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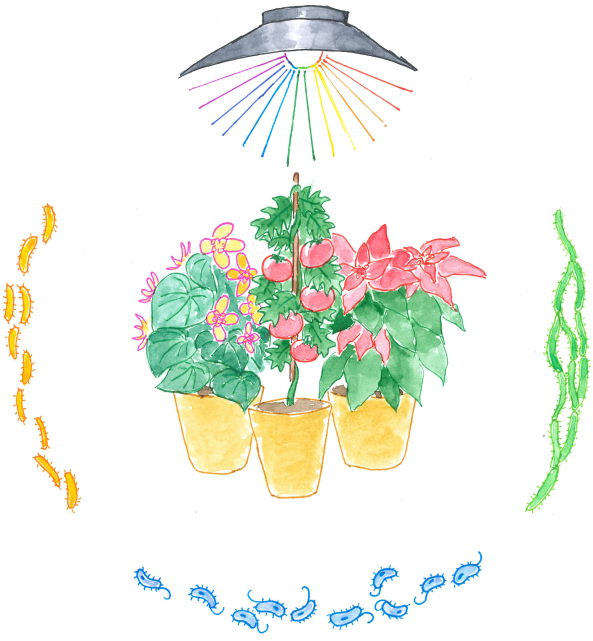
Bacterial Delight: Deciphering how visible light affects bacterial biological control agents in the phyllosphere of greenhouse crops

Maria Hellström



Bacterial Delight: Deciphering how visible light affects bacterial biological control agents in the phyllosphere of greenhouse crops

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Abstract

Light can be an enabler of life but also a stressor depending on the organism and the environment. When introducing biological control agents (BCAs) to the phyllosphere of greenhouse crops, several parameters need to be met to reach high efficacy. The newly applied invaders in the harsh phyllosphere environment need to successfully attach, compete and reproduce whilst also acting against a pathogen to be regarded as effective. Light has been found to induce biosurfactant and biofilm formation in non-phototrophic bacterial BCAs. These induced mechanisms could be key in aiding the introduction of non-phototrophic BCAs. In this thesis, the effects of placement in the canopy, leaf type, visible light treatment, exposure dose and sole carbon utilisation were investigated with respect to three non-phototrophic bacterial BCAs (*Bacillus amyloliquefaciens*, *Pseudomonas chlororaphis*, *Streptomyces griseoviridis*). Both greenhouse and laboratory experiments were used to decipher how visible light affects these BCAs. Exposure dose, light distribution in combination with a wavelength were found to affect the BCAs when introduced in the phyllosphere of greenhouse crops. Based on viable counts *S. griseoviridis* was recovered at high rates irrespective of the light treatment, whereas *P. chlororaphis* and *B. amyloliquefaciens* preferred the green and white light treatments. It was found that different wavelengths influence all three BCAs' sole carbon utilisation, where two BCAs, *B. amyloliquefaciens* and *P. chlororaphis*, had higher respiration rates under the blue spectrum, and *S. griseoviridis* preferred the red spectrum. In the case of *P. chlororaphis,* this was further translated when cascade effects were noted in the citrate cycle as an effect of the increased source utilisation under blue light treatment.

Keywords: begonia, biofilm formation, ddPCR, exposure dose, light emitting diode (LED), phenotypic microarray, poinsettia, sole-source carbon utilisation, tomato

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Bakteriell glädje: Hur påverkar synlig ljus bakteriella biologiska växthusmedel i fyllosfären hos växthusplantor

Sammanfattning

Ljus kan vara en drivfaktor men också en stressfaktor, beroende på organism och den miljö den befinner sig i. När biologiska bekämpningsmedel (BCA) introduceras i växthusgrödors fyllosfär måste flera parametrar uppfyllas för att uppnå hög effektivitet. De nya introducerade inkräktarna i den tuffa miljön i fyllosfären måste lyckas fästa, konkurrera och föröka sig samtidigt som de verkar mot en patogen för att anses vara effektiva. Det har visat sig att ljus inducerar biosurfactant- och biofilmsbildning i icke-fototrofa bakteriella BCA. Dessa inducerade mekanismer kan vara viktiga för att underlätta införandet av icke-fototrofa BCA.

I denna avhandling undersöktes effekterna av placering i bladskärmen, bladtyp, ljusets våglängd, exponeringsdos och kolutnyttjande, baserat på tre icke-fototrofa bakteriella BCA (*Bacillus amyloliquefaciens*, *Pseudomonas chlororaphis*, *Streptomyces griseoviridis*). Både växthus- och laboratorieexperiment användes för att ta reda på hur synligt ljus påverkar dessa BCA. Exponeringsdos och ljusspridning, i kombination med våglängd, påverkade BCA som introducerats i fyllosfären hos växthuskulturer. Baserat på levande celltal, återfanns *S. griseoviridis* i hög grad oavsett ljusbehandling, medan *P. chlororaphis* och *B. amyloliquefaciens* främjades vid exponering till grönt och vitt ljus. Det visade sig att val av våglängd påverkade kolanvändningen av alla tre BCA, där *B. amyloliquefaciens* och *P. chlororaphis*, hade högre respirationstakt vid exponering till blå spektrum, medan *S. griseoviridis* föredrog rött spektrum. För *P. chlororaphis* noterades kaskadeffekter i citronsyrecykeln på grund av det ökade källutnyttjandet under den blå behandlingen.

Nyckelord: begonia, biofilm formation, ddPCR, exponeringsdos, julstjärna, LED, kolutnyttjande, tomat

Βακτηριακή χαρά: Πώς επηρεάζει το ορατό φως τους μικροβιακούς ανταγωνιστές στη φυλλόσφαιρα των φυτών θερμοκήπιου

Περίληψη

Το φως μπορεί να είναι πηγή ζωής αλλά και παράγοντας άγχους, ανάλογα με τον οργανισμό και το περιβάλλον. Κατά την εισαγωγή βιολογικών παραγόντων ελέγχου (BCAs) στη φυλλόσφαιρα καλλιεργειών θερμοκηπίου, πρέπει να πληρούνται ορισμένες παράμετροι για να επιτευχθεί υψηλή αποτελεσματικότητα. Οι νέο εισαγόμενοι οργανισμοί στο δύσκολο περιβάλλον της φυλλόσφαιρας πρέπει να προσκολληθούν επιτυχώς, να ανταγωνιστούν, να αναπαραχθούν και ταυτόχρονα να δράσουν ενάντια σε παθογόνα για να θεωρηθούν αποτελεσματικοί. Έχει βρεθεί ότι το φως επάγει τον σχηματισμό βιοεπιφανειοδραστικών ουσιών και βιομεμβρανών σε μη φωτοτροφικούς βακτηριακούς BCAs. Αυτοί οι επαγόμενοι μηχανισμοί θα μπορούσαν να είναι καθοριστικοί για τη διευκόλυνση της εισαγωγής μη φωτοτροφικών BCAs.

Στην παρούσα διπλωματική εργασία, διερευνήθηκε πως τρεις μη φωτοτροφικοί βακτηριακοί BCAs (*Bacillus amyloliquefaciens*, *Pseudomonas chlororaphis*, *Streptomyces griseoviridis*) επηρεάζονται από τη θέση τους πάνω στο φυτό, από τον τύπο του φυλλώματος, από τα διαφορετικά μήκη κύματος του ορατού φωτός και από τη χρήση ελάχιστων θρεπτικών συστατικών. Πραγματοποιήθηκαν πειράματα τόσο στο θερμοκήπιο όσο και στο εργαστήριο για να αποσαφηνιστεί πώς το ορατό φως επηρεάζει αυτούς τους BCAs. Διαπιστώθηκε ότι η δόση έκθεσης, η κατανομή του φωτός σε συνδυασμό με το μήκος κύματος επηρεάζουν τους BCAs όταν εισάγονται στη φυλλόσφαιρα καλλιεργειών θερμοκηπίου. Βάσει βιώσιμων μετρήσεων, το *S. griseoviridis* ανιχνεύθηκε σε υψηλά ποσοστά ανεξάρτητα από το μήκος κύματος, ενώ τα *P. chlororaphis* και *B. amyloliquefaciens* προτίμησαν το πράσινο και λευκό φως. Διαπιστώθηκε ότι διαφορετικά μήκη κύματος επηρεάζουν τη χρήση ελάχιστων θρεπτικών συστατικών και των τριών BCAs. Τα *B. amyloliquefaciens* και *P. chlororaphis* παρουσίασαν υψηλότερους ρυθμούς αναπνοής στο μπλε φως, ενώ το *S. griseoviridis* προτίμησε το κόκκινο φως.

Λέξεις-κλειδιά: δόση έκθεσης, ddPCR, LED, μπιγκόνια, ντομάτα, σχηματισμός βιοϋμενίου

Dedication

To my family.

Ἰθάκη

Σὰ βγεῖς στὸν πηγαιμὸ γιὰ τὴν Ἰθάκη, νὰ εὔχεσαι νἄναι μακρὺς ὁ δρόμος, γεμάτος περιπέτειες, γεμάτος γνώσεις.

Τοὺς Λαιστρυγόνας καὶ τοὺς Κύκλωπας, τὸν θυμωμένο Ποσειδῶνα μὴ φοβᾶσαι, τέτοια στὸν δρόμο σου ποτέ σου δὲν θὰ βρεῖς, ἂν μέν᾽ ἡ σκέψις σου ὑψηλή, ἂν κλεκτὴ

συγκίνησις τὸ πνεῦμα καὶ τὸ σῶμα σου ἀγγίζει. Τοὺς Λαιστρυγόνας καὶ τοὺς Κύκλωπας, τὸν ἄγριο Ποσειδώνα δὲν θὰ συναντήσεις, ἂν δὲν τοὺς κουβανεῖς μὲς στὴν ψυχή σου, ἂν ἡ ψυχή σου δὲν τοὺς στήνει ἐμπρός σου.

Νὰ εὔχεσαι νά ΄ναι μακρὺς ὁ δρόμος.
Πολλὰ τὰ καλοκαιρινὰ πρωΐα νὰ εἶναι ποὺ μὲ τί εὐχαρίστηση, μὲ τί χαρὰ θὰ μπαίνεις σὲ λιμένας πρωτοειδωμένους

νὰ σταματήσεις σ᾽ ἐμπορεῖα Φοινικικά, καὶ τὲς καλὲς πραγμάτειες ν᾽ ἀποκτήσεις, σεντέφια καὶ κοράλλια, κεχριμπάρια κ᾽ ἔβενους, καὶ ἡδονικὰ μυρωδικὰ κάθε λογῆς, ὅσο μπορεῖς πιὸ ἄφθονα ἡδονικὰ μυρωδικά.

Σὲ πόλεις Αἰγυπτιακὲς πολλὲς νὰ πᾷς, νὰ μάθεις καὶ νὰ μάθεις ἀπ᾽ τοὺς σπουδασμένους. Πάντα στὸ νοῦ σου νἄχῃς τὴν Ἰθάκη. Τὸ φθάσιμον ἐκεῖ εἶν᾽ ὁ προορισμός σου.

Ἀλλὰ μὴ βιάζῃς τὸ ταξείδι διόλου. Καλλίτερα χρόνια πολλὰ νὰ διαρκέσει. Καὶ γέρος πιὰ ν᾽ ἀράξῃς στὸ νησί, πλούσιος μὲ ὅσα κέρδισες στὸν δρόμο, μὴ προσδοκώντας πλούτη νὰ σὲ δώσῃ ἡ Ἰθάκη.

Ἡ Ἰθάκη σ᾽ ἔδωσε τ᾽ ὡραῖο ταξίδι.
Χωρὶς αὐτὴν δὲν θἄβγαινες στὸν δρόμο. Ἄλλα δὲν ἔχει νὰ σὲ δώσει πιά.

Κι ἂν πτωχικὴ τὴν βρῇς, ἡ Ἰθάκη δὲν σὲ γέλασε.
Ἔτσι σοφὸς ποὺ ἔγινες, μὲ τόση πείρα, ἤδη θὰ τὸ κατάλαβες ἡ Ἰθάκες τί σημαίνουν

- Κ. Π. Καβάφης (1911)

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Alsanius, B.W., **Hellström, M.**, Bergstrand, K.-J., Vetukuri, R., Becher, P.G., Karlsson, M.E. (2024) The power of light from a non-phototrophic perspective: a phyllosphere dilemma. Frontiers in Photobiology, Sec. Photoecology and Environmental Photobiology, 2, 1432066 https://doi.org/10.3389/fphbi.2024.1432066

II. **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

III. 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/https://doi.org/10.3389/fmicb.2023.1087639

IV. **Hellström, M.**, Karlsson, M.E, Kleman, I., Vetukuri, R., Becher, P.G., Alsanius, B.W. (Manuscript) Light spectrum effects on non-phototrophic *Pseudomonas chlororaphis* in the phyllosphere: Ecological implications for sole carbon utilisation and niche realisation.

Papers I and III are reproduced with the permission of the publishers. Paper II is open access.

The contribution of Maria Hellström to the papers included in this thesis was as follows:

I. Description of contribution to paper I.

   Participated in the investigation and visualization of the manuscript. Wrote the original draft with the co-authors. All authors contributed to the final version of the article after editions and revisions.

II. Description of contribution to paper II.

   Contributed to the conception and designed the study together with MK and BA. Performed the experiments together with MK and IK. Collected data with MK, IK, AKR, JD, EM and LW. Analysed the data and wrote the first draft. BA and MH acquired funding. All authors contributed to the article and approved the submitted version.

III. Description of contribution to paper III.

   Assisted MK with the experiments. Participated in the writing of the manuscript. All authors contributed to the final version of the article after editions and revisions.

IV. Description of contribution to paper IV.

   Contributed to the conception and design of the study together with MK and BA. Performed the experiments and collected data together with IK. Analysed the data and wrote the first draft with the input of the co-authors. All authors contributed to the manuscript.

List of other publications

Work not contained in the thesis such as popular scientific articles, conference articles and other scientific articles:

I. **Hellström, M.**, Karlsson, M.E., Vetukuri, R., Becher, P. G. and Alsanius, B.W. (2024). Utilizing light exposure to its fullest: how light quality can aid biocontrol introduction in greenhouse horticulture – A pilot study. Acta Horticulturae. Accepted.

II. **Hellström, M.**, Karlsson, M., Kleman, I., Bergstrand, K.-J. and Alsanius, B.W. (2023). Artificial light quality changes colonization ability of biocontrol agents under greenhouse conditions. Acta Horticulturae. 1377, 299-306.

III. **Hellström, M.**, Karlsson, M., Bergstrand, K.-J., Alsanius, B. (2021). Bekämpning i nytt ljus. Viola, 1. 12-13.

IV. Kleman, I., **Hellström, M.**, Rosberg, A.K., Becher, P.G. and Mogren, L. (2023). Simple quality prediction measurements for stored onions. Acta Horticulturae. 1364, 183-186.

V. Alsanius, B.W., Karlsson, M.E., **Hellström, M.**, Bergstrand, K.-J., and Rosberg, A.K. (2024). Integrated production in new light: light quality in greenhouse horticulture and its impact on the phyllosphere microbiome. Acta Horticulturae. Accepted.

VI. Alsanius, B.W., Khalil, S., Karlsson, M., **Hellström, M.**, Vendrame, M. and Rosberg, A.K. (2023). The two sides of the coin: sustainability and controlled environment horticulture. Acta Horticulturae. 1377, 427-438.

List of tables

List of figures

Abbreviations

ANOVA	Analysis of variance
AUC	Area under the curve
B. amyloliquefaciens	*Bacillus amyloliquefaciens*
BSL	Biosafety level
B. cinerea	*Botrytis cinerea*
BCA	Biological control agent
BBCH-scale	Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie scale
BLUF	Blue-light sensor proteins using flavin adenine dinucleotide
BphP	Bacteriophytochrome
cDNA	Complementary deoxyribonucleic acid
CEA	Control environment agriculture
CFU	Colony forming units
CRY	Cryptochrome
DNA	Deoxyribonucleic acid
ddPCR	Digital droplet polymerase chain reaction
FIR	Far infrared radiation
hpi	Hours post inoculation

HPS lamps	High pressure sodium lamps
KEGG	Kyoto encyclopedia of genes and genomes
LED	Light emitting diode
lm	Lumen
LMM	Linear mixed model
LOV	Light oxygen voltage
lx	Luminous flux per unit area
NIR	Near-infrared radiation
PAR	Photosynthetic active radiation
P. chlororaphis	*Pseudomonas chlororaphis*
PCR	Polymerase chain reaction
PHY	Phytochromes
PHR	Photolyase
PM	Phenotypic microarray
PPFD	Photosynthetic photon flux density
PYP	Photosensory yellow protein
Rho	Rhodopsin
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
S. griseoviridis	*Streptomyces griseoviridis*
SDG	Sustainability development goals
SLU	Swedish University of Agricultural Sciences
TSA	Tryptic soy agar
TSB	Tryptic soy broth
UV	Ultraviolet

1. Introduction

When discussing visible light, we tend to take an anthropocentric approach to its effect and perception. What our eyes distinguish as red or white light may not be what a plant or a bacterial cell living on a leaf perceives. It is often said that beauty, or in this case light, is in the eyes of the beholder, and this thesis aimed to disentangle if and how light may be perceived by non-phototrophic biological control agents (BCAs) both *in-vitro* and *in-vivo*, post application to the phyllosphere of greenhouse grown crops.

Light is a strong abiotic environmental cue capable of affecting several aspects of the lifestyle of both phototrophic and non-phototrophic organisms (Alsanius et al., 2019; Canessa et al., 2013; Kraiselburd et al., 2017). Light can, among others, induce biofilm and biosurfactant production in non-phototrophic bacteria (Alsanius et al., 2021; Beattie et al., 2018; Fessia et al., 2024), which in the case of bacterial BCAs could be key in increasing their efficacy in controlled environment agriculture (CEA) (Alsanius et al., 2020).

Bacterial BCAs tend to thrive under controlled laboratory conditions but do not perform as well when applied via foliar spray or in the field (Milner et al., 1997; Salvatierra-Martinez et al., 2018). Increasing BCA establishment on the crop through directed biofilm or biosurfactant production could allow for a decreased reliance on chemical pesticides. Thus, BCAs have the potential to be a game changer in integrated pest management practices (Galli et al., 2024). This is important from an environmental perspective and due to the increased need for fresh produce globally. In 2022, 132 kg of fresh fruit and vegetables were discarded per person within the EU, where 7.6% was lost in production, most often due to plant diseases (EUROSTAT, 2024).

Investigating how the combination of light and limited nutrients, which have the potential to affect BCA mechanisms, could allow for improved crop protection strategies. This could be vital in addressing the global food security challenges that are to come, especially as the global population is projected to keep increasing, peaking at approximately 10.3 billion in the mid-2080s (UN, 2024).

2. Background

2.1 Light

Light can be defined and measured in an array of ways. Be it in the anthropocentric way of lumen, lm, which is a measure of the perceived power of visible light produced by a light source, or as irradiance, which is measured in W m^{-2} (Alsanius et al., 2024; Bell & Rose, 1981). Thimijan and Heins (1983) provided conversion constants and procedures to interconvert photometric, radiometric, and quantum light units for sunlight and other electrical light sources used within horticulture. Depending on the light source and the wavelength of interest, luminous flux (lx) could for example be converted into irradiance (W m^{-2}).

When discussing light within the plant community, light is usually defined as photosynthetically active radiation (PAR). It is measured in photosynthetic photon flux density (PPFD) stated in µmol m^{-2} s^{-1} and is based on the photosynthetically active spectrum of 400 – 700 nm utilised by plants (McCree, 1972). Similarly, light intensity, can also be stated in µmol m^{-2} s^{-1}. Within plant studies, the light environment is usually defined using PPFD and irradiance (W m^{-2}).

When comparing the amount of both photons (m^{-2} s^{-1} nm^{-1}) and the amount of irradiance (W m^{-2}) produced by the sun, it is clear that the photon flux curve peaks at approximately 700 nm, whereas the energy output peaks at 500 nm (Figure 1). Both curves offer insights into how the solar spectrum can be measured using different units, resulting in similarities and differences.

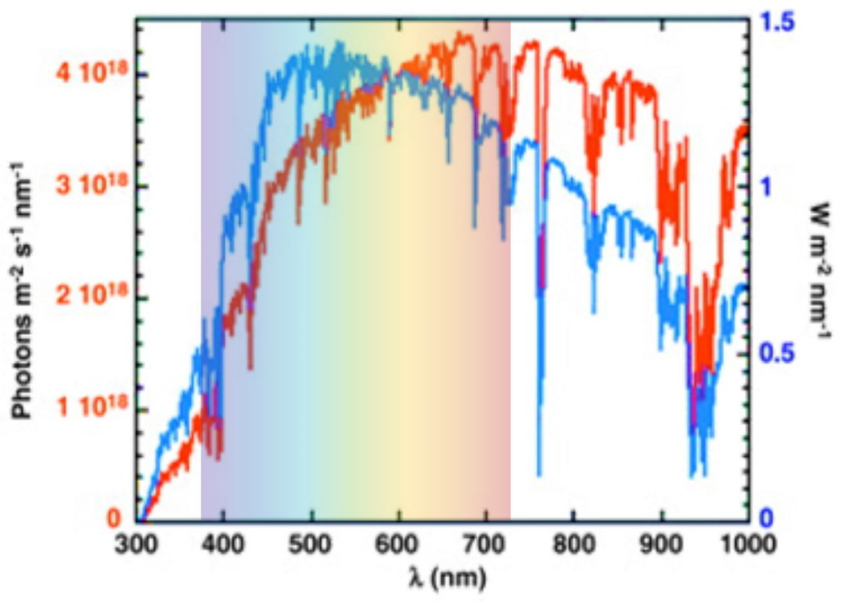

Figure 1: Solar spectrum where the blue curve is the energy output spectrum and the red curve is the photon flux spectrum. Adapted from Chen and Blankenship (2011) using Biorender.com.

2.1.1 Light and exposure dose

The impact of exposure time and wavelength, resulting in different energy levels, i.e. exposure dose (Paper I, Alsanius et al. (2024)), is not always considered in microbial and plant studies. Due to the inverse relationship between photon energy and wavelength, as noted in Planck's law (described further down), shorter wavelength photons require less time to supply a given energy when compared to longer wavelength photons (Chen & Blankenship, 2011). Apparent differences can be noted in the total exposure dose, when exposure time and wavelength are considered. This is prominent when comparing the total energy of a blue light source at 420 nm and a red light source at 660 nm (Figure 2). These differences could also be calculated. In the other example below when comparing a blue light source at 420 nm at an intensity of 100 µmol m^{-2} s^{-1}, with a red light source at 660 nm a 36.2% higher energy transfer in J m^{-2} s^{-1} would occur.

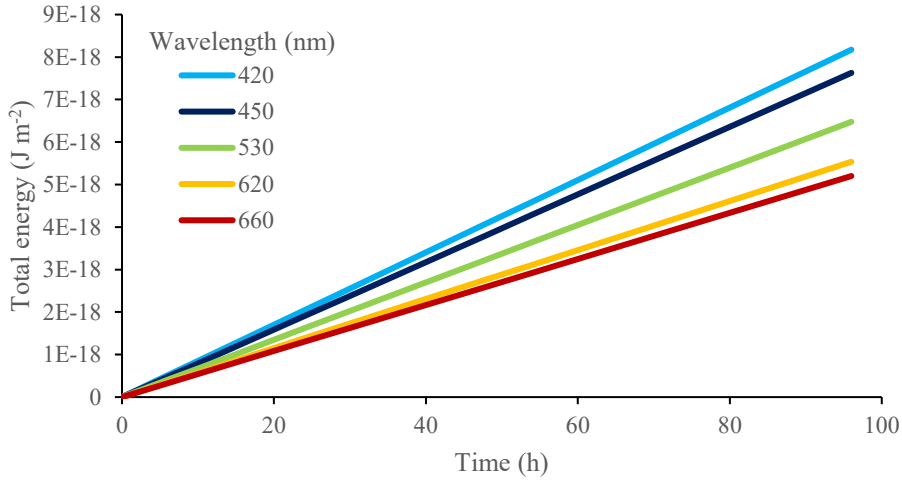

Figure 2: Exposure doses for the five wavelengths across the phenotypic microarray 96 h light experiments all at a light intensity of 50 µmol m^{-2} s^{-1} (Source Paper IV).

Equation used to calculate exposure dose:

To calculate the energy output of a light source with either the wavelength of 420 or 660 nm at an intensity of 100 µmol m^{-2} s^{-1}, Plank's law could initially be used:

$$E = \frac{hc}{\lambda}$$

E is the energy of the photon in joules

h is Planck's constant (approx. 6.62607015×10^{-34} J s)

c is the speed of light (approx. 299792458 m s^{-1}) in a vacuum

λ is the wavelength of the light in meters

Energy (J) of one photon of 660 nm: $E_{660} = 3.01 \times 10^{-19}$ J

Energy (J) of one photon of 420 nm: $E_{420} = 4.72 \times 10^{-19}$ J

If the intensity is set at 100 μmol m^{-2} s^{-1} = 6.022 x 10^{19} photons m^{-2} s^{-1}
Total energy output = E_{photon} (J) x Photons (m^{-2} s^{-1})

For a 660 nm lamp at 100 μmol m^{-2} s^{-1} = 3.01 x 6.022 = 18.13 J m^{-2} s^{-1}

For a 420 nm lamp at 100 μmol m^{-2} s^{-1} = 4.72 x 6.022 = 28.42 J m^{-2} s^{-1}

2.1.2 Photosensory proteins - sensing in microbes and plants

Plants can sense the direction and duration of a lighting incident. They can sense the quality and quantity of light emitted and use it as a signal for optimised growth and development based on the ambient light conditions throughout their life cycle (Batschauer, 1998). Microorganisms can similarly sense light even if they may not all be phototrophic. Plants and microorganisms can respond to light due to possessing a plethora of photosensory receptors, some of which they share (Table 1) (Batschauer, 1998; Mandalari et al., 2013). In non-phototrophic bacteria, photosensory receptors can affect functions in environmental adaptation, growth and development (Alsanius et al., 2019; Möglich et al., 2010). They can be classified into six families according to the chemical structure of their chromophores. They are BLUF (Blue-Light sensing Using Flavin) and LOV (Light, Oxygen and Voltage) proteins, cryptochromes, phytochromes, rhodopsins and xanthopsins (van der Horst et al., 2007).

Plants are known to use light as an energy source to photosynthesise. Phototrophic microorganisms such as purple bacteria, which possess photoactive yellow proteins (PYP), can also convert light into energy (Imamoto & Kataoka, 2007). In the case of non-phototrophic microorganisms, light could be viewed as a stressor able to invoke positive or negative metabolic reactions, such as switching from a sessile lifestyle to a motile one (Shah et al., 2016; Wilde & Mullineaux, 2017; Wu et al., 2013). Light has also been found to induce phenotypic placidity in several non-phototrophic species (Alsanius et al., 2021; Fessia et al., 2024; Gharaie et al., 2017).

Table 1: A compilation of plants, non-phototrophic bacteria and non-phototrophic fungal pathogens with known photosensory proteins. The photosensory protein acronyms are as follows: Blue-Light sensing Using Flavin (BLUF), bacteriophytochrome (BphP), cryptochrome/photolyase (Cry/PHR), Light, Oxygen and Voltage (LOV), phytochrome (PHY) and rhodopsin (Rho). The X does not represent the number of genes encoding the specified photosensory proteins.

Species	**Photosensory proteins**					
	BLUF	**BphP**	**Cry/ PHR**	**LOV**	**PHY**	**Rho**
Plants						
Arabidopsis thaliana			X		X	
Solanum lycopersicum			X		X	
Bacteria						
Bacillus spp.				X		
Pseudomonas spp.		X	X	X	X	
Streptomyces spp.			X			
Xanthomonas spp.	X			X	X	
Fungi						
Botrytis cinerea		X	X	X	X	X

References: (Batschauer, 1998; Carrau et al., 2023; Giliberto et al., 2005; Hatfield et al., 2023; Hauser et al., 1997; Kobayashi et al., 1989; Losi & Gärtner, 2021; Moyano et al., 2020; Schumacher, 2017; Wu et al., 2013; Yu et al., 2010)

Several photoreceptors such as cryptochrome/photolyase (Cry/PHR), BLUF and LOV proteins (Wilde & Mullineaux, 2017) are affected by wavelengths in the blue spectrum, 380 – 500 nm. Microbial rhodopsins can exhibit a wide range of absorption maxima from approximately 480 to 600 nm (Ernst et al., 2014). Phytochromes (PHY) absorb light in the red to far-red spectrum (Wilde & Mullineaux, 2017).

In some cases, light could be detrimental to the organism, for example, *B. cinerea* has been shown to have repressed conidiation when exposed to blue light (Imada et al., 2014; Schumacher, 2017). Another foliar pathogen, powdery mildew, could have reduced sporulation, inhibited germination, infection and colony expansion under UV-B and 630-690 nm treatments (Suthaparan & Stensvand, 2024; Suthaparan et al., 2014). Little is though known, about how visible light can affect the lifestyle choices of non-phototrophic microorganisms, such as introduced BCAs in the phyllosphere (Alsanius et al., 2019).

2.2 Greenhouse production

There are many advantages to protected cultivation using greenhouses, polytunnels or control environment agriculture (CEA). The extended growing season, increased productivity, better resource efficiency and disease management are some advantages (Geilfus, 2019; Gruda & Tanny, 2014; Wittwer & Castilla, 1995). By regulating the light, humidity, temperature and nutrient supply, crops can be grown based on their optimal needs, resulting in high crop quality and uniformity (Geilfus, 2019; Gruda & Tanny, 2014).

Large portions of vegetables and ornamental crops are currently produced in greenhouses. In 2023, nearly 80% of all cherry and grape tomatoes imported to the US were produced in greenhouses (Davis et al., 2024). The global horticultural market value has been evaluated at 35.6 billion US dollars for 2023 and is forecasted to grow by another 9% by 2032 (*Global Greenhouse Horticulture Market Size (2024-2032)*, 2024).

2.2.1 Lighting technology in greenhouse systems

Greenhouse systems have several different technological aspects, but one of the greatest advantages of growing crops in a greenhouse is that the light can be steered (Bergstrand et al., 2017; Pennisi et al., 2020; Schipper et al.,

2023). This is because complimentary lighting may be necessary due to natural limitations, as in the case of northern climates (Modarelli et al., 2022; Nemali, 2022). Two leading lighting technologies in greenhouses are light emitting diodes (LED) and high pressure sodium (HPS) lamps. While LEDs are more energy efficient, HPS lamps produce more heat, which could be beneficial when used in colder environments (Bergstrand et al., 2015). The HPS lamps could influence the greenhouse climate due to their higher output of photosynthetic active radiation (PAR) and near-infrared radiation (NIR). This could therefore affect the thermal convection exchange with the air, and higher operating temperatures allow for an increased emission of far infrared radiation (FIR) (Bergstrand et al., 2017; Katzin et al., 2020). In contrast to HPS lamps, light spectrum can be easily manipulated in LED lamps. Although this may allow for an enhanced adaptation of the light environment concerning crop needs, growers seem reluctant to abandon HPS based lighting due to higher initial costs associated with LED light installations (Nelson & Bugbee, 2014).

2.2.2 Common pathogens

Due to the intensive nature of greenhouse cropping systems and the microclimatic conditions present, there is an increased susceptibility to fungal diseases (both fungal-like organisms and true fungi) (Bardin & Gullino, 2020; Jarvis, 1989). Two of the most common pathogens found in greenhouse settings are grey mould (*Botrytis cinerea*) and powdery mildew, an obligate pathogen (Bardin & Gullino, 2020; Elad et al., 1999; Tronsmo et al., 2020). Grey mould can occur wherever tomato is grown and is a major disease in greenhouse systems (Jones et al., 2014). They have a wide host range and can cause disease in both edible and ornamental plants of high value (Daughtrey & Buitenhuis, 2020; Gard & Gilardi, 2020). *B. cinerea* is usually considered a weak pathogen, but due to being easily spread by conidia and being an efficient saprophyte, it only requires a short period of high humidity to germinate (Jones et al., 2014).

