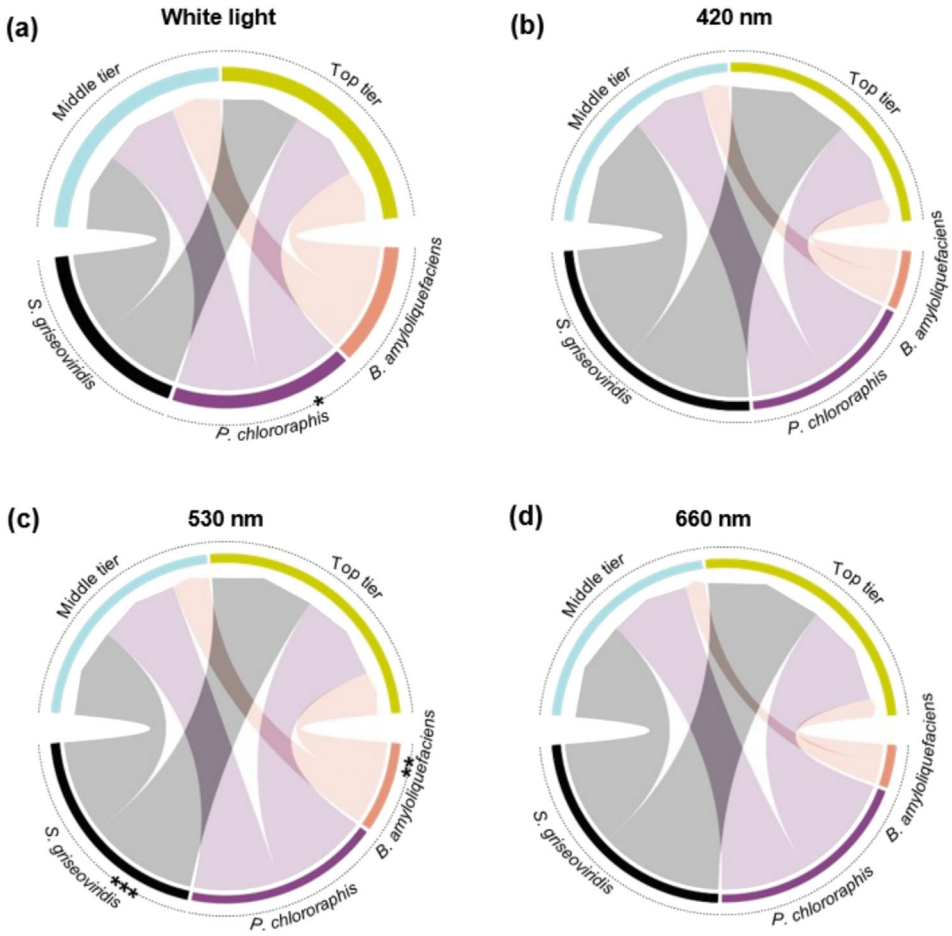

FIGURE 4 Average log colony forming units (CFU + 1) biological control agents cm^{-2} fresh tomato leaves at different placements in the canopy (middle tier in light blue and top tier in yellow) after a total of 48-h exposure to poly- (a: white light) or monochromatic (b: blue: 420 nm, c: green: 530 nm, d: red: 660 nm) light ($n = 288$). The three organisms: *Streptomyces griseoviridis* CBS904.68: black, *Pseudomonas chlororaphis* 50083: purple and *Bacillus amyloliquefaciens* DSM7: orange, were re-isolated from tomato leaves. Linear mixed models were used to determine statistical significances between interaction of the viable count when compared with placement and wavelength per organism, $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. The thickness of the arrows convey the total amount of colonies enumerated per organism per light treatment and placement.

