



Lauren Farwell

The Epidemiology and Management of *Cladosporium* on Raspberry

Environment and Agrifood Theme
Centre for Soil, Agrifood and Biosciences

PhD
Academic Year: 2019 - 2024

Supervisor: Prof. Naresh Magan & Prof. Xiangming Xu
Associate Supervisor: Prof. Angel Medina-Vaya
January 2024



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This thesis is submitted in partial fulfilment of the requirements for
the degree of Doctor of Philosophy

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Abstract

Cladosporium is a genus of fungi that causes dark mycelial lesions on raspberries, rendering fruit unmarketable. As *Cladosporium* is an opportunistic pathogen, its prevalence varies across years, and there is insufficient research on its epidemiology and management. This study aimed to investigate the epidemiology of *Cladosporium* on raspberries and to elucidate which management strategies could be effective.

Isolates of *Cladosporium* were obtained from raspberries, with *C. cladosporioides* being the most predominant, and was therefore used in further experiments. The susceptibility of raspberry fruit development was determined, with ripening and ripe fruit found to be susceptible to skin lesions; this stage of development was subsequently targeted in management studies.

The effects of ripening and fruit location (fruit location within a tunnel vs. across a farm) on the inoculum load on the surface of raspberries were investigated using metabarcoding studies. The fungal and bacterial microbiomes were also investigated to better elucidate the ecology of *Cladosporium* on the fruit surface. *Cladosporium* was more abundant on green fruit than ripening fruit; perhaps due to organisms such as *Rouxiella* present on the fruit's surface. Fungal α diversity increased during ripening while bacterial diversity decreased. The *Cladosporium* inoculum load in the air was also investigated, with more spores trapped inside a raspberry polytunnel than an open field.

The efficacy of commercial Biological Control Agents (BCAs) and resistant varieties to control *Cladosporium* skin lesions were tested across two years in field applications. The *Trichoderma* sp. was the most effective at reducing the incidence of *Cladosporium* on raspberry than compared to the other tested products. The variety resistance study was inconclusive, but results indicate further testing will reveal which varieties are more susceptible.

This research will inform potential management strategies against *Cladosporium* on raspberries that will aid growers in reducing food waste sustainably.

Keywords:

Microbiome; Inoculum; Biological Control; Susceptibility; Meta-Barcoding; Resistance; *Rubus ideaus*.

Acknowledgements

I want to first thank everyone in the NIAB pathology team who supported me throughout my PhD, especially during the COVID-19 pandemic, which made it very hard to find people in person! I want to thank Prof. Xiangming Xu for his patience and knowledge and for answering my endless questions. I am so grateful to have had you as a supervisor and to have had the chance to learn from you, so thank you for putting up with me! I also really want to thank Dr. Tom Passey and Dr. Greg Deakin for their endless support and for taking the time to show me everything I needed to carry out this project.

I also want to thank both Georgina Fagg and Sarah Cohen for not only being lovely lab managers, but great friends to me over the years and for supporting me throughout this PhD. There are so many people who stepped in to help me, so here's a big list of even more people I want to give a big hug as thanks: Dr. Mat Papp-Rupar, Dr. Netsai Mhalanga, Jen Kingsnorth, Joyce Robinson, Karen Cooper, and many more! A huge thank you to the farm team at NIAB East Malling for stepping in to help every time my irrigation failed (which always seemed to be at 5 pm on a Friday!); you were all such a big help. I also want to thank the lovely people at Cranfield University, including Dr. Carol Verheecke-Vaessen and Prof. Angel Medina-Vaya, for always taking the time to help me whenever I needed anything. You have always been so uplifting and kind.

Thanks to Richard Harnden for helping me to join the CTP PhD programme. I'm so glad you gave me the chance to further my research career. I also want to thank my industry representative, Harriet Duncalfe, for all the insight she provided during this project to help make something the industry would benefit from.

I want to thank all the NIAB East Malling PhD students for making our PhD cohort so welcoming and fun, and for being there for each other. In particular, I really want to thank Ece, Cindayniah and Hayden for always having the time to talk when I needed it and for all the laughs we had, even when times got hard. You guys kept me going when Naresh passed away, and I struggled to readjust. I don't know what I would have done without you!

A big thank you to my family for supporting me throughout my PhD, it was already a big step to be the first to go to university, and I truly didn't know how far I would have come all those years ago. In particular, I want to thank my nephews Ajay and Selasie, you always made me want to be the best version of myself that I can be.

I must also not understate the importance of coffee in both powering me through some rough field experiments, long lab days, and during my write up. I hope I can try and reduce my crippling caffeine addiction in the following months after submission...

Finally, I want to dedicate this thesis to both Adrian Harris and the memory of Prof. Naresh Magan. Adrian, thank you for your love and support throughout this endeavour. You were my rock during all the times I didn't feel like I was going to make it, and I'm so happy to have had you by my side for this journey. Words aren't enough to say what you mean to me. Naresh, I really wish you were here to see how it all came together. I remember being really nervous about meeting you, and Chris told me that you would always be on my side no matter what, and he was completely right. Despite COVID-19 hitting this project 6 months in so I couldn't see you as much as I'd hoped, you always made the time to help me when I needed it. You were always so much fun to talk to, and also brutally honest when you needed to be. I hope if you had read this, you would have approved (and someday, I hope I'm winning at science!).

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List of Abbreviations

μL	Microlitre
ACT	Actin region
ADONIS	A permutation MANOVA using F-tests based on the sequential sum of squares
ASV	Amplicon Sequence Variant
a_w	Water Activity
BCA	Biological Control Agent
BH	Benjamini-Hochberg
<i>Ca.</i>	<i>Circa</i>
cm	Centimetre
CO ₂	Carbon Dioxide
<i>e.g.</i>	<i>Exempli Gratia</i>
<i>etc.</i>	<i>Et Cetera</i>
g	Grams
hr	Hour
IPM	Integrated Pest Management
ITS	Internal Transcribed Spacer region
kb	Kilobase
KCl	Potassium Chloride
Km	Kilometres
L/ha	Litres per Hectare
M	Million
m	Metre
MA	Modified Atmosphere
Min	Minute
mL	Millilitre
mm	Millimetre
ms ⁻¹	Metre per Second
N	Nitrogen
NaCl	Sodium Chloride
nM	Nanomoles
NMDS	Non-Metric Multidimensional Scaling

O ₂	Oxygen
PC	Principal Component
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
qPCR	Quantitative Polymerase Chain Reaction
RH	Relative Humidity
RPM	Rotations per Minute
s	Seconds
S.E.	Standard Error of the Means
Sp.	Species
Spp.	Plural of Species
SWD	Spotted Winged Drosophila (<i>Drosophila suzukii</i>)
TEF1α	Trans-Elongation Factor 1α
UV	Ultra-Violet
V	Volts
v.	Version
vs.	<i>Versus</i>
x g	Times Gravity

1 Introduction

1.1 The Problem

Raspberry (*Rubus ideaus*) production in the U.K. is a profitable business, estimated at bringing £128.6 M (million) to the market in 2022 (DEFRA, 2023). Raspberries are delicate fruits that are susceptible to a wide range of fungal diseases, including *Botrytis*, *Rhizopus* and *Mucor* (Williamson *et al.*, 1991). Despite thorough research of many pathogens impacting raspberry fruits, some have been overlooked. One such disease is the genus *Cladosporium*, containing hundreds of species with some ubiquitous within the environment (Harvey, 1967) and found on a wide variety of economically important crops (Bensch *et al.*, 2012). In recent years, U.K. growers have reported *Cladosporium* being present on raspberries (O'Neill *et al.*, 2012), causing black mycelial growth and green skin lesions once sporulation has occurred on the fruit surface (Fig. 1-1). The incidence of *Cladosporium* can vary greatly across years, potentially explaining why research on this fungal pathogen is lacking. However, *Cladosporium* can be highly abundant in years conducive to its development; one farm in Kent, U.K., had over 50% of raspberries infected with *Cladosporium* post-harvest in 2010 (O'Neill *et al.*, 2012). *Cladosporium* has also been reported on raspberries pre-harvest, with $32\% \pm 4.2\%$ of raspberries surveyed infected at one site in Maryland, U.S.A. (Swett *et al.*, 2019).

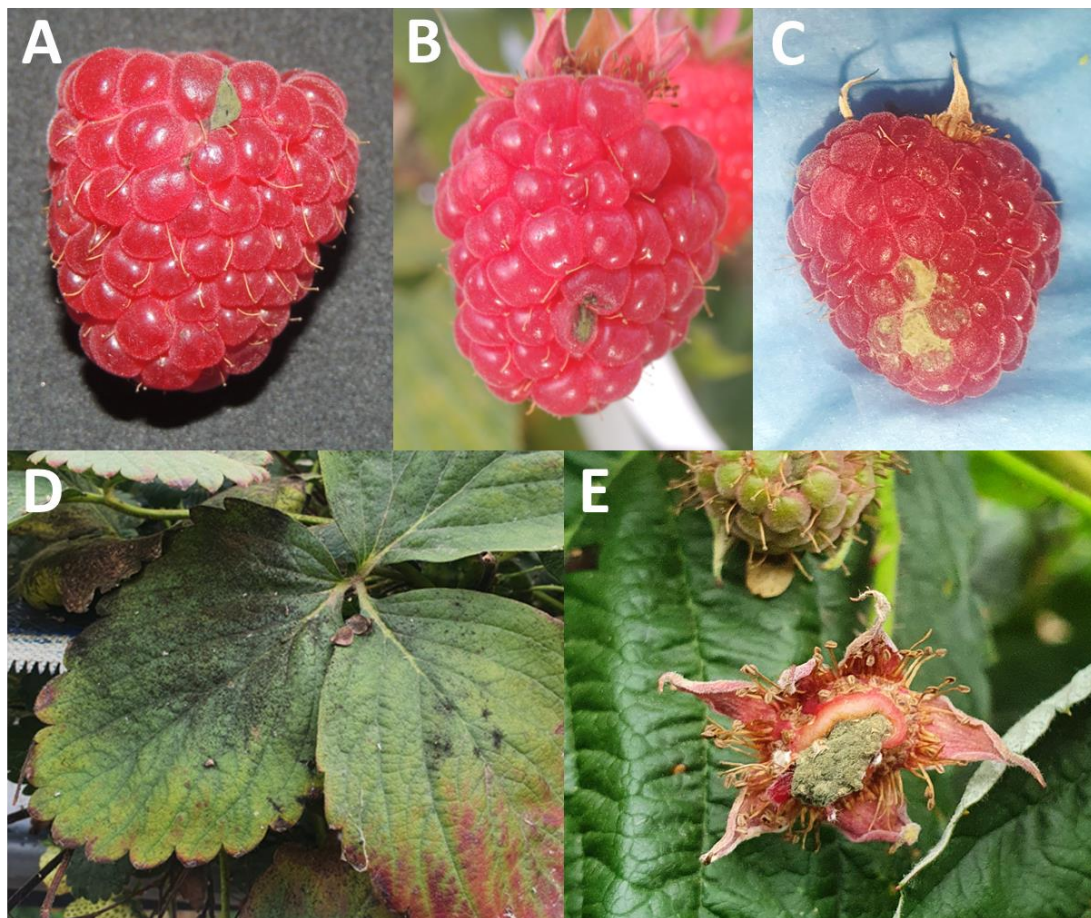


Figure 1-1. A), B) and C) *Cladosporium* skin lesions on raspberries. D) *Cladosporium* mycelial growth on aphid honeydew on strawberry leaves. E) *Cladosporium* growth on a raspberry receptacle.

The presence of *Cladosporium* on fruit can cause mass product rejection from suppliers, causing large amounts of food waste and losses for growers. Due to the current lack of research, it is difficult to know when to implement control measures against *Cladosporium*; current research has suggested *Cladosporium* can infect under-ripe fruit (Swett *et al.*, 2019), but it is unknown if green fruits are also susceptible. To develop successful management strategies, further work underpinning the epidemiology of *Cladosporium* on raspberry is needed to combat this disease.

1.2 *Cladosporium* on Horticultural Crops

Cladosporium spp. have been reported on multiple horticultural crops (Table 1-1), with frequently reported symptoms being lesions on the fruit surface and leaf spot.

Table 1-1. *Cladosporium* species isolated from horticultural crops and their associated symptoms.

<i>Cladosporium</i> species	Crop Species	Symptoms	Reference
<i>C. herbarum</i>, <i>C. passiflorae</i> & <i>C. subuliforme</i>	Passion Fruit (<i>Passiflora edulis</i>)	Warting of the fruit surface, scab lesions on fruit and leaf spots.	(Barbosa <i>et al.</i> , 2001; Rosado <i>et al.</i> , 2019).
<i>C. subuliforme</i>	Pepper (<i>Capsicum annuum</i>)	Yellow leaf spots.	(Ramos-Garcia <i>et al.</i> , 2016).
<i>C. halotolerans</i>, <i>C. ramotenellum</i> & <i>C. cladosporioides</i>	Grape Vine (<i>Vitis vinifera</i>)	Grape bunch rot.	(Lorenzini & Zapparoli, 2015; Solairaj <i>et al.</i> , 2020).
<i>C. cladosporioides</i>	Tomato (<i>Solanum lycopersicum</i>)	Leaf spot.	(Robles-Yerena <i>et al.</i> , 2019).
<i>C. oxysporum</i>	Bird Cherry (<i>Prunus nepalensis</i>)	Severe leaf blight.	(Baiswar <i>et al.</i> , 2011).
<i>C. cladosporioides</i>, <i>C. tenuissimum</i>, <i>C. macrocarpum</i> & <i>C. limoniforme</i>.	Strawberry (<i>Fragaria</i> × <i>ananassa</i>)	Blossom blight, skin lesions and sooty mycelia on the leaf surface.	(Ayoubi <i>et al.</i> , 2017; Nam <i>et al.</i> , 2015).

<i>C. cladosporioides</i>	Mandarin (<i>Citrus unshiu</i>)	Spots on the fruit.	(Tashiro <i>et al.</i> , 2013).
<i>C. cladosporioides</i>, <i>C. pseudocladosporioides</i>, <i>C. asperulatum</i>, <i>C. aggregatocicatricatum</i> & <i>C. ramotenellum</i>.	Pear (<i>Pyrus communis</i>)	Lesions on the fruit surface.	(Temperini <i>et al.</i> , 2018).

While *Cladosporium* can heavily impact raspberry yields, other important soft fruit crops have also been affected by *Cladosporium* fruit rot. This includes strawberries (*Fragaria × ananassa*) which have had mycelial growth on the fruit surface (Ayoubi *et al.*, 2017), but have also been reported to suffer from blossom blight (Nam *et al.* 2015). This is an important indicator that flowering tissues may be particularly susceptible to *Cladosporium* infection, and may require monitoring post-pollination when material senescens and is particularly vulnerable to saprophytic fungi.

In regards to top fruit production, there have been recent reports of *Cladosporium* impacting pears kept within cold stores, whereby dark mycelial patches form on the pear surface (Temperini *et al.*, 2018; Sardella *et al.*, 2016). This has been speculated to occur due to abrasions on the fruit surface allowing *Cladosporium* species to access nutrition and grow as a result (Sardella *et al.*, 2016). It may be that abrasions are necessary for *Cladosporium* spp. to colonise top fruits, as they often have thicker skin than that of soft fruits.

On other crops such as peppers (*Capsicum annuum*), tomatoes (*Solanum lycopersicum*) and bird cherry (*Prunus nepalensis*), various *Cladosporium* spp. were reported to cause spots and blight on leaves (Ramos-Garcia *et al.*, 2016; Robles-Yerena *et al.*, 2019; Baiswar *et al.*, 2011). As these reports seem to be limited in the literature, it may indicate that either *Cladosporium* spp. rarely cause disease on these hosts, or there are specific conditions that need to be met for disease to occur (such as correct environmental conditions or pathogen

specificity). On soft fruits such as strawberries, it seems *Cladosporium* spp. prefer to saprophytically colonise decaying material such as petals (Nam *et al.*, 2015). However, *Cladosporium* spp. are well known for germinating and growing on materials such as walls inside homes if conditions are conducive to growth (Bensch *et al.*, 2018). Perhaps *Cladosporium* can similarly grow superficially on plant surfaces in the correct conditions.

The most substantial study investigating *Cladosporium* on raspberry was performed in the U.S.A., with a primary focus on how the pest *Drosophila suzukii* (also referred to as Spotted Winged Drosophila; SWD) interacts with raspberries to exacerbate *Cladosporium* infections (Swett *et al.*, 2019), and discussed finding cases of *Cladosporium* pre-harvest (as above). The incidence of *Cladosporium* varied largely within the study; significant differences in *Cladosporium* incidence occurred across years, but not between farm sites or positions in the canopy when the data were consolidated. The reasons why the incidence of *Cladosporium* varied so much pre-harvest are still mostly unknown, but potentially, differences in the climate between years may have impacted *Cladosporium* development. Interestingly, when their results were analysed separately based on the locations and dates, they also found an effect of canopy location on the incidence of *Cladosporium* at one site in 2016, with fruit at the top of the canopy having a lower incidence of *Cladosporium* compared to other areas. It may be that spores from higher up the plant were being dislodged during picking, causing more rot on the lower branches. It is also important to note that raspberries ripen from the top of the plant downwards, with the senescing receptacles perhaps providing material for saprophytic fungi such as *Cladosporium* to colonise (Fig. 1-1E). They also found a substantial effect of fruit ripeness at picking on the post-harvest incidence of *Cladosporium*, with a higher incidence of $51\% \pm 11\%$ occurring when the fruit was picked red-ripe, compared to $16\% \pm 9\%$ when the fruit was picked under-ripe. It is, therefore, likely that raspberries are more susceptible to *Cladosporium* when ripe due to the skin of the fruit being weaker and more prone to breaking, providing nutrients for infection.

They also demonstrated that wounding similar to that caused by SWD increased *Cladosporium* infection; other research has found SWD to prefer fruit which visually contrasts from the surrounding foliage (Little *et al.*, 2017). This may lead to ripe fruit being damaged by SWD more frequently, and subsequently having a higher incidence of *Cladosporium* skin lesions. The softening of fruit skin during ripening has been attributed to pectin being broken down and reductions in cellulose in ripe berries (Stewart *et al.*, 2001); softer fruit skin may be more readily broken, and allow colonisation of saprophytic fungi. The authors also proposed that the lengthening of the growing season may be partially responsible for the increase in *Cladosporium* disease on raspberries, where conditions are more favourable for fungi in humid autumn months and SWD may be more prevalent. Further research is needed to assess the effect of extending the growing season on disease development in terms of inoculum dynamics, and weather conditions for fruit infection.

The final horticultural crop experiencing heavy losses to *Cladosporium* skin lesions are grapes. In wine grapes, *Cladosporium* becomes more of a problem when the fruits are left to ripen and mature (Briceño & Latorre, 2007). Currently, no reports of *Cladosporium* infections on grapes have been made in the U.K., perhaps as the conditions are unfavourable for *Cladosporium* development. Similar to raspberries, wounding of the fruit also increases the development of infection. Furthermore, skin lesions appear to only develop on the skin surface, with hyphae rarely penetrating further into the fruit (Briceño & Latorre, 2008). Investigations into the practice of removing leaves from vines to increase air flow near harvest found that it increased the incidence of *Cladosporium* rot on grapes (Latorre *et al.*, 2011). This increase in incidence may be due to a higher amount of rain and *Cladosporium* airborne inoculum falling directly onto grape bunches, with the increased water making the bunches more humid and facilitating fungal growth. As raspberries are mostly grown under cover, it may be that reducing foliage within the crop canopy has a different effect, highlighting how management practices impact the risk of pathogens and need further investigation.

1.3 *Cladosporium* Spore Dispersal and Germination

Cladosporium is an ascomycete fungus and belongs to the Fungi Imperfecti group as there is currently no known sexual stage in its life cycle (Samson, 2019). They, therefore, have a relatively simple asexual lifecycle (Fig. 1-2).

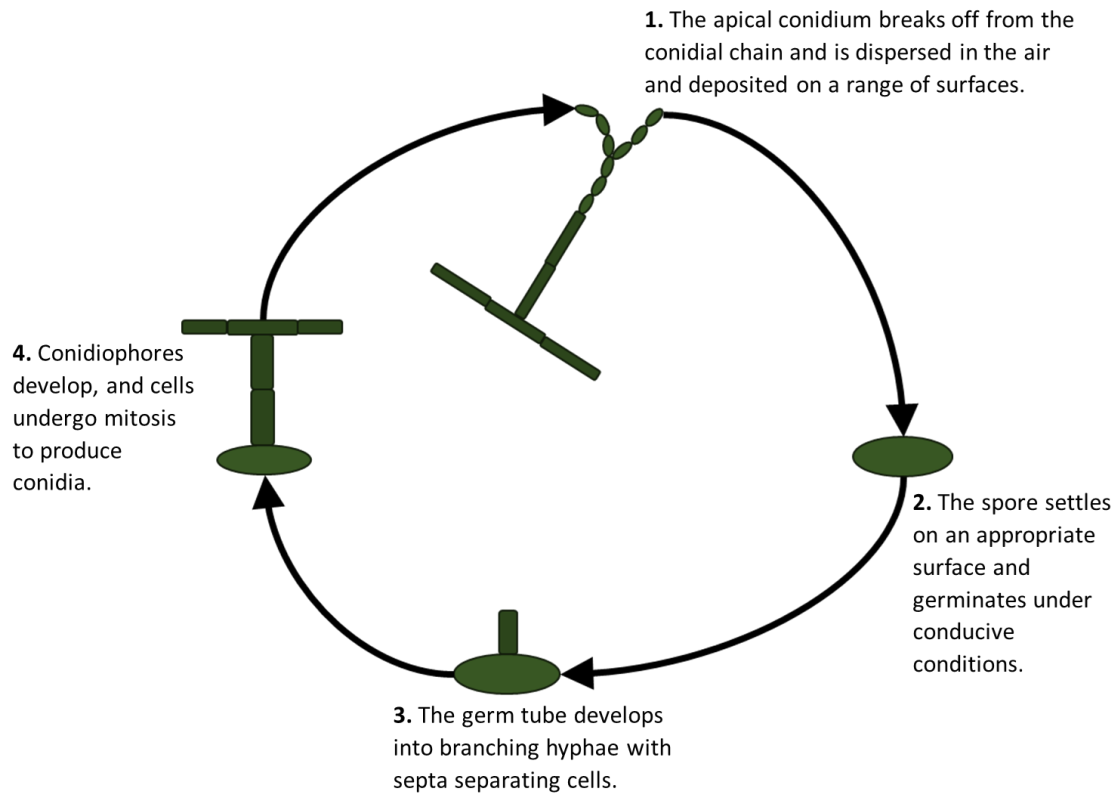


Figure 1-2. The asexual lifecycle of most *Cladosporium* species.