Cultural methods such as climate control within the greenhouse could be used to control the spread of these pathogens to some degree (Kruidhof & Elmer, 2020). This could be difficult at times as the pathogen could have similar needs to the plants. In the case of *B. cinerea*, the humid climate within a tomato canopy at night is adequate for disease development (Jones et al., 2014). *Oidium neolycopersici*, one of the casual agents of powdery mildew

in tomatoes has a conidial germination temperature range from 10-35 °C and can infect with little free moisture under high humidity (Jones et al., 2014). Due to legal restrictions on crops with continuous harvest, such as tomato and cucumber, options for chemical control are limited. What could be beneficial for greenhouse horticulture is the improved efficiency of microbial BCAs.

2.3 Biological control agents

Biological control agents (BCAs) are living organisms that can control and suppress pathogens (Stenberg et al., 2021; Tariq et al., 2020). Some known BCAs include parasitoids, bacteria and fungi (van Lenteren et al., 2020). Bacterial BCAs have shown promising plant health promoting and protection results in greenhouse settings, such as *Bacillus amyloliquefaciens*, which is both a biostimulant and a BCA (Luo et al., 2022; Salvatierra-Martinez et al., 2018).

Microbial BCAs can have an array of modes of action (Legein et al., 2020). These can be divided into direct or indirect mechanisms (Figure 3). Direct modes of antagonism require that the BCAs and the plant pathogens occupy the same niches (Sylla, 2013). Indirect modes of action can be driven by induced reactions within the host as a consequence of interacting with the microorganism. One of the most important direct modes of action that may control a plant pathogen is that of competition. Competitiveness of the introduced BCA in the new habitat is a main requirement in order to successfully combat pathogens. Competition for resources and space may allow BCAs to limit or inhibit pathogen establishment (Kinkel & Lindow, 1997). Similarly as important is antibiosis, e.g. the production of antimicrobial metabolites, as noted in the case of several *Pseudomonas* spp., one of these is phenazine-1-carboxylic acid produced by *P. chlororaphis* (Chi et al., 2024; Milner et al., 1997). Another example is *Streptomyces griseoviridis*, which can express hyperparasitism (Authority et al., 2020; Warsito & Kusumawati, 2020). Quorum sensing and quenching is another form of direct disease suppression whereby the BCA can directly affect a pathogen by modulating the pathogen's behaviour (Legein et al., 2020). Other direct modes of action include the production of biosurfactants, which are compounds that reduce surface and interfacial tension, and biofilm formation, which allows for the adaption and survival of microorganisms in

fluctuating environments (Chew & Yang, 2016; Costa et al., 2018; Dunne, 2002). The production of microbial volatile organic compounds (VOCs) (Weisskopf et al., 2021), is also another mode of action.

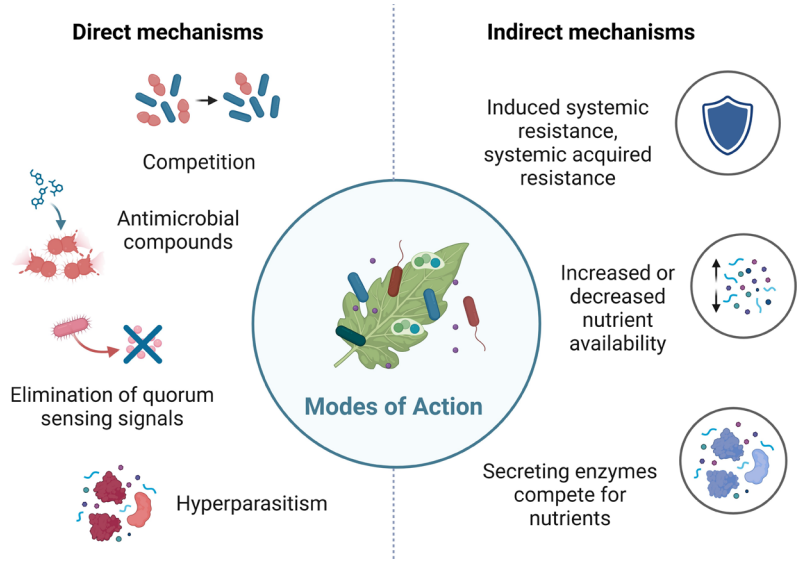

Figure 3. Examples of direct and indirect modes of action that can either be caused or used by bacterial biological control agents *(Illustration: M. Hellström, using BioRender.com).*

The main indirect mode of action is induced systemic resistance in the plant, which includes the production of precursors for plant hormones or other substances that can control phytopathogens. In many cases, BCAs are often equipped with multiple modes of action, an example being *Bacillus amyloliquefaciens*, which is capable of producing secondary metabolites and inducing systemic responses (Beris et al., 2018; Li et al., 2015). All modes of actions employed by the three BCAs used in the thesis can be found further down in Table 2, segment 4.1 Biological control agents.

2.4 Phyllosphere and the phylloplane

The term phyllosphere was first coined by Last (1955) and Ruinen (1956). The etymology of the word comes from the Ancient Greek words "φύλλον" meaning leaf, and "σφαῖρα" meaning sphere. Specifically, the leaf surface can be defined as the phylloplane (Andrews & Harris, 2000; Lindow &

Brandl, 2003). “A plant does not live alone but rather as an assemblage of interacting organisms” (Andrews & Harris, 2000).

The phyllosphere is often described as an unwelcoming environment, prone to fluctuations of several abiotic factors such as temperature, humidity, light (wavelength) intensity and exposure, all of which can affect the microbial community composition and function in the phyllosphere (Andrews & Harris, 2000; Juniper, 1991; Lindow & Brandl, 2003; Thapa & Prasanna, 2018). This microbial habitat could have a great effect on how well the introduction of BCAs goes. Successful BCA establishment could be explained through different ecological concepts taking plant architecture and leaf morphology into account. The concepts that pertain most for this thesis will be discussed below.

2.4.1 Ecological concepts in the phyllosphere

Several ecological concepts can affect the function and structure of native or introduced microorganisms in the phyllosphere. At any given moment, there is a constant shift in the relative abundance of taxa within the microbial communities in the phyllosphere (Koskella, 2020). This is because of intra- and inter-species interactions occurring across ecological and evolutional timescales. One main driver of these changes could be defined as competitive exclusion (Grime, 1973), as several species compete for the limited space and nutrients at all times, due to the diverse nature of the phyllosphere microbiome. Leaves have been found to have a carrying capacity of 10^7 individual cells per cm^2 and therefore competition is prone to occur (Remus-Emsermann & Vorholt, 2014). This competition will catalyse reactions and cause the organisms with the greatest phenotypic plasticity to outcompete the others (Turcotte & Levine, 2016).

Microorganisms differ in capacity to utilise nutrients, as depicted by the resource saturation limitation theory (Goelzer & Fromion, 2011), e.g., if they are an *r*- or *K*-strategists (Andrews & Harris, 1986; Pettersen et al., 2021). *r*-strategists use readily available nutrients, have a shorter life span, proliferate in uncrowded conditions and have an initially high population density under crowded conditions to compensate for their high mortality (Andrews & Harris, 1986). On the other hand, *K*-strategists survive under nutrient deficient conditions; have a long and low growth phase, especially in uncrowded conditions. The population density dynamics of *K*-strategists

tend to be of high equilibrium due to their competitive nature. They are more tolerant of inhibitory chemicals than *r*-strategists.

Microbial invasion is an ecological concept that involves an intruder entering an unfamiliar environment, establishing itself in it and later dispersing into another environment if a new nutrient pool becomes available (Mallon et al., 2015). Invasion of any alien microorganisms, including BCAs, not only comes along with intrusion of an already inhabited environment but also causes a disturbance by competing for both space and resources. The phyllosphere comprises of microhabitats (Leveau, 2019; Leveau & Lindow, 2001) and one leaf (the phylloplane) could be viewed as a lone island. Similarly, as noted in the invasion concept the theory of island biogeography is determined by three processes: immigration, evolution, and extinction (Whittaker et al., 2017). In both contexts, the newly introduced BCAs must successfully establish and reproduce before dispersing.

Due to the variability of the phyllosphere microbiota, several niches could occur in the phylloplane and as such lead to niche partitioning and realisation. Niche partitioning or differentiation is a form of coexistence that is driven by a lower interspecific nutrient competition when compared to the higher intraspecific competition (Griffin & Silliman, 2011). This is as the species can consume different forms of a limiting resource or use the same limiting resource at different times. What limits the population growth of a species is the species itself, within niche partitioning, and it thus "acts to promote the long-term coexistence of competing species" (Griffin & Silliman, 2011).

When it comes to the introduction of BCAs in the phyllosphere they do not necessarily get to choose their niche but in order to survive, they need to conform to the niches present before constructing one. Niche conformance is when an organism adjusts their phenotype in a given environment to match its surroundings, thus enhancing the organism's fitness (Müller et al., 2020). Niche construction is the process where the environment is modified by the organism and influences one or several selection pressures of another organism, which could be beneficial from the perspective of an introduced BCA in the phyllosphere.

In most cases, there is an overlap in ecological concepts, and as such, several could be present in any given system.

2.4.2 Crop stand, leaf types – plant architecture

One of the largest hurdles when introducing microorganisms to the phyllosphere of a crop is that of the topography of the leaf itself (Andrews, 1992; Mechaber et al., 1996). The topography of a leaf refers to leaf contours. This is especially true as a leaf is not a homogenous habitat (Remus-Emsermann & Vorholt, 2014). Vast differences have been noted in the topography of older leaves when compared to young leaves. Older leaf surfaces tend to have less regular patterns of surface morphology and lack broad elevated surfaces. In contrast, young leaf surfaces are notably characterised by a pattern of plateaus with elevation differences (Mechaber et al., 1996). The age of the leaf could determine how well an introduced microorganism in the phyllosphere establishes itself. *Escherichia coli* O157:H7 and *Salmonella enterica* have been found to have an increased ability to colonise young leaves of romaine lettuce when compared to leaves from the middle of the crop are older (Brandl & Amundson, 2008).

Due to a leaf's vastness from a microorganism's perspective, several microhabitats can occur within it (Leveau & Lindow, 2001). These microhabitats can experience fluctuations in irradiation and temperatures, in the availability of nutrients and the competition from native microbiota (Lindow & Brandl, 2003; Vorholt, 2012). The organism's ability to adapt, attach and use the available nutrients, irrespective of the leaf morphology, is fundamental for the successful establishment of microorganisms in the phyllosphere. The introduced microorganisms need to adapt to the number and position of stomata, the density of the veins and trichomes, and the cuticle presence of hydathodes (Andrews, 1992). When entering the phyllosphere, the introduced microbial cell or spore is first met by the cuticle, a protective hydrophobic layer, the aerial epidermis of all land plants (Yeats & Rose, 2013), which may hinder the introduction. The introduced BCAs must be able to adapt to the plant surface's physical and chemical environment. Schreiber et al. (2005) found that epiphytic bacteria such as *Pseudomonas graminis* were found to alter leaf surface permeability due to biosurfactant production that resulted in reduced barrier properties of waxy leaves of *Hedera* and rough *Prunus* leaves. Trichome density has been associated with a higher bacterial diversity when studied on tomatoes (Kusstatscher et al., 2020).

Another plant architectural aspect that could affect the introduction of microorganisms in the phyllosphere is the leaf placement within the canopy.

In the case of a trellising crop such as tomato, there will be a large difference in the irradiation gradient within the canopy. Top leaves will experience higher light exposures that could affect the overall photosynthetic rate and thus affect the temperature within the canopy. These temperature differences could be partially due to plant density or the use of either LED or HPS lamps (Kim et al., 2019; Westreenen et al., 2020). The light environment will differ greatly within the canopy due to absorption, reception and scattering (Schipper et al., 2023). Differences could also occur within the leaf due to the reflection occurring in the intercellular air pockets and the absorption in the chlorophylls and carotenoids (Vogelmann & Gorton, 2014).

2.5 Challenges of biological control agents in the phyllosphere

When introducing bacterial BCAs in the canopies of greenhouse crops, several challenges must be overcome. As previously mentioned, the phyllosphere is rather heterogeneous, especially compared to the rhizosphere (Andrews, 1992; Remus-Emsermann & Vorholt, 2014). Similarly, to when applied to the rhizosphere, BCAs in the phyllosphere need to compete with the native microbiota, find and realise a niche in order to survive. Leaf morphology, e.g., cuticle thickness, number of stomata and trichomes, may have an effect on BCA establishment. Morphologically, leaves can vary immensely between species, as in the case of begonias (waxy) when compared to tomato (trichome dense, non-waxy) (Gausman & Allen, 1973).

Due to the nature of the topography of the leaves microhabitats can occur. Differences in these microhabitats could lead to local leaf surface wettability differences, which could affect BCA spray effectiveness by determining droplet adhesion or spread (Mechaber et al., 1996). These microhabitats could be crucial in how well the introduced BCAs survive in the phyllosphere.

Environmental parameters such as temperature have been found to be drivers of the population dynamics of arthropods, bacteria and fungi (Kruidhof & Elmer, 2020). Choosing BCAs that can adapt and survive within the canopy of a crop in a greenhouse setting, irrespective of the temperature gradient, may be challenging. Environmental factors like light, humidity, and temperature are often steered within greenhouse production based on optimal plant growth parameters (Huché-Thélier et al., 2016). These parameters are

often assumed to be optimal for introduced BCAs. Despite the decisive role of light in greenhouse production, little is known about the effect of visible light on the lifestyle choices of microorganisms in the plant environment (Alsanius et al., 2019). The use of light when introducing BCAs to the phyllosphere could be key in overcoming challenges that are associated with their introduction.

3. Aims and objectives

Against the mentioned background, the primary aim of this thesis was to study the effect of visible light on non-phototrophic biological control agents (BCAs), *in-vitro* and *in-vivo*. The research focussed on determining if light distribution and exposure dose could affect the establishment of the BCAs in the canopy of greenhouse grown crops. It was also to study if sole carbon utilisation of non-phototrophic BCAs could be affected by different light treatments.

The main questions of the thesis were:

a) Could BCA establishment be promoted by modifying the light environment?
b) Does the addition of a sole carbon source in combination with light aid the introduction?
c) How does the combination of light and sole carbon source affect BCAs' lifestyle decisions?

This was further divided into several objectives to:

- Examine if different leaf types could affect BCA establishment in two greenhouse crops under white light
- Disentangle the realities of plants and microorganisms in the phyllosphere with respect to the light environment (Paper I)
- Determine if different light treatments and intensities created within a canopy can affect BCA establishment in a greenhouse crop (Paper II)
- Determine if exposure dose can impact BCA establishment in a greenhouse crop (Paper I and II)

- Investigate how the sole carbon utilisation of the three BCAs is affected by light treatments (Paper III)
- Explore if there are any ecological implications for *P. chlororaphis* when exposed to minimal medium conditions and varying light treatments (Paper IV)
- Examine what different exposure doses, minimal medium conditions and light treatments do to *P. chlororaphis* when applied on detached leaves (Paper IV)

4. Materials and methods

4.1 Biological control agents

Information about the biological control agents (BCAs) used in this thesis can be found in Table 2. All BCAs were equipped with spontaneous antibiotic resistance after pre-culturing on agar plates containing lower doses of antibiotics over time. All isolates were stored as cryocultures at -80 °C. In preparation for any experiment, the BCAs were pre-cultured on full-strength tryptic soy agar (TSA; BD 236950, Becton, Dickinson & Company Sparks, USA) plates and grown as stated in Table 2.

Table 2: Information about the biological control agents (BCA) used in the thesis: Species, strains, specific antibiotic resistance, growing conditions, commercial products currently approved in Sweden and its use.

Species	***Bacillus amyloliquefaciens***	***Pseudomonas chlororaphis***	***Streptomyces griseoviridis***
Strain	DSM7	50083	CBS904.68
Antibiotic resistance	Streptomycin 100 µg mL^{-1}	Ampicillin 100 µg mL^{-1}	
Growing conditions	25 °C for 48 h, with no shaker	25 °C for 24 h, set on 200 rpm shaking when in broth	
Growing medium	Full-strength tryptic soy broth (TSB) or tryptic soy agar (TSA) supplemented with the respective antibiotic		

Commercial products	Taegro®	Cedomon®	Mycostop®
Registered use of the commercial products	Against grey mould and powdery mildew diseases in greenhouse vegetable and strawberry crops, fungal diseases in vineyards	For seed priming against fungal diseases	Either as seed priming or foliar application against fungal diseases in greenhouse grown herbs, vegetables or ornamentals
Modes of action	• Secondary metabolites and antibiotics e.g. bacillomycin D, surfactins, acetoin • Competition • Induced systemic resistance	• Secretion of secondary metabolites and antibiotics e.g proteases, siderophores, phenazine, N-acyl homoserine lactones	• Root colonisation • Hyperparasitism; produces chitin and glucanas • Antibiotic production e.g. streptomycin • Secondary metabolites e.g. heptaene polyene

References used for the modes of action: Arrebola et al. (2019), Authority et al. (2020), Chi et al. (2024), Chowdhury et al. (2015), Dimopoulou et al. (2019), Lahdenperä et al. (1991), Luo et al. (2022), Puopolo et al. (2011).

4.2 Experimental systems and plant material

All experiments were conducted in the Vegetum greenhouses (Paper II and IV) or in the biosafety level (BSL) 2 laboratory (Paper III and IV) in Alnarp, at the Swedish University of Agricultural Sciences (SLU).

Table 3: Overview of the different systems, experiment/paper, biological control agents (BCAs) and lighting treatments used in the thesis.

System	Experiment/ Paper	BCAs	Light treatments							
			Dark	**400 nm**	**420 nm**	**450/ 460 nm**	**530 nm**	**600 nm**	**660 nm**	**White light**
Greenhouse (*in-vivo*)	Begonia and tomato	*								*
Greenhouse (*in-vivo*)	Large-scale tomato (II)	*			*		*		*	*
Laboratory (*in-vitro*)	Omnilog and KEGG analysis (III & IV)	*	*		*	*	*	*	*	
Greenhouse (*in-vivo*)	Detached leaves (IV)	*	*		*		*		*	*
Greenhouse (*in-vivo*)	Poinsettia (IV)			*	*	*	*	*	*	*

An overview of all the light combinations and systems can be found in Table 3 and a list with information about the plant material can be found in Table 4. Due to the removal of the tomato cultivar 'Picolino F_1' from the market, a switch to the commercial cultivar 'Cappricia^{RZ} F_1' was made.

Table 4: Species of plant materials used, why they were chosen and their origin.

Plant material	Chosen because of:	Origin
Tomato *(Solanum lycopersicum* L.) cv. 'Picolino F_1'	• Highly susceptible to grey mould (*Botrytis cinerea*) • High number of trichomes	Seeds were purchased from Olssons Frö AB
Tomato *(Solanum lycopersicum* L.) cv. 'Cappricia^{RZ} F_1'	• Commercial cultivar that is susceptible to grey mould (*Botrytis cinerea*) • High number of trichomes	Seeds were purchased from Semenco AB
Begonia (*Begonia* × *hiemalis*) cv. 'Rebecca' and 'Blitz'	• Cuticle properties (waxy) • Low number of trichomes	Purchased as rooted cuttings from K.E. Petterssons nurseries, Helsingborg, Sweden
Poinsettia (*Euphorbia pulcerima*)	• Compact crop architecture that aided the study of light distribution in the canopy	Purchased from Plantagen AB, Lund, Sweden

All tomato plants were grown from seed in 96-well plug trays in growing medium (K-jord, Hasselfors Garden, Sweden) in the university greenhouses. The temperature was set to 22 ℃ ± 2 ℃, with the ventilation onset set to 25 ℃ and a relative humidity of 60%. The seedlings were re-potted at a density of one plant per pot (3.375 L) in the same growing medium that was supplemented with fertilizer (5g L^{-1})(Basacote® Plus 3M 16-8-12(+2+TE), Compo Expert, Germany). Similarly, the rooted begonia seedlings were also re-potted in the same growing medium containing the added fertiliser.

All crops used throughout the thesis were exposed to a photoperiod of 14 h under high pressure sodium lamps (HPS lamps, Philips Greenpower 400 W, Philips, Eindhoven, Netherlands) until they were to be used for an experiment. For the specific mono- and polychromatic light treatments LED lamps (10.5 – 390 W) (Heliospectra AB, Gothenburg, Sweden) were used at an intensity of 50 µmol m^{-2} s^{-1}. Plants were watered as required.

Effect of leaf morphology a comparison between Begonias and Tomatoes

In a small-scale experiment, the three BCAs were applied by foliar application to two begonia cultivars (Table 3) and to one tomato cv. 'Picolino F_1'. The BCAs were prepared as described in Paper II. The bacterial densities were set to log 8 CFU mL^{-1} in 0.085% NaCl. The BCAs were applied using hand-held spray bottles until runoff from leaves was noted, as described in Wilson and Lindow (1992). The plants were left to dry for 10 min before being placed under the full spectrum lighting.

Several leaves per plant, three replicates per measurement, were harvested after 2, 4, 6 and 24 hours post inoculation (hpi). Leaves were weighed and then macerated (Smasher; bioMérieux, Inc., 100 Rodolphe Street, Durham, NC 27712, U.S.A.). The tomato samples were macerated for 30 s, and the begonia samples were macerated for 60 s, all in 50 mL Tris-buffer (0.01M, pH 8, Tris(hydroxymethyl)aminomethane hydrochloride, Merck, Germany). The samples were enumerated by viable counts.

Large greenhouse experiment (Paper II)

In paper II, 65-day-old tomato plants were exposed to four distinct light treatments (420, 530 or 660 nm and white light) once they had reached phenological stage 6, based on the BBCH scale (Feller et al., 2001). Plants were placed at a density of approximately 20 plants m^{-2}, with edge plants surrounding the entire perimeter. The bacterial inoculum was prepared as described in Paper II, Hellström et al. (2024). The bacterial inoculums were sprayed (Figure 4) until runoff was observed and left to dry for 10 min.

Leaves were harvested from two placements within the canopy at 0, 4, 8, 12, 24 and 48 hpi after being exposed to one wavelength at a light intensity of 50 µmol m^{-2} s^{-1}. Six replicates were harvested per measurement and placement in the canopy. The leaf samples were macerated for 30 s, all in 50 mL of 0.1 M TRIS buffer. The samples were enumerated via viable counts and digital droplet PCR (ddPCR), which will be discussed further down. The experiments were conducted during the course of one year. Blackout screens were used throughout the 48-hour light treatments to shield from any natural sunlight.

Figure 4: Application of BCAs by Maria Karlsson, to the phyllosphere of tomato plants prior to a light treatment *(Photograph: M. Hellström).*

Carbon source utilisation (Paper III and IV)

Phenotypic microarray (PM) was used to study the effect of light on sole carbon utilisation of the three BCAs, based on procedures described by Gharaie et al. (2017) and Alsanius et al. (2021). Two sole carbon source panels: PM1 and PM2, with a total of 190 sole carbon sources, with six replicates, were used per BCA and light treatment. The plates were prepared as described in Paper III, Karlsson et al. (2023). The panels were exposed to a total of six light treatments (Table 2) and were exposed over the course of 96 h at an intensity of 50 µmol m^{-2} s^{-1}, per light treatment. The sole carbon utilisation was quantified as the colour change of tetrazolium blue due to respiration. It was measured within the OmniLog incubator (OmniLog, catalog number 93182, Biolog Inc., United States), using the internally controlled camera system. The PM panels under the dark treatment were kept in the incubator throughout the experiment and monitored every fifteen minutes. The PM panels that were light treated were physically moved from under their respective lamps for the readings that occurred at 0, 6, 10, 24, 30, 48, 54, 72 and 96 hpi. All output values were expressed in OmniLog units.

Detached leaf assays (Paper IV)

To study the effect of the addition of a sole carbon source, exposure dose and light quality, detached leaf assays were used (Figure 5). Tomatoes were grown as previously described, and young leaves were harvested high in the crop canopy on the same day as the experiments. *P. chlororaphis* was pre-cultured and suspended in four sole carbon sources (20mM D-arabinose, 20mM D-ribose, 20mM uridine, 20% glucose) selected based on the biofilm data acquired in Paper III and in sterile distilled water. The full procedure can be found in Paper IV.

Five light regimes were tested: darkness, white light, 420, 530 or 660 nm. The regimes involving the four light qualities were tested at two intensities: 50 or 100 µmol m^{-2} s^{-1}. The inoculated leaves were exposed to light for either 30, 60, 120 or 240 min.

Figure 5: Detached leaf assays used to detect if biofilm could occur on tomato leaves exposed to different light treatments. This was post application of *P. chlororaphis* in combination with sole-carbon sources. All drops were added randomly (*Photograph: M. Hellström)*.

Post light treatment the leaves were stained using a modified method described by Carrau et al. (2023). The leaves were immersed for 20 minutes in 0.1% crystal violet solution (Sigma-Aldrich Co., MO 63103, USA). Post

immersion leaves were washed carefully with distilled water and left to dry. Biofilm occurrence was scored using an arbitrary visual scale (Paper IV, Table S1).

Light spectrum distribution (Paper II and IV)

Spectral irradiance ($\mu W\ cm^{-2}\ nm^{-1}$) of the four light treatments (420, 530, 660 nm and white light) used in the Paper II, were measured at two placements in the tomato canopy, top tier and middle tier, using a JAZ spectrometer (Ocean Optics, USA).

Similarly, the spectral irradiance was also measured across 1 m^2 of 36 poinsettias (Figure 5) at three distinct heights. Measurements were taken across 12 points across the poinsettias (Paper IV, Figure S2b). More specifically measurements occurred at 0.22 m next to the plant, under the top leaf at 0.22 m, under the leaves at 0.15 m and under the leaves 0.11 m. The intensity of all tested wavelengths (Table 3; 400, 420, 450, 530, 600, 660 nm) was set at 50 $\mu mol\ m^{-2}\ s^{-1}$.

Figure 6: An example of one of the light treatments (660 nm) used to study the light distribution across and within the area of 36 poinsettias *(Photograph: M. Hellström)*.

4.3 Analyses

Detailed information about the plant and microbial analyses performed can be found in Papers II-IV.

4.3.1 Plant analyses

Non-destructive plant physiological responses to the several light treatments were conducted throughout Papers II and IV. Measurements included: photosystem efficiency and stomatal conductance (L.MAN-LCpro, ADC BioScientific Ltd., United Kingdom), chlorophyll content (PAM-2500 chlorophyll fluorometer, Heins Walz GmbH, Effeltrich, Germany). Destructive methods were used to quantify the leaf surface area (cm^2) of control plants in Paper II.

4.3.2 Culture dependent microbial analyses

Viable counts were used to enumerate the BCAs for all *in-vitro* applications (begonia and tomato experiment and Paper II). The plant samples were serially diluted in 0.85% NaCl and plated on the TSA plates containing the respective antibiotic (Table 3, only for Paper II). The drop plate method was used in Paper II, and viable counts were assessed as colony forming units per centimetre leaf area (Log CFU+1 cm^{-2}). In the case of the begonias and tomato experiment, viable counts were assessed as colony forming units per gram leaf fresh weight (Log CFU g^{-1}).

In paper III, biofilm formation was assessed as described by Alsanius et al. (2021), whereby the microbial suspension was removed from each well found in the PM plates, the plates were washed, and 100 µl of 1% crystal violet solution was added. After incubating for 15 min the plates were washed with distilled water and left to dry. The next day, 100 µl of 95% ethanol was added one hour before the spectrophotometric measurements at 550 nm (Expert 96TM spectrophotometer, AsysHiTech). Similarly, based on a method by Gharaie et al. (2017), biosurfactant production was determined using a drop collapse test.

4.3.3 Culture independent microbial analyses

For each measurement taken throughout the large greenhouse experiment, six samples for each light treatment and placement in the canopy were extracted for DNA and RNA analyses, as described in Paper II. The samples were analysed using digital droplet PCR (ddPCR) with their respective 16S rRNA primers (Paper II, Table 1).

4.3.4 Statistical analyses

Statistical analyses were performed primarily in R-Studio (R Core Team, 2021) (Papers II-IV).

In Paper II, a linear mixed model approach, LMM, 'lmer4' (Bates et al., 2015), was used with a random factor set as the plant to compensate for the dual measurements that occurred per plant (leaves were harvested at two placements). Several analyses of variance (ANOVA) were used to analyse the viable counts and the biofilm data in Papers II and III.

In Paper III, the carbon utilisation data was extracted using the OmniLog PM kinetic analysis software. The data was further analysed using R-Studio's 'opm' package (Vaas et al., 2013). The KEGG pathways were created with data extracted from Paper III using the 'BiobManager' packages (Morgan & Ramos, 2024). More detailed descriptions of the statistical analyses used can be found in each paper.

5. Selected results and discussion

Plants are often considered holobionts (Berg et al., 2017; Vandenkoornhuyse et al., 2015). Microbial communities residing in the phyllosphere have been found to mediate plant health, contribute to several ecosystem functions and increase resilience against biological and environmental stressors (Manikandan et al., 2024; Vacher et al., 2016). Limited studies have examined how visible light affects the native or introduced organisms such as BCAs in the phyllosphere of greenhouse grown crops. This is even though light is a decisive environmental factor affecting all living organisms (Tierney et al., 2017). This thesis aimed to decipher if light quality (wavelength), distribution and exposure dose could affect non-phototrophic BCAs. Also, if different wavelengths could invoke altered sole carbon utilisations in the three non-phototrophic microorganisms.

Based on the work conducted throughout this thesis, it was made evident that visible light, be it in a greenhouse or a laboratory setting, could affect the lifestyle choice of the non-phototrophic bacterial BCAs (Papers II-IV). This could be due to all three belonging to species with known photosensory proteins (Table 1). Moving forward, the focus will be answering the objectives in section 3.

5.1 Impact of leaf morphology

Leaf morphological properties could play a crucial role in the foliar application of bacterial BCAs. Leaf surfaces are not homogenous; differences can occur due to trichome and vein densities, the number and positions of stomata, cuticle structure, wax composition, and the presence of hydathodes (Andrews & Harris, 2000; Remus-Emsermann & Vorholt,

2014). In order to establish effectively, BCAs must be able to adapt to these variations. Phyllosphere bacteria can use various colonisation strategies such as leaf habitat modification, aggregation and ingression (Beattie & Lindow, 1999).

In the begonia and tomato experiment, large differences in re-isolation counts occurred for most BCAs under the white light treatment. Begonia leaves had lower numbers for all introduced BCAs after six hours post inoculation (hpi), whereas tomato leaves resulted in higher numbers for two of the three organisms (Figure 6). This could be due to the distinct differences in the two crops' leaf thicknesses and cuticle compositions (Gausman & Allen, 1973).

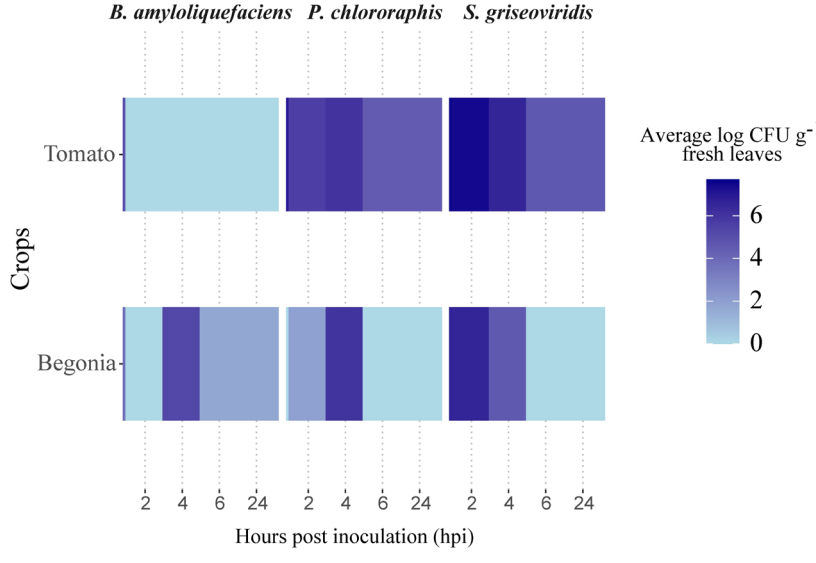

Figure 7: Average log colony forming units (CFU) g^{-1} fresh leaves of begonia and tomato post biological control agent (BCA) application

By visual comparison, it was noticeable that the begonia leaves were waxy with few trichomes when compared to the dull trichome-dense tomato leaves. The microscopic structure of the cuticle could have influenced BCA attachment. Waxy cuticles are often hydrophobic, functioning as a physical barrier and self-cleaning surface (Yeats & Rose, 2013), which could have deterred BCA attachment in begonias. A mechanism that could have aided BCA attachment could be biosurfactant production, which was noted when

the three BCAs were exposed to darkness and capric acid *in-vitro* (Table 1, Paper III). *S. griseoviridis* was the most prominent two hpi on both crops, though no colonies were re-isolated past six hpi on the begonia leaves.