registered for foliar application in Sweden, whereas the other two are primarily used to treat seed- and soil-borne diseases (Table S10). Though all three have shown promise in the phyllosphere (Mina et al., 2020; Raio et al., 2011; Vergnes et al., 2020). In the present study, all BCAs belong to bacterial families that are known to harbour photosensory proteins. *Pseudomonas* spp. are recognised for harbouring multiple photosensory proteins that function in the blue spectrum, including the light oxygen voltage *LOV-domain (Purcell et al., 2007), bacterial phytochrome (Hatfield et al., 2023) and cryptochrome/photolyase (Losi & Gärtner, 2021). *P. chlororaphis* was expected to perform better under the 420 nm treatment (Alsanius et al., 2021; Wu et al., 2013). This was partially the case as the highest average log +1 CFU cm^{-2} over time was observed under the white light treatment, which in our experiment had several peaks within the blue spectrum (Figures 2–4). The 420 nm treatment did not result in neither higher CFU nor higher absolute quantification values when compared with the other light treatments for *P. chlororaphis*, but an increase was observed in the CFU counts between 24 and 48 hpi (Figures 3 and 5a,b). Under the 530 nm treatment, a substantial increase in the cDNA of *P. chlororaphis* was noted after 48 hpi (Figure 5b), indicating that the bacterium potentially adapted over time, suggesting phenotypic plasticity in both tiers. This could be due to green light penetrating the leaf more effectively than the other wavelengths (Lanoue et al., 2022; Terashima et al., 2009) and thus may have had the greatest effect of all treatments irrespective of where the bacteria were on the leaves. When compared with the absolute DNA quantification, a decrease was otherwise noted over time under most treatments, highlighting how it only conveys the total amount of both live and dead cells, which in turn may not correlate to the bacterium's metabolic activity (Figure 5a).

Photosensory proteins have been found in the LOV domain in several *Bacillus* spp. such as *Bacillus subtilis* (Gomelsky & Hoff, 2011). Yu and Lee (2013) studied the effect of light on *B. amyloliquefaciens* JBC36 and its biocontrol efficacy when exposed to either 458, 524 or 645 nm at several intensities ranging from 40 to 240 µmol m^{-2} s^{-1}. They found, among others, that red light affected cell thickness on swarming plates and caused a higher swarming motility rate. This could possibly account for the lack of growth observed in the *B. amyloliquefaciens* under the 660 nm treatment in this study (Figure 3). Higher viable counts of *B. amyloliquefaciens* were enumerated under the white light treatment (Figures 2 and 3). In our case, the white light used had multiple peaks within the blue spectrum (403–458 nm, Table S1), with smaller peaks in the green, red and far-red spectrums (Figure 2a). It is tempting to speculate that the large discrepancies in the peaks could signify that photosensory proteins found in the blue spectrum, such as the LOV domain and cryptochrome/photolyase, may have been activated. To link the results to the expression of the respective photosensory proteins, transcriptome analyses would be required.