As the majority of *Cladosporium* species have no known sexual stage, they are assumed to asexually reproduce *via* conidiogenesis. New spores produced at the tip of conidial chains on the conidiophore and become detached by the action of wind or rain droplet impactation (Fig. 1-3); they are then carried by the wind where they can reach heights of 10 km in the air (Hirst & Stedman, 1967) to be deposited over a range of distances. They produce copious amounts of conidia to increase their chances of being deposited on material where they may eventually germinate and grow when conditions are suitable (Harvey, 1970). Currently, no research has focused on how long *Cladosporium* spores can survive and remain viable within the environment. This is an important avenue of investigation to

determine how long *Cladosporium* spores can remain latent so when conditions are suitable, they can germinate and subsequently cause disease on plant material.

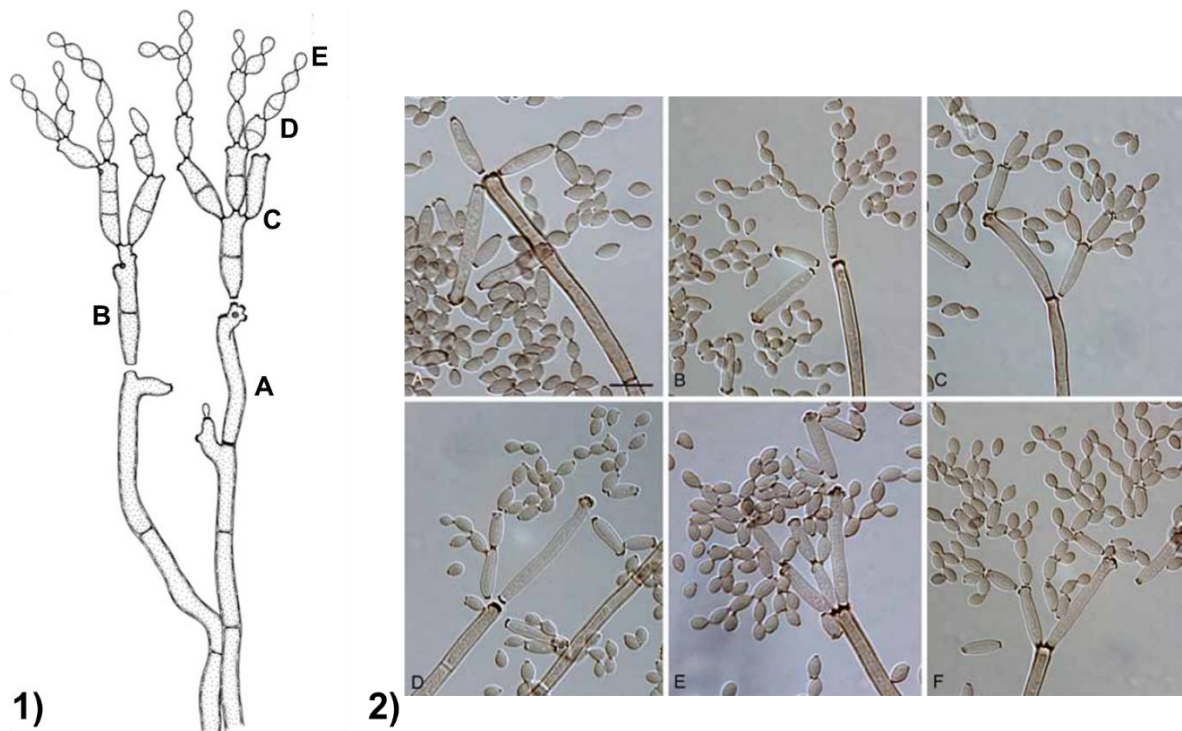


Figure 1-3. 1) An overview of the structure of *Cladosporium* species conidia showing A) a ramoconidium still attached to the conidiophore, B) a ramoconidium already seceded without cladosporioid basal hilum, C) secondary ramoconidia at the base of the chain, D) intercalary conidia (within the chain) and E) small terminal conidia (with either one apical hilum or none). 2) A-F Examples of conidia being released from *Cladosporium cladosporioides*. Scale bar = 10 µm. Both images adapted from Bensch *et al.*, 2012.

Cladosporium conidiogenesis and spore release are profoundly influenced by abiotic factors. Of particular importance are factors such as wind/air movements which are responsible for the spread of airborne spores within the crop canopy. Previous research has focused on *Cladosporium* airborne spores due to their importance as an allergen (Olsen *et al.*, 2023). It is important to understand the

fluxes in spore loads throughout the production season to predict when the disease risk will be highest, so that control strategies can be implemented. While the factors below are reviewed independently, it is important to remember they will be interacting to influence the germination, growth and sporulation of *Cladosporium* species.

1.3.1 Temperature

Investigations on strains of *C. cladosporioides* and *C. herbarum* isolated from cereal ears suggested they had germination temperature ranges from 5 - 35 °C, with no germination observed at 40 °C. Optimum mycelial growth for *C. cladosporioides* occurred at 15 - 27.5 °C and for *C. herbarum* at 25 - 30 °C (Magan & Lacey, 1984a). A range of strains of these two species isolated from grapes were found to grow optimally at 20 °C for both species, with a range of growth from 0 - 30 °C (Briceño & Latorre, 2008). Some *Cladosporium* species have been reported to grow at temperatures as low as -6 °C (Gravesen, 1979). If *Cladosporium* can grow at temperatures this low, it raises concerns for the use of cool-chain management as a post-harvest control strategy. However, growth at around 0 °C is very slow and due to the fast turnaround from picking soft fruit to fruit being taken to the market, the risk of excessive *Cladosporium* growth before reaching consumers is likely quite low. In addition, these studies suggest that *Cladosporium* strains may be differentially affected by temperature; hence, investigations into the intra-species variation may be warranted.

Many studies have observed that the number of airborne *Cladosporium* spores initially increases with temperature, with spore numbers peaking between 20 - 25 °C, and then decreases with increasing temperature (Hjelmroos, 1993; Hyde & Williams, 1953; Kurkela, 1997; Şakiyan & Inceoğlu, 2003). It has been suggested that the temperature threshold for spore release of *Cladosporium* is around 16 °C (Hjelmroos, 1993; O'Connor *et al.*, 2014). Extreme temperatures, which may occur more frequently with climate change, have also been examined. Under elevated temperatures (> 35 °C) there was a significant reduction in spore production for *C. oxysporum*; the opposite was observed for *C. cladosporioides*

(Damialis *et al.*, 2015). This highlights that the impact of climate change on *Cladosporium* species life cycles may differ considerably.

1.3.2 Water Activity and Relative Humidity

Relative Humidity (RH) is the pressure of water vapour in the air relative to the highest possible saturation the air can hold depending on the temperature, and is therefore used when referring to humidity in the atmosphere. This measure is important in referring to moisture around the ripening fruit surface or in the canopy, which can influence the development of fungal pathogens. Water Activity (a_w) is best measured as the amount of water available for microbial growth, which is directly related to the equilibrium relative humidity at a standard temperature and vapour pressure (Magan, 2007) and is therefore used when referring to the humidity on the surface of a material.

Humidity has a significant impact on fungal germination and growth, often with decreasing humidity inhibiting fungal development. The range of a_w required for germination of *C. cladosporioides* on wheat ears is 0.995 - 0.90 at 5 °C, 0.995 - 0.86 at 20 - 25 °C, and 0.995 - 0.90 a_w at 30 °C. For *C. herbarum*, the corresponding values were: 0.995 - 0.90, 0.995 - 0.95, and 0.995 - 0.90 a_w (Magan & Lacey, 1984b). Thus, the range of a_w levels required for germination varies with temperature and individual species or potentially strains. Strains grown on wheat-based media had germination occur at a minimum of 0.85 a_w for *C. herbarum* and 0.86 a_w for *C. cladosporioides* at optimum temperatures (Magan & Lacey, 1984a). The optimum range of growth for *C. cladosporioides* and *C. herbarum* strains isolated from grapes was at > 0.98 a_w (Briceño & Latorre, 2008), corroborating with previous research (Magan & Lacey, 1984a). The minimum a_w for mycelial growth has been recorded as 0.90 a_w for *C. herbarum* and 0.88 a_w for *C. cladosporioides* (Magan & Lacey, 1984a). When *C. sphaerospermum* was subjected to RHs of 75% and 43%, growth was reduced than compared to higher RHs, and when reduced to 11% growth was inhibited completely (Tang *et al.*, 2015), indicating species of *Cladosporium* are likely sensitive to low humidities, but more work is required on other species within the genus.

Spore release is also significantly affected by RH. Under a constant temperature of 21 °C in a growth chamber, the number of spores released from cultures of *C. herbarum* increased when the RH dropped substantially from 91% to 29% (Pady *et al.*, 1969). However, the exact effect of RH on spore release may vary with individual *Cladosporium* species. For example, more spores of *C. herbarum* were released in dry air than other species of *Cladosporium* in lab trials (Harvey, 1970). In the same study, more spores from all the species were released in mist laden air than in dry and humid air. However, this difference may be due to differences in the spore loads among the six species, and the species could not be compared directly in the results due to differences in incubation times.

Similar trends in RH have been observed for airborne inoculum under field conditions. Multiple studies have found a negative correlation between spore counts and RH in field experiments (Ballero *et al.*, 1992; Grinn-Gofroń & Strzelczak, 2013; Kurkela, 1997). The interpretation of such field experiments, however, is complex due to the confounding effects of many other environmental factors such as rainfall.

1.3.3 Rainfall

Rain events can have a complex effect on the number of airborne spores. Rain can aid conidia breaking off and being released, especially from infected crop debris. Rain droplets can also deposit spores present in the air, which may then develop on conducive surfaces, including plant parts.

The effect of rainfall on *Cladosporium* spores differs depending on the level of precipitation. Light showers can result in rainout events with spores in the air adhering to water droplets and being deposited, decreasing airborne spore concentration. However, stormy weather conditions with high winds can increase the spore inoculum by dispersing spores from *Cladosporium* colonies (Rich & Waggoner, 1962). Airborne spore counts were negatively correlated with the amount of rainfall, but with peak spore concentrations occurring on days when there were light rain events (O'Connor *et al.*, 2014). Heavy rainfall lasting for several hours may decrease the spore load as spores adhere to the water

droplets. Often, after such rainfall events the airborne spore load increases again, up to 14 hours afterwards (Kurkela, 1997), possibly due to high moisture facilitating fungal growth and spore production (Hjelmroos, 1993). Thus, measurements of airborne spore loads need to be timely to obtain accurate information on the relative populations present as an inoculum for the infection of crops. However, the majority of U.K. raspberries are now grown under polytunnels using drip irrigation systems. It is unknown if the majority of airborne spores are sourced from the outside of polytunnels, hence the importance of rainfall events on the inoculum within polytunnels still needs to be investigated. If the airborne spore source is more important from within polytunnels, rain events may not be a significant environmental factor impacting disease risk. This is also supported by recent studies finding precipitation only had a weak association with *Cladosporium* spore counts in Poland and Denmark (Grinn-Gofroń *et al.*, 2018; Olsen *et al.*, 2020).

1.3.4 The Role of Wind in Spore Dispersal

The primary source of spore dispersal of *Cladosporium* species is *via* wind. Both laboratory and field experiments suggest that very low wind speeds can result in conidia dispersing into the air. For example, when *Cladosporium* was exposed to 1.0 ms^{-1} velocity air, spores were broken off and released from conidial chains (Pasanen *et al.*, 1991). Wind speeds were also positively correlated with the number of *Cladosporium* spores in field conditions, with the most efficient release of spores occurring between $0.5\text{-}1.0 \text{ ms}^{-1}$ winds (Kurkela, 1997). More turbulent wind speeds may result in a significant increase in spores being released from *Cladosporium* colonies, but prevents them from settling if speeds remain high. In the context of raspberry production, wind movement will vary depending on the polytunnel and glasshouse infrastructure with predominant air movement at the openings and edges of tunnels. During fruit picking, the crop canopy is likely to be shaken repeatedly, and may result in spikes of spore dispersal.

Wind direction will also impact spore movement and subsequent deposition behaviour. Thus, more spores are often detected downwind when using spore traps, which can increase the potential inoculum load in those areas (O'Connor

et al., 2014). It is also important to consider what vegetation may surround raspberry growing systems, as it has been shown that the harvest of grains can increase spore loads in the air locally (Olsen *et al.*, 2019). Depending on the growing system (enclosed polytunnels or glasshouses versus open ended polytunnels), the effects of local vegetation management may alter inoculum loads around raspberry plants differently, and are worth further investigation.

1.3.5 Diurnal and Seasonal Patterns

Cladosporium spore release follows a circadian rhythm; more spores are released during the daytime than at night, and with a peak often reported around noon (O'Connor *et al.*, 2014; Olsen *et al.*, 2020; Stępańska & Wołek, 2009), associated with rising temperatures and a decrease in RH in the day (see 1.3.2). Currently, nothing in the literature has investigated if diurnal spore patterns are affected by horticultural environments such as glasshouses and polytunnels. Air sampling in Cork, Ireland and Worcester, England varied slightly in the timing of peak spore numbers (O'Connor *et al.*, 2014), likely due to variations in vegetation, climate and perhaps *Cladosporium* species present.

Cladosporium spore numbers also follow a seasonal pattern with studies reporting a peak in number in the summer months around July and August, and then dropping down in the winter months (Martínez-Bracero *et al.*, 2022; Olsen *et al.*, 2020). Potentially, the harvest of summer wheat during these months may also contribute towards these higher spore loads (Eversmeyer & Kramer, 1987; Olsen *et al.*, 2019). It is important to note that these periods of higher spore numbers coincide with the peak production of raspberries in the U.K. Raspberry growers may have wheat fields adjacent to raspberry cultivation, hence preventative measures such as reducing humidity within polytunnels and applying Biological Control Agents (BCAs) might help protect raspberry crops during wheat harvesting. The current trends in spore release over seasons may change as climate change continues. In Poland, the drier and warmer weather caused *Cladosporium* spores to peak earlier and extended the period when higher numbers of spores were in the atmosphere (Kasprzyk *et al.*, 2016). However, in France, patterns in *Cladosporium* spore numbers over the years

have varied with location (Sindt, Besancenot & Thibaudon, 2016). Hence, future studies are needed to further understand how the changing climate will affect airborne spores across geographical locations.

1.3.6 Indoor and Horticultural Environments

Within the last 10 years, raspberry production moved from within open-air soil to mainly growing raspberries under polythene in coir pots with fertigation. This has allowed the growing season of raspberries to be extended later into the year, and while the increase in productivity has helped improve yields, the new environment may favour some pathogens and pests, such as *Cladosporium*. Despite the clear impact polytunnels have had on horticultural husbandry in the U.K., there is a lack of studies investigating how they impact the pathogenic spore loads within them. Studies researching if airborne spore movement has changed within polytunnels are vital to predict how plant pathogens behave to predict disease outbreaks. On the other hand, investigations into how air movement within glasshouses impacts pathogen dispersal have begun. Air movement within glasshouses was shown to be an important factor in dispersing *Botrytis* spores (Boulard *et al.*, 2010), and similarly the movement of pickers through tomato glasshouse alleyways was postulated to have spread powdery mildew along the rows (Sokolidi *et al.*, 2023).

Comparisons between indoor and outdoor environments vary in their findings of *Cladosporium* spores, with some finding no significant differences in the number of spores inside and outside (Pei-Chih *et al.*, 2000), whereas other studies have found a higher number of spores outdoors than indoors (Li & Kendrick, 1995). The latter study argued that variation in venting and air exchange with the outdoors would impact spores found inside, and the differences in the vegetation outdoors would impact how many spores are present. These are factors also relevant to polytunnels that are designed to promote airflow to prevent a build-up of humidity. This may allow the movement of airborne inoculum and subsequent deposition onto raspberry plants. In addition, as polytunnels both retain heat and often have an increased RH, they may become suitable environments for *Cladosporium* to thrive, and have likely increased the disease risk on raspberry fruits. The environment inside polytunnels may affect various *Cladosporium*

species differentially, as species composition may differ between the outdoor and indoor environments. For example, Segers *et al.* (2015) found that *C. sphaerospermum* was the most common species dwelling in building environments, despite *C. cladosporioides* being often regarded as the most common airborne *Cladosporium* species (Harvey, 1967). It may, therefore, be useful to compare the *Cladosporium* species composition impacting raspberries within polytunnels and in field plants.

1.4 Microbiome Studies and *Cladosporium*

Microbiome studies utilise modern sequencing techniques to identify the microorganisms (fungi, bacteria, *etc.*) within a selected environment to help understand how their populations change over time. Sequencing can be of select DNA barcode regions or whole genome sequencing. In plant pathology, these methods enable the study of pathogens within their ecological communities. This allows us to understand how the pathobiome forms during crop development. We can also identify if any potential antagonists occur within communities that may be useful, for instance as BCAs. Prior to microbiome studies, specific species and strains of pathogens could be studied utilising qPCR to determine how inoculum loads change through various factors (Markakis *et al.*, 2009). qPCRs are now used in conjunction with microbiome studies to provide a more complete understanding of how pathogen loads are shaped by the environment.

1.4.1 Abiotic Factors

The natural fluctuations in the environment not only impact host plants growth and health, but that of the organisms on and within such plants too. Many reviews have covered the impacts of abiotic factors on plant microbiomes, hence for the purpose of this introduction the factors impacting the microbiome will be noted, but not elaborated on in great detail. The review by (Hussain *et al.*, 2018) is recommended for a thorough explanation of these factors.

The environment impacts both the host plants and the microorganisms directly, but can also indirectly impact microorganisms by affecting host resource

acquisition. For instance, nutrient uptake being impaired in drought or flooding conditions or water uptake in extreme hot and cold temperatures. Plants will recruit microorganisms to aid their resource acquisition in such adverse conditions, particularly in the rhizosphere (Pantigoso *et al.*, 2022). Other factors impacting plants and their microbiomes are high salinity within soils (Santos *et al.*, 2021), and exposure to heavy metals (Phurailatpam *et al.*, 2022), which are arguably unlikely to impact raspberries which are grown on fertigation.

Interactions between plants, their microbiomes and how they are affected by the environment are complex. Climate change is making weather more volatile, with plants and their microbiomes being subjected to more extreme weather events. It is predicted that as a result of these adverse climatic conditions, plant microbiomes will evolve to survive the new conditions they exist within, alongside their hosts evolving to facilitate beneficial interactions with the microorganisms upon them (Trivedi *et al.*, 2022). Geographical location of plant material is also repeatedly found to be an important factor impacting plant microbiomes. Within horticultural research, apple fruit microbiomes have been demonstrated to significantly differ across countries (Abdelfattah *et al.*, 2021) and across farms on a local scale on apple leaf scars (Papp-Rupar *et al.*, 2024). Arguably, the differences in local vegetation and microclimate contributes towards these differences in microbiomes.

Another factor significantly affecting compositions of fruit microbiomes are the husbandry practices of growers, in particular whether plants are grown with conventional or organic management practices. In apples, genes expressed by microorganisms were found to centre on plant defence in organic orchards (such as isoquinoline and alkaloids biosynthesis and aromatic compounds), whereas conventional orchards had more gene expression contributing towards the production of antibiotics (Bartuv *et al.*, 2023). In tomatoes, the fruit bacterial microbiome composition was significantly different between hydroponically grown plants versus soil grown plants (Escobar Rodríguez *et al.*, 2021). Organic and conventional raspberry production was found to significantly impact fruit microbiomes; organic raspberries were found to be dominated by one bacterial

genus, where it may be expected that the microbiome would be diverse when not subjected to pesticide sprays. However, fruit grown under Integrated Pest Management (IPM) practices were found to have a more diverse bacterial microbiome (Sangiorgio *et al.*, 2021). Management practices can clearly impact the fruit microbiome, which can impact risk of disease development. Therefore, further research is warranted to understand how other husbandry practices, such as polytunnel venting and fertigation regimes may impact the microbiome of various horticultural crops.

1.4.2 Biotic Factors

The host plant cultivar has been shown to affect the composition of microbiomes across horticultural crops. In strawberries, three cultivars were shown to have significantly different fungal and bacterial microbial communities, which authors argue is due to the excretion of chemicals that recruit microorganisms (Sangiorgio *et al.*, 2022). Three raspberry cultivars were also shown to differ in their bacterial microbiomes; a white fruiting cultivar had a larger difference in the fruit microbiome than the more traditional red fruiting cultivars (Perpetuini *et al.*, 2019). Currently, no comparison between cultivars in raspberries have been made for fungal microbiomes. Researchers theorise that the microbiomes may have co-evolved with their respective hosts in regards to species and cultivars (Mesny *et al.*, 2023), and evidence of such has been found in apples whereby fungal and bacterial microbiomes have hybridised during domestication, but also different species of apple display phyllosymbiosis (microbial communities being linked to their host phylogeny; Abdelfattah *et al.*, 2022). More work is needed to find if similar has occurred in soft fruits, as selecting for beneficial and robust microbiomes may be warranted in future breeding efforts.