Trichomes, on the other hand, may have provided refuge for the BCAs. The relatively thin leaf cuticle could have facilitated access to carbon sources essential for BCA survival in tomatoes. On the other hand, a dense trichome layer, like the cuticle, could act as a physical barrier, complicating BCA introduction and re-isolation. This could be the case for *B. amyloliquefaciens* as no colonies were re-isolated from tomato leaves. Trichomes can discourage attachment or trap BCAs, lowering bacterial plate counts. Tomato trichomes are predominantly glandular and secrete metabolites, often deterring pathogens, fungi, and herbivorous insects (Kortbeek et al., 2021). These findings raise intriguing questions about whether wetting agents, which reduce surface tension and counteract the hydrophobic effects of cuticle waxes, could enhance BCA establishment in the phyllosphere of waxy crops such as begonias.

5.2 Impact of light quality, exposure dose and placement in the canopy

The objective of Paper I; Alsanius et al. (2024), was to discuss if the realities of plants and microorganisms were the same concerning the light environment. It was identified that there is a discrepancy in how well the light environment is described with respect to bacteria and light. As previously mentioned, light can be an enabler or a stressor, depending on the organism. Plants, being phototrophic, require light as an energy source. Non-phototrophic bacteria and fungal pathogens can on the other hand, sense light via several photosensory proteins (Table 1) (Ohlendorf & Möglich, 2022). The primary finding of the opinion note was that accurate reporting of light parameters is crucial for reproducibility. Information that should be stated in bacteria-light interactions are light intensity, photoperiod, wavelength and exposure dose. Proper understanding and definitions of light measurement methods are vital for reliable scientific communication and clinical efficacy.

The impact of light quality, exposure dose and canopy placement were studied for three non-phototrophic BCAs in the phyllosphere of greenhouse grown tomato plants (Paper II). Several factors must align for BCA establishment when introduced into the harsh phyllosphere environment.

The newly introduced BCAs must exhibit strong invasive capabilities. According to the invasion concept, an invader should initially disperse, establish, grow and spread whilst expressing a mode of action against a pathogen (Alsanius et al., 2020; Mallon et al., 2015). Four traits are associated with increased invasion aptitudes: high growth rates, good dispersal capability, phenotypic plasticity and genetic diversity (Mallon et al., 2015). Since good dispersal is a trait of a successful invader, two of the three BCAs used (*B. amyloliquefaciens* and *S. griseoviridis*) belong to sporulating species (Daza et al., 1989; Galli et al., 2024), which could aid them when found in suboptimal environments. *Bacillus* spp. are known for their ability to produce long-lasting resistant endospores (Galli et al., 2024) and *Streptomyces* spp. produce over two-thirds of naturally occurring antibiotics (Bubici, 2018). Furthermore, *Pseudomonas* spp. are readily abundant in the phyllosphere of tomato and have several species and strains with antagonistic properties (Flores et al., 2023; Galli et al., 2024). In a study by Murillo-Roos et al. (2022), they found that when growing two *Pseudomonas* spp. as a pair, cooperative niche exploration was observed. If this were to occur in the phyllosphere when, introducing a BCA such as *P. chlororaphis* this could be most advantageous.

Different light qualities and intensities have been found to induce antibiotic formations, biosurfactant production, biofilm formation and increased virulence in non-phototrophic microorganisms (Alsanius et al., 2021; Bonomi et al., 2016; Kahl et al., 2022; Wu et al., 2013). Many of these modes of action could allow for increased BCA establishment and efficacy.

Our study found that exposure dose, light quality (wavelength) and light distribution within the canopy were decisive factors in how well the chosen BCAs were re-isolated from the phyllosphere of tomatoes. This was determined both via viable counts and through absolute quantification of cDNA and DNA by ddPCR, the latter allowed for the comparison between active and non-active bacterial cells. Different light quality preferences were observed for the three BCAs (Figure 8).

S. griseoviridis showed generalist behaviour by having the highest re-isolation counts under all light treatments (Figure 3, Paper II). This was irrespective of placement in the canopy for all but the 530 nm treatment, with a significant difference between the top and middle tier (Figure 4, Paper II). In high light intensities, green light has been found to penetrate deeper into the canopy, leading to more uniform light distribution within leaves,

potentially increasing photosynthetic efficiency (Liu & van Iersel, 2021). This uniformity in light distribution could possibly explain why all three BCAs thrived under the green light treatment (Figure 8). Phenotypic plasticity was noted for *P. chlororaphis* under the 530 nm treatment, as there was a substantial increase in the cDNA copies between 12 hpi and 48 hpi indicting that the organism adapted to the light environment (Figure 5b, Paper II).

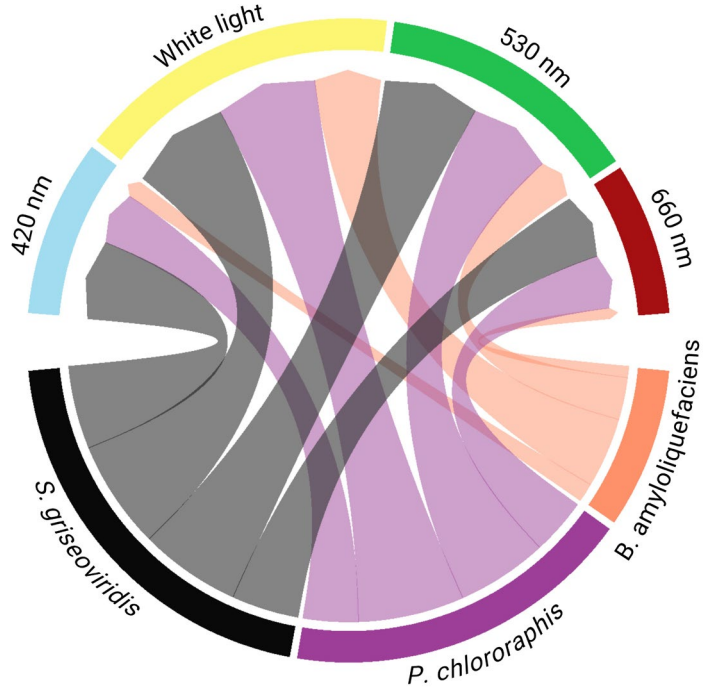

Figure 8: Average log CFU +1 cm^{-2} of the three biological control agents (BCAs; *S. griseoviridis*, *P. chlororaphis*, *B. amyloliquefaciens*) re-isolated from tomato leaves after 48 h of exposure to poly- (monochromatic (LED; blue: 420 nm, green: 530 nm, red: 660 nm).

All three organisms had the highest re-isolation counts under the white light treatment. Interestingly, the white light treatment used in the greenhouse experiments had distinct peaks in the blue spectrum (Figure 2 and Table S1, Paper II). The high re-isolation counts could be partially

explained due to the activation of photosensory proteins found in the blue spectrum, such as LOV and Cry/PHY proteins, which have been identified in *Bacillus*, *Pseudomonas* and *Streptomyces* species (Table 1). In order to determine if this is so, further transcriptomic analyses would be required.

The lowest combined re-isolation counts occurred under the 420 and the 660 nm treatments (Figure 8). This is unfortunate as red wavelengths have been found to suppress powdery mildews by suppressing conidia formation (Suthaparan & Stensvand, 2024) and *B. cinerea* has been shown to be affected negatively by 405 nm treatments (Imada et al., 2014). What could possibly help is the combination of a sole carbon source together with a blue or red wavelength that would induce biofilm formation in the BCA, as noted in the *in-vitro* experiments in Paper III, Karlsson et al. (2023). This would allow for a multipurpose light effect in a greenhouse setting.

Based on the results from Paper II no one recipe could be found even though the time of sampling and wavelength, exposure dose, did lead to significant statistical differences for all BCAs (Table 2, Paper II). A canopy effect (comparison of placement and light treatment) was observed for *P. chlororaphis* under the white light treatment and canopy effects occurred for *B. amyloliquefaciens* and *S. griseoviridis* under the 530 nm treatment. This indicated that exposure dose, wavelength and placement in the crop canopy could affect the establishment of bacterial BCAs in the phyllosphere of greenhouse grown tomato.

5.3 Impact of light quality under low nutrient availability

When introducing bacterial BCAs to the phyllosphere, they need to successfully compete for limited space and nutrients. Organic nutrient availability in the phyllosphere governs the ease in which microorganisms can colonise. Several factors can influence nutrient extrusion in the phyllosphere, such as temperature, UV radiation and the relative humidity (Leveau, 2019; Leveau, 2006). Microorganisms in the phyllosphere have been primarily found to utilise sugars diffused through the plant apoplast, though the cuticle often impedes this diffusion (Van Der Wal & Leveau, 2011). Within greenhouse production, light is often modified to suit the needs of the crops grown, leading to optimised photosynthetic activity, biomass production and plant architecture (Morrow, 2008). Kong et al. (2024) found that medium intensity of white light resulted in the highest

contents of leaf soluble sugars, cellulose, and free amino acids in lettuce. Light intensity also modulated the functional composition of the phyllosphere prokaryotic community. As light is a global regulator for both plants and microorganisms a focus on investigating what the different light qualities could induce in the non-phototrophic BCAs based on phenotypic microarray was used, Paper III. The results were later used to explore if there are any ecological implications for *P. chlororaphis* and if the combination of sole carbon sources and light could induce biofilm formation, Paper IV.

A total of 190 carbon sources were tested under six light treatments (at intensities of 50 µmol m^{-2} s^{-1}) for a total of 96 hours. The highest carbon utilisation based on the AUC values occurred for *B. amyloliquefaciens* under darkness and the 460 nm treatment (Figure 9).

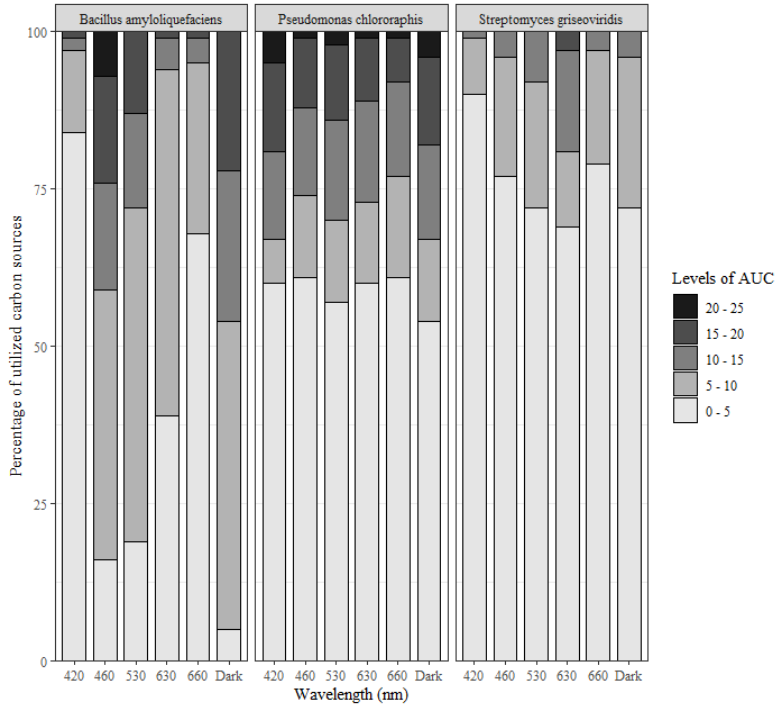

Figure 9: Percentage of carbon sources utilised under light regimes, at an intensity of 50 µmol m^{-2} s^{-1}, for *B. amyloliquefaciens*, *P. chlororaphis*, *S. griseoviridis*. Area under the curve (AUC) values ranged from 0 to 25,000. (Originally found as Figure 2 in Paper III)

Moreover, *B. amyloliquefaciens* exhibited generalist behaviour under 460 nm by utilising most of the available carbon sources (Figure 1, Paper III). *S. griseoviridis* had low carbon utilisation throughout all light treatments. A medium respiration was only noted for 3% of the tested carbon sources.

In the case of *P. chlororaphis* most carbon sources could be utilised irrespective of the treatment, though a higher proportion was noted under the 420 nm treatment (Figure 9). This was also observed in the reaction norms and the KEGG pathway analyses, where a higher proportion of the sole carbon sources were used under the 420 nm treatment (Figure 1, 3 and 4, Paper IV). When looking closer into cascade effects within key biochemical pathways such as the citrate cycle, a clear effect of the 420 nm treatment with increased use of succinate and malate (Figure 3, Paper IV). Succinate is a carbon source which is preferred by *P. aeruginosa* and has been linked with increased biofilm formation (McGill et al., 2021; Riquelme & Prince, 2021). In a study by Gharaie et al. (2017), they found that for another non-phototrophic *Pseudomonas* sp. DR 5-09, blue light had the most pronounced effect on substrate utilisation patterns.

Based on the results, phenotypic plasticity was noted for all three BCAs as adaptations occurred over time as a result of the sole carbon source utilisation with respect to the light treatments. The phenotypic plasticity observed was strain, light treatment and source dependent (Figure S1 and S2, Paper III). The BCAs' ability to use the limited nutrients present could be indicative of whether light could induce *r*- and *K*-strategies (Andrews & Harris, 1986). Being that the concentrations of the sole carbon sources found in the PM plates are approximately 10 – 20 mM, an increase in utilisation could possibly indicate a shift within the BCA. This is, as an r-strategist would grow rapidly when there is an abundance of nutrients (Pettersen et al., 2021). On the other hand, in a competitive environment, the slower-growing *K*-strategist will dominate, as its dietary needs are not as stringent as those of *r*-strategists. By examining the invasion and the resource saturation limitation theories, what could determine if a BCA is successful in establishing in the phyllosphere is if they can shift between being an *r*- or a *K*-strategist. Based on the experimental set-up the three BCAs were only exposed to low nutrient availability and as such, any growth could indicate that they were acting like a *K*-strategist.

Only capric acid was found to induce biosurfactant production in all three BCAs under different light spectra at a light intensity of 50 µmol m^{-2} s^{-1}

(Table 1, Paper III). Biosurfactant production is an essential mechanism for the establishment of microorganisms in a new environment. Choosing a sole carbon source that could, in combination with light, induce biosurfactant production could perhaps enable the BCA to invade and better attach to the surface of interest such as in the case of begonia leaves. Other studies have shown that other organic compounds such as amino acids could enhance biosurfactant production (Alsanius et al., 2021; Guerra-Santos et al., 1986).

Another essential mechanism of a good BCA is that of biofilm formation, as it allows for enhanced resilience of bacterial communities residing in the film due to the diverse ecological niches present (Gómez-Pérez et al., 2024). In the case of the introduction of BCAs in the phyllosphere niche construction and realisation could be most beneficial as this would allow for a better establishment whilst also affecting the pathogen negatively. Biofilm formation was detected for all three BCAs under several light treatments (Table 2, Paper III). Most occurred for *P. chlororaphis*, the only one were biofilm formation was seen under all light treatments. Selected sources based on these results, D-ribose resulted in biofilm formation under the red and dark treatments, uridine led to biofilm formation under 460 nm, were used to study biofilm occurrence when in combination with *P. chlororaphis* on detached leaf assays (Figure 10).

Biofilm formation as assessed through crystal violet staining, occurred under all light treatments irrespective of drop placement as the carbon sources were added in a random fashion. No effect of light intensity or exposure length was observed (Table S2, Paper IV). The glucose treatment resulted in the darkest biofilms when compared to the other selected carbon sources (Figure 10). Biofilm formation was noted even when only sterile distilled water was used in combination with *P. chlororaphis*. This could indicate that leaf wetness or humidity may play a key role in the establishment of microorganisms on a leaf.

Restricted leaf water activity has been reported to restrict microbial growth in food preservation, were decreased activity was found to prolong the lag phase, ultimately decreasing the cell growth rate (Tapia et al., 2020). Similarly, in the phyllosphere, the water content plays a fundamental role in plant-microbe interactions, though all the interactivities are not yet understood (Aung et al., 2018). In a study by Monier and Lindow (2004), they found that under moist and humid conditions bacterial aggregates were found at higher rates near the base of glandular trichomes. This could

potentially explain why, in the case of the trichome-dense tomato leaves used in the detached assay, a biofilm formation could be detected when only sterile distilled water was used in combination with the bacterium.

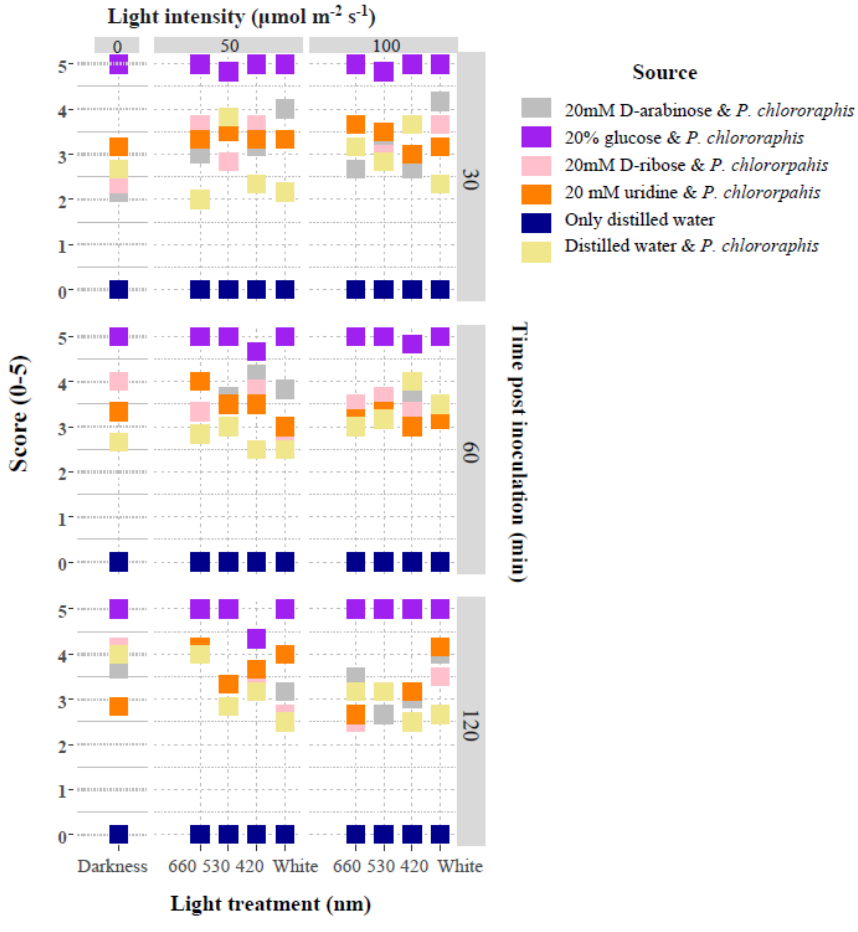

Figure 10: Average scores for all drops that were crystal violet treated. Detached tomato leaves exposed to five light conditions (monochromatic 660 nm, 530 nm, 420 nm; polychromatic white; darkness) under two intensities (50 µmol m^{-2} s^{-1}; 100 µmol m^{-2} s^{-1}) and for three time points post inoculation (30 min, 60 min, 120 min) of *P. chlororaphis*. Six substrates (four sole carbon sources (D-arabinose, glucose, D-ribose, uridine), control treatments: water with the bacterium, water without bacterium) were added randomly on each leaf with three biological replicates used. (Originally found as Figure 5 in Paper IV).

6. Conclusions

The main findings of the thesis were that:

- Combining a cultural control together with a biological could offer a new integrated pest control strategy
- Light spectrum modification is capable of inducing biosurfactant and biofilm in non-phototrophic bacterial BCAs
- No general light recipe could be identified for the three BCAs
- Light distribution can vary within the crop canopy
- Application to the canopy may lead to different results depending on the bacterium and plant species used
- Light and nutrient preferences can be species dependent
- Combining cultural dependent and culture independent methods could complement one another when evaluating BCA re-isolation from the phyllosphere
- Light could trigger cascade effects in key biochemical pathways e.g. carbon pathway and citrate pathway in non-phototrophic bacteria

7. Future perspectives

There are still a number of questions when it comes to understanding the true effect of light manipulation to suit the needs of non-phototrophic microorganisms, in the phyllosphere of greenhouse crops. The phyllosphere is a complex ecosystem and the need for BCAs that can invade and can realise a niche in the phyllosphere will continue to grow. Especially based on our needs for a more sustainable crop production.

Some aspects that could be looked into are:

- Using transcriptomics to decode which mechanisms light treatments and exposures affect within a bacterial BCA
- Consider how light treatments affect leaf exudates on crops of interest
- Compare leaf exudates to phenotypic microarray data
- Examine if the temporal differences in the humidity and temperature within the canopy of a trellising crop like the tomato can vary depending on light treatment
- Compare different white lights to determine if similar results can be obtained when combining other ratios of blue and green wavelengths
- Apply ecological concepts when identifying potential BCAs

- ❖ Investigate if light together with a nutrient source could aid the exploitation of multiple modes of action when using consortia

- ❖ Explore the consequences of introducing BCAs to the environment from an ecological perspective

8. Sustainability reflections

According to Santos et al. (2022), “sustainable agriculture requires the recruitment of bacterial agents to reduce the demand for mineral fertilizers and pesticides such as bacterial endophytes.” This thesis may not pertain to bacterial endophytes but rather epiphytes, but the sentiment is the same. In order to reduce our dependencies on both pesticides and mineral fertilisers, bacterial agents could and should be used instead. In an ever growing population, the demand for more and safer products will continue to expand. As mentioned in the introduction the world population will be peaking at approximately 10.3 billion in approximately 50 years. This may seem too far into the future, but now is the time to try to find solutions. One possibility could be combining microbiome research together with breeding programs to identify microorganisms that could both stimulate and protect the crop of interest.

Several sustainability development goals (SDGs) have been covered both directly and indirectly by this thesis. Some of these goals include SGD 3: Good health and well-being, which could be achieved by avoiding the use of chemical pesticides needed to combat fungal infections in greenhouse environments and as such target 3.9 could be met. Other goals include SDG 11: Sustainable Cities and Communities and 12: Responsible Consumption and Production, which are sustainability-oriented and interlinked. Lastly, two other SDGs, that this thesis could fall under include 13: Climate Action and 14: Life below water. By using biological control agents to eliminate unwanted organisms in a greenhouse setting, there will be a decreased need for chemical pesticides, which in turn would decrease the leaching of chemicals into the water ecosystems.

There is still a lot to uncover, but this thesis may be a small part of the bigger picture. Even the smallest of successes could bring about changes.

References

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/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*. 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

Andrews, J. H., & Harris, R. F. (1986). r- and K-Selection and Microbial Ecology. In K. C. Marshall (Ed.), *Advances in Microbial Ecology* (pp. 99-147). Springer US. https://doi.org/10.1007/978-1-4757-0611-6_3

Andrews, J. H., & Harris, R. F. (2000). The Ecology and Biogeography of Microorganisms on Plant Surfaces. *Annual Review of Phytopathology*, *38*(1), 145-180. https://doi.org/10.1146/annurev.phyto.38.1.145

Arrebola, E., Tienda, S., Vida, C., de Vicente, A., & Cazorla, F. M. (2019). Fitness Features Involved in the Biocontrol Interaction of *Pseudomonas chlororaphis* With Host Plants: The Case Study of PcPCL1606 [Mini Review]. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00719

Aung, K., Jiang, Y., & He, S. Y. (2018). The role of water in plant–microbe interactions. *The Plant Journal*, *93*(4), 771-780. https://doi.org/https://doi.org/10.1111/tpj.13795

Authority, E. F. S., Anastassiadou, M., Arena, M., Auteri, D., Brancato, A., Bura, L., Carrasco Cabrera, L., Chaideftou, E., Chiusolo, A., Crivellente, F., De Lentdecker, C., Egsmose, M., Fait, G., Greco, L., Ippolito, A., Istace, F., Jarrah, S., Kardassi, D., Leuschner, R.,…Villamar-Bouza, L. (2020). Peer

review of the pesticide risk assessment of the active substance *Streptomyces* strain K61. *EFSA Journal*, *18*(7), e06182. https://doi.org/https://doi.org/10.2903/j.efsa.2020.6182

Bardin, M., & Gullino, M. L. (2020). Fungal Diseases. In M. L. Gullino, R. Albajes, & P. C. Nicot (Eds.), *Integrated Pest and Disease Management in Greenhouse Crops* (pp. 55-100). Springer International Publishing. https://doi.org/10.1007/978-3-030-22304-5_3

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

Batschauer, A. (1998). Photoreceptors of higher plants. *Planta*, *206*(4), 479-492. http://www.jstor.org/stable/23385489

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

Beattie, G. A., & Lindow, S. E. (1999). Bacterial Colonization of Leaves: A Spectrum of Strategies. *Phytopathology®*, *89*(5), 353-359. https://doi.org/10.1094/phyto.1999.89.5.353

Bell, C. J., & Rose, D. A. (1981). Light measurement and the terminology of low. *Plant, Cell & Environment*, *4*(2), 89-96. https://doi.org/https://doi.org/10.1111/j.1365-3040.1981.tb01043.x

Berg, G., Köberl, M., Rybakova, D., Müller, H., Grosch, R., & Smalla, K. (2017). Plant microbial diversity is suggested as the key to future biocontrol and health trends. *FEMS Microbiology Ecology*, *93*(5). https://doi.org/10.1093/femsec/fix050

Bergstrand, K.-J., Asp, H., Larsson Jönsson, E. H., & Schüssler, H. K. (2015). Plant developmental consequences of lighting from above or below in the production of Poinsettia. *European Journal of Horticultural Science*, *80(2)*, 51-55. https://doi.org/DOI: 10.17660/eJHS.2015/80.2.1

Bergstrand, K. J., Asp, H., & Schüssler, H. K. (2017). Different light spectra is affecting growth and morphology of transplants of *Solanum lycopersicum*.

Beris, D., Theologidis, I., Skandalis, N., & Vassilakos, N. (2018). *Bacillus amyloliquefacien*s strain MBI600 induces salicylic acid dependent resistance in tomato plants against Tomato spotted wilt virus and Potato virus Y. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-28677-3

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*. https://doi.org/10.15252/embr.201541691

Brandl, M. T., & Amundson, R. (2008). Leaf Age as a Risk Factor in Contamination of Lettuce with *Escherichia coli* O157:H7 and *Salmonella enterica*.

Applied and Environmental Microbiology, *74*(8), 2298-2306. https://doi.org/10.1128/aem.02459-07

Bubici, G. (2018). *Streptomyces* spp. as biocontrol agents against *Fusarium* species. *CABI Reviews*, *2018*, 1-15. https://doi.org/doi:10.1079/PAVSNNR201813050

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

Carrau, A., Tano, J., Moyano, L., Ripa, M. B., Petrocelli, S., Piskulic, L., Moreira, L. M., Patané, J. S. L., Setubal, J. C., & Orellano, E. G. (2023). A novel BLUF photoreceptor modulates the Xanthomonas citri subsp. citri–host plant interaction. *Photochemical & Photobiological Sciences*, *22*(8), 1901-1918. https://doi.org/10.1007/s43630-023-00420-6

Chen, M., & Blankenship, R. E. (2011). Expanding the solar spectrum used by photosynthesis. *Trends in Plant Science*, *16*(8), 427-431. https://doi.org/10.1016/j.tplants.2011.03.011

Chew, S. C., & Yang, L. (2016). Biofilms. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of Food and Health* (pp. 407-415). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-384947-2.00069-6

Chi, S. I., Akuma, M., Xu, R., Plante, V., Hadinezhad, M., & Tambong, J. T. (2024). Phenazines are involved in the antagonism of a novel subspecies of *Pseudomonas chlororaphis* strain S1Bt23 against *Pythium ultimum*. *Scientific Reports*, *14*(1), 20517. https://doi.org/10.1038/s41598-024-71418-y

Chowdhury, S. P., Hartmann, A., Gao, X., & Borriss, R. (2015). Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42 – a review [Review]. *Frontiers in Microbiology*, *6*. https://doi.org/10.3389/fmicb.2015.00780

Costa, J. A. V., Treichel, H., Santos, L. O., & Martins, V. G. (2018). Chapter 16 - Solid-State Fermentation for the Production of Biosurfactants and Their Applications. In A. Pandey, C. Larroche, & C. R. Soccol (Eds.), *Current Developments in Biotechnology and Bioengineering* (pp. 357-372). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-444-63990-5.00016-5

Daughtrey, M., & Buitenhuis, R. (2020). Integrated Pest and Disease Management in Greenhouse Ornamentals. In M. L. Gullino, R. Albajes, & P. C. Nicot (Eds.), *Integrated Pest and Disease Management in Greenhouse Crops* (pp. 625-679). Springer International Publishing. https://doi.org/10.1007/978-3-030-22304-5_22

Davis, W. V., Weber, C., Wechsler, S., Wakefield, H., & Lucier, G. (2024). *Vegetables and pulses outlook: April 2024*. (VGS-372). Retrieved from https://www.ers.usda.gov/publications/pub-details/?pubid=109066

Daza, A., Martín, J. F., Dominguez, A., & Gil, J. A. (1989). Sporulation of Several Species of *Streptomyces* in Submerged Cultures after Nutritional Downshift. *Microbiology*, *135*(9), 2483-2491. https://doi.org/https://doi.org/10.1099/00221287-135-9-2483

Dimopoulou, A., Theologidis, I., Liebmann, B., Kalantidis, K., Vassilakos, N., & Skandalis, N. (2019). *Bacillus amyloliquefaciens* MBI600 differentially induces tomato defense signaling pathways depending on plant part and dose of application. *Scientific Reports*, *9*(1), 19120. https://doi.org/10.1038/s41598-019-55645-2

Dunne, W. M. (2002). Bacterial Adhesion: Seen Any Good Biofilms Lately? *Clinical Microbiology Reviews*, *15*(2), 155-166. https://doi.org/10.1128/cmr.15.2.155-166.2002

Elad, Y., Bélanger, R. R., & Kohl, J. (1999). Biological Control of Diseases in the Phyllosphere. In (pp. 338-352). Springer Netherlands. https://doi.org/10.1007/0-306-47585-5_24

Ernst, O. P., Lodowski, D. T., Elstner, M., Hegemann, P., Brown, L. S., & Kandori, H. (2014). Microbial and Animal Rhodopsins: Structures, Functions, and Molecular Mechanisms. *Chemical Reviews*, *114*(1), 126-163. https://doi.org/10.1021/cr4003769

EUROSTAT. (2024). *Food waste and food waste prevention - estimates*. Retrieved from https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Food_waste_and_food_waste_prevention_-_estimates#Amounts_of_food_waste_at_EU_level

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). https://doi.org/10.1007/s00203-024-03836-5

Flores, S. S., Cordovez, V., Oyserman, B., Stopnisek, N., Raaijmakers, J. M., & van't Hof, P. (2023). The Tomato's Tale: Exploring Taxonomy, Biogeography, Domestication, and Microbiome for Enhanced Resilience. *Phytobiomes Journal*, *8*(1), 5-20. https://doi.org/10.1094/PBIOMES-09-23-0091-MF

Galli, M., Feldmann, F., Vogler, U. K., & Kogel, K.-H. (2024). Can biocontrol be the game-changer in integrated pest management? A review of definitions, methods and strategies. *Journal of Plant Diseases and Protection*. https://doi.org/10.1007/s41348-024-00878-1

Gard, B., & Gilardi, G. (2020). IPM for Protecting Leafy Vegetables Under Greenhouses. In M. L. Gullino, R. Albajes, & P. C. Nicot (Eds.), *Integrated Pest and Disease Management in Greenhouse Crops* (pp. 567-595).