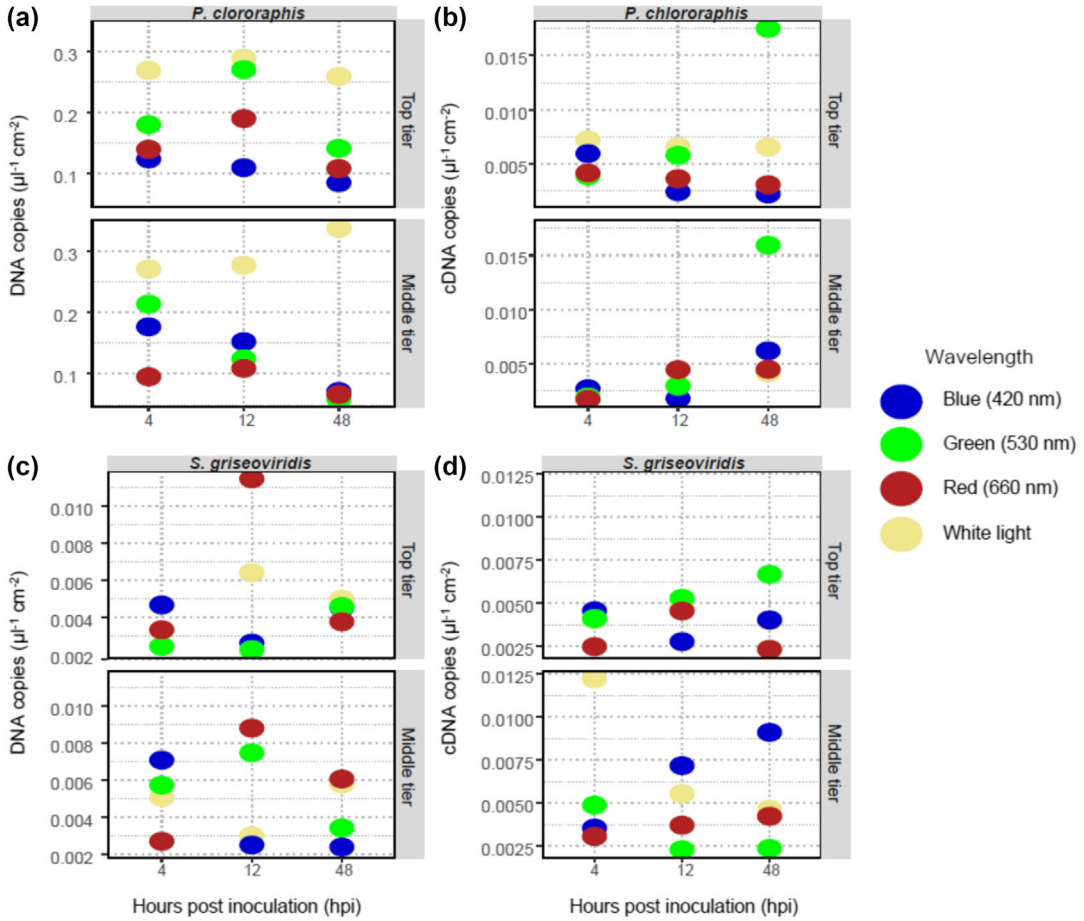

FIGURE 5 Mean number of copies of either DNA (a, c) or cDNA (b, d) for *Pseudomonas chlororaphis* 50083 and *Streptomyces griseoviridis* CBS904.68 copies (μl^{-1} cm^{-2}) at both tiers, top and middle tiers (standard deviations: Table S8). Samples were taken at three time points (4, 12 and 48 h post inoculation) under four light treatments (white light, 420, 530 and 660 nm), at times hidden by other points on the graphs, when extracted from the treated tomato leaves (DNA: $n = 226$, cDNA: $n = 180$, six replicates per treatment). The leaf surface area was accounted for and thus the data is presented as an absolute quantification of DNA or cDNA copies in μl^{-1} cm^{-2} per sample. All external light was blocked throughout the experiment.

By employing light as a stimulus in the form of several different wavelengths, differential preferences occurred. This can partly explain the strains' divergent environmental requirements and capacity to adapt to various environmental conditions (phenotypic plasticity), for example, nutritional factors as previously demonstrated by Karlsson et al. (2023) and biochemical pathways involved (Alsanius et al., 2021).

S. griseoviridis showed generalist behaviour as it had a high average log CFU + 1 cm^{-2} under all four light treatments (Figures 3 and 4). Light has been shown to invoke carotenogenesis in several *Streptomyces* species at a transcriptional level (Elías-Arnanz et al., 2011). *Streptomyces griseus* has shown to employ photolyase PhrB proteins, which are reactive to visible light within the UV-blue spectrum (Kobayashi et al., 1989).

Within a leaf, multiple microhabitats can be found (Leveau & Lindow, 2001), causing differences in irradiation, fluctuating temperatures and concomitantly nutritional conditions affecting competition from native microorganisms (Lindow & Brandl, 2003; Vorholt, 2012). These differences could have an effect on how well the BCAs acclimatise to their new environment when applied to the phyllosphere. The interaction between placement and wavelength and time of sampling were found to be significant when compared with average log CFU + 1 cm^{-2} for both *P. chlororaphis* and *S. griseoviridis* (Table 2). Significant differences occurred for the top tier under the white light treatment for *P. chlororaphis* and under 530 nm, top tier, for *B. amyloliquefaciens* and *S. griseoviridis* (Figure 4a,c). These differences could be due to the correlation between light intensity and how different wavelengths have more or less energised the photons striking the leaf surface (Table S5) and could therefore affect the top-tier BCA more. When time was accounted for, no placement effect could be stated for *B. amyloliquefaciens* and *S. griseoviridis* (Table 2). Placement did not lead to any significant effects on the quantification of either the DNA or cDNA of *S. griseoviridis* (Table S9) when all parameters were accounted for, indicating that the two quantification methods complement one another. The interaction between wavelength and placement was found to be significant at a *p*-value > .05 when