Variation in the microbiome can also occur on more acute scales, such as the different organs of a plant. Current studies have indicated that on both apples and strawberries, microbiome compositions differ between plant organs, and in strawberries the microbiome of organs within the soil were more dissimilar to the strawberry leaves (Longa *et al.*, 2022; Sangiorgio *et al.*, 2022). Further work on strawberries demonstrated that as organelles develop, a significant proportion of

the microbiome from the previous stage of development is transferred, and transfer occurred between the soil and fruit (Olimi *et al.*, 2022). This may differ from U.K. growing systems where most strawberries are grown in coir bags, and not in the ground. No studies on raspberry compartments have yet to be performed, but a larger separation may occur between organs due to the difference in plant architecture. Currently, studies on fruit microbiomes are shifting from using macerated fruit that has no separation between epiphytes and endophytes, to separating out plant organs. For instance, one study on blueberries found that both the mycobiome composition and diversity were significantly different between the skin and pulp (Szymanski *et al.*, 2023), indicating that further separation of fruit organs is needed across all fruits. In the case of *Cladosporium*, which is speculated to primarily develop on the fruit surface, it may be essential in the future to differentiate between plant organs which are exposed to different environmental conditions and host their own ecological niches. Differences have been recorded in the mycobiome on the epicarp of raspberries as they ripen, with diversity of fungal species peaking in the ripening stages and decreasing at the green and ripe stages of fruiting. However, this research sampled different stages of fruit ripening at different dates, therefore confounding the ripening stage with the dates of sampling (Jones *et al.*, 2022). Repeats of this experiment sampling all stages of fruit ripening simultaneously would be beneficial to understand how *Cladosporium* behaves on the fruit surface. It would also be beneficial to include bacteria within these studies, as they interact with fungi within these niches.

1.4.3 Utilising Microbiome Studies to Investigate BCAs

Microbiome experiments are increasingly important in regards to finding new BCAs and assessing the efficacy of their applications. Since next generation sequencing has become more readily available to researchers, they have been utilising this tool to screen healthy but disease susceptible plants for microorganisms that may confer resistance to pathogens (Collinge *et al.*, 2019). It has been argued however, that only screening healthy plants limits the resources for finding new BCAs, for instance successful control of stone fruit

diseases with *Rhizobium rhizogenes* K84 was only made possible by screening diseased plants (Ellis, 2017). Regardless of what material is screened, microbiome studies provide a powerful tool to find potential new BCAs. For instance, this technique has been used on blueberries and wild cultivars of raspberry to screen for potential BCAs (Chacón *et al.*, 2022; Pylak *et al.*, 2020). Arguably, it may be better to screen commercial plantings of raspberries, where microorganisms may be adapted to the environmental niches of polytunnels compared to the cooler conditions of wild raspberries. For BCAs to be effective within commercial settings, more research is needed to understand their ecology within commercial plantings. For instance, microbiome studies have been used to understand how *Bacillus subtilis* disperses on plants under protection vs. an open field, finding that there was less dispersal of this BCA under protection, perhaps due to the lack of droplets from rain to disperse the bacteria (Wei *et al.*, 2016).

1.4.4 *Cladosporium* in Microbiome Studies

Investigations into the microbiomes of soft fruit have found *Cladosporium* is often a prevalent genus, with one study on macerated strawberries finding *Cladosporium* had a relative abundance of 37.8% (Abdelfattah *et al.*, 2016). Another study investigating the epicarp of raspberries, strawberries, blueberries and cherries combined found *Cladosporium* was also the most abundant genus (Jones *et al.*, 2022). For *Cladosporium* to be so prevalent across fruit crops, but to only be considered a significant problem in raspberries, suggests that there is a niche within raspberries that allows it to exhibit pathogenic symptoms, instead of remaining as a neutral microorganism. There are multiple reasons this may occur, such as increased susceptibility to pest damage from *Drosophila suzukii*, the fruit architecture allowing transpiring air to collect around the fruit, fruit being softer, *etc.* Previous research on grapes found *Cladosporium* does not often produce hyphae that penetrate into the fruit tissues, and therefore preferentially colonises the grape surface (Briceño & Latorre, 2008), suggesting that *Cladosporium* spores landing on the fruit surface are an important inoculum source. *Cladosporium* spores are also highly melanised, with increased Ultra

Violet (UV) radiation causing *C. cladosporioides* to become darker (Bland *et al.*, 2022); it is speculated this darkening can help protect the hyphae and spores from UV radiation (Grishkan, 2011), indicating *Cladosporium* is adapted for a niche of surviving on surfaces and dispersing in the air.

1.5 Potential Control Methods

There are multiple potential methods that could be utilised to control *Cladosporium* on raspberry, however it is important to note that some of these methods may be applied both pre-harvest or post-harvest. Traditionally, only post-harvest control methods have been discussed regarding *Cladosporium* spp. on raspberry, however, as Swett *et al.* (2019) found *Cladosporium* to be present pre-harvest, it may be important to consider methods of control earlier in fruit development. To develop a successful IPM strategy for *Cladosporium* spp. on raspberry, understanding their ecology is vital to determine which approaches will be effective. Growers can then implement practices as needed depending on if preventative measures can be used, or an intervention approach is required (Fig. 1-4).

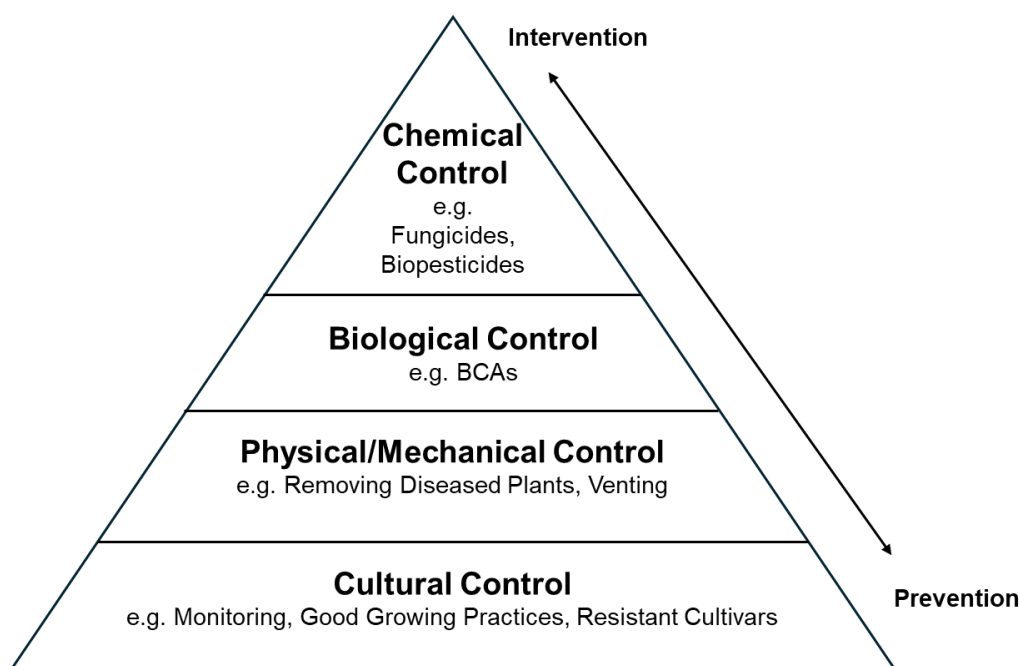


Figure 1-4. An IPM pyramid for pathogen control, including examples of control for each level. Adapted from (Singh *et al.*, 2017).

1.5.1 Fungicides

In recent years, there has been a movement away from chemical fungicides within horticulture due to the associated health and environmental concerns (Wightwick *et al.*, 2010). The lack of new fungicides being produced for the market also puts growers at risk of fungicide resistance developing as fewer products can be rotated (Deising *et al.*, 2008). Strains of *Cladosporium* isolated from raspberries have been found to be resistant to multiple fungicidal agents such as caspofungin, fluconazole and flucytosine (Farian & Wójcik-Fatla, 2022), eluding to the need for fungicides to be used judiciously and in conjunction with other management strategies.

In the past, pre-harvest applications of fungicides such as benlate (Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) and elvaron (Dichlofluanid) were shown to reduce the amount of *Cladosporium* that developed on freshly picked raspberries (Dennis, 1975), however, strains of benlate resistant *Cladosporium* were detected by the end of the season. Post-harvest *Cladosporium* infection of pears was significantly reduced when treated with fungicides pre-harvest

compared to post-harvest treatments (Lutz *et al.*, 2017), indicating that reducing the viable inoculum earlier in development may be a useful post-harvest control strategy. Using fungicides on fruits in the U.K. is risky due to the strict regulations of residues on soft fruit. Even with careful pre-harvest applications with time to allow chemicals to degrade before picking, due to the continuous fruiting of raspberries that require multiple picks weekly, it is difficult to apply fungicides safely close to harvest. It may therefore be better to look at safer alternatives to fungicides for a more strategic and sustainable control strategy for *Cladosporium* on raspberry.

1.5.2 Natural Plant Extracts

Natural plant extracts are compounds that can be a suitable alternative to fungicides as they are food safe, and can consequently be applied to crops close to harvest. Several plant-extracts such as thymol, carvacrol, eugenol and menthol were found to reduce both spore germination and mycelial growth of one strain of *Cladosporium* in *in vitro* assays (Abbaszadeh *et al.*, 2014). Plant extracts from garlic, ginger, onion, eucalyptus and neem were also found to significantly reduce *C. cladosporioides* growth in plate assays (Mengal *et al.*, 2020). As research from the U.S.A. indicated that *Cladosporium* rot of raspberry can be caused by a complex of *Cladosporium* species (Swett *et al.*, 2019), it would be beneficial to test a range of species for the efficacy of plant extracts. Further testing of such compounds is also needed in field trials to determine if the products are reliable when within the environment.

1.5.3 Biological Control Agents

For this introduction, a biopesticide is defined as a compound derived from a living organism whereas Biological Control Agents (BCAs) are living organisms that are antagonistic against plant pathogens. As previously discussed, BCAs are another safer alternative to fungicides for application close to harvest. BCAs use multiple mechanisms to inhibit the growth of pathogens, including emitting noxious substances, hyper-parasitism and out-competing the pathogens for resources (Köhl *et al.*, 2019).

Studies have shown that *Cladosporium* species can be controlled in plate assays with various BCAs, including the fungi *Trichoderma harzianum* (Lone *et al.*, 2012), *Hanseniaspora opuntiae* (Ruiz-Moyano *et al.*, 2016) and *Chaetomium globosum* (El-Dawy *et al.*, 2021). While *H. opuntiae* has been demonstrated to reduce *Cladosporium* infections on apple, currently no testing has investigated if any BCAs work on soft fruits against *Cladosporium*. This step in taking BCAs into field testing is vital, as plate assays show antagonism in unnatural conditions and in the absence of other organisms. It is also critical that fruit quality is not visually affected, which would impact the economic value of the product.

It is often recommended BCAs are applied on over-cast days where temperatures are not extreme and humidity is high. When conditions are unfavourable for their survival, biopesticides may be considered instead. As these are active compounds often isolated from BCAs, they can be applied in a wider range of environmental conditions. For example, the volatiles released from *Trichoderma* spp. were tested against isolates of *Cladosporium* and shown to be effective at reducing mycelial growth (Rolim *et al.*, 2019). Further work investigating the efficacy of secondary metabolites from a range of BCAs is therefore warranted to determine if any may have a commercial use.

An additional note, is that species of *Cladosporium* are often tested as BCAs themselves against other plant pathogens (Islam, 2022; Köhl *et al.*, 2015). Evidently, *Cladosporium* spp. can exist in great abundance on a wide range of horticultural crops without creating visible symptoms, but the physiology of raspberry plants may make them uniquely vulnerable to *Cladosporium* spp.

1.5.4 Irradiation and UV Treatment

There are different sources of irradiation that can be used on fresh produce to reduce food wastage microorganisms. One of the most commonly used is UV treatment. Treatment can be pre-harvest, in the form of robotics that move a UV light source through a planting to irradiate fruits (Onofre *et al.*, 2021), or can be used post-harvest on areas such as packaging conveyor lines. However, surface treatment alone may not be adequate as raspberry fruits are not placed within

punnets as a single layer. Without treatment of the entire surface area, the efficacy may be limited. In addition, using UV light commercially requires significant safety procedures, as UV light is damaging unless completely enclosed. The cost of using UV equipment and implementing necessary safety would also come at a large cost that may not be accessible to all growers or packhouses. Studies of *C. cladosporioides* and *C. herbarum* isolated from blueberries found these species to be highly resistant to UV treatment in growth cabinets (Latorre *et al.*, 2012). As previously mentioned, *Cladosporium* spp. are resistant to UV due to their pigmentation. UV-light may inhibit other beneficial phyllosphere microbiota, reducing competition of *Cladosporium* with other resident microorganisms, and may exacerbate problems in raspberries. Therefore, further investigations on how UV affects the ecology of *Cladosporium* on soft fruits may be warranted.

The use of gamma radiation has been trialled on raspberries, with less decay present on gamma-treated than untreated raspberries (Tezotto-Uliana *et al.*, 2013). However, they did not identify specific rots present in this study. In a different study, exposure of raspberries to gamma radiation controlled most rot species except *Cladosporium* (Verde *et al.*, 2013). Gamma irradiation is currently not recommended for fresh fruit in the E.U., although it has been successfully applied to spices in countries such as India. In the U.K., it is unlikely that gamma irradiation could be used for the treatment of any food products due to safety concerns.

1.5.5 Cool-Chain Management

A common method to reduce the growth of post-harvest rots is by controlling the fruit temperature immediately after harvest, during packaging, storage, transport and display at the supermarket. The main aim is to keep the fruit as cool as possible during distribution to minimise colonisation and germination of post-harvest rot pathogens and to maintain the required shelf life of the product (Shashi *et al.*, 2018). Cool chain management was effective at controlling the incidence of *Botrytis* rot of raspberries; however, in the same study, *Cladosporium* was found to be unaffected by cool chain management at one site

in Kent (O'Neill *et al.*, 2012). This could be because a number of *Cladosporium* species are able to grow, albeit slowly, even at $< 5 - 10^{\circ}\text{C}$ (Briceño & Latorre, 2008). Hence, combatting *Cladosporium* outbreaks may need to focus more on reducing the inoculum load pre-harvest.

1.5.6 Modified Atmosphere

Modified atmosphere (MA) storage has been widely used to increase the shelf-life of perishables. This usually involves increasing the CO_2 levels to $> 25\%$, reducing the O_2 concentration to $< 1 - 2\%$ and using N to balance these changes. Studies of MA storage have been carried out with raspberries, with a significant reduction in visible rots reported (Forney *et al.*, 2015; Goulart *et al.*, 1992). Often, these studies looked at the overall levels of decay, but not how specific fungal pathogens were affected by the MA treatment. The effects of MA on growth of *Cladosporium* species on raspberry is largely unknown; although one study suggested that *Cladosporium* species may be relatively resistant to control by MA storage (Magan & Lacey, 1984b).

1.5.7 Canopy Management

Another alternative approach to managing *Cladosporium* is to alter the environment and make conditions less favourable for pathogens. Canopy management is a well-established practice used for grapes; the canopy is thinned to improve air movement and decrease RH to help reduce *Botrytis* rot at harvest (Silvestroni *et al.*, 2018). However, recent research showed that this management strategy in grapes caused an increase in *Cladosporium* rots (Latorre *et al.*, 2011). Perhaps pruning caused some partial damage to fruit to facilitate *Cladosporium* infections. The exposure to the air may have allowed more spores to land on the fruit overtime and increase the risk of disease. A similar practice is used on raspberries to reduce the RH within plantings to thereby reduce cane *Botrytis*. While an improvement was seen in canes, the incidence of *Botrytis* on fruits was not reduced (O'Neill *et al.*, 2009). Thus, further investigation is warranted on how managing the canopy of raspberries impacts the disease risk of *Cladosporium*.

1.5.8 Vector Control

As previously discussed, damage from SWD can create entry points for pathogenic fungi to develop (Lewis *et al.*, 2019). However, SWD is not the only pest closely associated with *Cladosporium*. Large outbreaks of aphids within crops can lead to an excess amount of honeydew, a sugary substance produced as a byproduct of feeding (Gordon *et al.*, 1997). While no studies have focused on aphid outbreaks in relation to raspberries, a clear link between an increased amount of *Cladosporium* with the presence of aphids has been found on wheat and barley (Ajayi & Dewar, 1983). While a clear link between increased aphids causing increased *Cladosporium* spores could not be proven in a recent study, the authors did suggest that more experiments are needed to link aphid presence with increased spore production (Magyar *et al.*, 2023). These studies highlight the need for a holistic IPM approach to managing *Cladosporium*, as good plant husbandry will reduce available resources for *Cladosporium* to colonise. Furthermore, when growers have multiple crops grown concurrently, while aphids may not cross between plant species, the honeydew from an outbreak in one area could produce an inoculum source for a more susceptible nearby species.

1.5.9 Resistant Varieties

Different cultivars of raspberries have innate differences in their susceptibility to diseases. Only one previous study has examined the susceptibility of different raspberry cultivars to *Cladosporium* (Knight, 1980); most of the cultivars used have since been superseded by more modern cultivars. While this study could not use statistical analyses, varieties did exhibit differences in susceptibility to *Cladosporium*, with Malling Orion and Malling Jewel having 40% and 20% of fruits infected post-harvest, respectively. Research is needed to elucidate both genetic and physiological mechanisms of resistance.

Fruit firmness varies between varieties and may make them more or less susceptible to damage (Giongo *et al.*, 2019). Differences in skin thickness may have a similar effect, but no research has investigated this. Volatiles released by different cultivars also vary, and were shown to aid in resistance to *Botrytis* (Eugenio *et al.*, 2010); volatiles may similarly impact *Cladosporium* development.

Raspberry lateral branching may impact air flow and the RH; similarly, high drupelet numbers will mean more crevices for air to collect around the fruit to facilitate infections. Finally, *Cladosporium* often colonises decaying material, therefore any petals and stigmata left from the flower may be more readily colonised if they senesce faster, increasing the risk of subsequent *Cladosporium* infections on the fruit surface.

Screening popular commercial cultivars for *Cladosporium* susceptibility/resistance will allow growers with severe problems to select alternative cultivars with some degree of resistance, thus minimising yield losses. Understanding what genetic changes are causing these differences in susceptibility/resistance may allow breeders to select efficiently for *Cladosporium* resistance.

1.6 Summary

Currently, research has only just begun to understand the epidemiology of *Cladosporium* on raspberries. It is vital that for research to be relevant to U.K. growers, the most frequent species of *Cladosporium* are isolated from U.K. fruits. Determining when fruit are susceptible to infection will allow a more targeted approach to management and provide a window of when to look for visible symptoms. Currently, while research has begun to investigate the microbiome of the epicarp, more is needed on raspberries, including investigating fungal and bacterial microbiomes together; this will provide insight on the communities *Cladosporium* spp. are existing within and how they may be impacted by such microorganisms. While many studies have investigated *Cladosporium* spores in the air, none have focused on how polytunnels impact the patterns of airborne spore numbers. This will offer a perspective on how airborne primary inoculum is impacted by polythene, and how venting will impact disease risk. Due to the fruiting habits of raspberries, chemical fungicides may not be an appropriate control method for *Cladosporium* spp. Instead, BCAs may be a viable alternative; studies have already found BCAs that can successfully reduce *Cladosporium* spp. mycelial growth in plate assays. Applications need to be made in field

conditions to determine if products still have efficacy for controlling *Cladosporium* spp. Finally, knowledge on the susceptibility of raspberry cultivars to *Cladosporium* is currently outdated. Newer varieties of raspberries need to be screened to determine if there are variations in susceptibility, which will provide growers with a potential cultural control method. While there is much to investigate for this disease, research will hopefully provide answers for the multitude of crops that experience problems with *Cladosporium* spp.

2 Aims and Objectives

2.1 Aims and Objectives

The aim of this project was to build an understanding of the epidemiology of *Cladosporium* infections on raspberries to identify potential management strategies. The objectives of this project were to (i) identify the dominant species of *Cladosporium* present on U.K. raspberries (ii) determine the susceptibility of fruit in relation to fruit maturity, (iii) understand the dynamics of *Cladosporium* and other microorganisms on the raspberry fruit surface, (iv) investigate if polytunnel infrastructure impacts the *Cladosporium* inoculum load in the air, (v) assess the efficacies of commercially available BCAs against *Cladosporium* spp., and (vi) determine if there are differences in susceptibility among modern raspberry varieties to *Cladosporium* infections.

2.2 Studies Conducted

The following studies were performed to meet the objectives:

(a) Identification of *Cladosporium* spp. Isolated from U.K. Raspberries

- Build phylogenetic trees of *Cladosporium* isolates based on the Trans-Elongation Factor 1α and Actin regions.
- Compare the results from the phylogenetic trees to the NCBI blast database.

(b) Determining the Stages of Raspberry Development Susceptible to *Cladosporium* Infections

- Inoculate raspberries with *C. cladosporioides* to determine what stages of fruit development are susceptible to skin lesions and stigmata infections.
- Determine if *C. cladosporioides* isolates differ in their abilities causing disease symptoms on raspberries.
- Determine if *C. cladosporioides* prefers superficially growing on the surface of stigmata.

(c) Investigating the Dynamics of *Cladosporium* Inoculum on Raspberries and in the Air

- Use amplicon sequencing to profile the fungal and bacterial microbiomes on the raspberry fruit surface to determine how community profiles change during ripening.
- Investigate how the raspberry surface microbiomes differ within polytunnels (the centre vs. the outer edge) and across a farm (tunnels spaces evenly over a field).
- Compare the number of airborne fungal and *Cladosporium* spores using qPCRs from inside a raspberry polytunnel to an open field in the day vs. night, and across the day.

(d) Assessing the Efficacy of Biological Control Agents and Resistant Varieties to Manage *Cladosporium* on Raspberry

- Determine if commercially available BCAs are effective at reducing *in vivo Cladosporium* mycelial growth.
- Investigate the efficacy of BCAs to reduce the incidence of *Cladosporium* on raspberries in field inoculations and applications.
- Assess if modern raspberry varieties differ in their susceptibility to *C. cladosporioides* skin lesions and stigmata infections.