Springer International Publishing. https://doi.org/10.1007/978-3-030-22304-5_20

Gausman, H. W., & Allen, W. A. (1973). Optical Parameters of Leaves of 30 Plant Species. *Plant Physiology*, *52*(1), 57-62. https://doi.org/10.1104/pp.52.1.57

Geilfus, C.-M. (2019). *Controlled Environment Horticulture: Improving Quality of Vegetables and Medicinal Plants*. Springer Nature Switzerland. https://doi.org/10.1007/978-3-030-23197-2

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

Giliberto, L., Perrotta, G., Pallara, P., Weller, J. L., Fraser, P. D., Bramley, P. M., Fiore, A., Tavazza, M., & Giuliano, G. (2005). Manipulation of the Blue Light Photoreceptor Cryptochrome 2 in Tomato Affects Vegetative Development, Flowering Time, and Fruit Antioxidant Content. *Plant Physiology*, *137*(1), 199-208. https://doi.org/10.1104/pp.104.051987

Global Greenhouse Horticulture Market Size (2024-2032). (2024). Market Data Forecast. Retrieved 7/12 from https://www.marketdataforecast.com/market-reports/greenhouse-horticulture-market

Goelzer, A., & Fromion, V. (2011). Bacterial growth rate reflects a bottleneck in resource allocation. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1810*(10), 978-988. https://doi.org/https://doi.org/10.1016/j.bbagen.2011.05.014

Gómez-Pérez, D., Zott, L. M., Schmid, M., & Chaudhry, V. (2024). Nature's Protectors: A Biofilm Perspective on Bacterial Disease Control in Plants. In P. Singh Chauhan, S. K. Tewari, & S. Misra (Eds.), *Plant-Microbe Interaction and Stress Management* (pp. 109-133). Springer Nature Singapore. https://doi.org/10.1007/978-981-97-4239-4_7

Griffin, J. N., & Silliman, B. R. (2011). Resource Partitioning and Why it Matters. *Nature Education Knowledge*, *3(10):49*. https://www.nature.com/scitable/knowledge/library/resource-partitioning-and-why-it-matters-17362658/

Grime, J. P. (1973). Competitive Exclusion in Herbaceous Vegetation. *Nature*, *242*(5396), 344-347. https://doi.org/10.1038/242344a0

Gruda, N., & Tanny, J. (2014). Protected Crops. In G. R. Dixon & D. E. Aldous (Eds.), *Horticulture: Plants for People and Places, Volume 1: Production Horticulture* (pp. 327-405). Springer Netherlands. https://doi.org/10.1007/978-94-017-8578-5_10

Guerra-Santos, L. H., Käppeli, O., & Fiechter, A. (1986). Dependence of *Pseudomonas aeruginosa* continous culture biosurfactant production on nutritional and environmental factors. *Applied Microbiology and Biotechnology*, *24*(6), 443-448. https://doi.org/10.1007/BF00250320

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). https://doi.org/10.1073/pnas.2309632120

Hauser, B. A., Pratt, L. H., & Cordonnier-Pratt, M.-M. (1997). Absolute quantification of five phytochrome transcripts in seedlings and mature plants of tomato (*Solanum lycopersicum* L.). *Planta*, *201*(3), 379-387. https://doi.org/10.1007/s004250050080

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*. https://doi.org/https://doi.org/10.1002/ppp3.10586

Huché-Thélier, L., Crespel, L., Gourrierec, J. L., Morel, P., Sakr, S., & Leduc, N. (2016). Light signaling and plant responses to blue and UV radiations—Perspectives for applications in horticulture. *Environmental and Experimental Botany*, *121*, 22-38. https://doi.org/10.1016/j.envexpbot.2015.06.009

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

Imamoto, Y., & Kataoka, M. (2007). Structure and Photoreaction of Photoactive Yellow Protein, a Structural Prototype of the PAS Domain Superfamily†. *Photochemistry and Photobiology*, *83*(1), 40-49. https://doi.org/10.1562/2006-02-28-ir-827

Jarvis, W. (1989). Managing diseases in greenhouse crops. *Plant Disease*, *73*(3), 190-194.

Jones, J. B., Zitter, T. A., Momol, M. T., Miller, S. A., & Society, A. P. (2014). *Compendium of Tomato Diseases and Pests*. American Phytopathological Society. https://books.google.se/books?id=r9LHoAEACAAJ

Juniper, B. E. (1991). The Leaf from the Inside and the Outside: A Microbe's Perspective. In J. H. Andrews & S. S. Hirano (Eds.), *Microbial Ecology of Leaves*. Springer-Verlag New York Inc.

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), e01407-01422. 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*. https://doi.org/https://doi.org/10.3389/fmicb.2023.1087639

Katzin, D., van Mourik, S., Kempkes, F., & van Henten, E. J. (2020). GreenLight – An open source model for greenhouses with supplemental lighting:

Evaluation of heat requirements under LED and HPS lamps. *Biosystems Engineering*, *194*, 61-81. https://doi.org/https://doi.org/10.1016/j.biosystemseng.2020.03.010

Kim, H.-J., Lin, M.-Y., & Mitchell, C. A. (2019). Light spectral and thermal properties govern biomass allocation in tomato through morphological and physiological changes. *Environmental and Experimental Botany*, *157*, 228-240. https://doi.org/https://doi.org/10.1016/j.envexpbot.2018.10.019

Kinkel, L. L., & Lindow, S. E. (1997). Microbial Competition and Plant Disease Biocontrol. In D. A. Andow, D. W. Ragsdale, & R. F. Nyvall (Eds.), *Ecological Interactions And Biological Control*. Westview Press, A Division of HarperCollins Publishers, Inc.

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

Kong, D., Ye, Z., Dai, M., Ma, B., & Tan, X. (2024). Light Intensity Modulates the Functional Composition of Leaf Metabolite Groups and Phyllosphere Prokaryotic Community in Garden Lettuce (*Lactuca sativa* L.) Plants at the Vegetative Stage. *International Journal of Molecular Sciences*, *25*(3).

Kortbeek, R. W. J., Galland, M. D., Muras, A., Van Der Kloet, F. M., André, B., Heilijgers, M., Van Hijum, S. A. F. T., Haring, M. A., Schuurink, R. C., & Bleeker, P. M. (2021). Natural variation in wild tomato trichomes; selecting metabolites that contribute to insect resistance using a random forest approach. *BMC Plant Biology*, *21*(1). https://doi.org/10.1186/s12870-021-03070-x

Koskella, B. (2020). The phyllosphere. *Current Biology*, *30*(19), R1143-R1146. https://doi.org/10.1016/j.cub.2020.07.037

Kraiselburd, I., Moyano, L., Carrau, A., Tano, J., & Orellano, E. G. (2017). Bacterial Photosensory Proteins and Their Role in Plant-pathogen Interactions. *Photochemistry and Photobiology*, *93*(3), 666-674. https://doi.org/10.1111/php.12754

Kruidhof, H. M., & Elmer, W. H. (2020). Cultural Methods for Greenhouse Pest and Disease Management. In M. L. Gullino, R. Albajes, & P. C. Nicot (Eds.), *Integrated Pest and Disease Management in Greenhouse Crops* (pp. 285-330). Springer International Publishing. https://doi.org/10.1007/978-3-030-22304-5_10

Kusstatscher, P., Wicaksono, W. A., Bergna, A., Cernava, T., Bergau, N., Tissier, A., Hause, B., & Berg, G. (2020). Trichomes form genotype-specific microbial hotspots in the phyllosphere of tomato. *Environmental Microbiome*, *15*(1). https://doi.org/10.1186/s40793-020-00364-9

Lahdenperä, M. L., Simon, E., & Uoti, J. (1991). Mycostop - A Novel Biofungicide Based on *Streptomyces* Bacteria. In A. B. R. Beemster, G. J. Bollen, M. Gerlagh, M. A. Ruissen, B. Schippers, & A. Tempel (Eds.), *Developments in Agricultural and Managed Forest Ecology* (Vol. 23, pp. 258-263).

Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-444-88728-3.50048-2

Last, F. T. (1955). Seasonal incidence of *Sporobolomyces* on cereal leaves. *Transactions of the British Mycological Society*, *38*(3), 221-239. https://doi.org/https://doi.org/10.1016/S0007-1536(55)80069-1

Legein, M., Smets, W., Vandenheuvel, D., Eilers, T., Muyshondt, B., Prinsen, E., Samson, R., & Lebeer, S. (2020). Modes of Action of Microbial Biocontrol in the Phyllosphere. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.01619

Leveau, J. H. (2019). A brief from the leaf: latest research to inform our understanding of the phyllosphere microbiome. *Current Opinion in Microbiology*, *49*, 41-49. https://doi.org/10.1016/j.mib.2019.10.002

Leveau, J. H. J. (2006). Microbia Communities in the Phyllosphere. In *Annual Plant Reviews Volume 23: Biology of the Plant Cuticle* (pp. 334-367). https://doi.org/https://doi.org/10.1002/9780470988718.ch11

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

Li, Y., Gu, Y., Li, J., Xu, M., Wei, Q., & Wang, Y. (2015). Biocontrol agent *Bacillus amyloliquefaciens* LJ02 induces systemic resistance against cucurbits powdery mildew [Original Research]. *Frontiers in Microbiology*, *6*(883). https://doi.org/10.3389/fmicb.2015.00883

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

Liu, J., & van Iersel, M. W. (2021). Photosynthetic Physiology of Blue, Green, and Red Light: Light Intensity Effects and Underlying Mechanisms [Original Research]. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.619987

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

Luo, L., Zhao, C., Wang, E., Raza, A., & Yin, C. (2022). *Bacillus amyloliquefaciens* as an excellent agent for biofertilizer and biocontrol in agriculture: An overview for its mechanisms. *Microbiological Research*, *259*, 127016. https://doi.org/https://doi.org/10.1016/j.micres.2022.127016

Mallon, C. A., Elsas, J. D. V., & Salles, J. F. (2015). Microbial Invasions: The Process, Patterns, and Mechanisms. *Trends in microbiology*, *23*(11), 719-729. https://doi.org/10.1016/j.tim.2015.07.013

Mandalari, C., Losi, A., & Gärtner, W. (2013). Distance-tree analysis, distribution and co-presence of bilin- and flavin-binding prokaryotic photoreceptors for visible light. *Photochemical & Photobiological Sciences*, *12*(7), 1144-1157. https://doi.org/10.1039/c3pp25404f

Manikandan, A., Anandham, R., Madhan, S., Raghu, R., Krishnamoorthy, R., & Senthilkumar, M. (2024). Exploring the Phyllosphere: Microbial Diversity, Interactions, and Ecological Significance in Plant Health. In P. Singh Chauhan, S. K. Tewari, & S. Misra (Eds.), *Plant-Microbe Interaction and Stress Management* (pp. 29-49). Springer Nature Singapore. https://doi.org/10.1007/978-981-97-4239-4_3

McCree, K. J. (1972). Significance of Enhancement for Calculations Based on the Action Spectrum for Photosynthesis 1. *Plant Physiology*, *49*(5), 704-706. https://doi.org/10.1104/pp.49.5.704

McGill, S. L., Yung, Y., Hunt, K. A., Henson, M. A., Hanley, L., & Carlson, R. P. (2021). *Pseudomonas aeruginosa* reverse diauxie is a multidimensional, optimized, resource utilization strategy. *Scientific Reports*, *11*(1), 1457. https://doi.org/10.1038/s41598-020-80522-8

Mechaber, W. L., Marshall, D. B., Mechaber, R. A., Jobe, R. T., & Chew, F. S. (1996). Mapping leaf surface landscapes. *Proceedings of the National Academy of Sciences*, *93*(10), 4600-4603. https://doi.org/10.1073/pnas.93.10.4600

Milner, J., Silo-Suh, L., Goodman, R. M., & Handelsman, J. (1997). Antibiosis and Beyond: Genetic Diversity, Microbial Communities and Biological Control. In D. A. Andow, D. W. Ragsdale, & R. F. Nyvall (Eds.), *Ecological Interactions And Biological Control*. Westview Press, A Division of HarperCollins Publishers, Inc.

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

Möglich, A., Yang, X., Ayers, R. A., & Moffat, K. (2010). Structure and Function of Plant Photoreceptors. *Annual Review of Plant Biology*, *61*(Volume 61, 2010), 21-47. https://doi.org/https://doi.org/10.1146/annurev-arplant-042809-112259

Monier, J.-M., & Lindow, S. E. (2004). Frequency, Size, and Localization of Bacterial Aggregates on Bean Leaf Surfaces. *Applied and Environmental Microbiology*, *70*(1), 346-355. https://doi.org/doi:10.1128/AEM.70.1.346-355.2004

Morgan, M., & Ramos, M. (2024). *BiocManager: Access the Bioconductor Project Package Repository* https://CRAN.R-project.org/package=BiocManager

Morrow, R. C. (2008). LED Lighting in Horticulture. *HortScience horts*, *43*(7), 1947-1950. https://doi.org/10.21273/hortsci.43.7.1947

Moyano, L., Carrau, A., Petrocelli, S., Kraiselburd, I., Gärtner, W., & Orellano, E. G. (2020). Bacteriophytochromes from *Pseudomonas syringae* pv. tomato DC3000 modulate the early stages of plant colonization during bacterial speck disease. *European journal of plant pathology*, *156*(3), 695-712. https://doi.org/10.1007/s10658-019-01918-5

Müller, C., Caspers, B. A., Gadau, J., & Kaiser, S. (2020). The Power of Infochemicals in Mediating Individualized Niches. *Trends in Ecology & Evolution*, *35*(11), 981-989. https://doi.org/10.1016/j.tree.2020.07.001

Murillo-Roos, M., Abdullah, H. S. M., Debbar, M., Ueberschaar, N., & Agler, M. T. (2022). Cross-feeding niches among commensal leaf bacteria are shaped by the interaction of strain-level diversity and resource availability. *The ISME Journal*, *16*(9), 2280-2289. https://doi.org/10.1038/s41396-022-01271-2

Nelson, J. A., & Bugbee, B. (2014). Economic Analysis of Greenhouse Lighting: Light Emitting Diodes vs. High Intensity Discharge Fixtures. *PLOS ONE*, *9*(6), e99010. https://doi.org/10.1371/journal.pone.0099010

Nemali, K. (2022). History of Controlled Environment Horticulture: Greenhouses. *HortScience*, *57*(2), 239-246. https://doi.org/10.21273/hortsci16160-21

Ohlendorf, R., & Möglich, A. (2022). Light-regulated gene expression in Bacteria: Fundamentals, advances, and perspectives [Review]. *Frontiers in Bioengineering and Biotechnology*, *10*. https://doi.org/10.3389/fbioe.2022.1029403

Pennisi, G., Pistillo, A., Orsini, F., Cellini, A., Spinelli, F., Nicola, S., Fernandez, J. A., Crepaldi, A., Gianquinto, G., & Marcelis, L. F. M. (2020). Optimal light intensity for sustainable water and energy use in indoor cultivation of lettuce and basil under red and blue LEDs. *Scientia Horticulturae*, *272*, 109508. https://doi.org/https://doi.org/10.1016/j.scienta.2020.109508

Pettersen, J. P., Gundersen, M. S., & Almaas, E. (2021). Robust bacterial co-occurence community structures are independent of r- and K-selection history. *Scientific Reports*, *11*(1), 23497. https://doi.org/10.1038/s41598-021-03018-z

Puopolo, G., Raio, A., Pierson, L. S., & Zoina, A. (2011). Selection of a new *Pseudomonas chlororaphis* strain for the biological control of *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Phytopathologia Mediterranea*, *50*(2), 228-235. http://www.jstor.org/stable/26458696

R Core Team. (2021). *A Language and Environment for Statistical Computing*. In R Foundation for Statistical Computing. https://www.R-project.org/

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

Riquelme, S. A., & Prince, A. (2021). *Pseudomonas aeruginosa* Consumption of Airway Metabolites Promotes Lung Infection. *Pathogens*, *10*(8), 957. https://www.mdpi.com/2076-0817/10/8/957

Ruinen, J. (1956). Occurrence of *Beijerinckia* Species in the 'Phyllosphere'. *Nature*, *177*(4501), 220-221. https://doi.org/10.1038/177220a0

Salvatierra-Martinez, R., Arancibia, W., Araya, M., Aguilera, S., Olalde, V., Bravo, J., & Stoll, A. (2018). Colonization ability as an indicator of enhanced biocontrol capacity—An example using two *Bacillus amyloliquefaciens* strains and *Botrytis cinerea* infection of tomatoes. *Journal of*

Phytopathology, *166*(9), 601-612. https://doi.org/https://doi.org/10.1111/jph.12718

Santos, R. M. D., Desoignies, N., & Rigobelo, E. C. (2022). The bacterial world inside the plant. *Frontiers in Sustainable Food Systems*, *6*. https://doi.org/10.3389/fsufs.2022.830198

Schipper, R., Van Der Meer, M., De Visser, P. H. B., Heuvelink, E., & Marcelis, L. F. M. (2023). Consequences of intra-canopy and top LED lighting for uniformity of light distribution in a tomato crop. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1012529

Schreiber, L., Krimm, U., Knoll, D., Sayed, M., Auling, G., & Kroppenstedt, R. M. (2005). Plant–microbe interactions: identification of epiphytic bacteria and their ability to alter leaf surface permeability. *New Phytologist*, *166*(2), 589-594. https://doi.org/10.1111/j.1469-8137.2005.01343.x

Schumacher, J. (2017). How light affects the life of *Botrytis*. *Fungal Genetics and Biology*, *106*, 26-41.

Shah, R., Pathak, G., Drepper, T., & Gärtner, W. (2016). Selective Photoreceptor Gene Knock-out Reveals a Regulatory Role for the Growth Behavior of *Pseudomonas syringae*. *Photochemistry and Photobiology*, *92*(4), 571-578. https://doi.org/https://doi.org/10.1111/php.12610

Stenberg, J. A., Sundh, I., Becher, P. G., Björkman, C., Dubey, M., Egan, P. A., Friberg, H., Gil, J. F., Jensen, D. F., Jonsson, M., Karlsson, M., Khalil, S., Ninkovic, V., Rehermann, G., Vetukuri, R. R., & Viketoft, M. (2021). When is it biological control? A framework of definitions, mechanisms, and classifications. *Journal of Pest Science*, *94*(3), 665-676. https://doi.org/10.1007/s10340-021-01354-7

Suthaparan, A., & Stensvand, A. (2024). Shedding the Light on Powdery Mildew: The Use of Optical Irradiation in Management of the Disease. *Annual Review of Phytopathology*, *62*(Volume 62, 2024), 289-308. https://doi.org/https://doi.org/10.1146/annurev-phyto-021622-115201

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

Sylla, J. (2013). *Phyllosphere of organically grown strawberries : interactions between the resident microbiota, pathogens and introduced microbial agents* (Publication Number 2013:85) Swedish University of Agricultural Sciences]. Alnarp. https://publications.slu.se/?file=publ/show&id=51858&lang=se

Tapia, M. S., Alzamora, S. M., & Chirife, J. (2020). Effects of Water Activity (a) on Microbial Stability as a Hurdle in Food Preservation. In *Water Activity in Foods* (pp. 323-355). https://doi.org/https://doi.org/10.1002/9781118765982.ch14

Tariq, M., Khan, A., Asif, M., Khan, F., Ansari, T., Shariq, M., & Siddiqui, M. A. (2020). Biological control: a sustainable and practical approach for plant disease management. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, *70*(6), 507-524. https://doi.org/10.1080/09064710.2020.1784262

Thapa, S., & Prasanna, R. (2018). Prospecting the characteristics and significance of the phyllosphere microbiome. *Annals of Microbiology*, *68*(5), 229-245. https://doi.org/10.1007/s13213-018-1331-5

Thimijan, R. W., & Heins, R. D. (1983). Photometric, Radiometric, and Quantum Light Units of Measure: A Review of Procedures for Interconversion. *HortScience*, *18*(6), 818-822. https://doi.org/10.21273/hortsci.18.6.818

Tierney, S. M., Friedrich, M., Humphreys, W. F., Jones, T. M., Warrant, E. J., & Wcislo, W. T. (2017). Consequences of evolutionary transitions in changing photic environments. *Austral Entomology*, *56*(1), 23-46. https://doi.org/https://doi.org/10.1111/aen.12264

Tronsmo, A. M., Collinge, D. B., Djurle, A., Munk, L., Yuen, J., & Tronsmo, A. (2020). *Plant Pathology and Plant Diseases*. CABI. https://books.google.se/books?id=wGgCEAAAQBAJ

Turcotte, M. M., & Levine, J. M. (2016). Phenotypic Plasticity and Species Coexistence. *Trends in Ecology & Evolution*, *31*(10), 803-813. https://doi.org/https://doi.org/10.1016/j.tree.2016.07.013

UN. (2024). *World Population Prospects 2024: Summary of Results (UN DESA/POP/2024/TR/NO. 9)*. https://desapublications.un.org/publications/world-population-prospects-2024-summary-results

Vaas, L. A. I., Sikorski, J., Hofner, B., Fiebig, A., Buddruhs, N., Klenk, H.-P., & Göker, M. (2013). opm: an R package for analysing OmniLog® phenotype microarray data. *Bioinformatics*, *29*(14), 1823-1824. https://doi.org/10.1093/bioinformatics/btt291

Vacher, C., Hampe, A., Porté, A. J., Sauer, U., Compant, S., & Morris, C. E. (2016). The Phyllosphere: Microbial Jungle at the Plant–Climate Interface. *Annual Review of Ecology, Evolution, and Systematics*, *47*(Volume 47, 2016), 1-24. https://doi.org/https://doi.org/10.1146/annurev-ecolsys-121415-032238

van der Horst, M. A., Key, J., & Hellingwerf, K. J. (2007). Photosensing in chemotrophic, non-phototrophic bacteria: let there be light sensing too. *Trends in microbiology*, *15*(12), 554-562. https://doi.org/https://doi.org/10.1016/j.tim.2007.09.009

Van Der Wal, A., & Leveau, J. H. J. (2011). Modelling sugar diffusion across plant leaf cuticles: the effect of free water on substrate availability to phyllosphere bacteria. *Environmental Microbiology*, *13*(3), 792-797. https://doi.org/10.1111/j.1462-2920.2010.02382.x

van Lenteren, J. C., Alomar, O., Ravensberg, W. J., & Urbaneja, A. (2020). Biological Control Agents for Control of Pests in Greenhouses. In M. L.

Gullino, R. Albajes, & P. C. Nicot (Eds.), *Integrated Pest and Disease Management in Greenhouse Crops* (pp. 409-439). Springer International Publishing. https://doi.org/10.1007/978-3-030-22304-5_14

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., & Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *New Phytologist*, *206*(4), 1196-1206. https://doi.org/10.1111/nph.13312

Vogelmann, T. C., & Gorton, H. L. (2014). Leaf: Light Capture in the Photosynthetic Organ. In (pp. 363-377). Springer Netherlands. https://doi.org/10.1007/978-94-017-8742-0_19

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

Warsito, M., & Kusumawati, I. (2020). Antimicrobial activity of *Streptomyces griseoviridis* K10 against ESBL *Escherichia coli*, MRSA, and other pathogenic microorganisms. IOP Conference Series: Earth and Environmental Science,

Weisskopf, L., Schulz, S., & Garbeva, P. (2021). Microbial volatile organic compounds in intra-kingdom and inter-kingdom interactions. *Nature Reviews Microbiology*, *19*(6), 391-404. https://doi.org/10.1038/s41579-020-00508-1

Westreenen, A. v., Zhang, N., Douma, J. C., Evers, J. B., Anten, N. P. R., & Marcelis, L. F. M. (2020). Substantial differences occur between canopy and ambient climate: Quantification of interactions in a greenhouse-canopy system. *PLOS ONE*, *15*(5), e0233210. https://doi.org/10.1371/journal.pone.0233210

Whittaker, R. J., Fernández-Palacios, J. M., Matthews, T. J., Borregaard, M. K., & Triantis, K. A. (2017). Island biogeography: Taking the long view of nature's laboratories. *Science*, *357*(6354), eaam8326. https://doi.org/10.1126/science.aam8326

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/doi:10.1128/aem.58.12.3908-3913.1992

Wittwer, S. H., & Castilla, N. (1995). Protected Cultivation of Horticultural Crops Worldwide. *HortTechnology horttech*, *5*(1), 6-23. https://doi.org/10.21273/horttech.5.1.6

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*. https://doi.org/10.1128/mBio.00334-13

Yeats, T. H., & Rose, J. K. C. (2013). The Formation and Function of Plant Cuticles. *Plant Physiology*, *163*(1), 5-20. https://doi.org/10.1104/pp.113.222737

Yu, X., Liu, H., Klejnot, J., & Lin, C. (2010). The Cryptochrome Blue Light Receptors. *The Arabidopsis Book*, *8*, e0135. https://doi.org/10.1199/tab.0135

Popular science summary

The use of chemical pesticides needs to be reduced to promote sustainability in both food and ornamental plant production. One possible alternative is using other living organisms that can negatively affect harmful pathogens. Organisms such as bacterial biological control agents (BCAs) have several tools that allow them to control pathogens. Some of these tools include the production of substances that either harm or are disliked by pathogens and, by occupying space, preventing pathogens from accessing the plant.

However, there are some challenges that beneficial bacteria must overcome to protect the plant. As the plant surface is not a welcoming environment, it is sometimes hard for the BCAs to establish. For the BCAs to work well on the harsh plant surface, they need to attach to the leaves, compete with other microbes and multiply. One thing that could help the introduced bacteria is light! Interestingly, light has been found to affect even organisms that do not use it as an energy source. Organisms that do not use light in this way are called non-phototrophic. Recently, it has been shown that light can help non-phototrophic bacteria form protective layers called biofilms and produce substances that improve their survival.

This thesis set out to look into how visible light affects three types of beneficial BCAs in both laboratory and greenhouse settings. By testing different wavelengths, we found that under nutrient-low conditions in the laboratory, two of the three bacterial species, *Bacillus amyloliquefaciens* and *Pseudomonas chlororaphis*, preferred the blue light treatments. In contrast, the third species, *Streptomyces griseoviridis*, preferred the red treatment. Biofilm formation could occur depending on the combination of nutrient, light treatment and bacterium. The same preferences were not seen when the three BCAs were introduced to tomato leaves in the greenhouse. All BCAs

preferred the green and white light treatments in the greenhouse setting. *S. griseoviridis* grew well regardless of the light treatment, and *B. amyloliquefaciens* disliked the red light treatment the most.

The results revealed that the type of visible light matters and that no one recipe could be used. By combining two non-chemical methods: a biological (BCA) and a cultural (light) control method, this offers a new integrated pathogen control strategy. These insights could help develop future beneficial bacterial controls in greenhouses and hopefully lead to more sustainable growing practices.

Populärvetenskaplig sammanfattning

Användningen av kemiska växtskyddsmedel behöver minska för att främja en hållbar livsmedels- och prydnadsväxtproduktion. Ett möjligt alternativ är att använda andra levande organismer, som bakterier, som kan påverka skadliga patogener negativt. Organismer som biologiska bekämpningsmedel (BCAs) har flera verktyg som gör det möjligt för dem att kontrollera växtskadegörare. Några av dessa verktyg inkluderar produktionen av ämnen som antingen skadar eller ogillas av patogener. Även genom att ta upp plats hindrar BCA växtskadegörare från att komma åt växten.

Det finns dock vissa utmaningar som nyttobakterierna måste klara av innan de kan skydda växten. Eftersom växtens yta inte är en välkomnande miljö kan det ibland vara svårt för BCAs att etablera sig. För att BCAs ska fungera effektivt mot växtskadegörare måste de fästa sig vid bladen, konkurrera med andra mikroorganismer och föröka sig.

En sak som skulle kunna hjälpa de introducerade bakterierna är ljus! Intressant nog har det visat sig att ljus påverkar även organismer som inte använder det som energikälla. Organismer som inte använder ljus på detta sätt kallas icke-fototrofa. Nyligen har det visats att ljus kan hjälpa icke-fototrofa bakterier att bilda skyddande lager, så kallade biofilmer, och producera ämnen som förbättrar deras överlevnad.

Denna avhandling undersökte hur synligt ljus påverkar tre typer av fördelaktiga BCAs i både laboratorie- och växthusmiljöer. Genom att testa olika våglängder fann vi att under näringsfattiga förhållanden i laboratoriet föredrog två av de tre bakteriearterna, *Bacillus amyloliquefaciens* och *Pseudomonas chlororaphis*, behandlingar med blått ljus. Däremot föredrog den tredje arten, *Streptomyces griseoviridis*, behandling med rött ljus. Bildandet av biofilmer kunde ske men det var beroende på kombinationen

av näring, ljusbehandling och bakterie. Samma preferenser observerades inte när de tre BCAs introducerades till tomatblad i växthuset. Alla BCAs föredrog behandlingar med grönt och vitt ljus i växthusmiljön. *S. griseoviridis* växte bra oavsett ljusbehandling, medan *B. amyloliquefaciens* ogillade mest behandlingen under rött ljus.

Resultaten visade att typen av synligt ljus spelade roll och att inget entydigt recept fanns. Genom att kombinera två icke-kemiska metoder: en biologisk (BCA) och en kulturell (ljus) erbjuds en ny integrerad strategi för växtskadegörarbekämpning. Dessa insikter kan hjälpa till att utveckla framtida användningar av nyttobakteriekontroller i växthusmiljöer och kan förhoppningsvis leda till mer hållbara odlingsmetoder.

Απλή επιστημονική περίληψη

Η χρήση χημικών φυτοφαρμάκων πρέπει να μειωθεί για να προωθηθεί η βιωσιμότητα τόσο στην παραγωγή τροφίμων όσο και καλλωπιστικών φυτών. Μια πιθανή εναλλακτική λύση είναι η χρήση άλλων ζωντανών οργανισμών που μπορούν να επηρεάσουν αρνητικά τους επιβλαβείς παθογόνους παράγοντες. Οργανισμοί όπως τα βακτήρια που λειτουργούν ως βιολογικοί έλεγχοι (BCAs) διαθέτουν διάφορα εργαλεία που τους επιτρέπουν να ελέγχουν τους παθογόνους παράγοντες. Ορισμένα από αυτά τα εργαλεία περιλαμβάνουν την παραγωγή ουσιών που είτε βλάπτουν είτε αποθαρρύνουν τους παθογόνους οργανισμούς, καθώς και την κατάληψη χώρου, εμποδίζοντας έτσι την πρόσβαση των παθογόνων στο φυτό.

Ωστόσο, υπάρχουν ορισμένες προκλήσεις που πρέπει να ξεπεράσουν τα ευεργετικά βακτήρια για να προστατεύσουν το φυτό. Η επιφάνεια του φυτού δεν είναι φιλόξενη και συχνά καθιστά δύσκολη την εγκατάσταση των BCAs. Για να λειτουργήσουν αποτελεσματικά στην απαιτητική επιφάνεια των φύλλων, τα BCAs πρέπει να προσκολληθούν στα φύλλα, να ανταγωνιστούν άλλους μικροοργανισμούς και να πολλαπλασιαστούν.

Κάτι που θα μπορούσε να βοηθήσει τα εισαγόμενα βακτήρια είναι το φως! Είναι ενδιαφέρον ότι έχει βρεθεί πως το φως επηρεάζει ακόμη και οργανισμούς που δεν το χρησιμοποιούν ως πηγή ενέργειας. Αυτοί οι οργανισμοί ονομάζονται μη φωτοτροφικοί. Πρόσφατα, έχει αποδειχθεί ότι το φως μπορεί να βοηθήσει τα μη φωτοτροφικά βακτήρια να σχηματίσουν προστατευτικές στρώσεις, που ονομάζονται βιομεμβράνες, και να παράγουν ουσίες που βελτιώνουν την επιβίωσή τους.