comparing the DNA quantification of both biological control agents together (Table S11), as it was for the DNA quantification of *P. chlororaphis* with a *p*-value > .05 (Table S12), which is concurrent to the comparisons of the CFU counts.

The large decrease in the cDNA versus the DNA concentration of *P. chlororaphis*, where the cDNA was approximately 90% less when compared with the DNA concentration, could be indicative of the small proportion of active cells over time (Figure 5a,b). The presence of high concentrations of cDNA for *P. chlororaphis* after 48 hpi under 530 nm indicates that the bacteria were not only present on the leaves but were also metabolically active. The differences noted in the cDNA versus the DNA counts highlight that both analyses are necessary to understand whether an organism is attached and active. The highest re-isolation counts for *P. chlororaphis* were in fact under the 530 nm and white light treatment (Figure 3), and as such, this difference between the two analysis methods shows that there could be viable but not culturable cells present. This would have been missed if a molecular method had not been used.

One way of controlling the effect of the wavelength applied is steering the total exposure dose. The exposure dose increases exponentially over time, and as such, the higher the intensity used, the shorter the exposure time needed. No one recipe of either light quality or exposure dose could be found for the introduced BCAs in the phyllosphere of greenhouse-grown tomatoes. Our results convey that the three BCAs did persist better under the white light treatment when delving into the total log CFU + 1 cm^{-2}, though this did not necessarily result in statistical differences. This could be of further interest as greenhouse growers already use white light in their production systems, as crops tend to favour a broad spectrum irradiance. There are though differences between different makes of white light lamps, and thus further research is necessary. By assessing how the non-phototrophic BCA used in this study reacts to light qualities and exposure doses, a deeper understanding could be reached, creating a platform for improved efficacy against pathogens. A better adhesion could be achieved by adapting the wavelength that the crops are exposed to when applying the BCAs.

5 | CONCLUSIONS

The results indicate that BCAs react to their light environment and that an appropriate light environment is needed for their establishment. Our results also convey that there is no general recipe for the three BCAs tested. They confirm that light quality is a decisive factor as white light and the 530 nm allowed for the highest re-isolation counts and absolute quantifications but that further transcriptomic analyses would be needed in order to make BCA-specific recommendations. We conclude that exposure dose is fundamental to the survival of the BCA strains in a new environment. Individual organism-specific adaptations need to be made for a successful introduction of BCAs to the phyllosphere of any crop.

AUTHOR CONTRIBUTIONS

Maria Hellström, Maria E. Karlsson and Beatrix W. Alsanius contributed to the conception of the study and the design. Maria Hellström, Maria E. Karlsson and Isabella Kleman performed the experiments. Maria Hellström, Maria E. Karlsson, Isabella Kleman, Anna Karin Rosberg, Julia Darlison, Emina Mulaosmanovic and Lena Will collected data. Maria Hellström conducted data analysis and wrote the first draft. Maria Hellström and Beatrix W. Alsanius rewrote the final draft. All co-authors critically reviewed the manuscript. Beatrix W. Alsanius and Maria Hellström acquired funding. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGEMENTS

This research was funded by the Swedish Farmers' Foundation for Agricultural Research (SLF) and the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS), both in Stockholm, Sweden, grant number R-18-25-006 ('Optimized integrated control in greenhouse systems sees the light'; PI: BA). The ddPCR analyses were funded by the Royal Swedish Academy of Agriculture and Forestry (KSLA), Stockholm, Sweden, grant number HORT2022-0010 ('Integrerad bekämpning av växthustomater i nytt ljus'; PI: MH).