2.3 Connection of the Chapters

The first study on identifying which species are most prevalent on raspberries feeds into determining when raspberries are susceptible and the management studies to use the most prevalent species as the inoculum. Determining when fruit are susceptible fits into investigating how the airborne and surface inoculum behaves to identify when fruit are susceptible and when the inoculum is present to cause infection, and when to apply the management strategies. The microbiome experiment of the fruit surface also provides insight on how potential BCAs behave naturally on the fruit surface. All the chapters combined gives us a deeper understanding of the epidemiology of *Cladosporium* on raspberry, and what potential management strategies will be effective for growers to utilise.

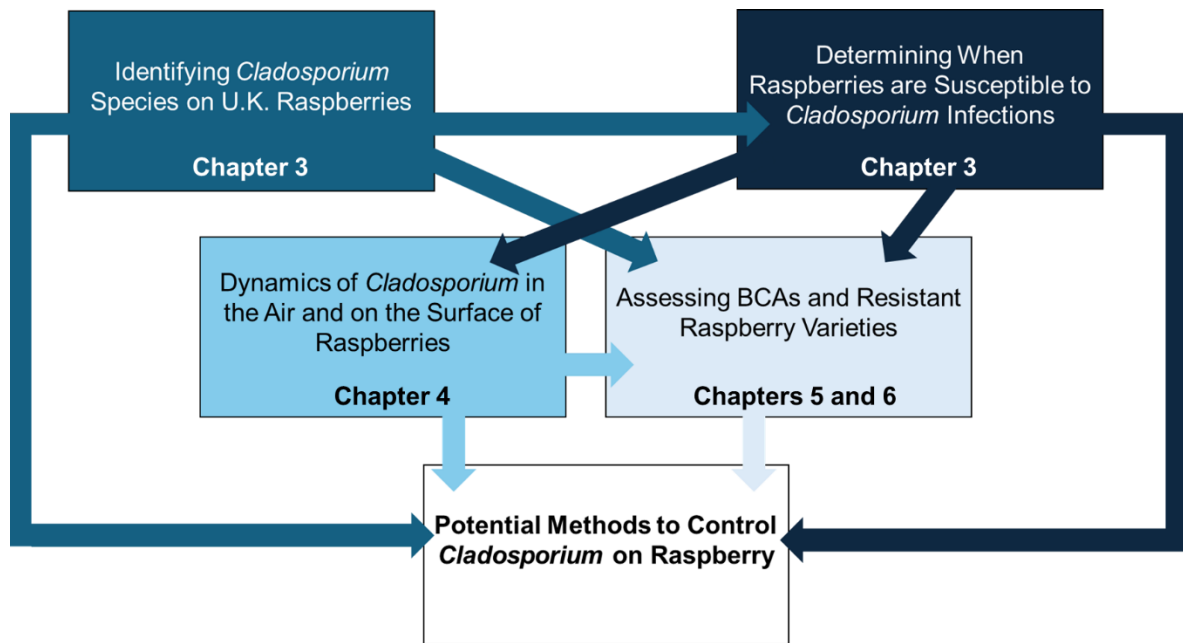


Figure 2-1. A schematical representation of each of the studies conducted to show how they link to inform potential methods to control *Cladosporium* on raspberry.

2.4 List of Submitted Work

- **“*Cladosporium* Species: The Predominant Species Present on Raspberries from the U.K. and Spain and Their Ability to Cause Skin and Stigmata Infections”**

L.H. Farwell, G. Deakin, A.L. Harris, G. Fagg, T. Passey, C. Verheecke-Vaessen, N. Magan & X. Xu.

Horticulturae.

Accepted 13 January 2023. *Horticulturae*, (2023). 9(2), 128; <https://doi.org/10.3390/horticulturae9020128>.

- **“Investigating the Inoculum Dynamics of *Cladosporium* on the Surface of Raspberry Fruits and in the Air”**

L.H. Farwell, M. Papp-Rupar, G. Deakin, N. Magan & X. Xu.

Environmental Microbiology.

Submitted 15 January 2024. In review.

- **“Assessing the Efficacy of Biological Control Agents in Plate Assays and Field Applications to Control *Cladosporium* on Raspberry”**

L.H. Farwell, A.L. Harris, G. Deakin, N. Magan & X. Xu.

In preparation.

3 *Cladosporium* Species: The Predominant Species Present on Raspberries from the U.K. and Spain and Their Ability to Cause Skin and Stigmata Infections

Authors: L.H. Farwell^{1,2}, G. Deakin², A.L. Harris², G. Fagg², T. Passey², C. Verheecke-Vaessen¹, N. Magan¹ and X. Xu².

¹ Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, Cranfield, U.K.

² NIAB—New Road, East Malling, West Malling, ME19 6BJ, U.K.

Published in *Horticulturae*.

Accepted 13 January 2023. *Horticulturae*, (2023). 9(2), 128;
<https://doi.org/10.3390/horticulturae9020128>.

3.1 Abstract

Raspberry production in the U.K. has moved rapidly in the last 10 years to under polythene, combined with a reduced availability of broad-spectrum fungicides. Hence, the incidence of previously less prevalent diseases, such as *Cladosporium*, has largely increased. This study aimed to identify the predominant *Cladosporium* species on raspberry and to understand the nature of its infection on raspberry fruit. Raspberries were collected from farms across the U.K. and Spain and incubated; fungal isolates were then isolated from typical *Cladosporium* lesions and identified to the species level based on the sequences of the trans elongation factor α and actin genes. *Cladosporium cladosporioides* was confirmed as the predominant species responsible for infecting raspberry fruit close to harvest on fruit from the U.K. and Spain, being present on 41.5% of U.K. fruit and 84.6% of Spanish fruit. Raspberries were subsequently inoculated at different developmental stages with *C. cladosporioides* isolates to determine the susceptibility to *Cladosporium* skin lesions and stigmata infections in relation to the developmental stage. Only the ripening and ripe raspberries were susceptible to *Cladosporium*, resulting in skin lesions. *Cladosporium* can colonise the stigmata of raspberries earlier in fruit development and future research is required to determine if such stigmata infections could cause subsequent skin lesion infections. This study has provided the necessary epidemiological information to develop effective management measures against the *Cladosporium* species.

3.2 Introduction

Raspberry (*Rubus ideaus*) is an economically important soft fruit crop in the U.K. worth approximately £128.6 M in 2022 (DEFRA, 2023). mostly grown under protection. Raspberries are delicate fruits susceptible to various fungal diseases, especially grey mould caused by *Botrytis cinerea*. Based on recent epidemiological knowledge of raspberry grey mould on fruits under protection (Xu *et al.*, 2011), a management strategy based on post-harvest cool-chain

management without fungicides was developed and able to control grey mould effectively (O'Neill *et al.*, 2012).

Recently, some U.K. raspberry growers reported significant losses in yield due to fruit rots caused by the *Cladosporium* species. Currently, there is insufficient epidemiological knowledge on raspberry fruit rot caused by the *Cladosporium* species to develop management strategies. One recent study in the U.S.A. examined the relationship between the *Cladosporium* species and *Drosophila suzukii* on raspberries; *C. cladosporioides*, *C. anthropophilum*, and *C. pseudocladosporioides* were found to be the predominant species (Swett *et al.*, 2019). In the U.K., it is unknown which predominant *Cladosporium* species are causing raspberry fruit rot. Outside of the U.K. growing season, raspberries are often sourced from nearby countries such as Spain and Morocco, whereby the different climates and growing conditions may also skew the species of *Cladosporium* present. It is therefore important to identify *Cladosporium* species both from U.K. fruits and imported fruits, such as those from Spain. Previous studies of *Cladosporium* raspberry rot have focused on post-harvest disease development (Dennis, 1975). For example, *Cladosporium* was found to be prevalent at one farm in Kent, U.K., where 55% of raspberries exhibited *Cladosporium* symptoms post-harvest (O'Neill *et al.*, 2012); however, the origin of this high post-harvest incidence of *Cladosporium* was unclear. In the U.S.A., Swett *et al.* (2019) showed that *Cladosporium* was present on fruit pre-harvest and on under-ripe raspberries when harvested and then incubated for 4 days.

If *Cladosporium* can infect raspberry fruits pre-harvest, the level of infection may depend critically on fruit age, as was found for *Botrytis* (Jersch *et al.*, 1989) and powdery mildew (Carisse & Bouchard, 2010) on strawberry. *Cladosporium cladosporioides* was reported to cause blossom blight of strawberries in Korea (Nam *et al.*, 2015) and the U.S.A. (Gubler *et al.*, 1999). There is no knowledge of whether *Cladosporium* species can similarly infect raspberry flower tissues, such as the stigmata, and become established on the fruit surface pre-harvest, resulting in yield losses. There is, therefore, an urgent need to understand the predominant *Cladosporium* species on raspberries and the susceptibility of

raspberry fruit at different developmental stages to infection by *Cladosporium*. This knowledge will facilitate better development of control strategies to minimise the impact of this fungal infection at both the pre- and post-harvest stage.

Thus, the objectives of this study were to (a) determine which were the predominant *Cladosporium* species present on U.K. and Spanish raspberry fruit; (b) investigate fruit susceptibility to *Cladosporium* in relation to fruit developmental stages; and (c) identify whether stigmata are susceptible to *Cladosporium* infection.

3.3 Materials and Methods

3.3.1 Determining *Cladosporium* Species Present on Raspberry Fruit

3.3.1.1 Isolate Collection

Raspberries were sent directly by U.K. growers or purchased from supermarkets in punnets (including fruit originating from Spain). The raspberries were incubated in polythene-bagged trays to maintain high humidity in ambient room conditions for four days to accelerate fungal growth. Fruit with typical *Cladosporium* lesions were dissected and the diseased parts were directly plated onto Potato Dextrose Agar (PDA; Oxoid, Basingstoke, U.K., CM0139) in 9 cm Petri dishes (supplemented with 9.3 g per 100 mL NaCl to inhibit bacterial growth). The fungal outgrowth that resembled the *Cladosporium* species was then sub-cultured onto fresh PDA medium to obtain pure cultures.

3.3.1.2 DNA Extraction, Amplification and Sequencing

DNA from those candidate *Cladosporium* isolates were extracted using a rapid fungal DNA extraction method with Sigma extraction and dilution buffers (Extract-N-Amp™, 2022). An approximately 1 mm × 1 mm piece of mycelium was taken from each isolate and placed in 30 µL of extraction buffer (Sigma-Aldrich, Gillingham, U.K., E7526) and heated to 95 °C for 5 mins. Then, 30 µL of dilution buffer (Sigma-Aldrich, Gillingham, U.K., D5688) was added, followed by 50 µL of sterile water. The final product was stored at –20 °C for subsequent downstream

processes. Once the DNA was extracted, PCR was performed on the trans elongation factor α (TEF1 α) (primer pair: EF1-728F and EF-2R; Table A-1) and actin (ACT) (primer pair: ACT-512F and ACT-783R; Table A-1) regions, as these have been shown to be accurate in identifying *Cladosporium* at the species level (Bensch *et al.*, 2012). The PCR cycle for TEF1 α was set as follows: 95 °C for 8 mins, 40 cycles of 95 °C for 15 s, 55 °C for 20 s, 72 °C for 1 min and, finally, 72 °C for 5 mins. For the ACT gene, it was set as follows: 94 °C for 5 mins, 45 cycles of 94 °C for 45 s, 52 °C for 30 s, 72 °C for 90 s and, finally, 72 °C for 6 mins, as described by Swett *et al.* (2019). The PCR products were run on a 1.5% agarose gel for 45 min at 100 V with a 1 kb pair ladder. If strong bands were seen, the products were diluted 1:10 and then sent to Eurofins (Ebersberg, Germany) for Sanger sequencing. The chromatograms were trimmed to remove poor end regions, and any sequences with signs of contamination were removed. For each isolate, the forward and reverse reads were combined for each gene to create a consensus sequence using Geneious v. 2019.2.1 (Auckland, New Zealand). Individual consensus sequences were then run through the nrBLAST database using the megaBLAST v. 2.12.0+ (Bethesda, MD, U.S.A.) programme for species identification based on the e-value ($< 10^{-80}$) and the percentage identity ($> 95\%$). If multiple species were found with similar probability, the species identity was resolved as ambiguous. In total, 54 isolates were sequenced for both ACT and TEF1 α regions.

3.3.1.3 Phylogenetic Analysis

Sequences from the BLAST searches with high similarity (e-value $< 10^{-80}$, percentage identity $> 95\%$) were added to our query sequences in the phylogenetic analyses as reference sequences (Table A-2). As some of the Centraalbureau voor Schimmelcultures (Westerdijk Institute, Utrecht, The Netherlands) *C. fusiforme* isolates were missing the 3' locus of the TEF1 α gene, they were excluded in the TEF1 α tree. In total, 73 sequences were used for the ACT phylogenetic tree at 215 base pairs long and 71 isolates for the TEF1 α tree at 508 base pairs long, with *Cercospora beticola* (CBS 116456) as the outgroup in both trees. Sequences were aligned separately for the ACT and TEF1 α genes using the online version of MAFFT v. 7 (Osaka, Japan)

(<https://mafft.cbrc.jp/alignment/server/index.html> (accessed on: 27 October 2022); Katok *et al.*, 2019) with the E-INS-I model. Alignments were visually inspected, and sequences were trimmed to remove the primers using the software MEGA v. X (Philadelphia, PA, U.S.A.; Kumar *et al.*, 2018). MEGA was also used to build maximum likelihood trees with 1000 bootstrap replicates for the two gene regions, in which a Kimura 2-parameter evolutionary model (Kimura, 1980) was used for the ACT and TEF1 α regions.

3.3.2 Susceptibility of Fruit to *Cladosporium* Skin Lesions

3.3.2.1 Experimental Design and Treatments

Inoculation experiments were conducted in an experimental raspberry plantation in a polytunnel in late summer 2019 and 2020 at NIAB, East Malling in Kent, U.K., (Longitude: 51.288350, Latitude: 0.454766). Potted plants of one proprietary raspberry variety (one of the most popular commercial varieties grown in the U.K. on which *Cladosporium* was previously reported by U.K. growers) were used and grown with an industry-standard fertigation regime for this specific cultivar. All three *C. cladosporioides* isolates used for inoculations were from U.K. farms and included in the phylogenetic analyses.

Fruits were classified into three developmental stages (green, ripening and ripe berries; Fig. 3-1) at the time of inoculation. Inoculations were conducted three times each year. On each plant, all fruits from one or more randomly selected branches with the widest range of fruit developmental stages were inoculated on each inoculation occasion unless otherwise specified. Any visible rotting fruits were removed prior to inoculation.



Figure 3-1. The three stages (green, ripening and ripe fruit) of raspberry fruit development inoculated in 2020 to investigate skin lesion development and stigmata infections by *Cladosporium*.

In 2019, for each fruit developmental stage, there was only one inoculation treatment without an un-inoculated control; fruits were inoculated with a mixture of three *C. cladosporioides* isolates. Fruits were inoculated on 29 August 2019, 5 September 19 and 12 September 2019. There were 12 pots (each with two canes) that were placed into four rows of three pots. Each row of plants was considered as one block since the three pots were fertigated by the same fertigation line. All 12 plants were inoculated on all three occasions—only a single branch was inoculated in each pot, with a new branch used at each inoculation date. The experimental design is displayed visually in figure 3-2.

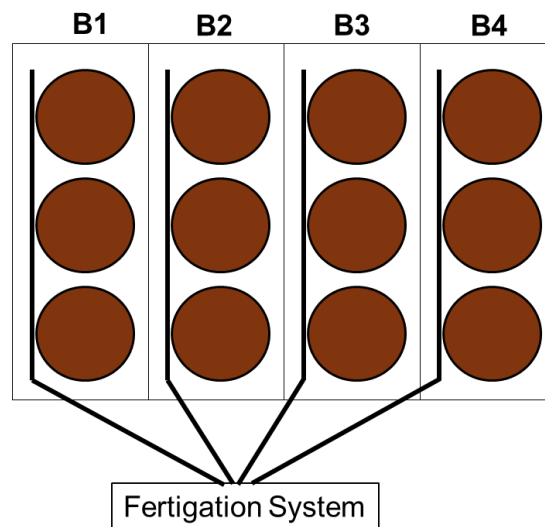


Figure 3-2. A diagram of the experimental design used in 2019 for the skin lesion experiment. Each circle represents one pot containing two canes, which all had the same *C. cladosporioides* inoculation. Rectangles around plots indicate blocks e.g. B1 = Block 1, B2 = Block 2, etc.

In 2020, there were four inoculation treatments: three isolates inoculated separately (but the same isolates used in 2019) and a control. Inoculations were conducted on 9 September 2020, 16 September 2020 and 23 September 2020. There were 32 pots, each with three canes, in two rows. Each row of 16 pots was divided into four blocks, with one block containing four consecutive pots. One of the four pots in each block was randomly assigned to one of the four inoculation treatments. In each pot, 2–3 branches with the widest range of developmental stages were randomly selected for inoculation on each inoculation date. The experimental design is displayed visually in figure 3-3.

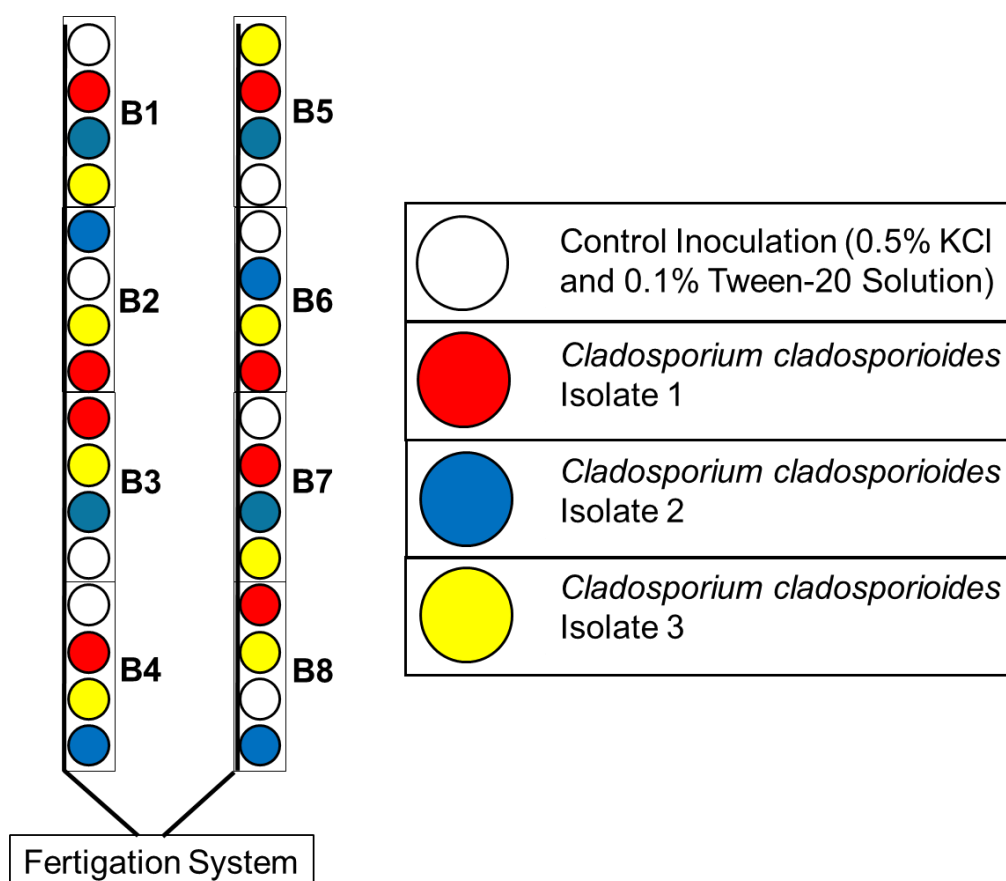


Figure 3-3. A diagram of the experimental design used in 2020 for the skin lesion and stigmata infections experiments. Each circle represents one pot containing three canes, with each pot being designated a different *C. cladosporioides* inoculation treatment. Rectangles around plots indicate blocks e.g. B1 = Block 1, B2 = Block 2, etc.

3.3.2.2 Fruit Inoculation

Cladosporium cladosporioides isolates were grown on PDA (Oxoid, Basingstoke, U.K., CM0139) for 7–10 days. Thirty millilitres of 0.5% KCl sterile water solution with 0.1% Tween-20 was placed onto the colony surface and agitated with a surface-sterilised glass rod, as recommended by Swett *et al.* (2019). The conidial suspension was decanted into a 25 mL universal bottle, and the concentration was determined using a haemocytometer and then adjusted to 10^7 conidia/mL using sterile water. To make the mixed inoculum in 2019, the same volumes of the adjusted conidial suspensions for the three isolates were mixed together. For the control treatment in 2020, 0.5% KCl and 0.1% Tween-20 solution was used

to inoculate fruits. Inoculations were carried out with a handheld sprayer to thoroughly wet individual fruit. Immediately after inoculation, each inoculated branch was covered with a polythene bag and plugged with a damp cotton bung. This was held in place with a cable tie to maintain high humidity inside the bag, which was left in place for 24 hr and then removed. All the inoculated fruit on the branch were then removed and placed in separate trays.

In 2019, immediately after removal, fruits were surface-sterilised by gently washing the fruit in 0.1% Tween-20 for 2 mins, 70% Ethanol for 30 s and, finally, 0.1% bleach for 2 mins, then left in a laminar flow hood for approximately 30 mins to dry, as described by Swett *et al.* (2019), and placed in surface-sterilised trays for incubation at 20 °C in 24 hr of light for four days before assessment.

In 2020, all the inoculated fruit on a single branch were removed into one punnet and then sterilised, as in 2019, but with an additional 2 mins sterile distilled water wash at the end. Fruits were then placed into punnets, covered with a polythene bag to maintain high humidity and incubated at 20 °C in 24 hr of light for four days.

3.3.2.3 Infection Assessment

In 2019, each individual fruit was assessed for its developmental stage and for the presence or absence of visual *Cladosporium* rot after incubation for four days. In 2020, each individual fruit was assessed for its developmental stage and the number of drupelets with visual *Cladosporium* symptoms on the following scale: 0—no drupelets infected, 1—one drupelet infected, 2—two drupelets infected, 3—three drupelets infected, 4—four drupelets infected, and 5—≥ 5 drupelets infected.