Αυτή η διπλωματική εργασία είχε ως στόχο να διερευνήσει πώς το ορατό φως επηρεάζει τρεις τύπους ευεργετικών βιολογικών παραγόντων ελέγχου (BCAs) τόσο σε εργαστηριακές όσο και σε θερμοκηπιακές συνθήκες. Δοκιμάζοντας διαφορετικά μήκη κύματος, διαπιστώθηκε ότι σε συνθήκες χαμηλών θρεπτικών στοιχείων στο εργαστήριο, δύο από τα τρία είδη βακτηρίων, *Bacillus amyloliquefaciens* και *Pseudomonas chlororaphis*, προτιμούσαν τις θεραπείες με μπλε φως. Αντίθετα, το τρίτο είδος, *Streptomyces griseoviridis*, προτιμούσε τη θεραπεία με κόκκινο φως. Ο σχηματισμός βιομεμβρανών μπορούσε να πραγματοποιηθεί ανάλογα με το θρεπτικό στοιχείο, τη θεραπεία με φως και το βακτήριο, κάτι που θα μπορούσε να είναι εξαιρετικά επωφελές.

Ωστόσο, οι ίδιες προτιμήσεις δεν παρατηρήθηκαν όταν οι τρεις BCAs εισήχθησαν σε φύλλα ντομάτας στο θερμοκήπιο. Σε αυτή τη ρύθμιση, όλοι οι BCAs προτίμησαν τις θεραπείες με πράσινο και λευκό φως. Το *S. griseoviridis* αναπτύχθηκε καλά ανεξάρτητα από τη θεραπεία φωτός, ενώ το *B. amyloliquefaciens* φάνηκε να αποφεύγει το κόκκινο φως.

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The power of light from a non-phototrophic perspective: a phyllosphere dilemma

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Plants and crop stands are considered holobionts, colonized by both autotrophic and by non-phototrophic heterotrophic microbiota. The dilemma in the exisiting body of studies is that the focus is primarily directed towards environmental specificties relevant for phototrophic organisms (predominantly plants), but does not take into account non-phototrophs. By definition, non-phototrophic heterotrophic bacteria do not use light as an energy source. Light energy and wavelength are rather used as a signal that can provoke shifts in both their metabolism and microbial lifestyle. Reaction and recovery time can vary between organisms and is dependent on the organism's physiological stage. The length of the lighting event affects the energy an organism is exposed to. We argue that to obtain a deeper and more distinct understanding of light exposure (irradiance, exposure length), quantity (light intensity), and quality (wavelength/spectral distribution, bandwidth at full-width half-maximum) related mechanisms on non-phototrophic bacteria in the phyllosphere, the light environment needs to be further strictly characterized. This includes information on the actual energy hitting planktonic or sessile non-phototrophic bacteria resident on and inside plants aboveground. Mapping the light environment in ecosystems aids in unraveling light-phyllosphere interactions and strengthens their transdisciplinary character. This issue is fundamental in order to revisit and repeat others' experimental approaches and findings but also to be able to translate findings into further action.

KEYWORDS

energy, exposure dose, irradiance, light intensity, non-phototrophic phyllosphere bacteria, phyllosphere environment

1 Introduction

Based on premises rather than experimental evidence, the phyllosphere is often characterized using atmospheric rather than boundary layer conditions (mainly temperature and to some extent relative humidity) as a basis of its description (Alsanius et al., 2019). Light conditions are often incomplete in microbial studies, such as day length, crop stand, light spectrum, and light intensity, are often ignored despite the overruling influence of light on primary (photosynthesis) and secondary plant processes (biomass formation, crop development, secondary metabolisms) which could influence the plant microbiota.

BOX 1 Definitions

Term	Definition	Unit*
Daily light integral	The number of photosynthetically active photons (photons in the PAR range) accumulated in a square meter during a day. The daily light integral measures light quantity.	mol m^{-2}
Exposure dose	Light irradiance multiplied with the length of exposure (exposure time) Exposure dose = Light irradiance (J or W m^{-2}) (where the light intensity is also taken into account) x Exposure time (s) An example can be found in the Supplementary Material S1	J m^{-2} or W m^{-2} s
Exposure length, exposure time	Duration of light treatment	s, min, h
Irradiance	Energy passing through a unit cross-sectional area per unit time.	W m^{-2}
Intensity	The number of photons at a certain period of time	
Light intensity (Quantum meter)	Number of photons hitting an area in unit time.	µmol m^{-2} s^{-1}
Light quality	See spectral distribution	
Photon flux	Number of photons hitting an area per unit time	
Photoperiod	Period of time each day during which an organism receives light; usually indicated by length of light and dark interruption period.	h
Photosynthetic Photon flux density (PPFD)	Number of the number of photosynthetically active photons striking a surface each second	µmol m^{-2} s^{-1}
Spectral distribution	Relative number of photons within the different wavelengths emitted from a light source. Spectral distribution reflects light quality.	
Visible light	Part of the electromagnetic radiation spectrum that can be observed by the human eye.	nm
Wavelength	The distance between two corresponding points in a light wave, an electromagnetical wave	nm

*for conversion between different units, please consult Goncalves dos Reis and Ribeiro (2020).

In crop science, visible light, ranging from 380 nm (violet) to 750 nm (far-red light), is usually in focus. Given the plants' ability to transform light into energy, light is usually expressed as photosynthetic active radiation (PAR; 400–700 nm) and photosynthetic photon flux density (PPFD; µmol m^{-2} s^{-1}). PAR and PPFD units have been applied in studies of interactions between plants and UV- or infrared-light. Plants and crop stands are considered holobionts, colonized by both autotrophic and by non-phototrophic heterotrophic microbiota. Although the latter ones do not directly depend on light as an energy source, there is growing evidence that their metabolism uses light (light quality: light spectral distribution; wavelength, exposure dose that can affect the circadian rhythm) as signals (Losi and Gärtner, 2021; Kahl et al., 2022; Wollmuth and Angert, 2023).

The interdependence of light quality and aerial fungal plant pathogens is well known (see Alsanius et al., 2019, see Beattie et al., 2018 and references in both). Several studies indicate that spectral distribution induced shifts in bacterial lifestyles (planktonic, sessile), metabolic activity and environmental stress responses can occur when exposed to different wavelengths and intensities (Wu et al., 2013; Gharaie et al., 2017; Alsanius et al., 2019; Alsanius et al., 2021; Kahl et al., 2022; Hatfield et al., 2023). Moreover, the presence or absence of light can impact biofilm formation, as can light quality influence respiration of non-phototrophic bacteria (Kahl et al., 2022); redox stages in zones affected by different previous lighting stimuli could be endured upon changes in light conditions. Thus, irradiance based measures, such energy (W m^{-2}) and exposure dose (W m^{-2} s^{-1}; J m^{-2}) rather than just photon density ought to be mentioned when describing the phyllosphere environment in relation to light exposure (see Box 1).

We postulate that

- To study light-phyllosphere interactions, non-phototrophic organisms' perception of light (radiation) must be considered
- PAR based descriptions of the light environment, only focuses on plants', and associated phototrophic organisms' ability to utilize light.

Thus energy based information must be used to study the fate of non-phototrophic organisms in the phyllosphere.

2 Plant-light interactions

Plants use light as a primary energy source via photosynthesis, but light also informs the plant about the time of day, time of year, and about its surroundings (e.g., if the plant is shaded by other plants) (Wassink and Stolwijk, 1956). The photosynthetically active spectrum is normally generalized to 400–700 nm in wavelength, the range of 380–710 nm has also been suggested (McCree, 1972). However, wavelengths shorter (Ultraviolet light, UV) and longer (Far red, FR, Infra-red, IR) than the photosynthetically active wavelengths will further affect the plant in several ways. For measuring photosynthetic light, special PAR-sensors (quantum sensors) have been developed, giving values in the unit µmol m^{-2} s^{-1} (Ryer, 1997). Information about light intensity in combination with daily photoperiod can be integrated into a daily light integral (DLI), expressed in mol m^{-2} day^{-1}, which is a commonly used unit to quantify photosynthetic light. Spectral distribution within the

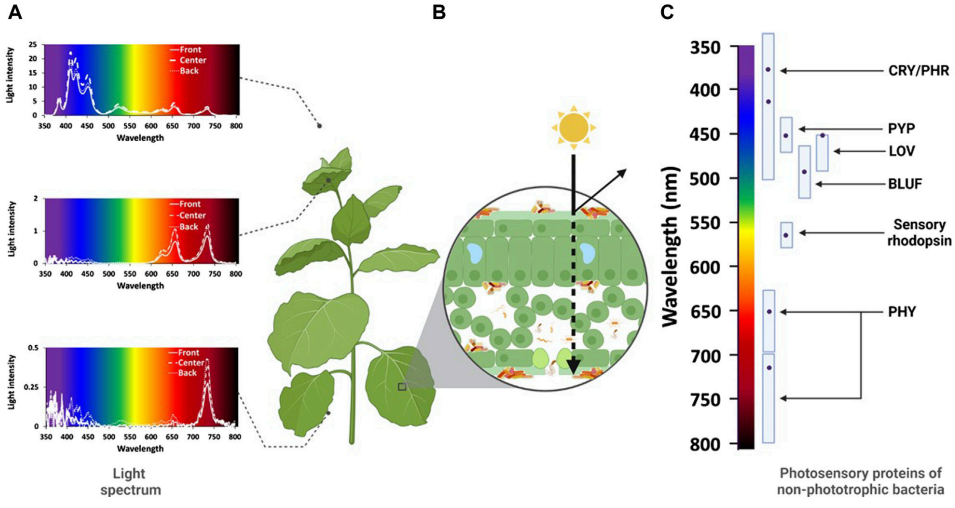


FIGURE 1
The plant holobiont as affected by light. **(A)** Light quantity (light intensity, irradiance) and light quality (spectral distribution) of white LEDs in the canopy of a Poinsettia (*Euphorbia pulcherima*) crop under greenhouse conditions. Light quantity and quality parameters were measured above the canopy as well as beneath the apical and basal leaves of Poinsettia plants placed directly under the LED (centre), or at 20 cm distance from the central plant (front, back). Light quantity decreases from the top to the bottom (note deviating y-axes). Differences also occurred with respect to the position of the plant in the crop stand. With respect to light quality, the light spectrum is distorted from high relative abundance of blue light above the canopy to higher relative abundance of red and far-red or just far-red bands beneath the apical and basal leaves, respectively. **(B)** Light quantity and quality hitting a leaf surface, light may be absorbed, refracted, or reflected. The leaf surface structure, but also the morphology and thickness of different leaf layers, the nature of pigments and their distribution play a key role for the transmittance of light through the leaf. **(C)** Spectral bands absorbed by different photosensory proteins (cryptochrome/photolyase, CRY/PHR; photosensory yellow protein, PYP; light-oxygen-voltage, LOV; sensor of blue light utilizing flavin adenine nucleotide, BLUF; Sensory rhodopsin; bacteriophotochrome, PHY) of non-phototrophic bacteria. The dots indicate the peak absorption within each wavelength spectrum for each photosensory protein. Recent results indicate that PHY also may act within the blue spectrum (Hatfield et al., 2023). (*Illustration: B. Alsanius and M. Hellström, supported by* Biorender.com).

photosynthetic light, and the amounts of UV-, FR- and IR-light can be measured using a spectroradiometer (Ryer, 1997), informing of the spectral irradiance for each wavelength in the units μmol m^{-2} s^{-1} or mW m^{-2}.

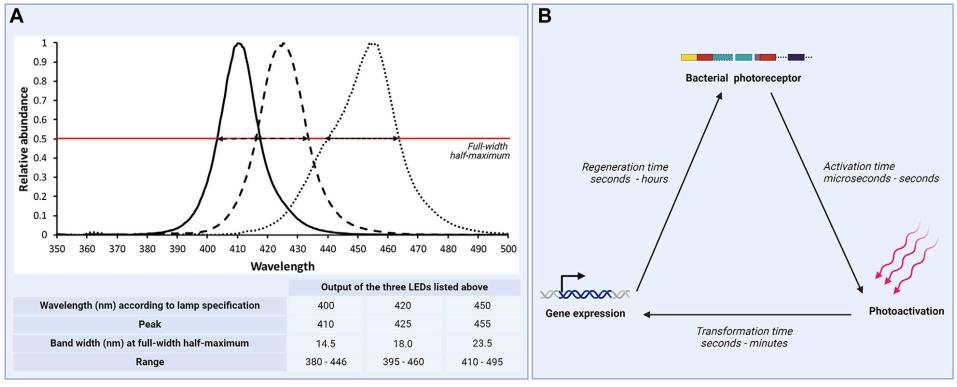
However, light measurements in growing systems are normally performed above the canopy. As soon as the light enters the canopy, the intensity and spectral distribution is altered due to absorption and reflection; this is a fact that is often overlooked when discussing the full lighting effect on and within plants (Figure 1).

3 Photoreception of non-phototrophic bacteria

Photosynthetic prokaryotes and some non-phototrophic bacteria are equipped with photosensory proteins. For non-phototrophic bacteria, in total, six different photosensory proteins have been identified with an array of absorption spectra within the visible and non-visible light spectrum (Figure 1). These include phytochromes (PHY) (Auldridge and Forest, 2011) absorbing red and far-red light, photoactive yellow proteins (PYP) (Kumauchi et al., 2008; Meyer et al., 2012), rhodopsins which are retinal binding receptors and provide light dependent ion transport (Ernst et al., 2014), blue light absorbing proteins cryptochrome/photolyase (Cry/PHR), blue light using flavin adenine dinucleotide (BLUF-FAD) and light oxygen voltage (LOV) domain (Gomelsky and Hoff, 2011; Wu et al., 2013).

The typical phytochrome architecture consists of three conserved domains: PAS (Per-ARNT.Sim), -GAF (cGMP phosphodiesterase/adenylate) and PHY (Phytochrome specific). In bacteria, biliverdin is used as a chromophore (Bhoo et al., 2001). The structure of PYP has a typical PAS domain and is often referred to as the prototype of proteins in this domain (Imamoto and Kataoka, 2007). The BLUF domain proteins can both be standalone BLUF domains or be coupled to phosphodiesterase (EAL) domains. Almost 70% of BLUF domains are not connected to EAL. The second most common structure of the BLUF domain is a BLUF-EAL combination (Kanazawa et al., 2010). The small photosensory protein LOV belongs to the PAS domain and is linked to histidine protein kinase (HisKa), di-guanylate cyclase (GGDEF) and EAL domains (Van der Horst et al., 2007).

Photosensing in bacteria can cause a cascade of reactions inside the cell. Signalling molecules and regulatory proteins can result in a change of gene regulation that can alter the behaviour and lifestyle of the bacteria involved. LOV, PYP and BLUF have under different



	Output of the three LEDs listed above		
Wavelength (nm) according to lamp specification	400	420	450
Peak	410	425	455
Band width (nm) at full-width half-maximum	14.5	18.0	23.5
Range	380 - 446	395 - 460	410 - 495

FIGURE 2
Exposure dose information - a fundamental detail in studies on light response of non-phototrophic bacteria. **(A)** Outputs of three LED devices within the violet-blue spectrum. Wavelengths specified for the LEDs were 400 nm, 420 nm, and 450 nm, respectively. Regauging the three LEDs displayed that peak relative abundance deviated from the specifications and that spectral width characteristics varied considerably between the three LEDs as expressed by bandwidth at full-width half-maximum (indicated by the red line) and spectrum range output. To calculate the exposure dose, these characteristics need to be described. **(B)** Response to electromagnetic radiation differs between different photoreceptors, bacterial organisms and strains, but also between different phases within. Activation time might range between microseconds to seconds, transition from photoactivation to gene expression (transition time) between seconds to minutes and regeneration time between seconds to hours. (*Illustration: B. Alsanius and M. Hellström, supported by* biorender.com).

light conditions shown to control the transition between planktonic single cell lifestyle into a sessile multicellular lifestyle. The key player in this is the second messenger c-di-GMP with the help by GGDEF and EAL, which is responsible in the synthesis and the hydrolysis of c-di-GMP (Hengge, 2009). If the concentration of c-di-GMP increases in the cell, the motility of the flagella is inhibited and thus the synthesis and excretion of the biofilm component is stimulated. There is also evidence that both LOV and PHY regulates swarming motility in the pathogen *Pseudomonas syringae* and that there is cross talk between these two proteins (Wu et al., 2013). In *Escherichia coli,* the BLUF-EAL protein YcgF does not hydrolyse c-di-GMP but instead binds to the repressor YcgE during blue light exposure. This leads to activation of both biofilm matrix production and of acid resistance genes and downregulation of adhesive curli fimbriae. Moreover, the expression of YcgF and YcgE was activated strongly at low temperatures (Tschowri et al., 2009).

Light can also change the utilization patterns of nutrients in bacteria and thus affect several metabolic pathways when bacteria are exposed to different light spectra (Müller et al., 2017; Alsanius et al., 2021; Karlsson et al., 2023). Recent results on *P. syringae* pv. *syringae* demonstrate that non-phototrophic bacteria employ light information to sense and prepare for environmental changes, such as water stress (Hatfield et al., 2023).

4 Discussion

In non-phototrophic bacteria, photoreceptors are globally regulating metabolic functional activities (Hatfield et al., 2023). Photoreceptors are thus high on the regulatory pyramid. Reaction and recovery time to light exposure can vary between seconds to minutes and even hours (Ernst et al., 2014; Liu et al., 2018). They deviate between different photoreceptor proteins and wavelengths, as well as on the intensity of emitted light (Figure 2). Furthermore, the light exposure and interval have been shown to affect the circadian rhythm in various bacteria (Kahl et al., 2022; Wollmuth and Angert, 2023), which have led to permanent metabolic changes. By not defining all possible driving factors, such as light intensity, wavelength and exposure time, a dilemma occurs with respect to understanding how they can affect microbial mechanisms, in particular of non-phototrophic bacteria within the phyllosphere. For example, when comparing the two wavelengths of 660 nm and 400 nm even though they have the same intensity (500 μmol m^{-2} s^{-1}) the total exposure dose is much higher in the latter of the two (see example shown in Supplementary Material S1). Some studies either state the wavelength used in treatment, *e.g.,* 420 nm, or just the colour perceived by the human eye, *e.g.*, blue, and the intensity at which it was used for (PPFD) (Huché-Thélier et al., 2016). This not only affects the reproducibility of experiments and results, comparability, generalization of data but also their translation to any applied setting. Thus, in the case of studying light-microbe and phyllosphere interactions a defined exposure dose is necessary.

Information about the light environment varies substantially in several published studies. A Scopus literature survey, spanning over a ten-year-period (2013–2023) and based on the search terms ("bacteria" OR "biocontrol*") AND ("light" OR "irradiation" OR "light quality") AND ("phyllosphere" OR "leaf") produced 21 eligible references and 29 individually assessed experiments (Alsanius et al., 2024). Most of the studies were conducted *in vitro*. A majority, but not all

studies, stated light quantity. Either light intensity (37.9%) or irradiance (48.3%) was used as a measure; no information was given on the intensity distribution (as displayed in Hoenes et al., 2015). Light quantity statements considered outputs from the light source but did not take material properties of vials or containers light transmittance into account, used for housing the organisms during the experiment (as displayed in Gharaie et al., 2017). The studies included various light sources delivering either mono- or polychromatic (white) light. Light quality parameters were poorly displayed and information on the spectral distribution was mostly lacking. In studies including monochromatic light, the peak value, but not the bandwidth, was often mentioned. The spectral composition in polychromatic light varies and information on the spectral range was not always sufficient. Most studies indicated exposure time. However, as light quantity and quality parameters were mostly poorly described and thus the recalculation of exposure dose is rather difficult.

As any environmental cue, light quality, exposure and dose can have large effects on the plant as a holobiont. To direct and manage photodependent responses in non-phototrophic plant colonizing bacteria, deeper insights are required into the pace of action caused by light within the crop stand. Reception, scattering and absorption of light differs greatly within the canopy as demonstrated by Schipper et al. (2023), but also within the plant organs, *e.g.*, leaf (Vogelmann and Björn, 1986; Vogelmann and Gorton, 2014; Müller et al., 2016) (Figure 1).

Leaves tend to absorb approximately 80% of the light they receive. Within this percentage, some of it is reflected due to light scattering within the intercellular air pockets inside a leaf. Further, several leaf organs can alter the spectral quality of the received light such as chlorophylls and carotenoids due to absorption. This alteration causes steep internal gradients within the leaf tissue and thus at different depths there are diverse light environments for chloroplasts. One other factor that can cause a light gradient is the leaf angle itself, this as light direction and quality is affected greatly by it (Vogelmann and Gorton, 2014) (Figure 1).

A majority of studies on light-non-phototrophic bacteria-phyllosphere interactions have only been conducted under *in vitro* conditions. Substantially fewer studies involve plants and crop stands. However, the experimental conditions (light intensity, wavelength, photoperiod, exposure dose, humidity, temperature) are not always stated (Alsanius et al., 2024). To apply and follow up photo-dependent bacterial mechanisms in crop stands, distinct characterization of plant canopy conditions are necessary. This is needed to better understand the plant holobiont and to foresee light related events in both natural and cultured crop stands. Thus, it is vital to re-evaluate findings presented in the literature from the perspective of light exposure dose and related parameters to get a deeper and more distinct understanding of the effect of light in light-microbe interactions, especially in the phyllosphere. This would lead to a clearer characterization of the ecosystems studied and allow for a richer understanding of why light quality and quantity can have the effect observed within the microbiota present or introduced to on a plant.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

BA: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Visualization, Writing–original draft, Writing–review and editing. MH: Investigation, Visualization, Writing–original draft, Writing–review and editing. K-JB: Investigation, Writing–original draft, Writing–review and editing. RV: Supervision, Writing–original draft, Writing–review and editing. PB: Supervision, Writing–original draft, Writing–review and editing. MK: Investigation, Supervision, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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References

Alsanius, B. W., Karlsson, M. E., Hellström, M., Bergstrand, K.-J., and Rosberg, A. K. (2024). Integrated greenhouse production in new light: prospects on improved LED-assisted biocontrol of foliar diseases. *Acta Hortic.*, 293–296. accepted. doi:10.17660/actahortic.2020.1296.38

Alsanius, B. W., Karlsson, M. E., Rosberg, A. K., Dorais, M., Naznin, T., Khalil, S., et al. (2019). Light and microbial lifestyle: the impact of light quality on plant-microbe interactions in horticultural production systems - a review. *Horticulturae* 5, 41. doi:10.3390/horticulturae5020041

Alsanius, B. W., Vaas, L. A. I., Gharaie, S., Karlsson, M. E., Rosberg, A. K., Wohanka, W., et al. (2021). Dining in blue light impairs the appetite of some leaf epiphytes. *Front. Microbiol.* 12, 725021. doi:10.3389/fmicb.2021.725021

Auldridge, M. E., and Forest, K. T. (2011). Bacterial phytochromes: more than meets the light. *Crit. Rev. Biochem. Mol. Biol.* 46, 67–88. doi:10.3109/10409238.2010.546389

Beattie, G. A., Hatfield, B. M., Dong, H., and Mcgrane, R. S. (2018). Seeing the light: the roles of red- and blue-light sensing in plant microbes. *Annu. Rev. Phytopathology* 56, 41–66. doi:10.1146/annurev-phyto-080417-045931

Bhoo, S. H., Davis, S. J., Karniol, B., and Vierstra, R. D. (2001). Bacteriophytochromes are photochromic histidine kinases using a biliverdin chromophore. *Nat. Rev. Microbiol.* 414, 776–779. doi:10.1038/414776a

Ernst, O. P., Lodowski, D. T., Elstner, M., Hegemann, P., Brown, L. S., and Kandori, H. (2014). Microbial and animal rhodopsins: structures, functions, and molecular mechanisms. *Chem. Rev.* 114, 126–163. doi:10.1021/cr4003769

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

Gomelsky, M., and Hoff, W. D. (2011). Light helps bacteria make important lifestyle decisions. *Trends Microbiol.* 19, 441–448. doi:10.1016/j.tim.2011.05.002

Goncalves Dos Reis, M., and Ribeiro, A. (2020). Conversion factors and general equations applied in agricultural and forest meteorology. *Agrometeoros* 27, 227–258. doi:10.31062/agrom.v27i2.26527

Hatfield, B. M., Lasarre, B., Liu, M., Dong, H., Nettleton, D., and Beattie, G. A. (2023). Light cues induce protective anticipation of environmental water loss in terrestrial bacteria. *Proc. Natl. Acad. Sci.* 120, e2309632120. doi:10.1073/pnas.2309632120

Hengge, R. (2009). Principles of c-di-GMP signalling in bacteria. *Nat. Rev. Microbiol.* 7, 263–273. doi:10.1038/nrmicro2109

Hoenes, K., Stangl, F., Stift, M., and Hessling, M. (2015). "Visible optical radiation generates bactericidal effect applicable for inactivation of health care associated germs demonstrated by inactivation of *E. coli* and B. subtilis using 405 nm and 460 nm light emitting diodes," in *Novel biophotonics techniques and applications III*. Editors A. AMELINK and I. A. VITKIN 95400T.

Huché-Thélier, L., Crespel, L., Gourrierec, J. L., Morel, P., Sakr, S., and Leduc, N. (2016). Light signaling and plant responses to blue and UV radiations—perspectives for applications in horticulture. *Environ. Exp. Bot.* 121, 22–38. doi:10.1016/j.envexpbot.2015.06.009

Imamoto, Y., and Kataoka, M. (2007). Structure and photoreaction of photoactive yellow protein, a structural prototype of the PAS domain superfamily. *Photochem. Photobiol.* 83, 40–49. doi:10.1562/2006-02-28-IR-827

Kahl, L. J., Eckartt, K. N., Morales, D. K., Price-Whelan, A., and Dietrich, L. E. P. (2022). Light/dark and temperature cycling modulate metabolic electron flow in *Pseudomonas aeruginosa* biofilms. *mBio* 13, e0140722. doi:10.1128/mbio.01407-22

Kanazawa, T., Ren, S., Maekawa, M., Hasegawa, K., Arisaka, F., Hyodo, M., et al. (2010). Biochemical and physiological characterization of a BLUF protein-EAL protein complex involved in blue light-dependent degradation of cyclic diguanylate in the purple bacterium Rhodopseudomonas palustris. *Biochemistry* 49, 10647–10655. doi:10.1021/bi101448t

Karlsson, M. E., Hellström, M., Flöhr, A., Bergstrand, K.-J., and Alsanius, B. W. (2023). The power of light: impact on the performance of biocontrol agents under minimal nutrient conditions. *Front. Microbiol.* 14, 1087639. doi:10.3389/fmicb.2023.1087639

Kumauchi, M., Hara, M. T., Stalcup, P., Xie, A., and Hoff, W. D. (2008). Identification of six new photoactive yellow proteins--diversity and structure-function relationships in a bacterial blue light photoreceptor. *Biochem. Photobiol.* 84, 956–969. doi:10.1111/j.1751-1097.2008.00335.x

Liu, Z., Zhang, J., Jin, J., Geng, Z., Qi, Q., and Liang, Q. (2018). Programming bacteria with light—sensors and applications in synthetic biology. *Front. Microbiol.* 9, 2692. doi:10.3389/fmicb.2018.02692

Losi, A., and Gärtner, W. (2021). A light life together: photosensing in the plant microbiota. *Photochem. Photobiological Sci.* 20, 451–473. doi:10.1007/s43630-021-00029-7

Mccree, K. J. (1972). Test of current definitions of photosynthetically active radiation against leaf photosynthesis data. *Agric. Meteorol.* 10, 443–453. doi:10.1016/0002-1571(72)90045-3

Meyer, T. E., Kyndt, J. A., Memmi, S., Moser, T., Colón-Acevedo, B., Devreese, B., et al. (2012). The growing family of photoactive yellow proteins and their presumed functional roles. *Photochem. Photobiological Sci.* 11, 1495–1514. doi:10.1039/c2pp25090j

Müller, D. B., Vogel, C., Bai, Y., and Vorholt, J. A. (2016). The plant microbiota: systems-level insights and perspectives. *Annu. Rev. Genet.* 50, 211–234. doi:10.1146/annurev-genet-120215-034952

Müller, G. L., Tuttobene, M., Altilio, M., Martínez Amezaga, M., Nguyen, M., Cribb, P., et al. (2017). Light modulates metabolic pathways and other novel physiological traits in the human pathogen *Acinetobacter baumannii*. *J. Bacteriol.* 199, e00011-17–e00017. doi:10.1128/jb.00011-17

Ryer, A. D. (1997). *The light measurement handbook*. Peabody, MA USA: International Light Technologies.

Tschowri, N., Busse, S., and Hengge, R. (2009). The BLUF-EAL protein YcgF acts as a direct anti-repressor in a blue-light response of *Escherichia coli*. *Genes Dev.* 23, 522–534. doi:10.1101/gad.499409

Van Der Horst, M. A., Key, J., and Hellingwerf, K. J. (2007). Photosensing in chemotrophic, non-phototrophic bacteria: let there be light sensing too. *Trends Microbiol.* 15, 554–562. doi:10.1016/j.tim.2007.09.009

Vogelmann, T. C., and Björn, L. O. (1986). Plants as light traps. *Physiol. Plant.* 68, 704–708. doi:10.1111/j.1399-3054.1986.tb03421.x

Vogelmann, T. C., and Gorton, H. L. (2014). "Leaf: light capture in the photosynthetic organ," in *The structural basis of biological energy generation*. Editor M. F. HOHMANN-MARRIOTT (Amsterdam: Springer).

Wassink, E., and Stolwijk, J. (1956). Effects of light quality on plant growth. *Annu. Rev. Plant Physiology* 7, 373–400. doi:10.1146/annurev.pp.07.060156.002105

Wollmuth, E. M., and Angert, E. R. (2023). Microbial circadian clocks: host-microbe interplay in diel cycles. *BMC Microbiol.* 123, 124. doi:10.1186/s12866-023-02839-4

Wu, L., Mcgrane, R. S., and Beattie, G. A. (2013). Light regulation of swarming motility in *Pseudomonas syringae* integrates signaling pathways mediated by a bacteriophytochrome and a LOV protein. *mBio* 4, 003344–e413. doi:10.1128/mBio.00334-13

Supplementary Material

Alsanius, B.W.*, Hellström, M., Bergstrand, K.-J., Vetukuri, R.R., Becher, P. G., Karlsson. M.E. The power of light from a non-phototrophic perspective: A phyllosphere dilemma. Front. Photobiol. - Photoecology and Environmental Photobiology, 2. DOI: 10.3389/fphbi.2024.1432066

Supplementary Material S1. Exposure dose example

The exposure dose of an LED set at 660 nm at a light intensity of 500 µmol m^{-2} s^{-1} vs and LED set at 400 nm at a light intensity of 500 µmol m^{-2} s^{-1}:

Energy of one photon

$$E = \frac{hc}{\lambda}$$

E is the energy of the photon in joules

h is Planck's constant (approx. 6.62607015×10^{-34} m^2 kg s^{-1})

c is the speed of light (approx. 299792458 m s^{-1}) in a vacuum

λ is the wavelength of the light in meters

Energy (J) of one photon of 660 nm:

$E_{660} = \frac{6.62607015 \times 10^{-34} \times 299792458}{6.6 \times 10^{-7}}$

$E_{660} = 3.01 \times 10^{-7}$ J

Energy (J) of one photon of 400 nm:

$E_{400} = 4.97 \times 10^{-7}$ J

Light intensity

Intensity (I) = 500 µmol m^{-2} s^{-1}

Area (A) = 1 m^2 (assuming light is falling on a square meter area)

Convert intensity to photons per second:

Photons s^{-1} = I x A

Photons s^{-1} = 500 x 10^{-6} x 1

Photons $s^{-1} = 5 \times 10^{-4}$
Total energy output
Total energy output = Photons $s^{-1} \times E_{photon}$ Total energy output (J m^{-2} s^{-1}): - For a 660 nm lamp at 500 µmol m^{-2} $s^{-1} = 1.50 \times 10^{-22}$ - For a 400 nm lamp at 500 µmol m^{-2} $s^{-1} = 2.48 \times 10^{-22}$
If the LEDs are switched on for 24 hours, the total exposure dose would be:
For a 660 nm lamp at 500 µmol m^{-2} s^{-1} • = 1.50×10^{-22} x (24 x 3600 s) • = 1.3×10^{-17} J m^{-2}
For a 400 nm lamp at 500 µmol m^{-2} s^{-1} • = 2.48×10^{-22} x (24 x 3600s) • = 2.15×10^{-17} J m^{-2}
The total exposure dose is approximate 44% lower for the 660 nm LED than for the 400 nm device.