CONFLICT OF INTEREST STATEMENT

No competing interests have been identified.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Maria Hellström https://orcid.org/0000-0003-2532-9913
Maria E. Karlsson https://orcid.org/0000-0002-7414-9022
Isabella Kleman https://orcid.org/0000-0002-0476-4820
Anna Karin Rosberg https://orcid.org/0000-0002-4851-7354
Julia Darlison https://orcid.org/0000-0003-3947-9699
Emina Mulaosmanovic https://orcid.org/0000-0003-3169-8336
Lena Will https://orcid.org/0009-0001-6024-7668
Beatrix W. Alsanius https://orcid.org/0000-0001-6300-2829

REFERENCES

Al_husnan, L. A., & Alkahtani, M. D. F. (2016). Molecular identification of *Streptomyces* producing antibiotics and their antimicrobial activities. *Annals of Agricultural Sciences*, *61*(2), 251–255. https://doi.org/10.1016/j.aoas.2016.06.002

Alsanius, B. W., Bergstrand, K. J., & Karlsson, M. E. (2020). Integrated greenhouse production in new light: Prospects on improved LED-assisted biocontrol of foliar diseases. *Acta Horticulturae*, *1296*, 293–296. https://doi.org/10.17660/ActaHortic.2020.1296.38

Alsanius, B. W., Hellström, M., Bergstrand, K.-J., Vetukuri, R., Becher, P., & Karlsson, M. E. (2024). The power of light from a non-phototrophic perspective: A phyllosphere dilemma. *Frontiers in Photobiology*, *2*. https://doi.org/10.3389/fphbi.2024.1432066

Alsanius, B. W., Karlsson, M. E., Rosberg, A.-K., Dorais, M., Naznin, T., Khalil, S., & Bergstrand, K.-J. (2019). Light and microbial lifestyle: The impact of light quality on plant–microbe interactions in horticultural production systems—A review. *Horticulturae*, *5*, 41. https://doi.org/10.3390/horticulturae5020041

Alsanius, B. W., Vaas, L., Gharaie, S., Karlsson, M. E., Rosberg, A.-K., Wohanka, W., Khalil, S., & Windstam, S. (2021). Dining in blue light impairs the appetite of some leaf epiphytes. *Frontiers in Microbiology*, *12*, 725021. https://doi.org/10.3389/fmicb.2021.725021

Andrews, J. H. (1992). Biological control in the phyllosphere. *Annual Review of Phytopathology*, *30*(1), 603–635. https://doi.org/10.1146/annurev.py.30.090192.003131

Baskins, S., Bond, J., & Minor, T. (2019). *Unpacking the growth in per capita availability of fresh market tomatoes*. (VGS-19C-01). USDA-ERS.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Beattie, G. A., Hatfield, B. M., Dong, H., & McGrane, R. S. (2018). Seeing the light: The roles of red- and blue-light sensing in plant microbes. *Annual Review of Phytopathology*, *56*(1), 41–66. https://doi.org/10.1146/annurev-phyto-080417-045931

Bonomi, H., Toum, L., Sycz, G., Sieira, R., Toscani, A., Gudesblat, G., Leskow, F., Goldbaum, F., Vojnov, A., & Malamud, F. (2016). *Xanthomonas campestris* attenuates virulence by sensing light through a bacteriophytochrome photoreceptor. *EMBO Reports*, *17*, 1565–1577. https://doi.org/10.15252/embr.201541691

Canessa, P., Schumacher, J., Hevia, M. A., Tudzynski, P., & Larrondo, L. F. (2013). Assessing the effects of light on differentiation and virulence of the plant pathogen *Botrytis cinerea*: Characterization of the white collar complex. *PLoS ONE*, *8*(12), e84223. https://doi.org/10.1371/journal.pone.0084223

Carlström, C. I., Field, C. M., Bortfeld-Miller, M., Müller, B., Sunagawa, S., & Vorholt, J. A. (2019). Synthetic microbiota reveal priority effects and keystone strains in the *Arabidopsis* phyllosphere. *Nature Ecology & Evolution*, *3*(10), 1445–1454. https://doi.org/10.1038/s41559-019-0994-z