3.3.2.4 Statistical Analyses

To estimate the effect of inoculation date, fruit development stage and their interaction on the number (incidence) of *Cladosporium* infections for the 2019 inoculation, a Binomial generalised linear mixed model (GLMM) was fitted to the data using the glmmTMB package (Brooks *et al.*, 2017) in R v. 4.0.3 (Vienna, Austria; R Core Team, 2021). In GLMM, residual errors were assumed to follow

a binomial distribution, assuming no over-dispersion. Row (block), date of inoculation and fruit development stage were added as fixed effect factors to the model. The plant (pot) was added as a random-effect factor to control for plant effects. An ordinal logistic regression model was fit to the 2020 skin lesion infection score data using the R “ordinal” package (Christensen, 2019). Five fixed-effect factors were used: block, inoculation date, *Cladosporium* isolate, *Cladosporium*-inoculated or not and fruit development stage. Fixed effects showed proportional odds, meeting the assumptions of ordinal regression. Green fruit (which all had no infection) were excluded from the analysis due to the complete separation causing converging issues with the model fit. The fitted ordinal model was used to generate estimated incidences of infections (either uninfected [score = 0] or infected [score ≥ 1]). The standard errors were also generated *via* the fitted ordinal model and adjusted by multiplying by the root of the estimated over-dispersion parameter. In all analyses, statistical significance was based on deviance analysis *via* a Chi-square test.

3.3.3 Susceptibility of Stigmata to *Cladosporium* Infection

3.3.3.1 Experimental Design

This experiment focused on stigmata infection in relation to fruit development stage (Fig. 3-1) and surface-sterilisation, which was used to indicate if *Cladosporium* hyphae could penetrate stigmata or only colonise the stigmata surface. The same experimental planting was used as for the 2020 fruit inoculation experiment. Three inoculations were performed on: 9 September 2020, 16 September 2020 and 23 September 2020 (the number of raspberries assessed for each treatment are shown in Table A-3). On each inoculation date, in addition to the fruit developmental stage and the four inoculation treatments (as for the skin lesion experiment), there was an additional treatment factor: with or without post-collection surface-sterilisation before incubation. Individual branches on each pot were randomly assigned to the “sterilised” or “unsterilised” treatment.

3.3.3.2 Fruit Inoculation

The same inoculation methods were used as described for the 2020 skin lesion experiments. All fruit on those selected branches were inoculated and picked 24 hr after inoculation. When appropriate, fruit were surface-sterilised as described for the 2020 skin lesion susceptibility experiment; fruit from one branch were then placed into a single punnet covered with a polythene bag and incubated for a further four days at 20 °C before assessment.

3.3.3.3 Assessment

Individual fruit were scored for the severity of stigmata infections on the following scale: 0—no stigmata infected, 1—1–20% of stigmata infected, 2—21–40% of stigmata infected, 3—41–60% of stigmata infected, 4—61–80% of stigmata infected, and 5—81–100% of stigmata infected.

3.3.3.4 Statistical Analyses

An ordinal logistic regression was used to analyse the data as for the 2020 skin lesion data with one additional fixed-effect factor (sterilisation vs. unsterilised).

3.4 Results

3.4.1 Determination of Predominant *Cladosporium* Species on Raspberry Fruit

In total, molecular analyses of 54 isolates from raspberry fruit were performed, with 41 from the U.K. and 13 from Spain. Molecular analyses showed that in the U.K., the most frequently present *Cladosporium* species was *C. cladosporioides*, present on 41.5% of ripe fruit. The next most common species were *C. sphaerospermum* and *C. europaeum*, present on 14.6% of ripe fruit. On the U.K. fruit, 9.8% of isolates were identified as *C. limoniforme* and 7.3% as *C. ramotellum*. Finally, 12.2% of the isolates from U.K. grown raspberries gave a mixture of species from the BLAST database; however, their identities closely matched *C. fusiforme*. Similarly, the predominant species present on fruit from

Spain was *C. cladosporioides*, present on 84.6% of fruit. The only other species present was *C. sphaerospermum*, present on 15.4% of fruit (Table 3-1).

Table 3-1. The percentage breakdown of *Cladosporium* species present on raspberries collected from the U.K. and Spain. The total number of isolates was 54, with 41 from the U.K. and 13 from Spain.

<i>Cladosporium</i> Species	U.K. Fruit	Spanish Fruit
<i>C. cladosporioides</i>	41.5	84.6
<i>C. fusiforme</i>	12.2	0.0
<i>C. sphaerospermum</i>	14.6	15.4
<i>C. europaeum</i>	14.6	0.0
<i>C. limoniforme</i>	9.8	0.0
<i>C. ramotellum</i>	7.3	0.0

Phylogenetic analysis of both the ACT and TEF1 α region corroborated with the species ID based on the nrBLAST database. Isolates showing close matches with *C. fusiforme* also closely matched a mixture of other *Cladosporium* species (however, with poor query coverage), which were also added to the phylogenetic analysis. These isolates, however, still formed a clade with the *C. fusiforme* reference sequences in the phylogenetic trees.

In both trees (Fig. 3-4 and Fig. 3-5), a mixture of *Cladosporium* isolates were found in individual farms; but for those isolates closely related to *C. fusiforme*, three of the five isolates originated from one farm in Kent.

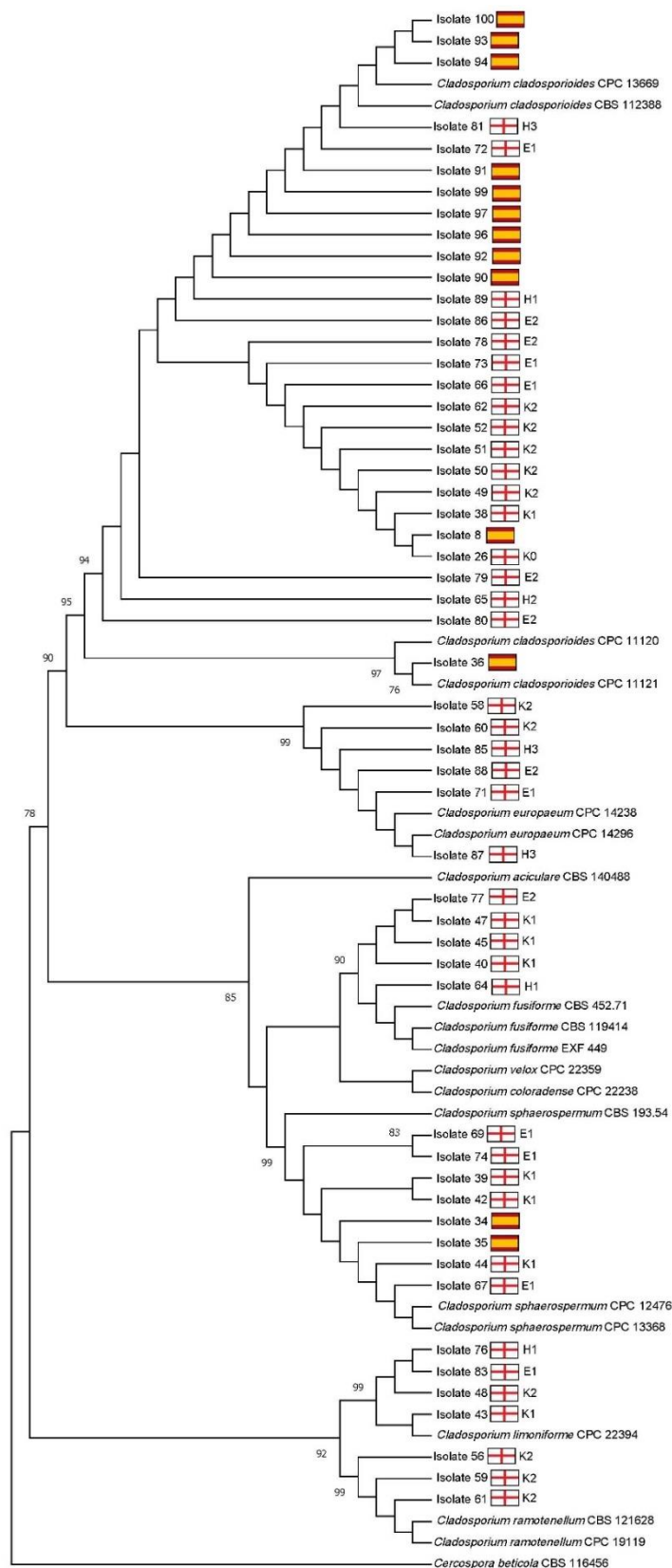


Figure 3-4. A phylogenetic tree of the ACT gene for 72 isolates of *Cladosporium*. The tree was created using maximum likelihood with a Kimura 2-parameter evolutionary model and 1000 bootstrap replications. The numbers above branches represent bootstrap values. The flags represent the country of origin, the codes represent areas within the country and numbers denote different farms. Area codes are as follows: E = Essex, K = Kent, H = Herefordshire.

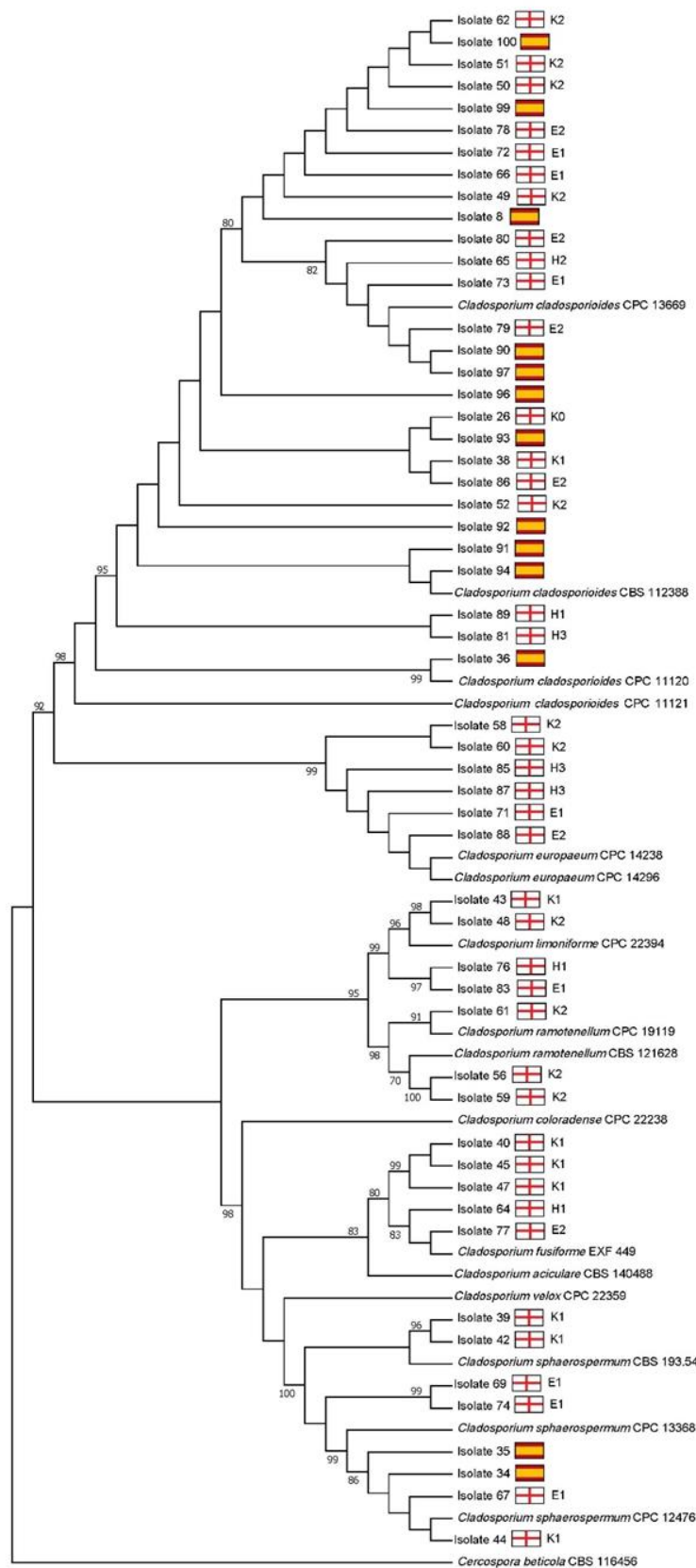


Figure 3-5. A phylogenetic tree of the TEF1 α gene for 70 isolates of *Cladosporium*. The tree was created using maximum likelihood with a Kimura 2-parameter evolutionary model and 1000 bootstrap replications. The numbers above branches represent bootstrap values. The flags represent the country of origin, the codes represent areas within the country and numbers denote different farms. Area codes are as follows: E = Essex, K = Kent, H = Herefordshire.

3.4.2 Fruit Susceptibility to *Cladosporium* Skin Lesions

In 2019, *Cladosporium* skin lesions were only present on fruit at the ripening and ripe stage. The overall incidence of fruit with *Cladosporium* skin lesions were 13.2% (s.e. $\pm 8.3\%$) on ripening fruit and 26.5% (s.e. $\pm 11.5\%$) on ripe fruit. There was no significant difference in the presence of *Cladosporium* on ripening and ripe fruit ($X^2(1, n = 36) = 1.06, p = 0.303$).

In 2020, *Cladosporium* skin lesions were present only on fruit at the ripening and ripe stages, with the overall incidence being 50.2% (s.e. $\pm 12.1\%$) of ripe fruit with skin lesions and 17.4% (s.e. $\pm 9.0\%$) of ripening fruit with skin lesions. Ripe fruit were more susceptible to *Cladosporium* than ripening fruit: the odds of fruit with *Cladosporium* skin lesions at a given severity score (odds = infected/healthy) increased to 5.14 (s.e. ± 1.3) ($p < 0.001$) times when fruit moved from the ripening to ripe stage. Inoculation led to more fruit with skin lesions with an overall incidence of *Cladosporium* of 36% (s.e. $\pm 9.9\%$) in inoculated and 20.4% (s.e. $\pm 13.2\%$) in the control ($p < 0.05$). There was also a significant difference in *Cladosporium* skin lesion scores amongst the three isolates ($p < 0.05$): isolate three caused significantly higher skin lesion severity scores when compared to isolate two.

3.4.3 Infection of Stigmata by *Cladosporium*

Stigmata infections occurred across all fruit developmental stages irrespective of surface-sterilisation (Fig. 3-6). There were significant differences in the severity scores between inoculation dates ($p < 0.001$), inoculated or uninoculated ($p < 0.001$), three *Cladosporium* isolates ($p < 0.001$) and surface-sterilisation ($p < 0.001$) on the severity of stigmata infections. There were no significant differences in stigmata infection scores across developmental stages ($p = 0.166$).

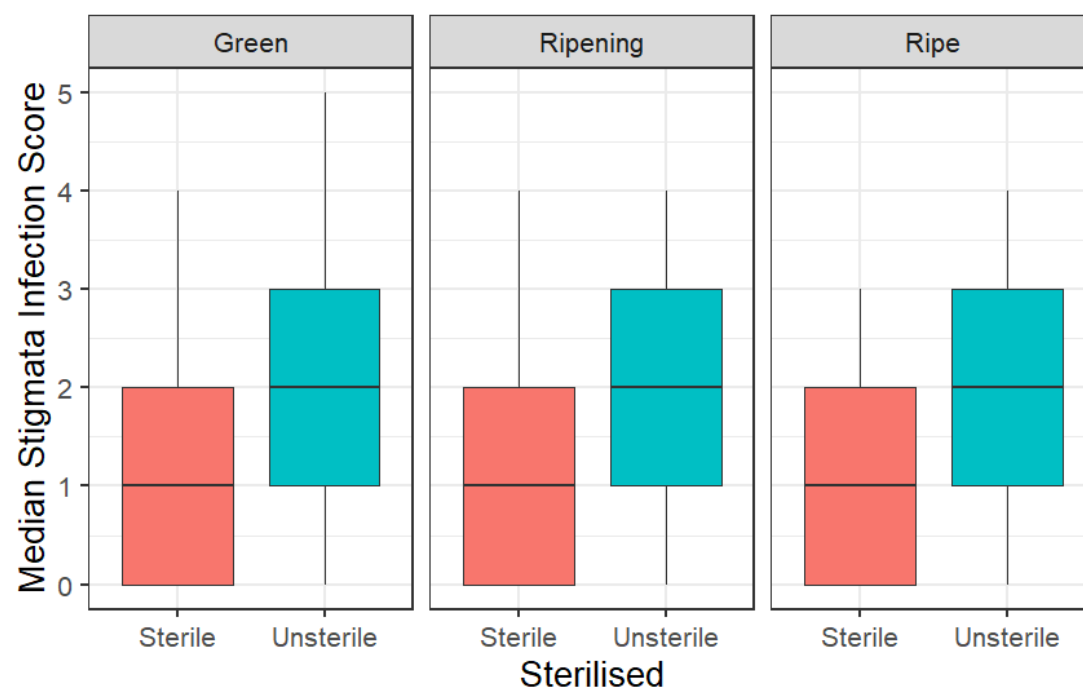


Figure 3-6. A boxplot of stigmata infection scores across the three developmental stages and between sterile vs. unsterile raspberry fruits; the black horizontal line is the median score.

There were higher incidences of *Cladosporium* on stigmata across all the developmental stages in the unsterilised treatments compared to the sterilised treatments (Table 3-2).

Table 3-2. The overall incidence (% fruit with *Cladosporium* present $\pm$ S.E.) of *Cladosporium* on stigmata across four raspberry developmental stages and between sterilisation treatments. S.E.: standard error of the means.

Sterilisation Treatment	Green	Ripening	Ripe
Sterilised	66.4 $\pm$ 6.9	74.5 $\pm$ 12.3	69.9 $\pm$ 16.5
Unsterilised	88.3 $\pm$ 6.8	87.0 $\pm$ 11.1	90.6 $\pm$ 8.6

The incidences of *Cladosporium* infections on stigmata were also higher across all developmental stages inoculated with isolate three vs. isolate one and two (Table 3-3).

Table 3-3. The overall incidence (% fruit with *Cladosporium* present $\pm$ S.E.) of *Cladosporium* on stigmata across raspberry developmental stages and between inoculums. S.E.: standard error of the means. Letters signify significant differences in overall incidence between isolates. Comparisons were not made between fruit stages.

Inoculum	Green	Ripening	Ripe
Control	65.7 $\pm$ 10.5 ^a	68.7 $\pm$ 14.3 ^a	70.1 $\pm$ 14.7 ^a
Isolate 1	79.1 $\pm$ 9.1 ^b	81.2 $\pm$ 10.7 ^b	82.2 $\pm$ 10.8 ^b
Isolate 2	81.0 $\pm$ 8.7 ^b	82.9 $\pm$ 10.1 ^b	83.9 $\pm$ 10.7 ^b
Isolate 3	87.9 $\pm$ 6.4 ^c	89.3 $\pm$ 7.3 ^c	89.9 $\pm$ 7.2 ^c

3.5 Discussion

This study has shown that *C. cladosporioides* is the predominant species infecting raspberry fruit in the U.K. and Spain, consistent with findings in the U.S.A. (Swett *et al.*, 2019). Several other *Cladosporium* species were also found on ripe raspberries, including *C. sphaerospermum*, *C. europaeum*, *C. limoniforme*, *C. ramotellum* and *C. fusiforme*. Of particular interest is the first isolation of *C. fusiforme* from plant material, suggesting the possibility of its presence on other crops. Three of the five isolates of this species were detected at one farm in Kent, indicating that this farm may provide an ecological niche favourable to this species. Some of these *Cladosporium* species were tested for their pathogenicity on other important horticultural crops, such as *C. limoniforme* on strawberries (Ayoubi *et al.*, 2017) and *C. cladosporioides*, *C. limoniforme* and *C. ramotellum* on pears and apples (Temperini *et al.*, 2018).

As *C. cladosporioides* was the most prevalent species found on raspberries, a large proportion of the primary inoculum may arise from airborne spores, as well as from crop debris (Ellis, 1971), as this species comprises a large proportion of the airborne fungal load (Harvey, 1967). Previous studies investigating the patterns of *Cladosporium* airborne spores have focused on indoor and outdoor environments (Pasanen *et al.*, 1992; Anees-Hill *et al.*, 2022). It may, therefore, be necessary to investigate how agricultural environments, e.g., inside polytunnels, impact the inoculum load and, hence, disease risk. There also appear to be differences among the three *C. cladosporioides* isolates in causing skin lesions and stigmata infections. Thus, in addition to inter-species differences in pathogenicity, further work is also needed to assess the intra-species variability in pathogenicity on raspberry fruit.

Present results indicate that ripe raspberries are most susceptible to *Cladosporium* skin lesions, followed by ripening fruit. In contrast, green fruit are not susceptible to *Cladosporium* skin lesions. Similarly, Swett *et al.* (2019) showed that ripe raspberries are most susceptible to *Cladosporium* infection. Due to ripe fruit being delicate and susceptible to attacks by pests such as *Drosophila suzukii*, wounds may provide entry points for *Cladosporium* species, as found in the U.S.A. (Swett *et al.*, 2019). In addition, manual fruit harvesting may cause small abrasions to the surface, allowing access for fungi such as *Cladosporium* to infect and cause visible symptoms post-harvest.

Fruit from the green developmental stage onwards appear susceptible to *Cladosporium* stigmata infections. The severity of stigmata infections decreased when the fruit were surface-sterilised, indicating a significant proportion of *C. cladosporioides* inoculum only colonised the stigmata surface. Thus, the stigmata of raspberries are susceptible to *C. cladosporioides* and this species grows more readily as a saprophyte on the stigmata surface. The present results are consistent with a previous finding that *Cladosporium* rarely invaded the internal tissues of the grape fruiting structure (Briceño & Latorre, 2008). *Cladosporium cladosporioides* can colonise or infect raspberry stigmata early in fruit development. However, further research is needed to study the dynamics of

Cladosporium spore survival on the fruit surface. It is unknown if *Cladosporium* spores could survive long enough to cause secondary infections from the stigmata onto the skin's surface. Similar secondary infections were observed with *Botrytis cinerea* on raspberries (Kozhar & Peever, 2018). *Cladosporium cladosporioides* was demonstrated to infect strawberry flowers (Nam *et al.*, 2015); therefore, similar investigations into the susceptibility of raspberry flowers to *Cladosporium* infections are needed.