II

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RESEARCH ARTICLE

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

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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

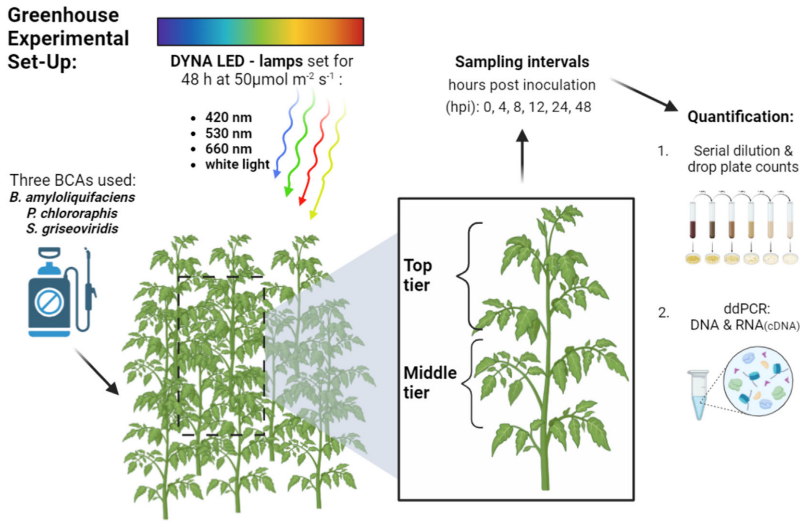

FIGURE 1 A schematic overview of the greenhouse experimental set-up where three biological control agents (BCAs) were sprayed onto tomato plants that were exposed to different light treatments for 48 h. Leaves were harvested from two placements (top and middle tier) in the canopy at 0, 4, 8, 12, 24 and 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

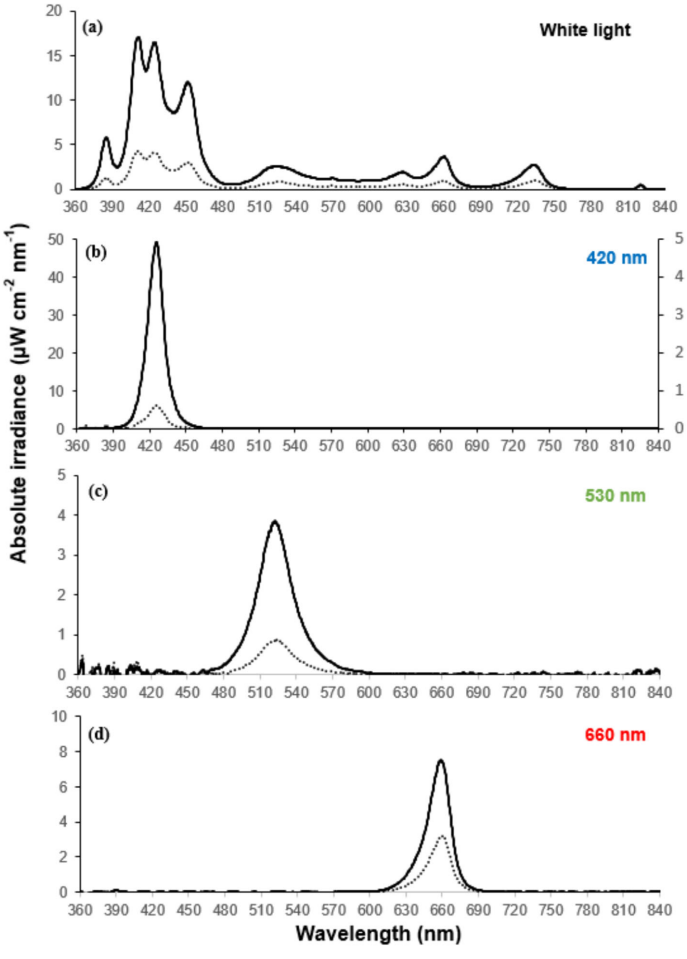

FIGURE 2 Spectral distribution of the four light treatments (DYNA LED lamps) used in the greenhouse experiments. Depicted is the absolute irradiance for both the top tier (solid line) and the middle tier (broken line) of the tomato canopy, (a) white light; (b) blue: 420 nm (note: varying y-axis); (c) 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,

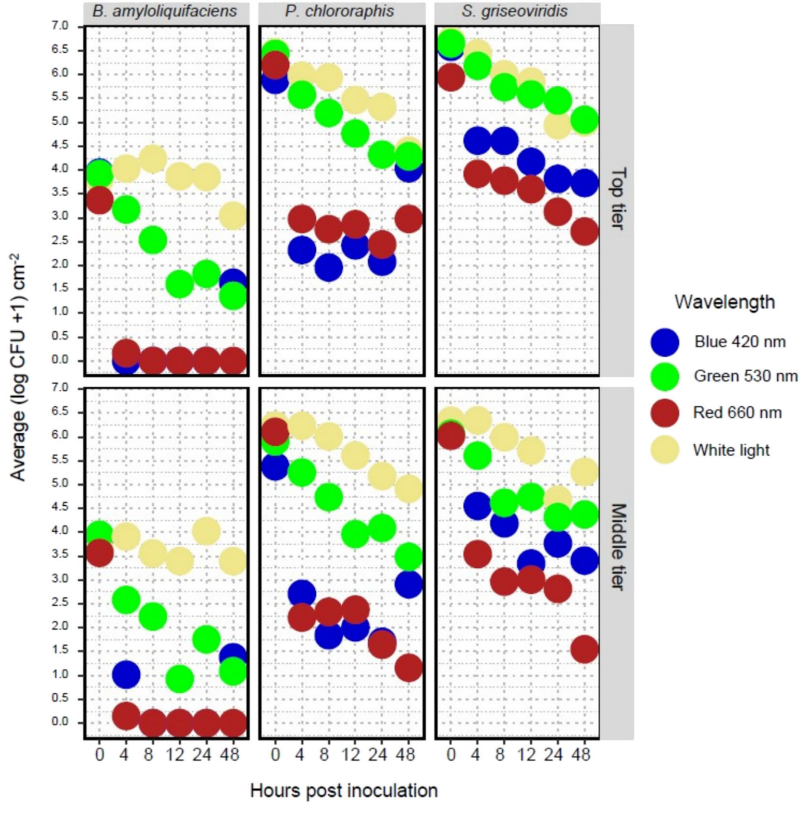

FIGURE 3 Average log colony forming units (CFU + 1) biological control agents cm^{-2} tomato leaves at two placements in the crop canopy; top tier and middle tier over time ($n = 36$, per organism, tier and light treatment, standard deviation, Table S6). The three introduced organisms via foliar spray 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

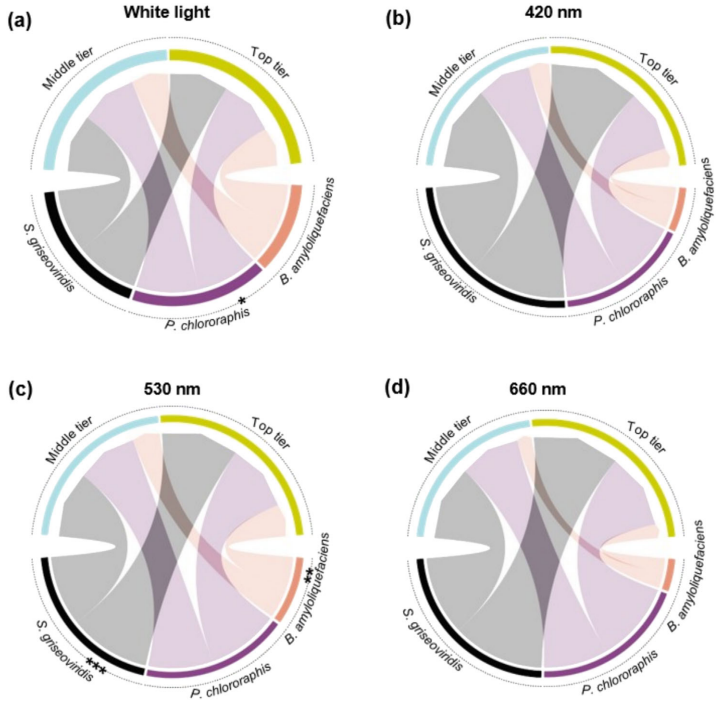

FIGURE 4 Average log colony forming units (CFU + 1) biological control agents cm^{-2} fresh tomato leaves at different placements in the canopy (middle tier in light blue and top tier in yellow) after a total of 48-h exposure to poly- (a: white light) or monochromatic (b: blue: 420 nm, c: green: 530 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.

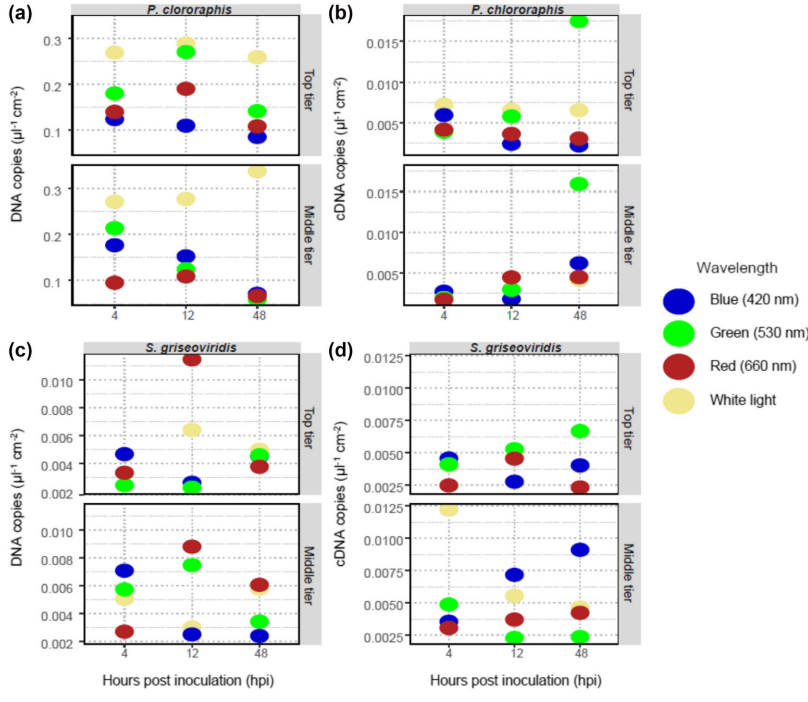

FIGURE 5 Mean number of copies of either DNA (a, c) or cDNA (b, d) for *Pseudomonas chlororaphis* 50083 and *Streptomyces griseoviridis* CBS904.68 copies (μl^{-1} cm^{-2}) at both tiers, top and middle tiers (standard deviations: Table S8). Samples were taken at three time points (4, 12 and 48 h post 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.

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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.

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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.

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Plants, People, Planet **Supporting Information**

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

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

The following Supporting Information is available for this article:

Figure S1 The relative spectral irradiance of the four light treatments.

Table S1 The breadth of each of the four light treatments determined by setting a target line and calculating the breadth of the peak at 0.5 of the relative spectral irradiance.

Table S2 Average absolute quantification of DNA and cDNA copies μl^{-1} of the undiluted and sprayer content for *P. chlororaphis* and *S. griseoviridis*.

Table S3 Mean values of six untreated tomato plants used to measure several plant parameters such as dry weight, surface area, leaf angle, photosynthetic rate, stomatal conductance, and chlorophyll content as well as photosystem efficiency for each light treatment.

Table S4 Means of physiological measurements of six untreated plants used for each light treatment and the average total of all the means.

Table S5 The total exposure dose for all light treatments (White light, blue: 420 nm, green: 530 nm, red: 660 nm) at both placements (Top and middle tier) for either 0, 4, 8, 12, 24 or 48 hours post inoculation.

Table S6 Average log CFU +1, cm^{-2} and standard deviations of the three BCAs (*B. amyloliquefaciens, P. chlororaphis, S. griseoviridis*) under all light treatments (White light, blue: 420 nm, green: 530 nm, red: 660 nm) at both placements (Top and middle tier) for either 0, 4, 8, 12, 24 or 48 hours post inoculation.

Table S7 Four-way Anova results for the three biological control agents (BCAs) (*B. amyloliquefaciens, P. chlororaphis, S. griseoviridis*). BCA, hours post inoculation, placement and wavelength were taken into account when compared to the average log CFU +1, biological control agents cm^{-2} tomato leaves during the 48 hour period.

Table S8 Average of copies DNA and cDNA and their standard deviations of the re-isolated *S. griseoviridis* (SG) and *P. chlororaphis* (PC), per light treatment and placement in the canopy, at 4, 12 and 48 hours post inoculation.

Table S9 ANOVA results based on a LLM for the ddPCR data, DNA and cDNA copies (μl^{-1} cm^{-2}) of samples of *S. griseoviridis* under four light treatments**.**

Table S10 Commercial products containing the three biological control agents used in the current study, which are approved for use in Sweden.

Table S11 ANOVA results for the ddPCR data, DNA and cDNA copies (μl^{-1} cm^{-2}) of samples of *P. chlororaphis* and *S. griseoviridis* under four light treatments.

Table S12 ANOVA results based on a LLM for the ddPCR data, DNA and cDNA copies (μl^{-1} cm^{-2}) of samples of *P. chlororaphis* under four light treatments.

Figure S1 The relative spectral irradiance of the four light treatments (DYNA LED lamps) (White light, Blue: 420 nm, Green: 530 nm, Red: 660 nm) used in the greenhouse experiments with a target line set at 0.5 of the relative spectral irradiance.

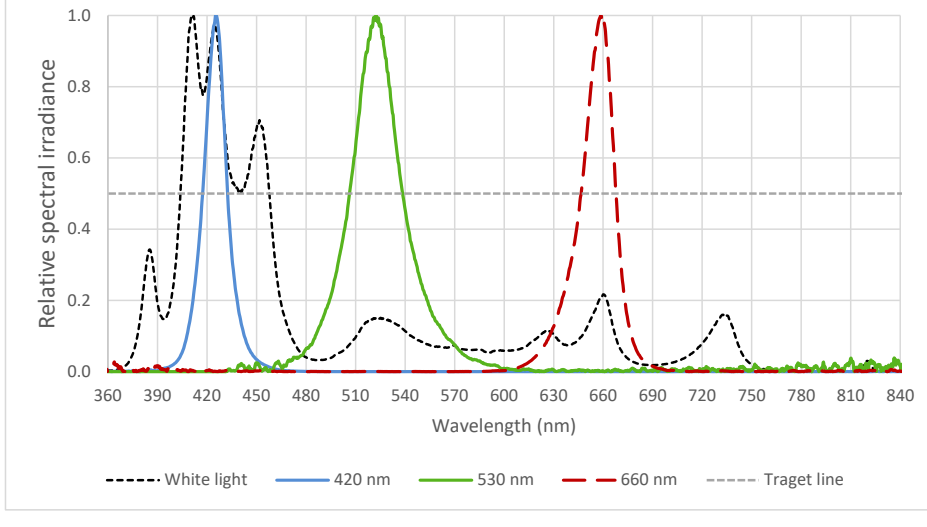


Table S1 The breadth of each of the four light treatments determined by setting a target line and calculating the breadth of the peak at 0.5 of the relative spectral irradiance. In the case of the full spectrum, 12 nm were omitted, as there is a dip between 444 – 456 nm, which happens to be at the target line.

	Range at 0.5 of the relative spectral irradiance (nm)	**Breadth of peak (nm)**
White light	403 - 458	43
Blue: 420 nm	416 - 432	16
Green: 530 nm	505 - 540	35
Red: 660 nm	645 - 668	23

Table S2 Average absolute quantification of DNA and cDNA copies (μl^{-1}) for *S. griseoviridis* and *P. chlororaphis* in both the pure undiluted culture and the sprayer content.

Species	**Type**	**average DNA copies (μl^{-1})**	**average cDNA copies (μl^{-1})**
Streptomyces griseoviridis	Pure culture	90.50	0.94
Streptomyces griseoviridis	Sprayer content	5.53	0.38
Pseudomonas chlororaphis	Pure culture	1807.33	1.60
Pseudomonas chlororaphis	Sprayer content	2.65	0.85

Table S3 Mean values of six untreated tomato plants used to measure the dry weight (g), surface area (cm^2), leaf angle (°), photosynthetic rate ($\mu mol\ m^{-2}\ s^{-1}$), stomatal conductance ($mol\ m^{-2}\ s^{-1}$), and chlorophyll content as well as photosystem efficiency (Fm/Fv) for each light treatment. Light intensities ($\mu mol\ m^{-2}\ s^{-1}$) monitored at the different leaf positions are displayed.

Light treatment	White light	White light	420 nm	420 nm	530 nm	530 nm	660 nm	660 nm
Placement	Top	Middle	Top	Middle	Top	Middle	Top	Middle
Light intensity	48.3 ± 6.8	18.8 ± 8.4	48.8 ± 16.7	20.0 ± 11.2	46.3 ±19.8	11.5 ±5.2	49.3 ± 3.1	21.5 ± 4.2
Dry weight	3.2 ± 0.7	5.3 ± 1.3	5.3 ± 1.9	5.8 ± 1.5	6.0 ± 2.4	8.0 ± 2.7	5.4 ± 0.7	4.1 ± 0.9
Surface area	97.8 ± 13.5	177.5 ± 29.2	133.8 ± 38.1	184.1 ± 51.9	169.7 ± 33.2	232.8 ± 37.1	119.7 ± 18.0	97.8 ± 15.7
Leaf angle	64.5 ± 10.6	70.3 ± 3.6	63.2 ± 12.4	73.0 ± 21.7	57.0 ± 8.6	85.8 ± 20.7	50.5 ± 9.9	71.5 ± 21.5
Photosynthetic rate	1.9 ± 0.6	0.5 ± 0.2	1.2 ± 0.5	0.8 ± 0.4	1.6 ± 0.4	0.3 ± 0.1	0.4 ± 0.5	-0.3 ± 0.1
Stomatal conductance	0.04 ± 0.02	0.01 ± 0.01	0.05 ± 0.02	0.02 ± 0.00	0.04 ± 0.04	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Photosystem efficiency	0.77 ± 0.03	0.76 ± 0.04	0.74 ± 0.05	0.78 ± 0.02	0.80 ± 0.03	0.75 ± 0.01	0.76 ± 0.02	0.74 ± 0.02

Table S4 Means of physiological measurements of six untreated plants used for each light treatment and the average total of all the means.

Wavelength (nm)	**Height Tot. (cm)**	**Height to the middle (cm)**	**Width (cm)**	**No. of leaves top**	**No. of leaves bottom**
White light	76.0 ± 3.4	39.1 ± 2.9	58.3 ± 7.2	7.0 ± 0.6	7.0 ± 0.9
420	98.5 ± 3.1	45.3 ± 3.3	72.0 ± 9.0	6.8 ± 1.0	6.3 ± 0.8
530	109.8 ± 6.2	52.0 ± 3.6	77.0 ± 5.4	7.5 ± 0.8	8.0 ± 0.6
660	66.8 ± 1.8	29.5 ± 3.4	51.2 ± 7.5	8.0 ± 0.9	5.3 ± 0.5

Table S5 The total exposure dose for all light treatments (White light, blue: 420 nm, green: 530 nm, red: 660 nm) at both placements (Top and middle tier) for either 0, 4, 8, 12, 24 or 48 hours post inoculation (hpi). The total energy calculations and R-script used to calculate the total energy can be found below.

Light treat-ment	**Place-ment**	**Light intensity (µmol m^{-2} s^{-1})**	**Intensity (m^{-2} s^{-1})**	**Total energy (J s^{-1} or W m^{-2})**	**Exposure dose (Wm^{-2} h) = Intensity (J s^{-1} or W m^{-2}) x Exposure Length (h)**					
					0	**4**	**8**	**12**	**24**	**48**
420 nm	Top	48.75	2.94E+19	13.88	0	55.54	111.08	166.62	333.24	666.48
420 nm	Middle	20	1.20E+19	5.70	0	22.79	45.57	68.36	136.71	273.43
530 nm	Top	46.25	2.79E+19	10.44	0	41.76	83.51	125.27	250.53	501.07
530 nm	Middle	11.5	6.93E+18	2.60	0	10.38	20.76	31.15	62.29	124.59
660 nm	Top	49.25	2.97E+19	8.93	0	35.71	71.41	107.12	214.24	428.47
660 nm	Middle	21.5	1.29E+19	3.90	0	15.59	31.17	46.76	93.52	187.05
White light	Top	48.33	-	12.72	0	50.88	101.77	152.65	305.30	610.61
White light	Middle	18.83	-	3.23	0	12.93	25.86	38.79	77.59	155.18

Calculations:

Energy of one photon

$$E = \frac{hc}{\lambda}$$

E is the energy of the photon in joules

h is Planck's constant (approx. $6.62607015 \times 10^{-34}$ J s)

c is the speed of light (approx. 299792458 m s^{-1}) in a vacuum

λ is the wavelength of the light in meters

Energy (J) of one photon of 420 nm:

$E_{420} = \frac{6.62607015 \times 10^{-34} \times 299792458}{4.2 \times 10^{-7}}$

$E_{420} = 3.01 \times 10^{-19}$ J

Energy (J) of one photon of 530 nm:

$E_{530} = 3.74 \times 10^{-19}$ J

Energy (J) of one photon of 660 nm:

$E_{660} = 4.72 \times 10^{-19}$ J

Light intensity

Intensity (I) = 500 µmol m^{-2} s^{-1}

1 µmol m^{-2} s^{-1} = 6.022 x 10^{17} photons m^{-2} s^{-1}

Convert intensity to photons m^{-2} s^{-1} :

50 µmol m^{-2} s^{-1} = 50 x 6.022 x 10^{17}

50 µmol m^{-2} s^{-1} = 3.011 x 10^{19} photons m^{-2} s^{-1}

Total energy output

Total energy output = E_{photon} (J) x Photons (m^{-2} s^{-1})

Total energy output (J m^{-2} s^{-1}):

- For a 660 nm lamp at 50 µmol m^{-2} s^{-1} = 3.01 x 10^{-19} x 3.011 x 10^{19} = 9.06 J m^{-2} s^{-1}
- For a 530 nm lamp at 50 µmol m^{-2} s^{-1} = 3.74 x 10^{-19} x 3.011 x 10^{19} = 11.26 J m^{-2} s^{-1}
- For a 420 nm lamp at 50 µmol m^{-2} s^{-1} = 4.72 x 10^{-19} x 3.011 x 10^{19} = 14.21 J m^{-2} s^{-1}

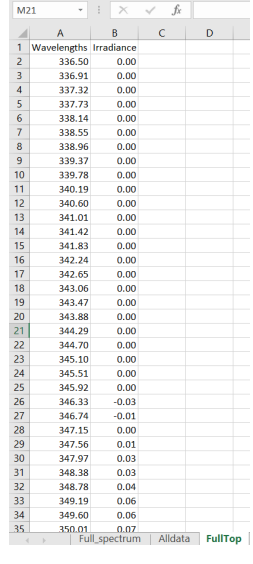
R-code used for the white light treatment:

```
library(readxl)
library(car)
library(tidyverse)
library(pracma)

#Top tier data for white light (full spectrum) treatment greenhouse exp.
dat <- read_excel("C:/Directory_name",sheet = "Sheet_nameFullTop")
dat
# Convert Irradiance to W/m^2/nm (since 1 µW/cm^2 = 0.01 W/m^2 and 1 nm = 1e-
9 m)
dat$Irradiance <- dat$Irradiance * 0.01
# Perform numerical integration using trapezoidal rule
total_energy <- trapz(x = dat$Wavelengths, y = dat$Irradiance)
total_energy

#Middle tier data for white light (full spectrum) treatment greenhouse exp.
dat2 <- read_excel("C:/Directory_name",sheet = "Sheet_nameMiddleTier")
dat2
# Convert Irradiance to W/m^2/nm (since 1 µW/cm^2 = 0.01 W/m^2 and 1 nm = 1e-
9 m)
dat2$Irradiance <- dat2$Irradiance * 0.01
# Perform numerical integration using trapezoidal rule
total_energy2 <- trapz(x = dat2$Wavelengths, y = dat2$Irradiance)
total_energy2
```

Here is a snapshot from the data file:



M21

	A	B	C	D
1	Wavelengths	Irradiance		
2	336.50	0.00		
3	336.91	0.00		
4	337.32	0.00		
5	337.73	0.00		
6	338.14	0.00		
7	338.55	0.00		
8	338.96	0.00		
9	339.37	0.00		
10	339.78	0.00		
11	340.19	0.00		
12	340.60	0.00		
13	341.01	0.00		
14	341.42	0.00		
15	341.83	0.00		
16	342.24	0.00		
17	342.65	0.00		
18	343.06	0.00		
19	343.47	0.00		
20	343.88	0.00		
21	344.29	0.00		
22	344.70	0.00		
23	345.10	0.00		
24	345.51	0.00		
25	345.92	0.00		
26	346.33	-0.03		
27	346.74	-0.01		
28	347.15	0.00		
29	347.56	0.01		
30	347.97	0.03		
31	348.38	0.03		
32	348.78	0.04		
33	349.19	0.06		
34	349.60	0.06		
35	350.01	0.07		

Full_spectrum | Alldata | FullTop

Table S6 Average (log CFU + 1) cm^{-2} and standard deviations of the re-isolated *B. amyloliquifaciens*, *P. chlororaphis*, *S. griseoviridis* per light treatment and placement in the canopy, throughout the 48-hour treatments.

Wavelength (nm)	Hour	Placement	Organism	Average (log CFU + 1) cm^{-2}	Standard deviation
White	0	Top tier	*B. amyloliquifaciens*	6.47	0.21
White	0	Middle tier	*B. amyloliquifaciens*	6.24	0.21
White	4	Top tier	*B. amyloliquifaciens*	3.80	0.17
White	4	Middle tier	*B. amyloliquifaciens*	3.64	0.12
White	8	Top tier	*B. amyloliquifaciens*	6.71	0.80
White	8	Middle tier	*B. amyloliquifaciens*	6.34	0.26
White	12	Top tier	*B. amyloliquifaciens*	5.98	0.43
White	12	Middle tier	*B. amyloliquifaciens*	6.23	0.27
White	24	Top tier	*B. amyloliquifaciens*	4.03	0.73
White	24	Middle tier	*B. amyloliquifaciens*	3.35	0.80
White	48	Top tier	*B. amyloliquifaciens*	6.45	0.17
White	48	Middle tier	*B. amyloliquifaciens*	6.34	0.20
420	0	Top tier	*B. amyloliquifaciens*	5.94	0.31
420	0	Middle tier	*B. amyloliquifaciens*	6.01	0.38
420					
420	4	Top tier	*B. amyloliquifaciens*	4.24	0.29
420	4	Middle tier	*B. amyloliquifaciens*	3.56	0.54
420	8	Top tier	*B. amyloliquifaciens*	6.01	0.28
420	8	Middle tier	*B. amyloliquifaciens*	5.99	0.24
420	12	Top tier	*B. amyloliquifaciens*	5.47	0.52
420	12	Middle tier	*B. amyloliquifaciens*	5.61	0.41
420	24	Top tier	*B. amyloliquifaciens*	3.87	0.91

420	24	Middle tier	*B. amyloliquifaciens*	3.40	1.74
420	48	Top tier	*B. amyloliquifaciens*	5.85	0.30
420	48	Middle tier	*B. amyloliquifaciens*	5.71	0.40
530	0	Top tier	*B. amyloliquifaciens*	5.32	0.59
530	0	Middle tier	*B. amyloliquifaciens*	5.18	0.44
530	4	Top tier	*B. amyloliquifaciens*	3.85	0.66
530	4	Middle tier	*B. amyloliquifaciens*	4.02	0.35
530	8	Top tier	*B. amyloliquifaciens*	4.94	1.12
530	8	Middle tier	*B. amyloliquifaciens*	4.68	1.39
530	12	Top tier	*B. amyloliquifaciens*	4.40	0.72
530	12	Middle tier	*B. amyloliquifaciens*	4.91	0.30
530	24	Top tier	*B. amyloliquifaciens*	3.04	0.45
530	24	Middle tier	*B. amyloliquifaciens*	3.40	0.61
530	48	Top tier	*B. amyloliquifaciens*	5.00	0.32
530	48	Middle tier	*B. amyloliquifaciens*	5.27	0.35
660	0	Top tier	*B. amyloliquifaciens*	5.89	0.56
660	0	Middle tier	*B. amyloliquifaciens*	5.38	0.45
660	4	Top tier	*B. amyloliquifaciens*	3.95	0.25
660	4	Middle tier	*B. amyloliquifaciens*	3.89	0.15
660	8	Top tier	*B. amyloliquifaciens*	4.62	0.34
660	8	Middle tier	*B. amyloliquifaciens*	4.55	0.36
660	12	Top tier	*B. amyloliquifaciens*	2.33	1.15
660	12	Middle tier	*B. amyloliquifaciens*	2.71	0.84
660	24	Top tier	*B. amyloliquifaciens*	0.00	
660	24	Middle tier	*B. amyloliquifaciens*	1.02	1.59
660	48	Top tier	*B. amyloliquifaciens*	4.62	0.34
660	48	Middle tier	*B. amyloliquifaciens*	4.55	0.36
White	0	Top tier	*P. chlororaphis*	1.96	0.99
White	0	Middle tier	*P. chlororaphis*	1.84	1.45
White	4	Top tier	*P. chlororaphis*	0.00	
White	4	Middle tier	*P. chlororaphis*	0.00	

White	8	Top tier	*P. chlororaphis*	4.61	0.42
White	8	Middle tier	*P. chlororaphis*	4.19	0.55
White	12	Top tier	*P. chlororaphis*	2.42	1.23
White	12	Middle tier	*P. chlororaphis*	2.01	1.08
White	24	Top tier	*P. chlororaphis*	0.00	
White	24	Middle tier	*P. chlororaphis*	0.00	
White	48	Top tier	*P. chlororaphis*	4.17	0.26
White	48	Middle tier	*P. chlororaphis*	3.35	0.36
420	0	Top tier	*P. chlororaphis*	2.08	1.11
420	0	Middle tier	*P. chlororaphis*	1.71	1.35
420	4	Top tier	*P. chlororaphis*	0.00	
420	4	Middle tier	*P. chlororaphis*	0.00	
420	8	Top tier	*P. chlororaphis*	3.81	0.58
420	8	Middle tier	*P. chlororaphis*	3.77	0.56
420	12	Top tier	*P. chlororaphis*	4.04	1.73
420	12	Middle tier	*P. chlororaphis*	2.91	1.97
420	24	Top tier	*P. chlororaphis*	1.64	0.52
420	24	Middle tier	*P. chlororaphis*	1.38	0.38
420	48	Top tier	*P. chlororaphis*	3.73	0.37
420	48	Middle tier	*P. chlororaphis*	3.41	0.53
530	0	Top tier	*P. chlororaphis*	6.44	0.38
530	0	Middle tier	*P. chlororaphis*	5.91	0.30
530	4	Top tier	*P. chlororaphis*	3.91	0.29
530	4	Middle tier	*P. chlororaphis*	3.95	0.17
530	8	Top tier	*P. chlororaphis*	6.66	0.25
530	8	Middle tier	*P. chlororaphis*	6.07	0.26
530	12	Top tier	*P. chlororaphis*	5.58	0.15
530	12	Middle tier	*P. chlororaphis*	5.25	0.39
530	24	Top tier	*P. chlororaphis*	3.17	0.21
530	24	Middle tier	*P. chlororaphis*	2.58	0.49
530	48	Top tier	*P. chlororaphis*	6.19	0.53

530	48	Middle tier	*P. chlororaphis*	5.61	0.40
660	0	Top tier	*P. chlororaphis*	5.20	0.29
660	0	Middle tier	*P. chlororaphis*	4.74	0.18
660	4	Top tier	*P. chlororaphis*	2.54	0.39
660	4	Middle tier	*P. chlororaphis*	2.24	0.65
660	8	Top tier	*P. chlororaphis*	5.74	0.29
660	8	Middle tier	*P. chlororaphis*	4.62	0.42
660	12	Top tier	*P. chlororaphis*	4.77	0.39
660	12	Middle tier	*P. chlororaphis*	3.96	0.27
660	24	Top tier	*P. chlororaphis*	1.61	0.33
660	24	Middle tier	*P. chlororaphis*	0.93	0.65
660	48	Top tier	*P. chlororaphis*	5.59	0.43
660	48	Middle tier	*P. chlororaphis*	4.74	0.46
White	0	Top tier	*S. griseoviridis*	4.77	0.39
White	0	Middle tier	*S. griseoviridis*	3.96	0.27
White	4	Top tier	*S. griseoviridis*	1.61	0.33
White	4	Middle tier	*S. griseoviridis*	0.93	0.65
White	8	Top tier	*S. griseoviridis*	5.46	0.50
White	8	Middle tier	*S. griseoviridis*	4.32	0.56
White	12	Top tier	*S. griseoviridis*	4.29	0.36
White	12	Middle tier	*S. griseoviridis*	3.49	0.19
White	24	Top tier	*S. griseoviridis*	1.36	0.34
White	24	Middle tier	*S. griseoviridis*	1.08	0.39
White	48	Top tier	*S. griseoviridis*	5.06	0.25
White	48	Middle tier	*S. griseoviridis*	4.37	0.31
420	0	Top tier	*S. griseoviridis*	6.20	0.25
420	0	Middle tier	*S. griseoviridis*	6.11	0.22
420	4	Top tier	*S. griseoviridis*	3.37	0.38
420	4	Middle tier	*S. griseoviridis*	3.57	0.47
420	8	Top tier	*S. griseoviridis*	5.95	0.33
420	8	Middle tier	*S. griseoviridis*	6.02	0.25

420	12	Top tier	*S. griseoviridis*	2.98	0.22
420	12	Middle tier	*S. griseoviridis*	2.22	0.55
420	24	Top tier	*S. griseoviridis*	0.17	
420	24	Middle tier	*S. griseoviridis*	0.15	
420	48	Top tier	*S. griseoviridis*	3.92	0.83
420	48	Middle tier	*S. griseoviridis*	3.54	0.22
530	0	Top tier	*S. griseoviridis*	2.78	0.35
530	0	Middle tier	*S. griseoviridis*	2.34	0.27
530	4	Top tier	*S. griseoviridis*	0.00	
530	4	Middle tier	*S. griseoviridis*	0.00	
530	8	Top tier	*S. griseoviridis*	3.78	0.68
530	8	Middle tier	*S. griseoviridis*	2.97	0.11
530	12	Top tier	*S. griseoviridis*	2.87	0.28
530	12	Middle tier	*S. griseoviridis*	2.38	0.30
530	24	Top tier	*S. griseoviridis*	0.00	
530	24	Middle tier	*S. griseoviridis*	0.00	
530	48	Top tier	*S. griseoviridis*	3.60	0.54
530	48	Middle tier	*S. griseoviridis*	3.01	0.62
660	0	Top tier	*S. griseoviridis*	2.44	0.47
660	0	Middle tier	*S. griseoviridis*	1.65	1.04
660	4	Top tier	*S. griseoviridis*	0.00	
660	4	Middle tier	*S. griseoviridis*	0.00	
660	8	Top tier	*S. griseoviridis*	3.13	0.55
660	8	Middle tier	*S. griseoviridis*	2.81	0.47
660	12	Top tier	*S. griseoviridis*	2.98	0.34
660	12	Middle tier	*S. griseoviridis*	1.16	1.28
660	24	Top tier	*S. griseoviridis*	0.00	
660	24	Middle tier	*S. griseoviridis*	0.00	
660	48	Top tier	*S. griseoviridis*	2.71	0.39
660	48	Middle tier	*S. griseoviridis*	1.55	1.23

Table S7 Four-way Anova results for the three biological control agents (BCAs) (*B. amyloliquefaciens*, *P. chlororaphis*, *S. griseoviridis*) BCA, hours post inoculation, placement and wavelength were taken into account when compared to the average log CFU + 1, biological control agents cm^{-2} tomato leaves during the 48 hour period. **, P < 0.05; **, P < 0.01; ***, P < 0.001*.