Cutolo, E. A., Guardini, Z., Dall'Osto, L., & Bassi, R. (2023). A paler shade of green: Engineering cellular chlorophyll content to enhance photosynthesis in crowded environments. *New Phytologist*, *239*, 1567–1583. https://doi.org/10.1111/nph.19064

Elías-Arnanz, M., Padmanabhan, S., & Murillo, F. J. (2011). Light-dependent gene regulation in nonphototrophic bacteria. *Current Opinion in Microbiology*, *14*(2), 128–135. https://doi.org/10.1016/j.mib.2010.12.009

Fan, X.-X., Xu, Z.-G., Liu, X.-Y., Tang, C.-M., Wang, L.-W., & Han, X.-L. (2013). Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and blue light. *Scientia Horticulturae*, *153*, 50–55. https://doi.org/10.1016/j.scienta.2013.01.017

Feller, C., Bleiholder, H., Buhr, L., Hack, H., Hess, M., Klose, R., Meier, U., Stauss, R., van den Boom, T., & Weber, E. (2001). Growth stages of mono-and dicotyledonous plants. In U., Meier (Ed.), *BBCH-monograph*. Federal Biological Research Centre for Agriculture and Forestry.

Fessia, A., Ponzio, R., Arcibia, L., Barros, G., & Nesci, A. (2024). Effects of different light wavelengths on *Bacillus subtilis* and *Bacillus velezensis*, two biocontrol agents isolated from the maize phyllosphere. *Archives of Microbiology*, *206*(3), 104. https://doi.org/10.1007/s00203-024-03836-5

Garrido-Sanz, D., Arrebola, E., Martínez-Granero, F., García-Méndez, S., Muriel, C., Blanco-Romero, E., Martín, M., Rivilla, R., & Redondo-Nieto, M. (2017). Classification of isolates from the *Pseudomonas fluorescens* complex into phylogenomic groups based in group-specific markers. *Frontiers in Microbiology*, *8*, 413. https://doi.org/10.3389/fmicb.2017.00413

Gharaie, S., Vaas, L. A. I., Rosberg, A. K., Windstam, S. T., Karlsson, M. E., Bergstrand, K.-J., Khalil, S., Wohanka, W., & Alsanius, B. W. (2017). Light spectrum modifies the utilization pattern of energy sources in *Pseudomonas* sp. DR 5-09. *PLoS ONE*, *12*(12), e0189862. https://doi.org/10.1371/journal.pone.0189862

Gomelsky, M., & Hoff, W. D. (2011). Light helps bacteria make important lifestyle decisions. *Trends in Microbiology*, *19*(9), 441–448. https://doi.org/10.1016/j.tim.2011.05.002

Gu, Z., Gu, L., Eils, R., Schlesner, M., & Brors, B. (2014). *circlize* implements and enhances circular visualization in R. *Bioinformatics*, *30*(19), 2811–2812. https://doi.org/10.1093/bioinformatics/btu393

Hatfield, B. M., Lasarre, B., Liu, M., Dong, H., Nettleton, D., & Beattie, G. A. (2023). Light cues induce protective anticipation of environmental water loss in terrestrial bacteria. *Proceedings of the National Academy of Sciences*, *120*(38), e2309632120. https://doi.org/10.1073/pnas.2309632120

Hoff, W. D., Horst, M. A., Nudel, C. B., & Hellingwerf, K. J. (2009). *Prokaryotic phototaxis* (pp. 25–49). Humana Press. https://doi.org/10.1007/978-1-60761-198-1_2

Imada, K., Tanaka, S., Ibaraki, Y., Yoshimura, K., & Ito, S. (2014). Antifungal effect of 405-nm light on *Botrytis cinerea*. *Letters in Applied Microbiology*, *59*(6), 670–676. https://doi.org/10.1111/lam.12330