Overall, it may be more economical to focus on managing *Cladosporium* infection when fruit are most susceptible to skin lesions, especially at the ripening stages of development. Improved husbandry and hygiene by removing decaying material may aid in reducing the inoculum load to prevent both skin and stigmata infections. The use of chemical fungicides to manage *Cladosporium* is limited unless there are effective products with a short half-life. As *Cladosporium* mainly grows on the stigmata surface, biological control agents may be able to compete effectively with *Cladosporium*, leading to much reduced incidences of raspberries with *Cladosporium* infections. The use of biocontrol agents against *Cladosporium* species was demonstrated in multiple studies. Examples include multiple species of *Trichoderma* inhibiting the growth of *C. herbarum* (Barbosa *et al.*, 2001) and *Chaetomium globosum* inhibiting the growth of multiple isolates of *C. cladosporioides* (El-Dawy *et al.*, 2021) during *in vitro* plate assays. Further research is needed to demonstrate the efficacy of such biological control agents in field conditions against the *Cladosporium* species.

Overall, the data obtained in this study will be beneficial in understanding the predominant *Cladosporium* species impacting raspberries and the growth stages of the ripening fruit most susceptible to *Cladosporium* infections. This will benefit agronomists and growers in more accurately assessing *Cladosporium* rot symptoms and identifying the most appropriate, and timing of, the application of control measures.

4 Investigating the Inoculum Dynamics of *Cladosporium* on the Surface of Raspberry Fruits and in the Air

Authors: L.H. Farwell^{1,2}, M. Papp-Rupar², G. Deakin², N. Magan¹ and X. Xu².

¹ Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, Cranfield, U.K.

² NIAB—New Road, East Malling, West Malling, ME19 6BJ, U.K.

Submitted to *Environmental Microbiology* (in review).

4.1 Abstract

Raspberry production is under threat from the emerging fungal pathogenic genus *Cladosporium*. We used amplicon-sequencing, coupled with qPCR, to investigate how fruit age, fruit location within a polytunnel, polytunnel location and sampling date affected the fruit epiphytic microbiome. Fruit age was the most important factor impacting the fungal microbiome, followed by sampling date and polytunnel location. In contrast, polytunnel location and fruit age were important factors impacting the bacterial microbiome composition, followed by the sampling date. The within-tunnel location had a small significant effect on the fungal microbiome, and no effect on the bacterial microbiome. As fruit ripened, fungal diversity increased and the bacterial diversity decreased. *Cladosporium* was the most abundant fungus of the fruit epiphytic microbiome, accounting for nearly 44% of all fungal sequences. Rotorod air samplers were used to study how the concentration of airborne *Cladosporium* inoculum (quantified by qPCR) varied between location (inside and outside the polytunnel) and time (daytime vs. nighttime). Quantified *Cladosporium* DNA was significantly higher during the day than the night and inside the polytunnel than the outside. This study demonstrated the dynamic nature of epiphytic raspberry fruit microbiomes and daily airborne *Cladosporium* inoculum within polytunnels, which will impact disease risks on raspberry fruit.

4.2 Introduction

Raspberries (*Rubus idaeus*) contribute significantly to the U.K. economy, worth £128.6 M in 2022 (DEFRA, 2023), but are vulnerable to fungal pathogens such as *Botrytis*, *Rhizopus*, *Mucor*, *Cladosporium*, and bacterial pathogens such as *Erwinia* and *Agrobacterium* (Williamson *et al.*, 1991). In recent years, U.K. raspberry production has shifted from open soil to coir-based substrate cultivation under polytunnels. However, there is a lack of knowledge on how the change in production systems may impact disease dynamics. *Cladosporium* is an opportunistic pathogen, and has been reported as a problem for U.K. raspberry growers in recent years (Farwell *et al.*, 2023). It is suspected to occur more

frequently in warm and humid environments (O'Neill *et al.*, 2012), such as those conditions within polythene tunnels. Furthermore, *Cladosporium* is one of the most abundant genera in airborne spore samples (Damialis *et al.*, 2017; Harvey, 1967) and is suspected to be an important primary inoculum source for colonising raspberries. *Cladosporium* is also frequently reported as being prevalent in plant tissues as an endophyte (Franco Ortega *et al.*, 2020; Wicaksono *et al.*, 2023), with the relative abundance being 37.8% in one study in homogenised strawberries (Abdelfattah *et al.*, 2016). It is currently unknown, however, how dominant *Cladosporium* is within the epiphytic microbiome of fruit, where disease development likely begins for skin lesions.

Pathogenic fungi and bacteria have various mechanisms for dispersal, including aerial dispersal and insect vectors (West, 2014). There is a knowledge gap regarding the impact of horticultural environments, such as polytunnels, on airborne inoculum dispersal. Air movement was shown to be an important factor in dispersing *Botrytis* spores in glasshouses (Boulard *et al.*, 2010). The spread of tomato powdery mildew (*Erysiphe neolycopersici*) in glasshouses may be facilitated by air movement due to workers moving down alleyways (Sokolidi *et al.*, 2023). The concentration of plant material and warmer conditions may provide an environment more conducive to *Cladosporium* survival and sporulation inside polytunnels. Hence, understanding if these environmental factors impact the number and dynamics of airborne inoculum is vital for assessing the disease risk under polythene. A method frequently used to assess the airborne inoculum is the use of qPCRs (Clark *et al.*, 2023), which can provide estimates of spore concentrations relatively quickly. The aerosol microbiome is known to have a complex relationship with temperature, relative humidity and rainfall (de Groot *et al.*, 2021; Gusareva *et al.*, 2020). Variation in airborne spores likely impacts the phyllosphere microbiome, as we would expect microbes present in the air to land on and colonise exposed plant tissues, potentially affecting disease risk. Variations in climatic conditions can occur on a local scale; for instance, temperature and humidity vary considerably within a polytunnel (Hall *et al.*, 2020). The effect of polytunnels on both the inoculum load and the composition of

resident microbial communities need to be considered when developing and implementing disease management strategies for raspberry.

Survival and hence infectivity of plant pathogens, such as *Cladosporium*, may be greatly affected by the resident microbiome on plant tissue surfaces. As ripe fruits have a longer period of exposure to the environment and airborne microbes than immature fruits, they may have more complex communities of microorganisms. Fruit volatiles also change during ripening (Robertson *et al.*, 1995), with microbes on the fruit surface contributing towards this complex cocktail of volatiles (Sangiorgio *et al.*, 2022). Such plant-emitted volatiles can inhibit/induce recruitment, growth and survival of specific microbes, with some microbes adapted to exploit these plant defence volatiles for resource acquisition (Hammerbacher *et al.*, 2019). To fully understand the ecology of pathogens on the fruit surface, we need to understand how they interact with the resident microbes within a given environmental niche.

There are conflicting findings on the compositions of microbiomes on the surface of ripening fruit. For instance, one study found a higher bacterial microbial diversity on ripe strawberries, but no difference in the fungal diversity (Olimi *et al.*, 2022); no difference was found in the fungal microbiome composition between immature and ripe strawberries. However, another study found a significant difference in the fungal community between immature and mature strawberries (Abdelfattah *et al.*, 2016). Raspberry epicarp fruit microbiome compositions were observed to significantly vary across fruit ripening stages; a higher fungal diversity was found at the ripening stage of fruit development than on green and ripe fruit. However, as each raspberry development stage was sampled across different dates, the varying environmental conditions may have biased their observations (Jones *et al.*, 2022). In soft fruits, particularly raspberries, fruit firmness decreases during ripening (Giongo *et al.*, 2019), making fruit vulnerable to tissue damage. The Dipteran pest, *Drosophila suzukii*, also prefers ripe fruit due to a higher visual contrast with the surrounding foliage (Little *et al.*, 2017), leading to higher oviposition damage on ripening fruit. This damage may release sugars and

nutrients, affecting epiphytic communities and creating entry points for pathogens.

This study aims to 1) assess the extent of influence fruit age, location within the polytunnel, polytunnel location, and sampling times have on the microbial communities on the raspberry fruit surface using amplicon sequencing; and 2) investigate the diurnal pattern and within-day variations in the level of airborne *Cladosporium* spores inside and outside a polytunnel using qPCR.

4.3 Materials and Methods

4.3.1 Raspberry Fruit Epiphytic Microbiome

4.3.1.1 Experimental Design, Sampling and Fruit Washing

In the first experiment, the effect of four factors on the raspberry epiphytic microbiome were investigated: fruit age (green fruit, ripening fruit that were turning pink, and ripe fruit; Fig. 3-1), location within a polytunnel (the centre and the outer edge), polytunnel locations on a farm (four polytunnels at one farm) and sampling date (2/10/2021 and 9/10/2021). Raspberry fruits were collected from a commercial raspberry farm in Kent, England at the end of the growing season (October). Sampled polytunnels were separated by three buffer polytunnels. The same four polytunnels were sampled on each of the two sampling dates.

Within each tunnel, four rows of raspberries were grown, with fruit samples taken from an approximately 10 m long area in the central two rows at (a) the outer edge (both tunnel ends, ca. first 10 m) and (b) the middle of the tunnel (ca. 50 m from the tunnel entrance). For each outer edge sample, 20 fruits were collected for each of the three maturity stages; fruits from the two outer edges were then pooled into one sample. Similarly, 40 fruits were collected for each maturity stage from the central location.

In total, 48 samples were collected (two sampling dates x four polytunnels x two within-tunnel locations x three fruit ages). Fruits were placed into bleach-sterilised pipette boxes by snipping the tip of the petiole near the fruit with a sterilised pair

of scissors to avoid cross-contamination. Collected samples were immediately placed into polystyrene boxes containing ice packs and taken to the lab for processing.

Each sample of 40 fruits were transferred into individual Ziplock bags with 100 mL of Maximum Recovery Diluent (07233, Sigma-Aldrich), sealed, surrounded horizontally by ice, and placed on a platform shaker set to 120 RPM for 1 hr. All six samples from one tunnel collected on the same day were shaken concurrently. The bags were rotated 180° after 30 mins of shaking. After shaking, the wash liquid from each sample was filtered from the Ziploc bags through sterile muslin cloth into two sterile 50 mL falcon tubes to remove plant material and centrifuged at 3894 x g for 10 mins to produce a pellet. Most of the supernatant was pipetted off (leaving approximately 0.5 mL), then the pellet was resuspended in the remaining liquid and transferred to a 2 mL Eppendorf tube. The Eppendorfs were then spun at 16602 x g to produce the final pellet. Finally, the supernatant was removed, and samples were stored at -80 °C until further processing.

4.3.1.1 DNA Extraction, qPCR Estimation of Microbial Communities and Amplicon Sequencing

DNA was extracted from the pellets using the TRI reagent protocol (93289, Sigma-Aldrich; TRI Reagent® Protocol, 2023) with an additional 75% ethanol precipitation step after the trisodium-citrate washes to help remove salt contaminants and increasing centrifugation speed to 16000 x g. This method was used to maximise DNA yield as preliminary testing of DNA extraction kits containing spin columns gave very low DNA yields (data not shown). Samples were sent on dry ice to Novogene, U.K. (Cambridge, U.K.) for library prep and 16S and ITS amplicon sequencing (primers detailed in Table A-4) on the Illumina NovaSeq 6000 platform (San Diego, U.S.A.) to produce 250 nucleotide-paired reads.

The community size of bacteria and fungi in each sample was estimated with qPCR. The most abundant Amplicon Sequence Variants (ASVs) from the ITS and 16S sequences (see the bioinformatics section below) were synthesised into a synthetic gBlock™ gene (Integrated DNA Technologies INC.; Table A-5), which

were diluted and used as standard curves (in a 10-fold series dilution from 10^{-5} to 10^{-8}), and stored at -20°C . Samples were then run on 96 or 384 well plates with the standards, a non-template control and two dilutions of each sample (20 x and 80 x for ITS, 10 x and 40 x for 16S) with triplicates for each dilution. Each reaction was 10 μL total using SsoAdvanced Universal SYBR Green Supermix (1725270, Bio-Rad) with 2 μL of template DNA. The same primers were used as in the amplicon sequencing (Table A-4), with the 16S V3-V4 primers totalling a 200 nM concentration and the ITS1F primers totalling a 100 nM concentration. The same thermocycler setting was used as in Papp-Rupar *et al.* (2022) for both ITS1F and 16S V3-V4: denaturation for 5 mins at 94°C and 40 cycles of 60 s at 94°C , 10 s at 75°C , 30 s at 55°C , and 60 s at 72°C , and ending with a melt curve from 55 to 95°C .

Sample efficiencies were corrected using the same technique as Papp-Rupar *et al.* (2022); as the gBlocks™ were supplied with a known quantity of DNA and molecular weight, the copy number present in the reconstituted synthetic genes were calculated to estimate the efficiencies. Samples with efficiencies below 0.7 and above 1.3 were repeated with lower dilutions to reduce the effect of PCR inhibitors present in samples.

4.3.1.2 Bioinformatics and Statistical Data Analyses

Paired-end amplicon sequence data from Novogene were used to generate Amplicon Sequence Variants (ASVs) and counts tables. Primer sequences were removed from both filtered and unfiltered reads. Any read pairs with lengths less than 250 nucleotides in either of the reads, incorrect primer sequences, or adapters or primers within the sequence were removed. The remaining read pairs were then processed to produce reads for: (1) ASV creation; and (2) frequency table creation. For generating ASVs, read pairs were merged with a maximum of five differences in the overlapping region for 16S and zero for ITS, and a minimum merged length of 400 (16S) and 185 (ITS). Reads were filtered for quality with a maximum expected error rate (Edgar & Flyvbjerg, 2015) of 0.5 (16S) and 0.1 (ITS). The UPARSE pipeline (v. 11.0; Edgar, 2013) was then used on the filtered reads to produce ASVs; the pipeline automatically identifies and removes any

chimeral sequences. Taxonomic ranks were assigned to ASVs using the SINTAX algorithm (https://www.drive5.com/usearch/manual/sintax_algo.html), with bacterial sequences using the RDP Training Set 18 bacterial database (Cole *et al.*, 2014) and fungal sequences using the Unite v. 8.3 database (Kõljalg *et al.*, 2013). ASVs identified as originating from mitochondria or chloroplasts were removed before statistical analyses were performed.

In the bacterial samples, 12 samples were redacted (all samples from polytunnels 3 and 4 on 2/09/21) from the analysis due to an experimental error. The bacterial and fungal abundances were normalised by the absolute (16S/ITS) biomass in each sample using the qPCR copy number. An ANOVA was performed to assess if fruit age, within-tunnel location, polytunnels and sampling date affected the size of fungal and bacterial communities. The alpha diversity indices Chao1, Simpson, Shannon and Observed were calculated using the R package *vegan* v. 2.3-1 (Dixon, 2003). A permutation-based ANOVA was then performed on the rank of the diversity indices to determine the effects of sampling date, polytunnel, location within the tunnel and fruit age. The beta diversities were calculated as Bray-Curtis indices, then subjected to non-metric multidimensional scaling (NMDS) analysis with the R package *vegan*. Then, an ADONIS (a permutation MANOVA using F-tests based on the sequential sum of squares) was used to assess the importance of individual factors and their interactions in affecting Bray-Curtis indices. Principal Components Analysis (PCA) was also carried out to generate the first four Principal Component (PC) scores, which were then subjected to ANOVA to determine the effects of individual factors and their interactions. A differential abundance analysis was used to assess the effects of within-tunnel location, fruit age and sampling date on the abundance of individual ASVs using the R package DESeq2 v. 1.34.0 (Love *et al.*, 2014). In the differential analysis, we did not consider the three-way interactions between the three factors. *P*-values were adjusted using the Benjamini–Hochberg (BH) method (Benjamini & Hochberg, 1995) with the significance set at $p = 0.05$.

4.3.2 Fungal DNA and *Cladosporium* Spores Across Time Points and in Polytunnels vs. Outdoors

4.3.2.1 Experimental Design and Sample Collection

The effects of the time of day and the rotorod sampler location (inside a polytunnel and in a neighbouring open field) on the concentration of *Cladosporium* airborne spores were investigated. Air samples were collected between 10/10/2022 and 31/10/2022 from the centre of a raspberry polytunnel at NIAB East Malling in Kent, England, and 100 m away from the tunnel in an open field. Samples were taken at the end of the growing season.

Rotorod air samplers (Lacey & West, 2006; Fig. 4-1) were attached to a supported pole with rain covers attached at a height of 1.4 m, one inside the centremost pole of the polytunnel and one in the open field 100 m away. The rotorod samplers were wired to an automatic timer system attached to a 12 V battery that was swapped for a freshly charged battery every 24 hours to ensure the rotorod samplers maintained maximum speed.

The air was sampled from 10/10/2022 to 21/10/2022 in four periods: 08:00-12:00 (morning), 12:00-16:00 (afternoon), 16:00-20:00 (evening), and 20:00-08:00 (nighttime), resulting in 83 samples (11 days x four time periods x two locations; excluding five samples where the rotorod did not spin for the full sampling period). From 21/10/2022 to 31/10/2022, the air was sampled for two time periods: 08:00-20:00 (daytime) and 20:00-08:00 (nighttime), resulting in 40 samples (ten days x two time periods [daytime and nighttime] x two locations).

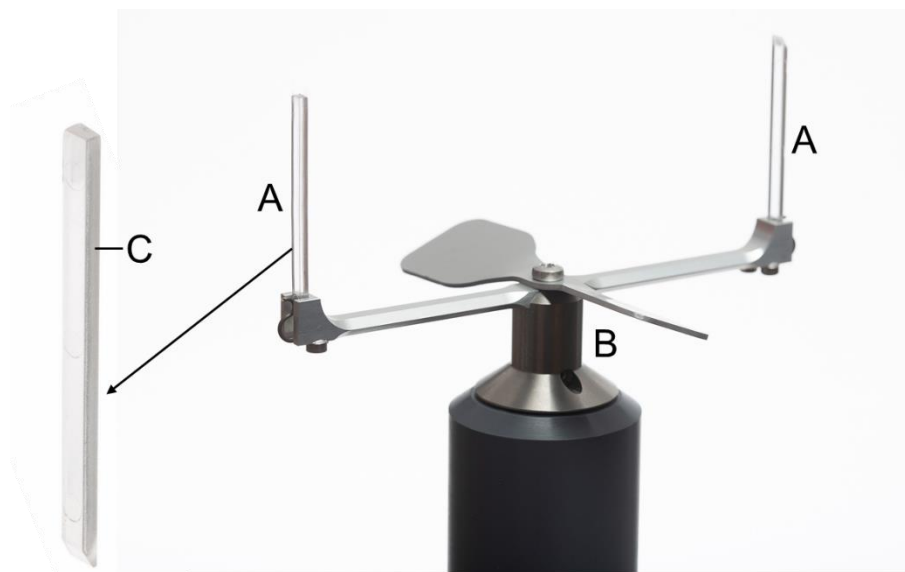


Figure 4-1. A diagram of the rotorod sampler, including A) two rotorod collecting arms, B) the motor that rotates the arms and C) a close up of the removable rotorod arms and the collecting edge where Vaseline is applied.

Before each sampling period, two plastic rotorod arms (AS-10-04, Agri Samplers Ltd.) were placed into the rotorod arm holders (Fig. 4-1). The collecting edge was then coated with a thin film of Vaseline using a finger in a sterilised glove. The rotorod was then ran for the allotted time of collection, and the rotorod arms were removed at the end of the designated sampling period with a pair of sterile tweezers. Both arms were placed into a single 2 mL screwcap tube and taken immediately to a -80 °C freezer for storage.

4.3.2.2 DNA Extraction and Airborne Spore Estimation *via* qPCR

The MasterPure Yeast DNA Purification Kit (LGC Biosearch Technologies) was used to extract DNA from the rotorod samples, where the Yeast Cell Lysis Solution was added to the screwcap tube containing the rotorod arms. We used the same method as Fraaije *et al.* (2021), where 0.5 g of glass beads (0.425 - 0.600 mm) were added, and the tubes vortexed at maximum speed for 2 mins before the MCP protein precipitation reagent was added. A standard curve was created using an isolate of *C. cladosporioides* (isolate #65 in Farwell *et al.*, 2023);

to generate the standard curve, DNA was extracted from 1 mL of a 5.7×10^7 spores/mL suspension of this specific strain and diluted in a 10-fold series dilution. This curve was used to estimate ITS copy number and *Cladosporium* spore count in each sample.

Samples were then diluted 10 x for the daytime-nighttime experiment and 5 x for the four-hour and nighttime experiment and ran with the same ITS1F primers and qPCR setup as detailed in section 4.3.1.2. to estimate the overall fungal DNA present (Table A-4). A pair of *Cladosporium* genus-specific primers (Zeng *et al.*, 2006; Table A-4) at a concentration of 400 nM were used to estimate the amount of *Cladosporium* spores in the samples. Thermocycler settings followed Zeng *et al.* (2006): denaturation set to 3 mins at 95 °C, 40 cycles of 95 °C for 10 s and 68 °C for 30 s, followed by a melt curve from 55 to 95 °C.

4.3.2.3 Statistical Analyses

To increase the number of samples in the daytime vs. nighttime experiment, the estimated ITS copy number and *Cladosporium* spore counts from the four-hour periods collected from 10/10/2022 to 21/10/2022 were summed, and the 12-hour nighttime estimates were included. Analyses were performed in R with the package glmmTMB v. 1.1.8 (Brooks *et al.*, 2017). A Generalised Linear Mixed Model (GLMM) was fitted to the data to account for correlations between samples taken over time, with residuals assumed to follow a negative binomial distribution to account for overdispersion in the dataset. The model included the fixed effects of rotorod sampler location and the time of day. The day of sampling was treated as a random effect to account for temporal correlation. The significance of fixed effects was tested using a type 2 ANOVA. Post-hoc z tests were then performed with the R package emmeans v. 1.8.7. (Lenth, 2023) with *p*-values corrected by the Tukey tests for multiple comparisons. For the daytime four-hour samples, all data were expressed at the number of estimated spores per hour.