Independent variable: Average log CFU + 1 biological control agents cm^{-2} tomato leaves	**df**	**Chisq**	**P-value**	
BCA	1926.15	2	**< 0.001**	***
Hour	1182.44	5	**< 0.001**	***
Placement	110.06	1	**< 0.001**	***
Wavelength	1419.56	3	**< 0.001**	***
BCA x Hour	37.59	10	**< 0.001**	***
BCA x Placement	11.91	2	**0.003**	**
Hour x Placement	7.24	5	0.20	
BCA x Wavelength	124.55	6	**< 0.001**	***
Hour x Wavelength	276.83	15	**< 0.001**	***
Placement x Wavelength	50.62	3	**< 0.001**	***
BCA x Hour x Placement	19.61	10	0.03	*
BCA x Hour x Wavelength	102.99	23	**< 0.001**	***
BCA x Placement x Wavelength	10.82	6	0.09	
Hour x Placement x Wavelength	41.99	15	**< 0.001**	***
BCA x Hour x Placement x Wavelength	19.35	22	0.62	

Table S8 Average of copies DNA and cDNA and their standard deviations of the re-isolated *S. griseoviridis* (SG) and *P. chlororaphis* (PC), per light treatment and placement in the canopy, at 4, 12 and 48 hours post inoculation.

Wavel-ength	Hour	Placement	Organism	Average Copies DNA	Standard deviation DNA	Average Copies cDNA	Standard deviation cDNA
White	4	Top tier	SG	0.0070	0.0044	0.0041	0.0017
White	4	Middle tier	SG	0.0051	0.0050	0.0122	0.0103
White	12	Top tier	SG	0.0064	0.0024	0.0047	0.0017
White	12	Middle tier	SG	0.0030	0.0011	0.0056	0.0043
White	48	Top tier	SG	0.0050	0.0040	0.0040	0.0040
White	48	Middle tier	SG	0.0058	0.0048	0.0047	0.0047
420	4	Top tier	SG	0.0047	0.0017	0.0046	0.0027
420	4	Middle tier	SG	0.0071	0.0057	0.0036	0.0011
420	12	Top tier	SG	0.0026	0.0015	0.0028	0.0000
420	12	Middle tier	SG	0.0025	0.0008	0.0072	0.0054
420	48	Top tier	SG	0.0045	0.0040	0.0040	0.0020
420	48	Middle tier	SG	0.0024	0.0015	0.0091	0.0035
530	4	Top tier	SG	0.0024	0.0006	0.0041	0.0029
530	4	Middle tier	SG	0.0057	0.0036	0.0049	0.0029
530	12	Top tier	SG	0.0022	0.0007	0.0053	0.0047
530	12	Middle tier	SG	0.0075	0.0028	0.0023	
530	48	Top tier	SG	0.0046	0.0017	0.0067	0.0053
530	48	Middle tier	SG	0.0034	0.0015	0.0024	
660	4	Top tier	SG	0.0033		0.0025	0.0021
660	4	Middle tier	SG	0.0027		0.0031	0.0022
660	12	Top tier	SG	0.0115	0.0116	0.0046	0.0016
660	12	Middle tier	SG	0.0088	0.0069	0.0037	0.0026
660	48	Top tier	SG	0.0038	0.0006	0.0023	0.0020
660	48	Middle tier	SG	0.0061	0.0013	0.0042	0.0031

White	4	Top tier	PC	0.2686	0.1412	0.0072	0.0033
White	4	Middle tier	PC	0.2705	0.1478	0.0019	0.0010
White	12	Top tier	PC	0.2883	0.1502	0.0066	0.0055
White	12	Middle tier	PC	0.2765	0.2488	0.0030	0.0025
White	48	Top tier	PC	0.2586	0.1924	0.0066	0.0049
White	48	Middle tier	PC	0.3369	0.3071	0.0042	0.0041
420	4	Top tier	PC	0.1243	0.0506	0.0060	0.0059
420	4	Middle tier	PC	0.1764	0.0968	0.0027	0.0018
420	12	Top tier	PC	0.1101	0.0286	0.0025	0.0008
420	12	Middle tier	PC	0.1523	0.0816	0.0018	0.0008
420	48	Top tier	PC	0.0855	0.0328	0.0022	0.0010
420	48	Middle tier	PC	0.0710	0.0423	0.0062	0.0031
530	4	Top tier	PC	0.1802	0.1106	0.0039	0.0025
530	4	Middle tier	PC	0.2134	0.1268	0.0019	0.0005
530	12	Top tier	PC	0.2699	0.1615	0.0058	0.0050
530	12	Middle tier	PC	0.1242	0.0729	0.0030	0.0039
530	48	Top tier	PC	0.1417	0.0303	0.0066	0.0049
530	48	Middle tier	PC	0.0584	0.0288	0.0042	0.0041
660	4	Top tier	PC	0.1401	0.0553	0.0042	0.0039
660	4	Middle tier	PC	0.0950	0.0253	0.0018	0.0005
660	12	Top tier	PC	0.1901	0.0663	0.0037	0.0030
660	12	Middle tier	PC	0.1088	0.0359	0.0045	0.0024
660	48	Top tier	PC	0.1087	0.0350	0.0031	0.0001
660	48	Middle tier	PC	0.0661	0.0296	0.0045	0.0039

Table S9 ANOVA results based on a LLM for the droplet digital PCR (ddPCR), DNA copies (μl^{-1} cm^{-2}), collected for the DNA and cDNA samples of *Streptomyces griseoviridis* CBS904.68 under four light treatments (Full spectrum, 420, 530 and 660 nm) and from 4, 12 and 48 hours post inoculation. **, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$*.

S. griseoviridis	Concentration DNA copies (μl^{-1} cm^{-2})			Concentration cDNA copies (μl^{-1} cm^{-2})		
	df	**Chisq**	**P-value**	**df**	**Chisq**	**P-value**
Wavelength	3	3.01	0.39	3	5.96	0.11
Placement	1	0.97	0.32	1	2.56	0.11
Hour	2	0.60	0.74	2	0.43	0.81
Placement x Wavelength	3	4.01	0.26	3	3.42	0.33
Hour x Wavelength	6	6.85	0.34	6	6.87	0.33
Hour x Placement	2	0.99	0.61	2	0.52	0.77
Hour x Placement x Wavelength	6	9.26	0.16	6	7.18	0.30

Table S10 Commercial products containing the three biological control agents used in the current study, which are approved for use in Sweden.

Commercial product	**Species**	**Proprietor**	**Source**
Cedomon	*Pseudomonas chlororaphis*	Koppert B.V.	(KEMI, 2024a)
Mycostop	*Streptomyces griseoviridis* K61	Danstar Ferment AG	(KEMI, 2024b)
Serenade ASO	*Bacillus amyloliquefaciens* QST 713	Bayer A/S	(KEMI, 2024c)

Table S11 ANOVA results based on a LLM for the droplet digital PCR (ddPCR) data, DNA copies (μl^{-1} cm^{-2}), collected for the DNA and cDNA absolute quantification of replicates of *Pseudomonas chlororaphis* 50083 and *Streptomyces griseoviridis* CBS904.68 under four light treatments (white light, 420, 530 and 660 nm) and from 4, 12 and 48 hpi. **, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.*

DNA copies per µl cm^{-2}	**df**	**Chisq**	**Pr(>F)**	**cDNA copies per µl cm^{-2}**	**df**	**Chisq**	**Pr(>F)**

Wavelength	3	29.45	**< 0.001**	***	Wavelength	3	11.86	**0.008**	**
Placement	1	1.30	0.25		Placement	1	0.09	0.76	
Hour	2	3.03	0.22		Hour	2	8.25	**0.016**	*
BCA	1	117.38	**< 0.001**	***	BCA	1	0.28	0.60	
Wavelength x Placement	3	8.67	**0.034**	*	Wavelength x Placement	3	2.10	0.55	
Wavelength x Hour	6	2.91	0.82		Wavelength x Hour	6	25.60	**< 0.001**	***
Placement x Hour	2	2.59	0.27		Placement x Hour	2	2.20	0.33	
Wavelength x BCA	2	18.46	**< 0.001**	***	Wavelength x BCA	3	5.64	0.13	
Placement x BCA	1	0.54	0.46		Placement x BCA	1	4.82	**0.028**	*
Hour x BCA	2	1.97	0.37		Hour x BCA	2	3.69	0.16	
Wavelength x Placement x Hour	6	6.94	0.33		Wavelength x Placement x Hour	6	6.09	0.41	
Wavelength x Placement x BCA	3	4.21	0.24		Wavelength x Placement x BCA	3	4.71	0.19	
Wavelength x Hour x BCA	6	2.06	0.91		Wavelength x Hour x BCA	6	14.79	**0.022**	*
Placement x Hour x BCA	2	1.97	0.37		Placement x Hour x BCA	2	2.35	0.31	
Wavelength x Placement x Hour x BCA	6	3.62	0.73		Wavelength x Placement x Hour x BCA	6	2.52	0.87	

Table S12 ANOVA results based on a LLM for the droplet digital PCR (ddPCR), DNA copies (μl^{-1} cm^{-2}), collected for the DNA and cDNA samples of *Pseudomonas chlororaphis* 50083 under four light treatments (Full spectrum, 420, 530 and 660 nm) and from 4, 12 and 48 hpi. **, P < 0.05; **, P < 0.01; ***, P < 0.001*.

P. chlororaphis	**Concentration DNA copies (μl^{-1} cm^{-2})**				**Concentration cDNA copies (μl^{-1} cm^{-2})**			
	df	**Chisq**	**P-value**		**df**	**Chisq**	**P-value**	
Wavelength	3	30.37	**< 0.001**	***	3	11.85	**0.008**	**
Placement	1	1.42	0.23		1	2.86	0.09	
Hour	2	3.12	0.21		2	12.64	**0.002**	**
Placement x Wavelength	3	9.44	**0.02**	*	3	3.61	0.31	
Hour x Wavelength	6	2.89	0.82		6	32.78	**< 0.001**	***
Hour x Placement	2	2.57	0.28		2	3.58	0.17	
Hour x Placement x Wavelength	6	7.42	0.28		6	1.72	0.94	

References

KEMI. (2024a). *Cedomon*. Kemikalieinspektionen - Swedish Chemical Agency Retrieved 5/9 from https://apps.kemi.se/BkmRegistret/Kemi.Spider.Web.External/Produkt/Details?produktId=9363&produktVersionId=21203

KEMI. (2024b). *Mycostop*. Kemikalieinspektionen - Swedish Chemical Agency. Retrieved 5/9 from https://apps.kemi.se/BkmRegistret/Kemi.Spider.Web.External/Produkt/Details?produktId=9651&produktVersionId=21478

KEMI. (2024c). *Serenade ASO*. Kemikalieinspektionen - Swedish Chemical Agency. Retrieved 5/9 from https://apps.kemi.se/BkmRegistret/Kemi.Spider.Web.External/Produkt/Details?produktId=14086&produktVersionId=20529

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The power of light: Impact on the performance of biocontrol agents under minimal nutrient conditions

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Background: The spectral distribution of light (different wavelength) has recently been identified as an important factor in the dynamics and function of leaf-associated microbes. This study investigated the impact of different wavelength on three commercial biocontrol agents (BCA): *Bacillus amyloliquefaciens* (BA), *Pseudomonas chlororaphis* (PC), and *Streptomyces griseoviridis* (SG).

Methods: The impact of light exposure on sole carbon source utilization, biofilm formation, and biosurfactant production by the selected BCA was studied using phenotypic microarray (PM) including 190 sole carbon sources (OmniLog®, PM panels 1 and 2). The BCA were exposed to five monochromatic light conditions (420, 460, 530, 630, and 660nm) and darkness during incubation, at an intensity of 50μmolm^{-2} s^{-1}.

Results: Light exposure together with specific carbon source increased respiration in all three BCA. Different wavelengths of light influenced sole carbon utilization for the different BCA, with BA and PC showing increased respiration when exposed to wavelengths within the blue spectrum (420 and 460nm) while respiration of selected carbon sources by SG increased in the presence of red light (630 and 660nm). Only one carbon source (capric acid) generated biosurfactant production in all three BCA. A combination of specific wavelength of light and sole carbon source increased biofilm formation in all three BCA. BA showed significantly higher biofilm formation when exposed to blue (460nm) and green (530nm) light and propagated in D-sucrose, D-fructose, and dulcitol. PC showed higher biofilm formation when exposed to blue light. Biofilm formation by SG increased when exposed to red light (630nm) and propagated in citraconic acid.

Conclusion: To increase attachment and success in BCA introduced into the phyllosphere, a suitable combination of light quality and nutrient conditions could be used.

KEYWORDS

biocontrol agent, biofilm formation, biosurfactant production, light quality, phenotypic microarray, sole carbon source utilization

1. Introduction

The encouraging results of microbial biocontrol agents (BCA) under laboratory and small-scale conditions do not always translate into consistent biocontrol efficacy in commercial settings. Rapidly declining numbers of introduced BCA is a recurring problem in seed, root, and foliar application. Various reasons may underlie this decline, such as problematic application techniques and poor adaptation of BCA to the commercial growing environment.

The phyllosphere harbors a variety of microorganisms that have a strong impact on plant fitness and support plant growth and survival, e.g., by improved nutrient provisioning and uptake, resilience to environmental stresses, or even disease defense (Vorholt, 2012). Organic nutrient availability on leaf surfaces is an important factor governing microbial colonization. Dissolved organic compounds exuded *via* the plant cuticle serve as energy sources to sustain the metabolism of associated microbes (Yeats and Rose, 2013). Ambient conditions, i.e., temperature, ultraviolet (UV) radiation, and relative humidity, affect nutrient extrusion (Leveau, 2009, 2019). Nutrients are not exuded evenly over the leaf surface, so microbial colonization of leaves is patchy. Moreover, the amount of exuded nutrients is finite and therefore introduced microbes need to compete with the leaf microbiota for these nutrients. Survival and establishment of BCA on the leaf surface is dependent on their ability to compete with the existing microbial community (Mallon et al., 2015). In order for BCA to co-exist with existing microbial species, limited niche overlap is needed (Chase and Myers, 2011; Hawkes and Connor, 2017). However, with respect to plant pathogens and BCA, niche overlap is essential for pathogen control.

Light (different wavelength) has recently been identified as an important factor for the dynamics and function of leaf-associated microbes (Alsanius et al., 2017, 2019, 2021; Gharaie et al., 2017). Recent studies have shown that non-phototrophic microbes can also respond phenotypically to differences in light quality (Wu et al., 2013; Beattie et al., 2018; Alsanius et al., 2021; Losi and Gärtner, 2021). Photosensory proteins in bacteria, such as blue light receptor proteins, could play a crucial role in sensing and responding to light (Losi et al., 2014). Respiration, growth rate, motility, and microbial lifestyle (planktonic, sessile) vary under different light quality levels, but are also affected by nutritional conditions (Gharaie et al., 2017; Alsanius et al., 2021). Beauregard et al. (2013) demonstrated that specific polysaccharides leaching from the plant serve as a cue for *Bacillus subtilis* to form biofilm on the root of *Arabidopsis thaliana*. Biosurfactant formation by BCA is a crucial mechanism to facilitate their dispersal on the leaf surface and biofilm formation is essential for their establishment (Alsanius et al., 2021). Thus, manipulation of light quality and nutritional factors might enable BCA to transition between planktonic and sessile lifestyles, and could be a key factor for optimized BCA performance on leaf surfaces.

In controlled-environment plant production, e.g., in greenhouses, artificial irradiation with mono- or polychromatic light sources with wavelength from 400 to 700 nm is used to optimize crop photosynthesis, biomass formation, and/or plant architecture (Morrow, 2008). Monochromatic blue and red light and polychromatic white light influence phyllospheric community structure (Vänninen et al., 2010; Alsanius et al., 2017). The lethal effect of UV-light on plant pathogens is well established (Newsham, 1997; Kadivar and Stapelton, 2003). However, other wavelengths within the visible light spectrum have also been demonstrated to affect the behavior of plant pathogens such as downy and powdery mildew (Reuveni and Raviv, 1997; Suthaparan et al., 2012, 2014) and grey mold (*Botrytis cinerea*; Nicot et al., 1996; Elad, 1997). Examples of light spectra-dependent performance have also been reported for non-pathogenic microorganisms, such as *Pseudomonas* sp. DR 5-09 (Gharaie et al., 2017) and *Bacillus amyloliquefaciens* (Yu and Lee, 2013; Rajalingam and Lee, 2017).

The aim of this study was to determine the effects of different wavelengths on utilization of different sole carbon sources by commercial BCA and their biosurfactant production and biofilm formation. The starting hypotheses were that: (i) different wavelength affects the substrate utilization pattern of the target strains; and (ii) different wavelength affects biofilm formation and biosurfactant production by the target strains.

2. Materials and methods

The study was conducted using three commercial BCA strains. *Bacillus amyloliquefaciens* DSM7 (BA) and *Pseudomonas chlororaphis* 50083 (PC) were purchased from DSMZ (Leibniz Institute, Braunschweig, Germany). *Streptomyces griseoviridis* CBS904.68 (SG) was purchased from Centraalbuureau voor Schimmelcultures, Utrecht, Netherlands.

2.1. Phenotypic microarray

Phenotypic microarrays (PM) were performed following procedures described by Gharaie et al. (2017) and Alsanius et al. (2021). In brief, the microarrays were performed at a density of six replicates per strain and treatment on two sole carbon source panels (PM01, PM02A) according to the Biolog standard protocols, using 190 different sole carbon sources. Bacteria were propagated overnight from cryoculture at 25°C on tryptic soy agar (TSA; DIFCO 236950, United States). Colony swabs were transferred to IF-0a GN medium (Biolog Inc., Hayward, CA, United States) and turbidity of the bacterial suspension was adjusted turbidimetrically (Biolog Inc., United States, catalog no. 3587) to 81% transmittance. Redox dye (dye mix A (catalog no. 74221) for PC; dye mix G (catalog no. 74227) for BA and SG; Biolog Inc., Haywood, United States) was then added. A 100 µl aliquot of the suspension was pipetted into each plate well and the plates were sealed with Greiner ViewSeal (Greiner Bio-one, 676070; Sigma Aldrich, Z617571-100EA, St. Louis, MO, United States; Gharaie et al., 2017). The panels were exposed to five monochromatic light-emitting diode (LED) light regimes (blue: 420 and 460 nm, green: 530 nm, red: 630 and 660 nm), while control panels were incubated in darkness for 96 h. Panels incubated in darkness were kept in the OmniLog incubator (OmniLog, catalog number 93182, Biolog Inc., United States) at 20°C during the entire incubation period. Panels exposed to the various light conditions were placed in lined cabinets (500 × 500 × 1,000 mm) and incubated at 20°C. Each cabinet was equipped with a LED lamp (Heliospectra Dyna, Heliospectra AB, Gothenburg, Sweden). Light intensity was adjusted to 50 µmol m^{-2} s^{-1}.

Sole carbon source utilization in each well of the PM panels was measured as color change in the added redox dye, using a computer-controlled camera system. Under dark conditions, color change was recorded automatically every 15 min, while under light exposure color change of the PM panels was recorded according to previously established growth curves. Readings were set to occur 0, 6, 10, 24, 30, 48, 54, 72, and 96 h post inoculation (hpi). Output values were expressed in OmniLog units.

2.2. Biosurfactant production

Biosurfactant formation was monitored using a drop collapse test. Aliquots of 20 µl from each well of the carbon source panels (PM01 and PM02A) were transferred to glass plates covered with parafilm and a template of the 96-well plate. After 2 min, each drop was scored from 0 to 2 (0 = convex, 1 = moderately convex and 2 = flattened drop; Gharaie et al., 2017).

2.3. Biofilm formation

Assessment of biofilm formation followed the procedure described by Alsanius et al. (2021). In brief, the microbial suspension was removed from the PM panels, the plates were washed, and 100 µl of 0.5% crystal violet solution (1% Crystal violet solution, V5265-500ML, Sigma-Aldrich) were added to each well, after which the plates were incubated for 15 min. The crystal violet solution was then removed and the plates were repeatedly washed and left to dry overnight. Finally, 100 µl of 95% ethanol were added to each well and the plates were left for 60 min before spectrophotometric determination of extinction at 550 nm (Expert 96TM spectrophotometer, AsysHiTech, Eugendorf, Austria).

2.4. Analysis and statistical calculations

The recorded carbon utilization data were exported as csv-files using OmniLog PM kinetic analysis software and then analyzed in R-studio using the *opm* package (Vaas et al., 2013; Göker et al., 2016; R Core Team, 2021), based on curve parameter maximum curve height (A) and area under curve (AUC). Analysis of variance (ANOVA) was used to analysis the biofilm formation data, followed by Tukey test, both performed in R-studio.

3. Results

3.1. Sole carbon utilization

For all three test-organisms, sole carbon utilization changed when the bacteria were exposed to different light regimes. No directionality was identified, since the impact of light on the respiration was increased on some carbon sources and decreased on other. For BA, the number of utilized carbon sources and intensity of utilization were generally highest on exposure to 460 and 530 nm (blue and green spectrum) and corresponded to the utilization pattern under dark conditions (Figure 1). Blue light exposure increased respiration by BA of 7% of the carbon sources to a level above AUC 20000 (Figure 2), which was not observed under dark conditions. Based on the KEGG database, the carbon sources for BA affected by light treatment were those responsible for amino sugar and nucleotide metabolism, and biosynthesis of secondary metabolites and antibiotics, and involved in the phosphotransferase system. BA exhibited generalist behavior with respect to almost all carbon sources tested when exposed to blue light (460 nm). In total, 39 carbon sources were utilized by BA under the 460 nm and 530 nm treatments and in the dark incubation. Two carbon sources (Tween 20 and Tween 40) were utilized under all wavelengths except 420 nm (Figure 3A; Supplementary Figures S1, S2).

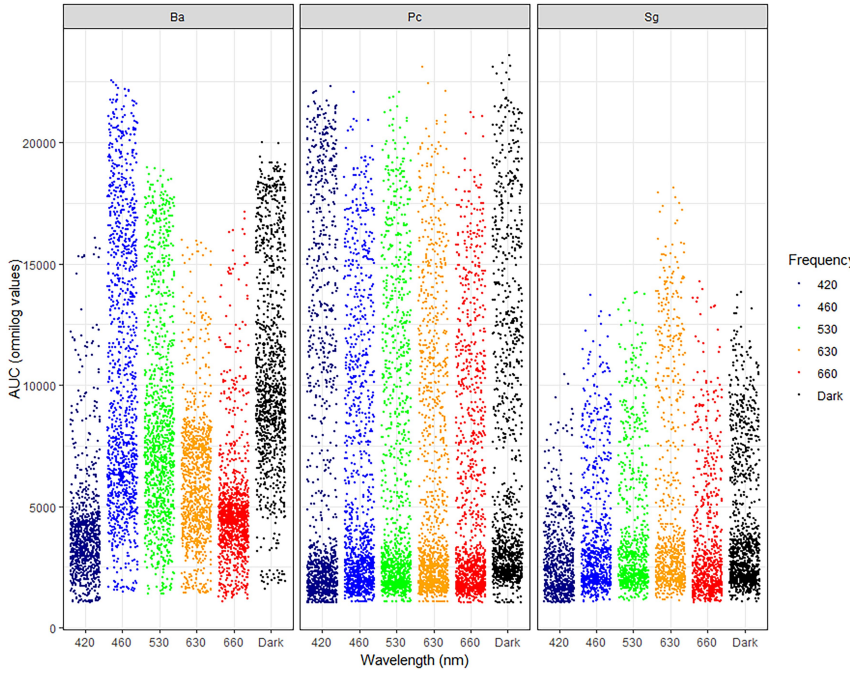

FIGURE 1
Jitter plot showing the area under the curve (AUC) for utilization of 190 sole carbon sources under different wavelengths (420, 460, 530, 630, and 660nm) and in dark conditions by *Bacillus amyloliquefaciens* (BA), *Pseudomonas chlororaphis* (PC), and *Streptomyces griseoviridis* (SG). Each dot represents maximum Omnilog value of each single carbon source.

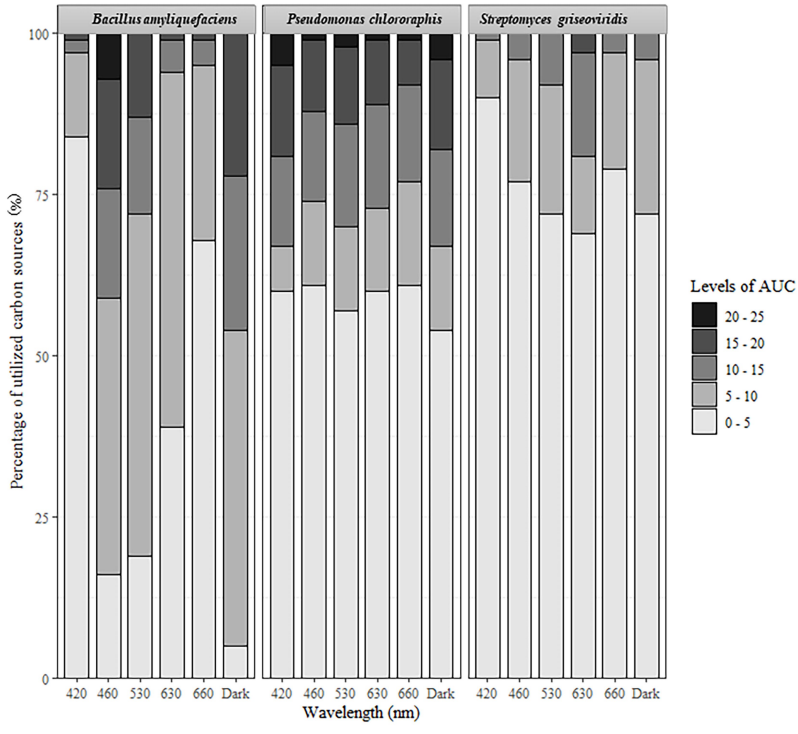

FIGURE 2
Percentage of carbon sources utilized under different light regimes. Area under the curve (AUC) values ranged from 0 to 25,000.

Sole carbon utilization by PC was less sensitive to the different light regimes tested, as multiple carbon sources were consumed under all light conditions. However, there was a trend for increased utilization in the near-blue spectrum of 420 nm (Figure 1), which increased utilization of 5% of the carbon sources (Figure 2). PC showed increased respiration for the sole carbon sources involved in biochemical processes mentioned above for BA, but also for carbon sources responsible for inositol phosphate metabolism and bacterial chemotaxi. In total, 27 carbon sources (L-glutamine, mucic acid, L-aspargine, inosine, D-mannitol, fumaric acid, D-saccharic acid, D-gluconic acid, Ala-Gly, D-serine, D,L-malic acid, L-alanine, citric acid, Gly-Glu, succrose, L-serine, D-malic acid, L-malic acid, D-trehalose, myo-inositol, L-aspartic acid, Gly-Pro, L-proline, D-mannose, *N*-acetyl-D-glucosamine, pyruvic acid, D-aspartic acid) were utilized by PC under all light treatments (Figure 3B; Supplementary Figures S1, S2).

In general, sole carbon source utilization by SG was very low for almost all carbon sources tested (Supplementary Figures S1, S2) under light and dark exposure. From this low utilization rate, an increase in utilization was found under exposure to the red spectrum (630–660 nm; Figure 1). Respiration by SG rose to an AUC level of 15,000 only on 3% of the carbon sources tested (inosine, α-D-glucose-1-phosphate, D-glucose-6-phosphate, D-galcturonic acid, D-glucoronic acid, D-fructose-6-phosphate; Figure 2). These carbon sources are involved in various metabolic pathways, such as starch and sugar metabolism, biosynthesis of secondary metabolites, ABC transport, and D-amino acid metabolism. Ten carbon sources were utilized under all light spectra and seven carbon sources were solely utilized under the red spectrum of 630 nm (Figure 3C; Supplementary Figures S1, S2).