Kahl, L. J., Eckartt, K. N., Morales, D. K., Price-Whelan, A., & Dietrich, L. E. P. (2022). Light/dark and temperature cycling modulate metabolic electron flow in *Pseudomonas aeruginosa* biofilms. *MBio*, *13*(4), e01407–e01422. https://doi.org/10.1128/mbio.01407-22

Karlsson, M. E., Hellström, M., Flöhr, A., Bergstrand, K.-J., & Alsanius, B. W. (2023). The power of light: Impact on the performance of biocontrol agents under minimal nutrient conditions. *Frontiers in Microbiology*, *14*, 1087639. https://doi.org/10.3389/fmicb.2023.1087639

Kobayashi, T., Takao, M., Oikawa, A., & Yasui, A. (1989). Molecular characterization of a gene encoding a photolyase from *Streptomyces griseus*. *Nucleic Acids Research*, *17*(12), 4731–4744. https://doi.org/10.1093/nar/17.12.4731

Lanoue, J., Little, C., Hawley, D., & Hao, X. (2022). Addition of green light improves fruit weight and dry matter content in sweet pepper due to greater light penetration within the canopy. *Scientia Horticulturae*, *304*, 111350. https://doi.org/10.1016/j.scienta.2022.111350

Leveau, J. H. J., & Lindow, S. E. (2001). Appetite of an epiphyte: Quantitative monitoring of bacterial sugar consumption in the phyllosphere. *Proceedings of the National Academy of Sciences*, *98*(6), 3446–3453. https://doi.org/10.1073/pnas.061629598

Lindow, S. E., & Brandl, M. T. (2003). Microbiology of the phyllosphere. *Applied and Environmental Microbiology*, *69*(4), 1875–1883. https://doi.org/10.1128/AEM.69.4.1875-1883.2003

Losi, A., & Gärtner, W. (2021). A light life together: Photosensing in the plant microbiota. *Photochemical & Photobiological Sciences*, *20*(3), 451–473. https://doi.org/10.1007/s43630-021-00029-7

Mina, D., Pereira, J. A., Lino-Neto, T., & Baptista, P. (2020). Screening the olive tree phyllosphere: Search and find potential antagonists against *Pseudomonas savastanoi* pv. *savastanoi*. *Frontiers in Microbiology*, *11*, 2051. https://doi.org/10.3389/fmicb.2020.02051

Modarelli, G. C., Paradiso, R., Arena, C., De Pascale, S., & Van Labeke, M. C. (2022). High light intensity from blue-red LEDs enhance photosynthetic performance, plant growth, and optical properties of red lettuce in controlled environment. *Horticulturae*, *8*(2), 114. https://doi.org/10.3390/horticulturae8020114

Purcell, E. B., & Crosson, S. (2008). Photoregulation in prokaryotes. *Current Opinion in Microbiology*, *11*(2), 168–178. https://doi.org/10.1016/j.mib.2008.02.014

Purcell, E. B., Siegal-Gaskins, D., Rawling, D. C., Fiebig, A., & Crosson, S. (2007). A photosensory two-component system regulates bacterial cell attachment. *Proceedings of the National Academy of Sciences*, *104*(46), 18241–18246. https://doi.org/10.1073/pnas.0705887104

R Core Team. (2021). A language and environment for statistical computing. In R Foundation for Statistical Computing. https://www.R-project.org/

Raio, A., Puopolo, G., Cimmino, A., Danti, R., Della Rocca, G., & Evidente, A. (2011). Biocontrol of cypress canker by the phenazine producer *Pseudomonas chlororaphis* subsp. aureofaciens strain M71. *Biological Control*, *58*(2), 133–138. https://doi.org/10.1016/j.biocontrol.2011.04.012

Remus-Emsermann, M. N. P., & Vorholt, J. A. (2014). Complexities of microbial life on leaf surfaces. *Microbe Magazine*, *9*(11), 448–452. https://doi.org/10.1128/microbe.9.448.1

Schlechter, R. O., Kear, E. J., Bernach, M., Remus, D. M., & Remus-Emsermann, M. N. P. (2023). Metabolic resource overlap impacts competition among phyllosphere bacteria. *The ISME Journal*, *17*, 1445–1454. https://doi.org/10.1038/s41396-023-01459-0