4.4 Results

4.4.1 Raspberry Fruit Epiphytic Microbiome

4.4.1.1 Microbial Abundance (qPCR), Overall Sequencing and ASV Generation

The abundance of epiphytic fungal microbiome (total fungal ITS copy numbers per 40 fruit) was significantly affected by sampling date ($F(1) = 8.46$, $p < 0.01$; Fig. 4-2).

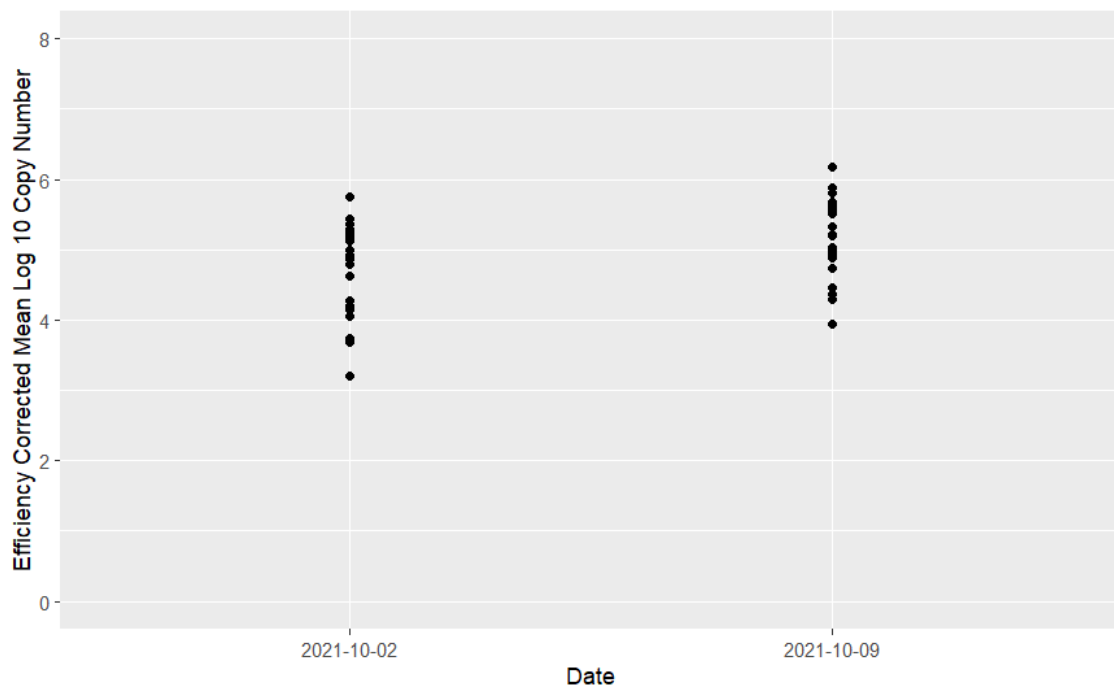


Figure 4-2. The efficiency corrected mean Log_{10} copy number quantitative PCR results for the ITS of fungal epiphytes on the surface of raspberries across the two sampling dates.

For the epiphytic bacterial microbiome abundance (total 16S copy numbers per 40 fruit) was significantly affected by fruit age ($F(2) = 3.45$, $p < 0.05$) and dates ($F(1) = 5.69$, $p < 0.05$; Fig. 4-3).

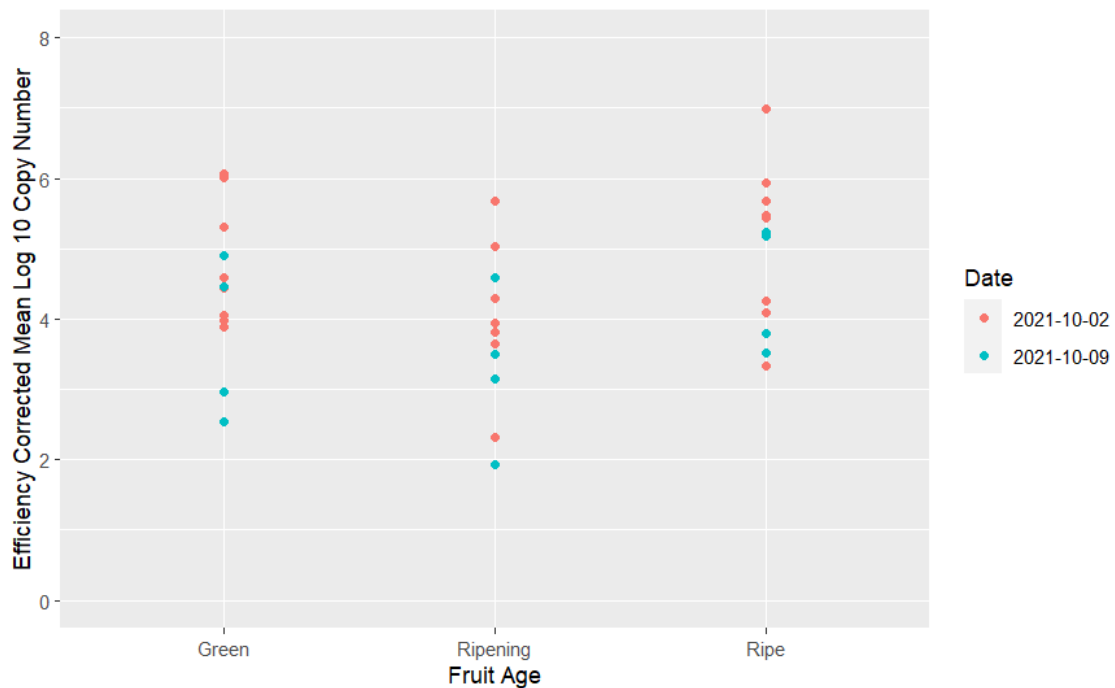


Figure 4-3. The efficiency corrected mean Log₁₀ copy number quantitative PCR results for the 16S of bacterial epiphytes on the surface of raspberries across fruit ages and two sampling dates.

For fungi, the number of raw reads per sample ranged from 47360 to 112216 (average reads = 88089.6). Out of the total 227 fungal ASVs, the percentage that could be identified to the phylum, class, order, family, genus and species level with at least 80% confidence was 94.2%, 52.4%, 52.4%, 52.3%, 51.5% and 2.3%, respectively. The dominating phylum is ascomycetes across all fruit stages and within-tunnel locations (Fig. 4-4A). The number of normalised reads per ASV ranged from 55.4 to 1411738, with an average of 18903.5. The genus with the greatest abundance in the top 10 fungal ASVs was *Cladosporium*, accounting for 43.9% of the total number of fungal reads.

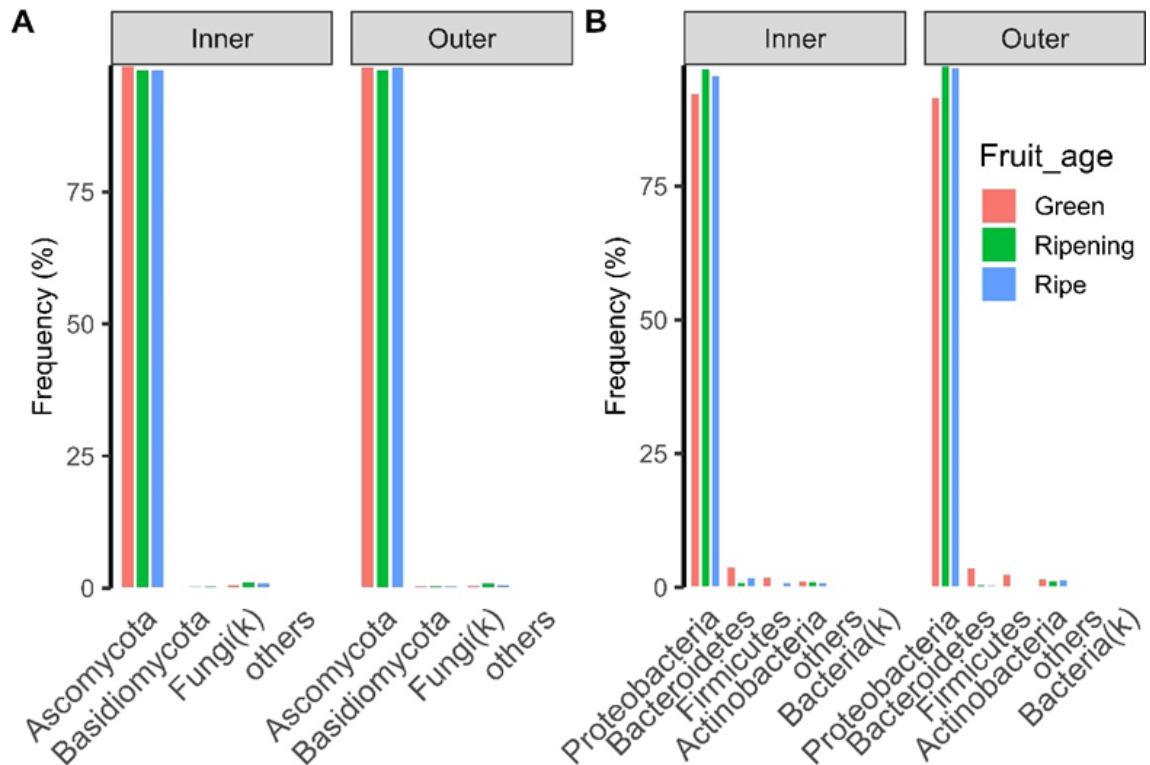


Figure 4-4. The percentage of A) fungal and B) bacterial ASVs assigned to each phylum at the 80% confidence level across fruit ages and within-tunnel locations.

For bacteria, the number of raw reads per sample ranged from 55492 to 103520 (average reads = 85933.3). Out of the total 679 bacterial ASVs, the percentage that could be identified to the phylum, class, order, family, and genus level with at least 80% confidence was 99.9%, 99.7%, 94.3%, 89.6%, and 49.3%, respectively. As for fungi, the bacterial microbiome was dominated by ASVs from a single phylum - proteobacteria (Fig. 4-4B). The number of normalised reads per ASV ranged from 34 to 601765 (average = 4815.1). The *Erwinia* genus had the greatest abundance in the top 10 bacterial ASVs, accounting for 23.2% of the total number of bacterial reads.

For both fungal and bacterial sequences, rarefaction curves indicate that the sequencing depth was acceptable (Fig. A-1).

4.4.1.2 Alpha Diversity of the Raspberry Fruit Epiphytic Microbiome

Fruit age significantly affected all fungal alpha diversity indices (Chao1, Simpson, and Shannon; $p < 0.01$). Both the Shannon and Simpson indices were significantly higher on ripening and ripe fruit, indicating greater diversity on ripening and ripe fruit (Fig. 4-5). Fruit age also significantly influenced the bacterial Shannon and Simpson diversity indices ($p < 0.05$); in contrast, higher diversity was observed on green fruit than on ripening and ripe fruit (Fig. 4-6). Sampling date affected the Shannon and Simpson indices for fungi ($p < 0.01$) and a significant interaction was found between the polytunnel and the sampling date (Shannon $p < 0.01$, Simpson $p < 0.01$), with two of the four tunnels appearing to have lower diversity scores on the 9/10/2021 than the 2/10/2021. There was no significant effect of date on the bacterial diversity indices. Within-tunnel location only affected fungal species richness, with fruit from the outer edge having a higher number of ASVs than fruit from the centre (Chao1: $p < 0.05$). However, there was a significant interaction in the bacterial Shannon index between within-tunnel location and fruit age ($p < 0.05$): outer green fruit had a higher diversity than inner green fruit, which was opposite to the diversity on the ripening and ripe fruit where the inner fruit has a higher diversity.

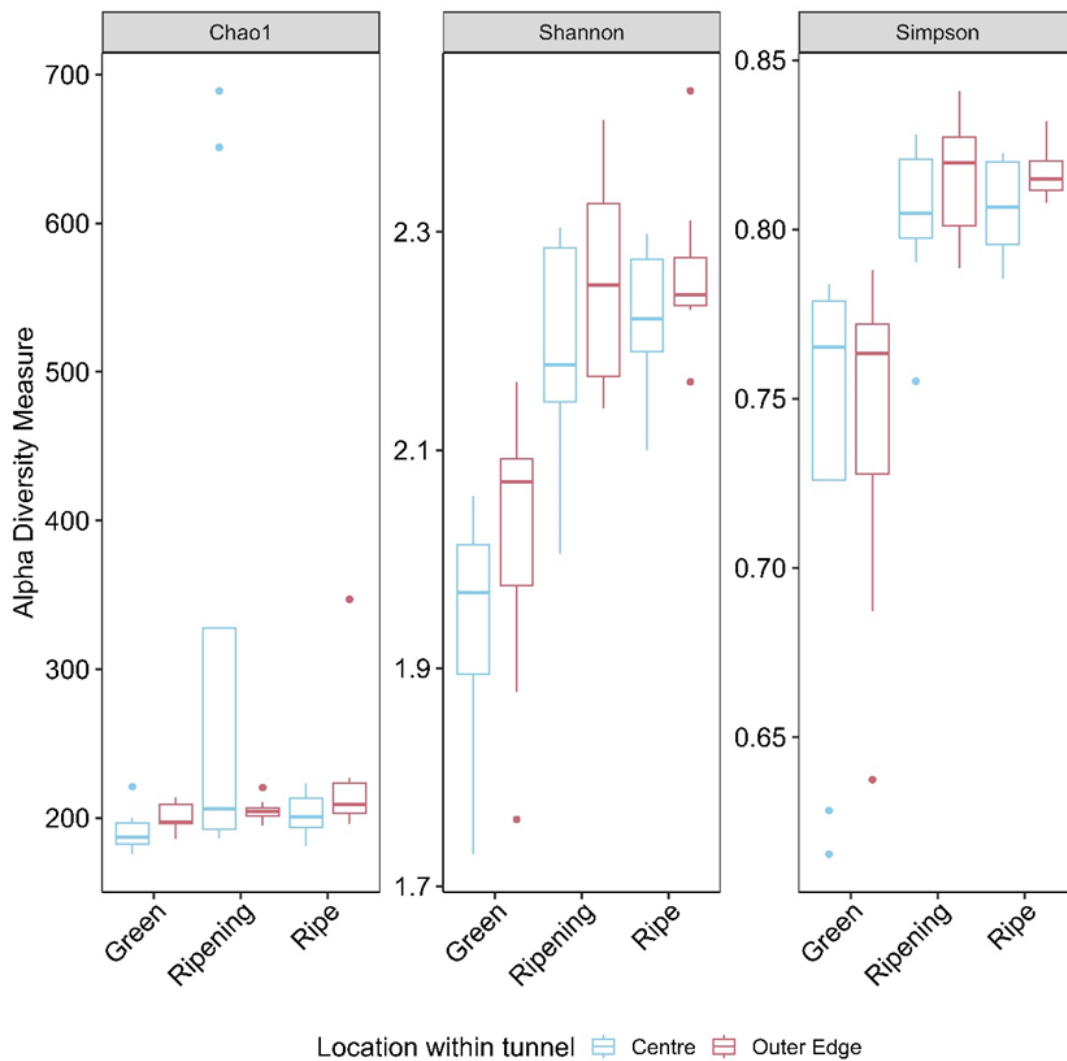


Figure 4-5. The alpha diversity measures for fungal Amplicon Sequence Variants (ASVs) of Chao1, Shannon and Simpson diversity indices. Four polytunnels were sampled across two repeats in time (2/10/2021 and 9/10/2021) which are pooled in the above diagram. Data is from a total of 48 samples (3 fruit ages x 2 within-polytunnel locations x 4 polytunnels on the farm x 2 sampling dates). The x-axis denotes fruit ripeness, and colour denotes location within the polytunnel.

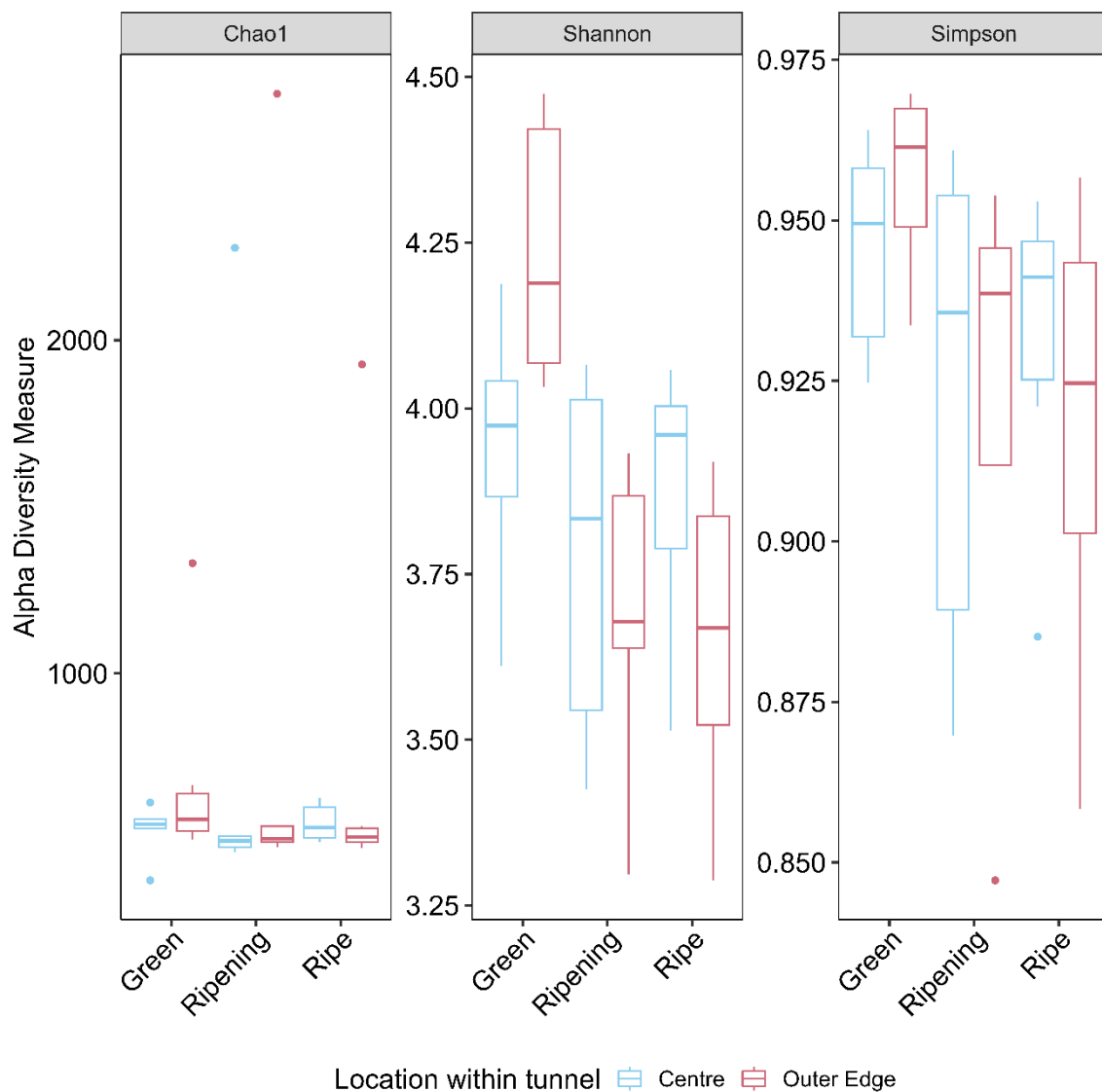


Figure 4-6. The alpha diversity measures for bacterial Amplicon Sequence Variants (ASVs) of Chao1, Shannon and Simpson diversity indices. Four polytunnels were sampled across two repeats in time (2/10/2021 and 9/10/2021) which are pooled in the above diagram. Data is from a total of 36 samples (3 fruit ages x 2 within-polytunnel locations x 4 polytunnels on the farm x 2 sampling dates; 12 samples redacted due to experimental error). The x-axis denotes fruit ripeness, and the colour denotes location within the polytunnel.

4.4.1.3 Beta Diversity of the Raspberry Fruit Epiphytic Microbiome

4.4.1.3.1 PCA

For fungi, the first four principal components (PCs) explained 18.7%, 12.1%, 9.1% and 6.4% of the total variance in the data, respectively (Fig. 4-7A). The polytunnel location in the field accounted for the highest variance in the first four PCs, contributing 13.8% to the total variability (Table 4-1), particularly within PC1 and 4. Sampling date, fruit age and within-tunnel location also contributed 5.9%, 8.3% and 10.8% to the overall variability, respectively (Table 4-1).