3.2. Biosurfactant production

Only one of the 190 carbon sources tested (capric acid) generated drop collapse in all three BCA, regardless of high or low respiration level (S2). Hence, different wavelengths had an effect on surface activity. Upon dark incubation, complete drop collapse was noted for BA and SG, whereas PC showed moderately convex droplets. No drop collapse was noted for the incubated capric acid suspension when BA was exposed to 530 nm or when PC was exposed to 630/660 nm. Moderate drop collapse was found after incubation of SG in capric acid solution exposed to red light (620 nm), but no drop collapse was seen on exposure to blue light (400, 420 nm; Table 1).

3.3. Biofilm formation

Bacterial biofilm formation was affected by light quality. Biofilm formation by BA was enhanced when this species was incubated under dark and red (660 nm) light conditions ($p < 0.01$; Figure 4). A combination of light quality and specific carbon source increased biofilm formation in some cases, e.g., BA showed significantly higher

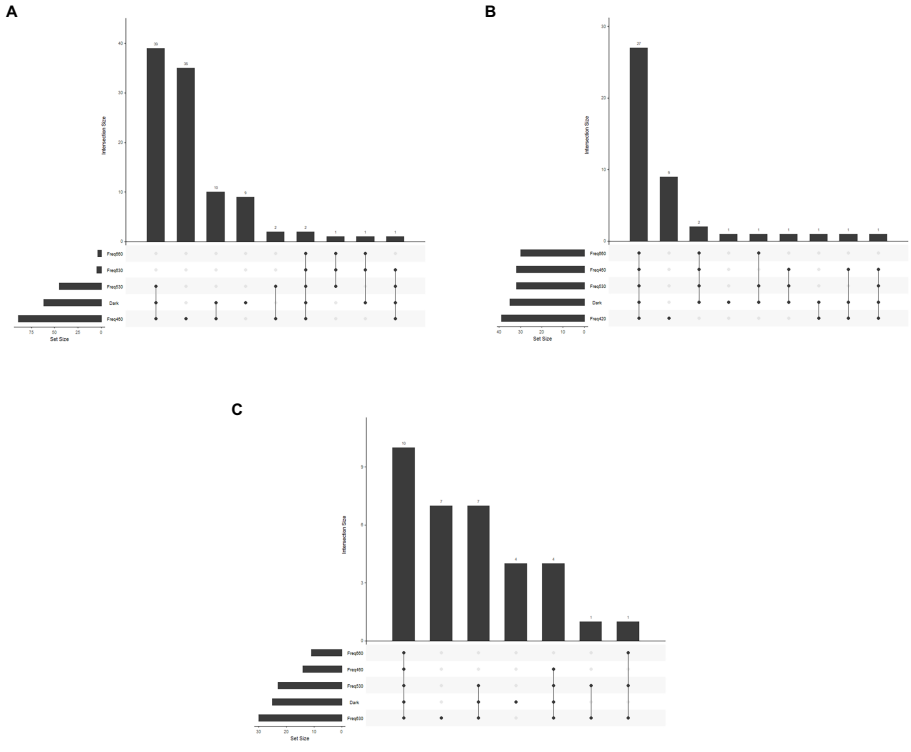

FIGURE 3
Upset plot of number of carbon sources utilized under different wavelengths and number of carbon sources utilized under the same wavelength. **(A)** *Bacillus amyloliquefaciens*, **(B)** *Pseudomonas chlororaphis*, and **(C)** *Streptomyces griseoviridis*.

TABLE 1 Drop collapse of sole capric acid suspensions incubated with ***Bacillus amyloliquefaciens, Pseudomonas chlororaphis*, or *Streptomyces griseoviridis*** for 96h under exposure to different light regimes (monochromatic LED at 400, 430, 460, 530, 620, and 660nm; dark conditions).

Wave length	*Bacillus amyloliquefaciens*	*Pseudomonas chlororaphis*	*Streptomyces griseoviridis*
Dark	2	1	2
400	2	2	1
430	2	2	1
460	2	2	2
530	0	2	0
620	2	0	0
660	2	0	2

0 = convex, 1 = moderately convex, and 2 = flattened drop.

biofilm formation when exposed to blue (460 nm) and green (530 nm) light and propagated in D-sucrose, D-fructose, and dulcitol, together with a very high respiration level (Table 2).

PC showed significantly ($p < 0.001$) higher biofilm formation when exposed to blue (400 nm) light compared with dark conditions (Figure 4). PC had higher respiration levels when grown on the carbon sources α-D-glucose, m-tartaric acid, uridine, D-malic acid, and D-ribose, where light quality seemed to be of minor importance (Table 2).

Biofilm formation by SG increased significantly under blue (400 nm) light ($p = 0.04$) compared with dark conditions (Figure 4). SG showed higher respiration levels and high biofilm formation when propagated on citraconic acid as the carbon source and exposed to red light (630 nm). Biofilm formation was still found when SG was exposed to 530 nm with L-alanine, 630 nm with L-rhamnose, and 420 nm with α-methyl-D-mannoside as the carbon source, despite low respiration level (Table 2).

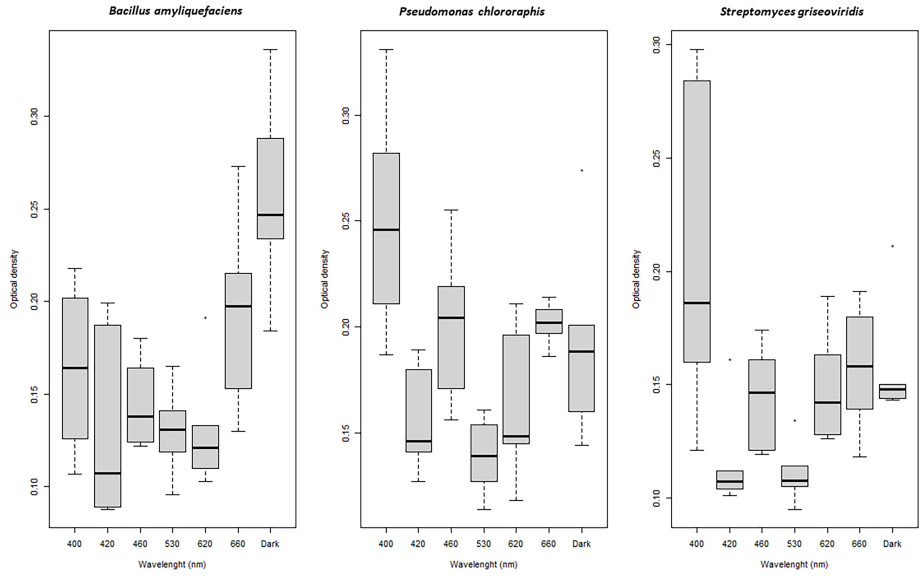

FIGURE 4
Boxplot of biofilm formation by *Bacillus amyloliquefaciens*, *Pseudomonas chlororaphis*, and *Streptomyces griseoviridis* after 96h of exposure to different wavelenghts (biofilm measured as optical density at 550nm).

4. Discussion

The results obtained in this study confirm that metabolism in non-phototrophic bacteria is altered by the prevailing light environment and that directionality of change is absent, as previously suggested by Gharaie et al. (2017) and Alsanius et al. (2021). From an ecological perspective, application of BCA to a crop can be seen as a microbial invasion, which is a widespread phenomenon in nature. This phenomenon follows a certain process, starting with (I) introduction, when the invader comes into a new environment, followed by (II) establishment of the invader and maintenance of a viable invader population. After establishment, the invader has to (III) disperse in the new environment and if successful it can displace or re-shape the resident microbial community (Mallon et al., 2015). Another important aspect is competition for resources. When using BCA to control plant pathogens, a certain niche overlap is desirable. In theory, niche overlap means that species have similar factors that regulate their population growth, such as nutrients and response to different stressors in the environment (Pastore et al., 2021). In the present study, we selected three commercially available BCA with known efficacy against powdery mildew and grey mold and investigated whether habitat manipulation could enhance establishment of the selected species. Based on sole carbon source utilization, the results demonstrated that the phenotypic plasticity of the selected BCA varies under minimal nutrient conditions and that directionality in phenotypic response is dependent on (i) the bacterial strain, (ii) the light spectrum, and (iii) the individual carbon source (Supplementary Figures S1, S2). In general, based on sole carbon source utilization, PC and BA showed high variable plasticity in response to the light spectrum and were particularly enhanced by blue light, while SG showed low plasticity, with low respiration rates and low sensitivity to the different spectra, although red light increased sole carbon source utilization by SG to some extent.

The findings for PC support previous findings by Gharaie et al. (2017) and Alsanius et al. (2021). However, it is worth noting that different species within the genus *Pseudomonas* differ in their carbon source utilization rate, with respect to intensity and maximum utilization. Thus, the impact of blue light needs to be determined separately for different pseudomonad strains before the insights in this study are used for secondary purposes, such as improved metabolite formation. This is also true for species within *Bacillus*. In contrast to *Bacillus thuringiensis* (Alsanius et al., 2021), in the present study BA displayed light sensitivity as assessed by respiration under minimal nutrient conditions. It is also worth noting that phenotypic plasticity is a rapid adaptation response to a threat in the environment, enabling growth and propagation (Chevin and Hoffmann, 2017). In this study, different wavelength and nutrient conditions changed and thus the bacteria needed to change their utilization pattern and adapt to the new challenging environment if they were to grow and survive.

Biosurfactant production and biofilm formation are essential mechanisms for dispersal and establishment of BCA on a given surface. Only one (capric acid) of the 190 sole carbon sources tested in this study induced biosurfactant production in all three BCA, but under different lighting conditions. However, other studies have reported effects of various organic compounds, such as carbohydrates and amino acids, in enhancing biosurfactant production (Guerra-Santos et al., 1986; Alsanius et al., 2021). In the study by Alsanius et al. (2021), no drop collapse was observed at lower respiration levels, whereas in our study drop collapse was observed at very low respiration rates for SG.

Different light spectra influence physiological responses and metabolic pathways in microorganisms, such as swarming motility,

TABLE 2 Directionality of biofilm formation by *Bacillus amyloliquefaciens*, *Pseudomonas chlororaphis*, and *Streptomyces griseoviridis* in the presence of selected sole carbon sources under exposure to different light regimes (light intensity: 50μmols^{-1} m^{-1}: monochromatic LED: 400, 420, 460, 530, 620, and 660nm; darkness).

Wave length	*Bacillus amyloliquefaciens*		*Pseudomonas chlororaphis*		*Streptomyces griseovirides*	
400			Glycolic acid	↓		
420					α-Methyl-D-Mannoside	↓
460	D-Sucrose	↑	α-D-Glucose	↑		
	Dulcitol	↑	m-Tartaric acid	↑		
			Uridine	↑		
530	D-Sucrose	↑	D-Malic acid	↑	L-Alanine	↓
	D-fructose	↑	δ-Amino valeric acid	↓		
			Citramalic acid	↓		
			Capric acid	↓		
620			D-Ribose	↑	Citraconic Acid	↑
			Sorbic Acid	↓	L-Rhamnose	↓
			δ-Amino valeric acid	↓		
			Citramalic acid	↓		
660	Dulcitol	↑	D-Ribose	↑		
			δ-Amino valeric acid	↓		
			Sorbic acid	↓		
			Citramalic acid	↓		
Dark			D-Ribose	↑		
			Sorbic acid	↓		

Arrows indicate the directionality of carbon source utilization after 96 h of incubation (OmniLog value; ↑ = high respiration, ↓ = low respiration).

biofilm formation, virulence, and antibiotic production (Kraiselburd et al., 2012; Yu and Lee, 2013; Müller et al., 2017). From an agriculture and horticulture point of view, biofilm formation and BCA dispersal are crucial for the control of microbial pathogens and for the overall utility of BCA. Therefore, it is important to determine the role of light in bacterial behavior. Non-phototrophic bacteria may be equipped with photosensory proteins, which are involved in controlling the transition between a planktonic lifestyle and a sessile multicellular lifestyle in biofilm (van der Horst et al., 2007; Purcell and Crosson, 2008). These blue-light receptors are linked to two protein domains (GGDEF and EAL) that have been shown to control this transition through cyclic di-GMP, a second messenger that stimulates the biosynthesis of adhesins and poly-saccharide matrix substances important for biofilm formation (Jenal and Malone, 2006). Organic compounds such as acyl-homoserine lactones (AHL) and phenazines have been shown to be strongly linked to biofilm formation (Rieusset et al., 2020).

The phyllosphere is often described as a challenging environment for microbiota, especially with respect to the availability of organic nutrients. We therefore applied a minimal nutrient approach to mimic such conditions. To translate the findings to greenhouse settings and improve the establishment and efficacy of BCA, challenge experiments need to be conducted in planta under greenhouse conditions. One approach could be to apply BCA together with a specific carbon source and light quality that trigger BCA dispersal and surfactant production, followed by another compound and light quality that enhance establishment and biofilm formation (Figure 5).

In conclusion, our results showed that the choice of wavelength affects the sole carbon source utilization pattern of all three target strains and metabolic responses to a particular wavelength was species-specific. Biosurfactant production and biofilm formation are important mechanisms for BCA to be successful. To use different wavelengths of light may enhance establishment of BCA on the leaf surface. The findings need to be validated under greenhouse conditions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MK and BA contributed to the conception of the study and the design, and wrote the original draft. AF contributed statistical support and helped in managing the opm package. MH and K-JB critically reviewed the manuscript. MK and MH performed the experiments. MK conducted data analysis. BA acquired funding. All authors contributed to the article and approved the submitted version.

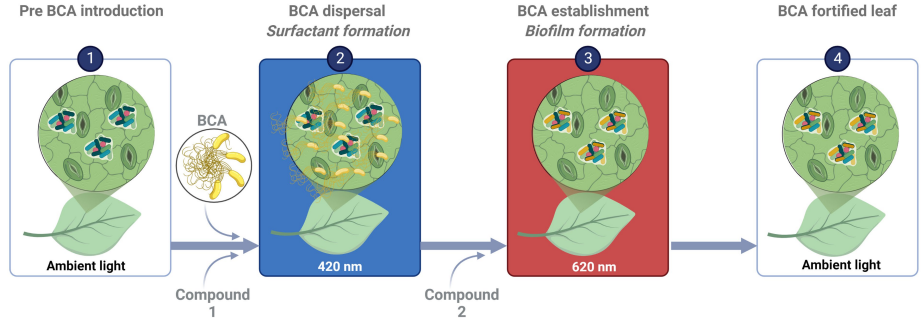

FIGURE 5
Schematic diagram illustrating putative areas of application for biocontrol agents (BCA). *(Illustration: B. Alsanius; "Created with BioRender.com." agreement number HJ24LJ7YZ0).*

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Conflict of interest

The authors declare no conflict of interest. This research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1087639/full#supplementary-material

References

Alsanius, B. W., Bergstrand, K.-J., Hartmann, R., Gharaie, S., Wohanka, W., Dorais, M., et al. (2017). Ornamental flowers in new light: artificial lighting shares the microbial phyllosphere community structure of greenhouse grown sunflowers (*Helianthus annuus* L.). *Sci. Hortic.* 216, 234–247. doi: 10.1016/j.scienta.2017.01.022

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

Alsanius, B. W., Vaas, L., Gharaie, S., Karlsson, M. E., Rosberg, A. K., Wohanka, W., et al. (2021). Dining in blue light impairs the appetite of some leaf epiphytes. *Front. Microbiol.* 12:725021. doi: 10.3389/fmicb.2021.725021

Beattie, G. A., Hatfield, B. M., Dong, H., and McGrane, R. S. (2018). Seeing the light: the roles of red- and blue-light sensing in plant microbes. *Annu. Rev. Phytopathol.* 56, 41–66. doi: 10.1146/annurev-phyto-080417-045931

Beauregard, P. B., Chai, Y., Vlamakis, H., Losick, R., and Kolter, R. (2013). Bacillus subtilis biofilm induction by plant polysaccharides. *Proc. Natl. Acad. Sci. U. S. A.* 110, E1621–E1630. doi: 10.1073/pnas.1218984110

Chase, J. M., and Myers, J. A. (2011). Disentangling the importance of ecological niches from stochastic processes across scales. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366, 2351–2363. doi: 10.1098/rstb.2011.0063

Chevin, L. M., and Hoffmann, A. A. (2017). Evolution of phenotypic plasticity in extreme environments. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 372:20160138. doi: 10.1098/rstb.2016.0138

Elad, Y. (1997). Effect of filtration of solar light on the production of conidia by field isolates of *Botrytis cinerea* and on several diseases of greenhouse-grown vegetables. *Crop Prot.* 16, 635–642. doi: 10.1016/S0261-2194(97)00046-X

Gharaie, S., Vaas, L., Rosberg, A. K., Windstam, S. T., Karlsson, M. E., Bergstrand, K.-J., et al. (2017). Light spectrum modifies the utilization pattern of energy sources in *Pseudomonas* sp. DR 5-09. *PLoS One* 12, 12:e0189862. doi: 10.1371/journal.pone.0189862

Göker, M., Hofner, B., Montero Calasanz, M. C., Sikorski, J., and Vaas, L. A. I. (2016). Opm: An R package for analysing phonotype microarray and growth curve data. Phenotype Microarray data.

Guerra-Santos, L. H., Käppeli, O., and Fiechter, A. (1986). Dependence of *Pseudomonas aeruginosa* continuous culture biosurfactant production on nutritional and environmental factors. *Appl. Microbiol. Biotechnol.* 48, 301–305. doi: 10.1128/aem.48.2.301-305.1984

Hawkes, C. V., and Connor, E. W. (2017). Translating phytobiome from theory to practice: ecological and evolutionary considerations. *Phytobiomes* 1, 57–69. doi: 10.1094/PBIOMES-05-17-0019-RVW

Jenal, U., and Malone, J. (2006). Mechanisms of cyclic-di-GMP signaling in bacteria. *Annu. Rev. Genet.* 40, 385–407. doi: 10.1146/annurev.genet.40.110405.090423

Kadivar, H., and Stapelton, A. E. (2003). Ultraviolet radiation alters maize phyllosphere bacterial diversity. *Microb. Ecol.* 45, 353–361. doi: 10.1007/s00248-002-1065-5

Kraiselburd, I., Alet, A. I., Tondo, M. L., Petrocelli, S., Daurelio, L. D., Monzón, J., et al. (2012). A LOV protein modulates the physiological attributes of *Xanthomonas axonopodis* pv. citri relevant for host plant colonization. *PLoS One* 7:e38226. doi: 10.1371/journal.pone.0038226

Leveau, J. H. J. (2009). Microbiology life on leaves. *Nature* 461, 741–742. doi: 10.1038/461741a

Leveau, J. H. J. (2019). A brief from the leaf: latest research to inform our understanding of the phyllosphere microbiome. *Curr. Opin. Microbiol.* 49, 41–49. doi: 10.1016/j.mib.2019.10.002

Losi, A., and Gärtner, W. (2021). A light life together: photosensing in the plant microbiota. *Photochem. Photobiol. Sci.* 20, 451–473. doi: 10.1007/s43630-021-00029-7

Losi, A., Mandalri, C., and Gärtner, W. (2014). From plant infectivity to growth patterns: the role of blue-light sensing in the prokaryotic world. *Plan. Theory* 3, 70–94. doi: 10.3390/plants3010070

Mallon, A. C., van Elsas, J. D., and Salles, J. F. (2015). Microbial invasions: the process, patterns and mechanisms. *Trends Microbiol.* 23, 719–729. doi: 10.1016/j.tim.2015.07.013

Morrow, R. C. (2008). LED lighting in horticulture. *HortScience* 43, 1947–1950. doi: 10.21273/HORTSCI.43.7.1947

Müller, G. L., Tuttobene, M., Altilio, M., Martínez Amezaga, M., Nguyen, M., Cribb, P., et al. (2017). Light modulates metabolic pathways and other novel physiologicaltraits in the human pathogen Acinetobacter baumannii. *J. Bacteriol.* 199, e00011–e00017. doi: 10.1128/JB.00011-17

Newsham, K. K. (1997). Ultraviolet-B radiation influences the abundance and distribution of phylloplane fungi on pedunculate oak. *New Phytol.* 136, 287–297. doi: 10.1046/j.1469-8137.1997.00740.x

Nicot, P. C., Mermier, M., and Vaissière, B. E. (1996). Differential spore production by *Botrytis cinerea* on agar medium and plant tissue under near ultraviolet light-absorbing polyethylene film. *Plant Dis.* 80, 555–558. doi: 10.1094/PD-80-0555

Pastore, A. I., Barabas, G., Bimler, M. D., Mayfield, M. M., and Miller, T. E. (2021). The evolution of niche overlap and competitive differences. *Nat. Ecol. Evol.* 5, 330–337. doi: 10.1038/s41559-020-01383-y

Purcell, E. B., and Crosson, S. (2008). Photoregulation in prokaryotes. *Curr. Opin. Microbiol.* 11, 168–178. doi: 10.1016/j.mib.2008.02.014

R Core Team (2021). *A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rajalingam, N., and Lee, Y. H. (2017). Effects of green light on the gene expression and virulence of the plant pathogen pseudomonas cichorii JBC1. *Eur. J. Plant Pathol.* 150, 223–236. doi: 10.1007/s10658-017-1270-1

Reuveni, R., and Raviv, M. (1997). Control of downy mildew in greenhouse-grown cucumbers using blue photoselective polyethylene sheets. *Plant Dis.* 81, 999–1004. doi: 10.1094/PDIS.1997.81.9.999

Rieusset, L., Rey, M., Muller, D., Vacheron, J., Gerin, F., Dubost, A., et al. (2020). Secondary metabolites from plant-associated pseudomonas are overproduced in biofilm. *Microb. Biotechnol.* 13, 1562–1580. doi: 10.1111/1751-7915.13598

Suthaparan, A., Stensvand, A., Solhaug, K. S., Torre, S., Mortensen, L. M., Gadoury, D. M., et al. (2012). Suppression of powdery mildew (*Podosphaera pannosa*) in greenhouse roses by brief exposure to supplemental UVB radiation. *Plant Dis.* 96, 1653–1660. doi: 10.1094/PDIS-01-12-0094-RE

Suthaparan, A., Stensvand, A., Solhaug, K. S., Torre, S., Telfer, K. H., Ruud, A. K., et al. (2014). Suppression of cucumber powdery mildew by supplemental UV-B radiation in greenhouses can be augmented or reduced by background radiation quality. *Plant Dis.* 98, 1349–1357. doi: 10.1094/PDIS-03-13-0222-RE

Vaas, L., Sikorski, J., Michael, V., Göker, M., Klenk, H. P., and Göker, M. (2013). Opm: an R package for analysing Omnilog® phenotype MicroArray data. *Bioinformatics* 29, 1823–1824. doi: 10.1093/bioinformatics/btt291

Van der Horst, M. A., Key, J., and Hellingwerf, K. J. (2007). Photosensing in chemotrophic non-phototrophic bacteria: let there be light sensing too. *Trends Microbiol.* 15, 554–562. doi: 10.1016/j.tim.2007.09.009

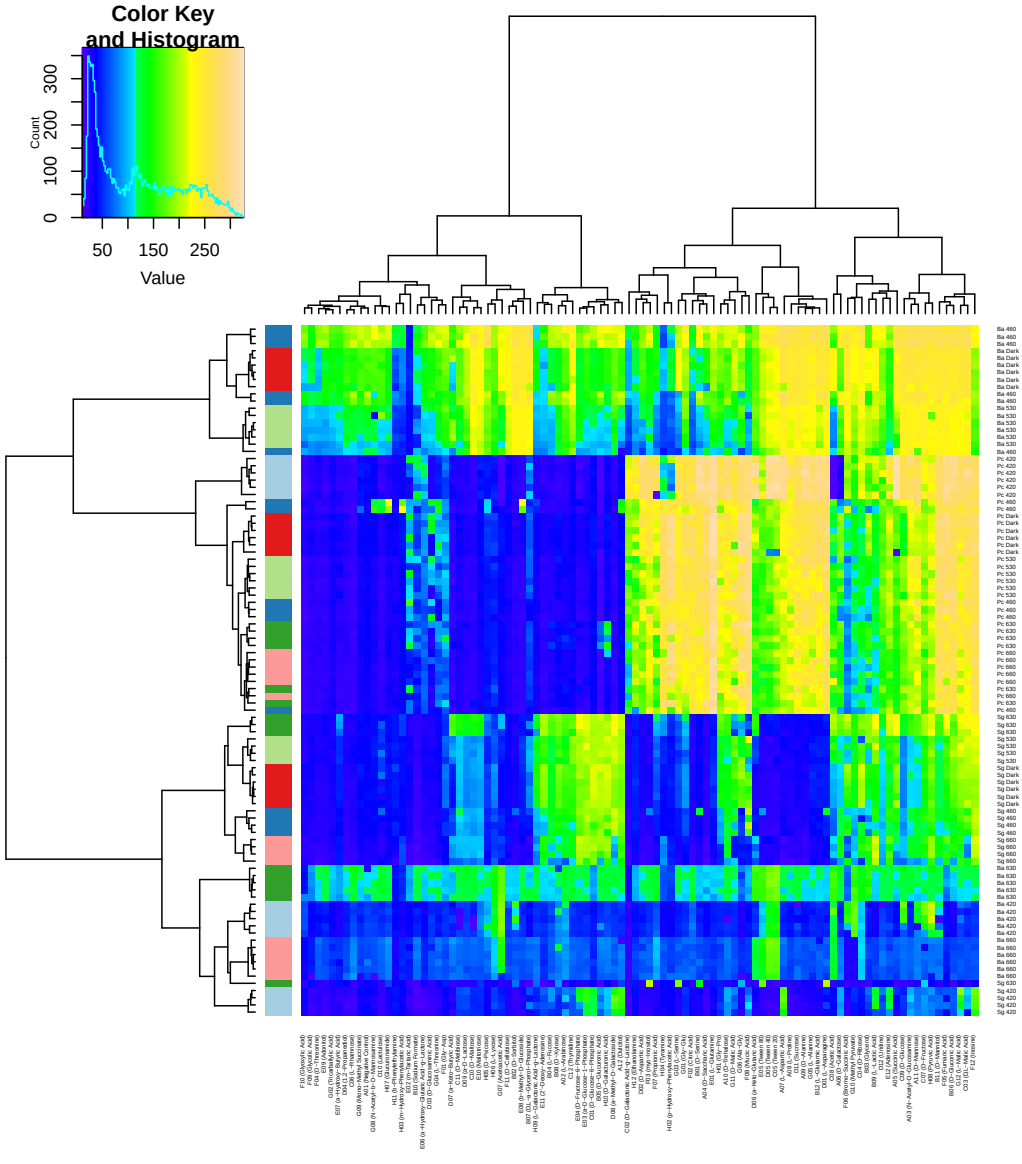
Vänninen, I., Pinto, D., Nissinen, A., Johansen, N., and Shipp, L. (2010). In the light of new greenhouse technologies: 1. Plant-mediated effects of artificial lighting on arthropods and tritrophic interactions. *Ann. Appl. Biol.* 157, 393–414. doi: 10.1111/j.1744-7348.2010.00438.x

Vorholt, J. A. (2012). Microbial life in the phyllosphere. *Nat. Rev. Microbiol.* 10, 828–840. doi: 10.1038/nrmicro2910

Wu, L., McGrane, R., and Beattie, G. (2013). Light regulation of swarming motility in pseudomonas syringae integrates signaling pathways mediated by a bacteriophytochrome and a LOV protein. *mBio* 4:e00334-13. doi: 10.1128/mBio.00334-13

Yeats, T. H., and Rose, J. K. C. (2013). The formation and function of plant cuticles. *Plant Physiol.* 163, 5–20. doi: 10.1104/pp.113.222737

Yu, S.-M., and Lee, Y. H. (2013). Effect of light quality on bacillus amyloliquefaciens JBC36 and its biocontrol efficacy. *Biol. Control* 64, 203–210. doi: 10.1016/j.biocontrol.2012.11.004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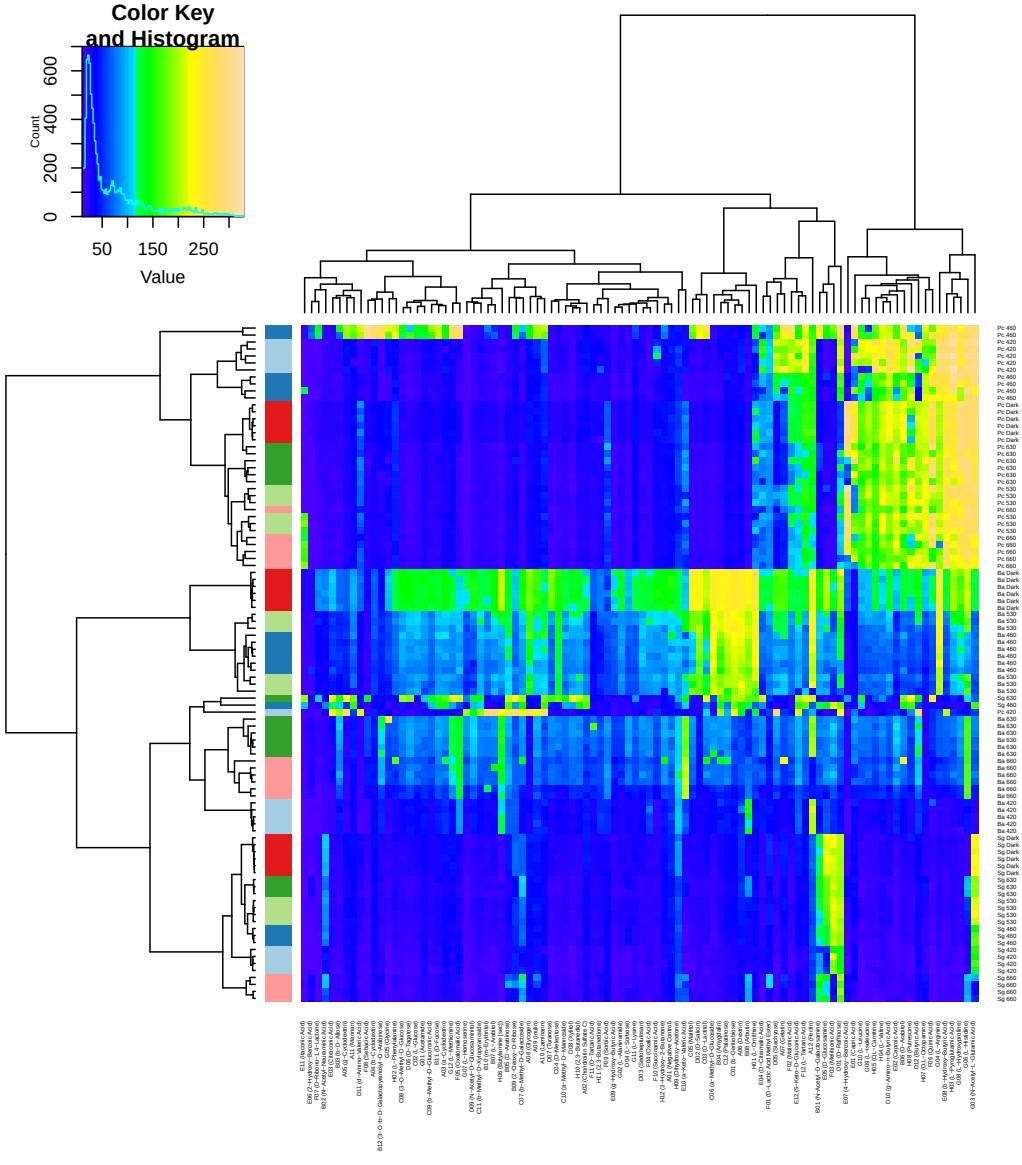
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Biological control agents (BCAs) can help decrease our reliance on chemical pesticides. Due to the harsh environment of leaves (phyllosphere), the introduction of BCAs is challenging, especially due to several factors such as fluctuating temperatures and limited nutrients. Light has been found to induce several mechanisms that can help non-light-dependent (non-phototrophic) bacteria. In this thesis, the effects of placement in the canopy, visible light treatment, exposure dose and sole carbon utilisation were investigated with respect to three non-phototrophic bacterial BCAs.

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