Slattery, R. A., Walker, B. J., Weber, A. P. M., & Ort, D. R. (2018). The impacts of fluctuating light on crop performance. *Plant Physiology*, *176*(2), 990–1003. https://doi.org/10.1104/pp.17.01234

Suthaparan, A., Stensvand, A., Solhaug, K. A., Torre, S., Telfer, K. H., Ruud, A. K., Mortensen, L. M., Gadoury, D. M., Seem, R. C., & Gislerød, H. R. (2014). Suppression of cucumber powdery mildew by supplemental UV-B radiation in greenhouses can be augmented or reduced by background radiation quality. *Plant Disease*, *98*(10), 1349–1357. https://doi.org/10.1094/pdis-03-13-0222-re

Terashima, I., Fujita, T., Inoue, T., Chow, W. S., & Oguchi, R. (2009). Green light drives leaf photosynthesis more efficiently than red light in strong white light: Revisiting the enigmatic question of why leaves are green. *Plant and Cell Physiology*, *50*(4), 684–697. https://doi.org/10.1093/pcp/pcp034

Vergnes, S., Gayrard, D., Veyssière, M., Toulotte, J., Martinez, Y., Dumont, V., Bouchez, O., Rey, T., & Dumas, B. (2020). Phyllosphere colonization by a soil *Streptomyces* sp. promotes plant defense responses against fungal infection. *Molecular Plant-Microbe Interactions*, *33*(2), 223–234. https://doi.org/10.1094/mpmi-05-19-0142-r

Vorholt, J. A. (2012). Microbial life in the phyllosphere. *Nature Reviews Microbiology*, *10*(12), 828–840. https://doi.org/10.1038/nrmicro2910

Wattiau, P., Renard, M. E., Ledent, P., Debois, V., Blackman, G., & Agathos, S. (2001). A PCR test to identify *Bacillus subtilis* and closely related species and its application to the monitoring of wastewater biotreatment. *Applied Microbiology and Biotechnology*, *56*(5), 816–819. https://doi.org/10.1007/s002530100691

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. Springer-Verlag. https://ggplot2.tidyverse.org

Wilde, A., & Mullineaux, C. W. (2017). Light-controlled motility in prokaryotes and the problem of directional light perception. *FEMS Microbiology Reviews*, *41*(6), 900–922. https://doi.org/10.1093/femsre/fux045

Wilson, M., & Lindow, S. E. (1992). Relationship of total viable and culturable cells in epiphytic populations of *Pseudomonas syringae*. *Applied and Environmental Microbiology*, *58*(12), 3908–3913. https://doi.org/10.1128/aem.58.12.3908-3913.1992

Wu, L., McGrane, R., & Beattie, G. (2013). Light regulation of swarming motility in *Pseudomonas syringae* integrates signaling pathways mediated by a bacteriophytochrome and a LOV protein. *MBio*, *4*(3), 10–1128. https://doi.org/10.1128/mBio.00334-13

Yu, S.-M., & Lee, Y. H. (2013). Effect of light quality on *Bacillus amyloliquefaciens* JBC36 and its biocontrol efficacy. *Biological Control*, *64*(3), 203–210. https://doi.org/10.1016/j.biocontrol.2012.11.004

Zheng, L., & Van Labeke, M.-C. (2017). Long-term effects of red- and blue-light emitting diodes on leaf anatomy and photosynthetic efficiency of three ornamental pot plants. *Frontiers in Plant Science*, *8*, 917. https://doi.org/10.3389/fpls.2017.00917

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hellström, M., Karlsson, M. E., Kleman, I., Rosberg, A. K., Darlison, J., Mulaosmanovic, E., Will, L., & Alsanius, B. W. (2024). Exposure dose, light distribution and wavelength affect the fate of introduced bacterial biological control agents in the phyllosphere of greenhouse grown tomato. *Plants, People, Planet*, 1–12. https://doi.org/10.1002/ppp3.10586