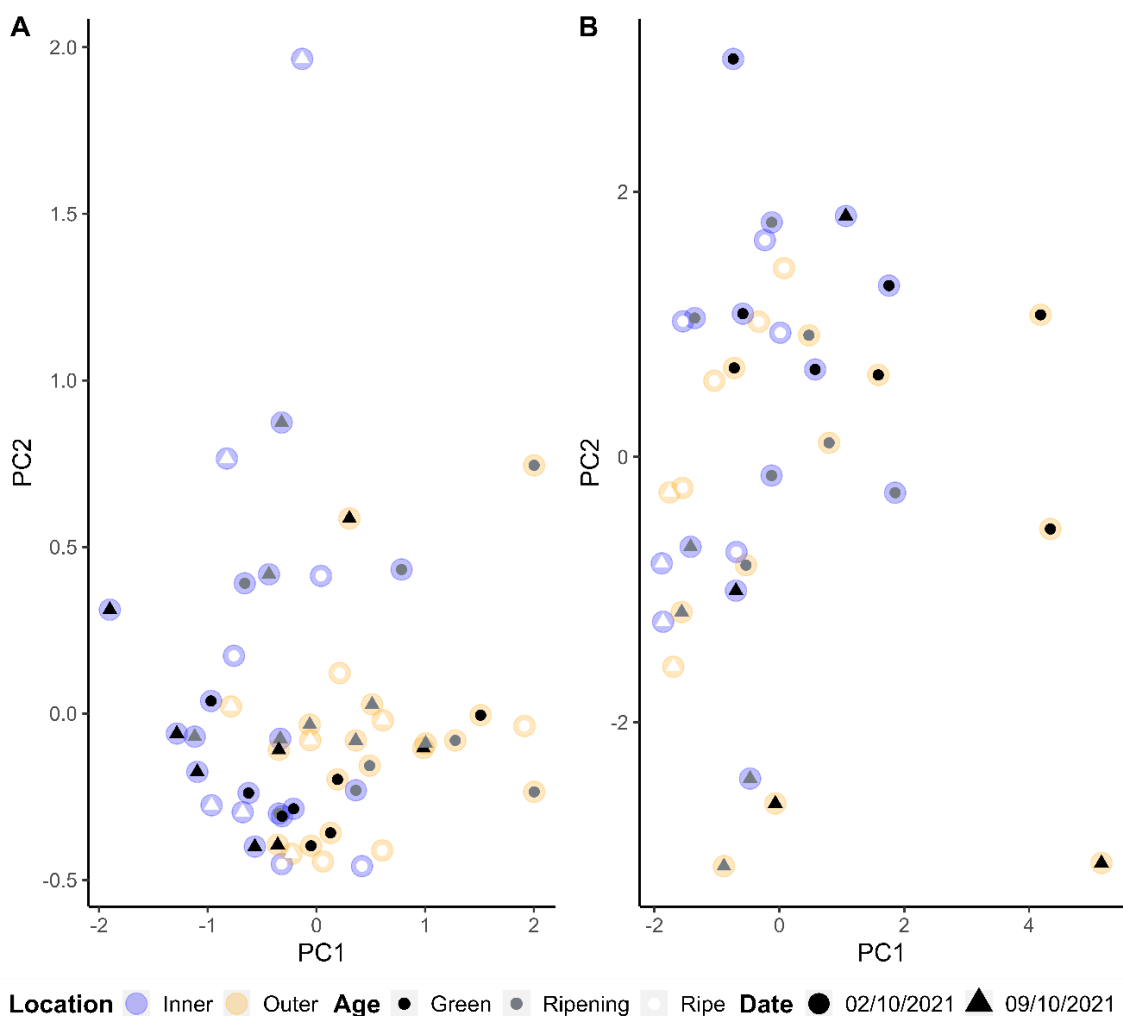


Figure 4-7. The first two Principal Components for the A) fungal and B) bacterial communities on raspberry fruits that were sampled at two time points (2/10/2021 and 9/10/2021), in two locations within polytunnels (centre vs. outer edge) and across three fruit development stages (green, ripening and ripe).

Table 4-1. Percentage of variance accounted for by the factors date, polytunnel, fruit age and within-tunnel location in the NMDS and PCA, and the associated F values for the first 4 principal components for fungal and bacterial microbiomes.

	Fungi					Bacteria				
	NMDS					NMDS				
	R ²	PC1	PC2	PC3	PC4	R ²	PC1	PC2	PC3	PC4
Date [A]	14.2***	52.26***	2.37	2.33	17.58***	5.3**	< 0.01	18.87***	3.17	37.96***
Polytunnel [B]	7.4**	2.77	0.94	7.62	12.15*	14.5*	2.1	4.19	1.89	8.75
Fruit Age [C]	21.8***	17.17***	1.2	0.87	14.68***	14.4***	13.83***	1.6	1.58	4.05*
Within-tunnel Location [D]	2.4**	15.31*	1.21	18.03*	9.95	5.3	1.07	3.56	0.02	< 0.01
[A] x [B]	3.3	1.21	0.42	4.51*	0.88	2.2	0.02	0.26	< 0.01	0.05
[A] x [C]	4.2	0.93	0.47	2.66	0.19	2.3	0.93	0.22	0.18	0.4
[A] x [D]	1.9	0.4	1.06	1.51	0.77	1.4	0.02	0.65	0.01	0.42
[C] x [D]	2.4	3.43*	0.9	0.8	0.11	5.8	4.24*	2.6	0.16	0.42
[A] x [C] x [D]	2.1	1.94	0.63	0.67	0.02	3.2	< 0.01	1.17	0.05	0.38
Residual	40.4					40.6				

*denotes $p \leq 0.05$, **denotes $p \leq 0.01$, *** denotes $p \leq 0.001$.

For bacteria, the first four PCs explained 17.5%, 15.4%, 11.6% and 8.8% of the overall variance, respectively (Fig. 4-7B). In contrast to fungi, fruit age was the most important factor driving epiphytic bacterial communities, accounting for 16.5% of the total variance (Table 4-1). Sampling date, polytunnel location within the field and within-tunnel location contributed 10.2%, 12.5%, and 3.8%, respectively to total variance (Table 4-1).

4.4.1.3.2 Bray-Curtis Indices

Samples were not distinctly separated for fungal composition, but there was a noticeable gradient of fruit age along the second dimension and a separation by within-tunnel locations (Fig. 4-8A). ADONIS permutational analysis highlighted the importance of fruit age in shaping epiphytic fungal microbiomes. Fruit age explained 21.8% of the variation ($p < 0.001$), followed by sampling date (accounting for 14.2% of the variation, $p < 0.001$). Other factors contributed to the variation, but to a lesser extent. Polytunnel accounted for 7.4% ($p < 0.01$), and

the within-tunnel location explained 2.4% ($p < 0.01$) of the variation in the Bray-Curtis indices (Table 4-1). None of the interaction terms were significant (Table 4-1).

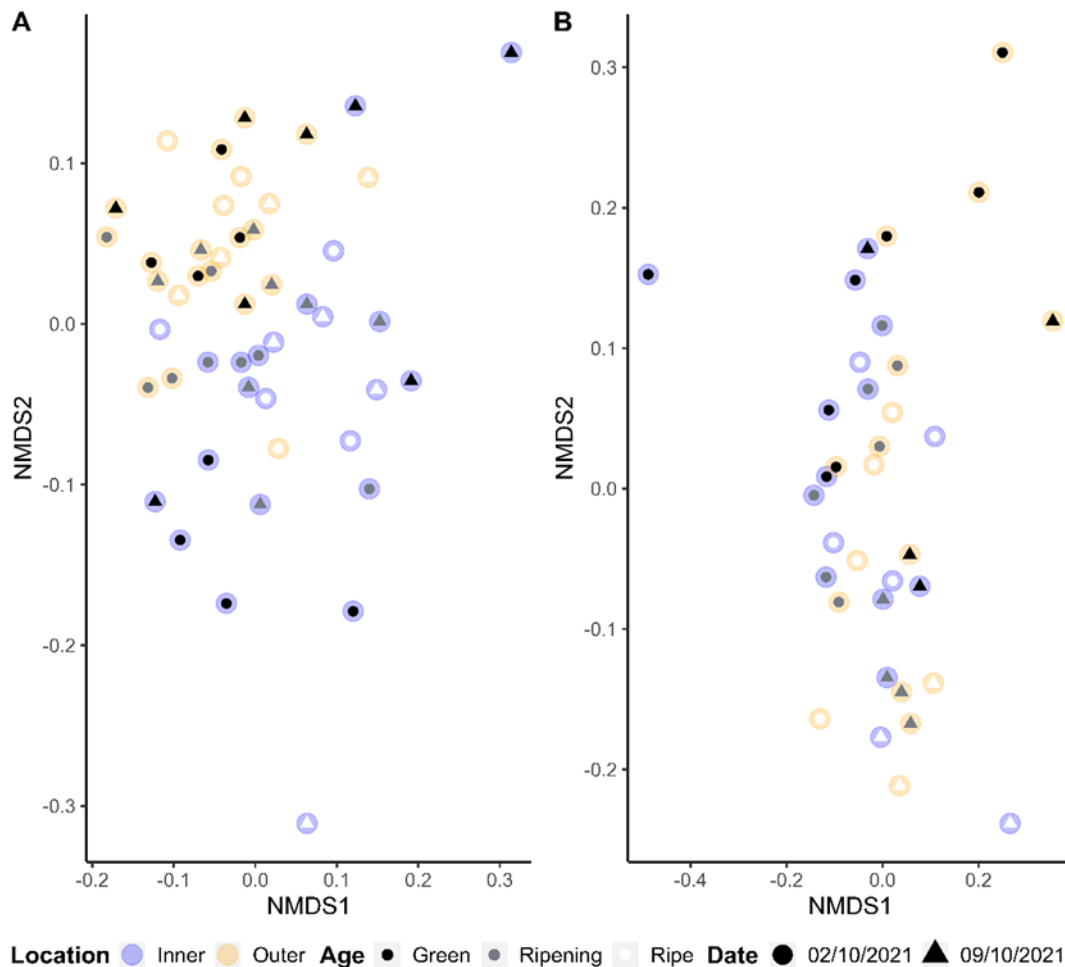


Figure 4-8. The first two dimensions of the NMDS analysis based on Bray-Curtis indices for A) fungi and B) bacteria from the surface of raspberries collected on two dates (2/10/2021 and 9/10/2021), from two locations within polytunnels (inner vs. outer) and across three ripening stages (green, ripening and ripe).

In contrast to the fungal microbiome, the bacterial NMDS plot (Fig. 4-8B) showed a clearer separation between sampling dates along the second dimension, explaining 5.3% of the variance ($p < 0.01$; Table 4-1). Fruit age accounted for 14.4% of the variability in the bacterial microbiome ($p < 0.001$), but there was no

significant difference between the two locations within a tunnel. None of the interaction terms were significant (Table 4-1). Poly tunnel location greatly impacted on the bacterial microbiome, explaining 14.5% of the variability ($p < 0.05$).

4.4.1.4 Differential Abundance Analysis

A total of 58 fungal ASVs significantly differed among the fruit ripening stages (Table A-6), 17 of which were identified at the species level. Of the 58 ASVs, 31 had a base mean larger than 20 (Table 4-2). Genera of interest include *Cladosporium*, which had a significantly higher abundance on green fruit than ripening and ripe fruit; the opposite was found for *Podosphaera* and *Epicoccum nigrum* (Table 4-2).

Table 4-2. Fungal ASVs that significantly differed ($p < 0.05$, BH adjusted) across raspberry fruit ages with a base mean read count of 20 or above. A positive $\log_2$ fold change indicates a higher relative abundance in the factor listed first in the comparison.

Factor Comparison	ASV ID	Taxonomy	Base Mean Read Count	$\log_2$ Fold Change
Green vs. Ripening	ASV57	<i>Ascomycota</i> (p)	27.56	0.91
	ASV1	<i>Cladosporium</i> (g)	29411.20	0.56
	ASV2	<i>Ascomycota</i> (p)	23509.46	-0.51
	ASV3	<i>Ascomycota</i> (p)	6959.27	-0.57
	ASV5	<i>Ascomycota</i> (p)	3877.30	-0.57
	ASV17	<i>Epicoccum nigrum</i> (s)	269.26	-0.66
	ASV32	<i>Ascomycota</i> (p)	42.66	-0.80
	ASV39	<i>Hypocreales</i> (o)	33.68	-0.80
	ASV40	<i>Hypocreales</i> (o)	31.10	-0.83

		ASV55	<i>Pithomyces chartarum</i> (s)	27.49	-0.84
		ASV46	<i>Vishniacozyma carnescens</i> (s)	27.67	-0.87
		ASV54	<i>Epicoccum nigrum</i> (s)	28.97	-0.91
		ASV58	<i>Sarocladium strictum</i> (s)	20.57	-0.93
		ASV37	<i>Vishniacozyma victoriae</i> (s)	42.90	-0.95
		ASV20	<i>Fungi</i> (k)	199.86	-1.02
		ASV18	<i>Fungi</i> (k)	246.23	-1.08
		ASV22	<i>Wallemia sebi</i> (s)	99.72	-1.12
		ASV24	<i>Fungi</i> (k)	84.37	-1.14
		ASV48	<i>Podosphaera</i> (g)	28.47	-1.40
Green vs. Ripe		ASV57	<i>Ascomycota</i> (p)	27.56	1.10
		ASV1	<i>Cladosporium</i> (g)	29411.20	0.50
		ASV40	<i>Hypocreales</i> (o)	31.10	-0.65
		ASV39	<i>Hypocreales</i> (o)	33.68	-0.82
		ASV58	<i>Sarocladium strictum</i> (s)	20.57	-1.12
		ASV48	<i>Podosphaera</i> (g)	28.47	-1.40
Ripening vs. Ripe		ASV54	<i>Epicoccum nigrum</i> (s)	28.97	0.95
		ASV22	<i>Wallemia sebi</i> (s)	99.72	0.93
		ASV24	<i>Fungi</i> (k)	84.37	0.91
		ASV20	<i>Fungi</i> (k)	199.86	0.82

ASV18	<i>Fungi</i> (k)	246.23	0.71
ASV31	<i>Entyloma dahlia</i> (s)	79.64	-1.42

(k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family, (g) denotes genus and (s) denotes species.

A total of 75 fungal ASVs significantly differed between the centre and the outer edge of tunnels (Table A-7). Twenty-four ASVs had base means larger than 20 (Table 4-3), including three ASVs from *Cladosporium*, one *Botrytis* ASV and two *Alternaria* ASVs (Table 4-3), which were more abundant in the outer edge than in the central location.

Table 4-3. Fungal ASVs that significantly differed ($p < 0.05$, BH adjusted) across locations within a polytunnel with a base mean read count of 20 or above. A positive $\log_2$ fold change indicates a higher relative abundance in the centre of the polytunnel vs. the outer edge.

ASV ID	Taxonomy	Base Read Count	Mean	Log ₂ Fold Change
ASV48	<i>Podosphaera</i> (g)	28.47		1.31
ASV53	<i>Neoerysiphe galeopsidis</i> (s)	30.95		1.3
ASV74	<i>Corynespora cassicola</i> (s)	27.79		1.24
ASV80	<i>Didymellaceae</i> (f)	25.11		1.08
ASV24	<i>Fungi</i> (k)	84.37		0.52
ASV20	<i>Fungi</i> (k)	199.9		0.33
ASV13	<i>Botrytis</i> (g)	466.8		-0.39

ASV7	<i>Cladosporium ramotenellum</i> (s)	2746	-0.45
ASV15	<i>Cladosporium</i> (g)	255.1	-0.55
ASV44	<i>Mycosphaerellaceae</i> (f)	30.24	-0.55
ASV58	<i>Sarocladium strictum</i> (s)	20.57	-0.58
ASV29	<i>Golovinomyces orontii</i> (s)	78.16	-0.6
ASV42	<i>Sarocladium kiliense</i> (s)	39.68	-0.89
ASV11	<i>Cladosporium iridis</i> (s)	610.8	-1
ASV37	<i>Vishniacozyma victoriae</i> (s)	42.9	-1.04
ASV35	<i>Pichia terricola</i> (s)	30	-1.16
ASV46	<i>Vishniacozyma carnescens</i> (s)	27.67	-1.23
ASV28	<i>Pichia terricola</i> (s)	44.68	-1.27
ASV45	<i>Sporidiobolus</i> (g)	29.79	-1.27
ASV69	<i>Alternaria</i> (g)	23.01	-1.41
ASV17	<i>Epicoccum nigrum</i> (s)	269.3	-1.46
ASV54	<i>Epicoccum nigrum</i> (s)	28.97	-1.52
ASV36	<i>Alternaria</i> (g)	96.73	-1.67
ASV55	<i>Pithomyces chartarum</i> (s)	27.49	-1.95

(k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family, (g) denotes genus and (s) denotes species.

Two hundred and eighty-seven bacterial ASVs differed in abundance among the three fruit ripening stages (Table A-8). Among these ASVs, 205 could be

identified to the genus level. A total 86 of the 287 ASVs had a base mean greater than 100 (Table 4-4). Thirty-seven *Pseudomonas* ASVs were present, with some showing higher prevalence on green fruit and others on the ripening and ripe fruit (Table 4-4).

Table 4-4. Bacterial ASVs that significantly differed ($p < 0.05$, BH adjusted) across raspberry fruit ages with a base mean read count of 100 or above. A positive $\log_2$ fold change indicates a higher relative abundance in the factor listed first.

Factor Comparison	ASV ID	Taxonomy	Base Mean Read Count	$\log_2$ Fold Change
Green vs. Ripening	ASV80	<i>Pedobacter</i> (g)	108.5	4.6
	ASV40	<i>Janthinobacterium</i> (g)	315.1	3.49
	ASV95	<i>Sphingobacterium</i> (g)	100.5	3.31
	ASV61	<i>Paenibacillus</i> (g)	143.9	3.23
	ASV47	<i>Paenibacillus</i> (g)	231.1	3.22
	ASV49	<i>Pseudomonas</i> (g)	199	3.04
	ASV54	<i>Pedobacter</i> (g)	212.98	2.83
	ASV43	<i>Pseudomonas</i> (g)	202.8	2.32
	ASV74	<i>Bacillus</i> (g)	205.1	2.24
	ASV38	<i>Enterobacterales</i> (o)	258.2	1.72
	ASV32	<i>Ewingella</i> (g)	421.7	1.67
	ASV36	<i>Pseudomonas</i> (g)	516.8	-0.79
	ASV8	<i>Pseudomonas</i> (g)	3286	-0.83
	ASV29	<i>Pseudomonas</i> (g)	493.1	-1.11

	ASV108	<i>Pseudomonas</i> (g)	114.5	-1.12
	ASV800	<i>Pseudomonadaceae</i> (f)	103.9	-1.31
	ASV31	<i>Pseudomonas</i> (g)	562.1	-1.46
	ASV806	<i>Gammaproteobacteria</i> (c)	104.8	-1.49
	ASV2	<i>Pseudomonas</i> (g)	5081	-1.56
	ASV21	<i>Rouxiella</i> (g)	655.1	-1.71
	ASV24	<i>Rouxiella</i> (g)	686.3	-2.13
	ASV48	<i>Rouxiella</i> (g)	279	-3.24
Green vs. Ripe	ASV80	<i>Pedobacter</i> (g)	108.5	6.75
	ASV54	<i>Pedobacter</i> (g)	213	4.92
	ASV62	<i>Janthinobacterium</i> (g)	145.7	4.72
	ASV95	<i>Sphingobacterium</i> (g)	100.5	4.58
	ASV77	<i>Pseudomonas</i> (g)	110.9	3.9
	ASV74	<i>Bacillus</i> (g)	205.1	3.24
	ASV40	<i>Janthinobacterium</i> (g)	315.1	2.8
	ASV43	<i>Pseudomonas</i> (g)	202.8	2.56
	ASV49	<i>Pseudomonas</i> (g)	199	2.34
	ASV41	<i>Pseudomonas</i> (g)	293.7	2.27
	ASV42	<i>Brucella</i> (g)	151.8	2.06
	ASV71	<i>Pseudomonas</i> (g)	111.5	1.98
	ASV15	<i>Stenotrophomonas</i> (g)	1055	1.96
	ASV52	<i>Pseudomonas</i> (g)	298.7	1.84
	ASV12	<i>Pseudomonas</i> (g)	2272	1.7

	ASV38	<i>Enterobacterales</i> (o)	258.2	1.7
	ASV64	<i>Pseudomonas</i> (g)	195.5	1.66
	ASV32	<i>Ewingella</i> (g)	421.7	1.56
	ASV743	<i>Pseudomonas</i> (g)	106.9	1.48
	ASV629	<i>Pseudomonas</i> (g)	124.5	1.47
	ASV18	<i>Luteibacter</i> (g)	662.7	1.45
	ASV526	<i>Pseudomonas</i> (g)	157.1	1.45
	ASV619	<i>Pseudomonadales</i> (o)	114.2	1.26
	ASV63	<i>Pseudomonas</i> (g)	524.2	1.22
	ASV3	<i>Pseudomonas</i> (g)	3831	1.21
	ASV113	<i>Pseudomonas</i> (g)	278.6	1.15
	ASV1207	<i>Gammaproteobacteria</i> (c)	165	0.9
	ASV55	<i>Pseudomonas</i> (g)	211.6	0.86
	ASV1230	<i>Gammaproteobacteria</i> (c)	207.2	0.85
	ASV22	<i>Pseudomonas</i> (g)	1015	-1.12
	ASV800	<i>Pseudomonadaceae</i> (f)	103.9	-1.15
	ASV2	<i>Pseudomonas</i> (g)	5081	-1.38
	ASV39	<i>Rhizobiaceae</i> (f)	175.1	-1.69
Ripening vs. Ripe	ASV48	<i>Rouxiiella</i> (g)	279	3.4
	ASV89	<i>Pseudomonas</i> (g)	113.7	2.24
	ASV15	<i>Stenotrophomonas</i> (g)	1055	2.12
	ASV54	<i>Pedobacter</i> (g)	213	2.09
	ASV77	<i>Pseudomonas</i> (g)	110.9	2.08

ASV927	<i>Gammaproteobacteria</i> (c)	129.3	1.77
ASV743	<i>Pseudomonas</i> (g)	106.9	1.68
ASV113	<i>Pseudomonas</i> (g)	278.6	1.55
ASV42	<i>Brucella</i> (g)	151.8	1.4
ASV17	<i>Yersiniaceae</i> (f)	1088	1.35
ASV5	<i>Yersiniaceae</i> (f)	3013	1.34
ASV699	<i>Gammaproteobacteria</i> (c)	188.1	1.29
ASV6	<i>Pseudomonas</i> (g)	2987	1.27
ASV747	<i>Gammaproteobacteria</i> (c)	300.9	1.23
ASV63	<i>Pseudomonas</i> (g)	524.2	1.21
ASV8	<i>Pseudomonas</i> (g)	3286	1.14
ASV619	<i>Pseudomonadales</i> (o)	114.2	1.14
ASV593	<i>Pseudomonas</i> (g)	143.9	1.1
ASV772	<i>Gammaproteobacteria</i> (c)	144.8	1.08
ASV29	<i>Pseudomonas</i> (g)	493.1	1.06
ASV55	<i>Pseudomonas</i> (g)	211.6	1.05
ASV108	<i>Pseudomonas</i> (g)	114.5	1.05
ASV762	<i>Gammaproteobacteria</i> (c)	173.4	1.03
ASV879	<i>Gammaproteobacteria</i> (c)	229.5	1
ASV23	<i>Rahnella</i> (g)	735.4	0.92
ASV1207	<i>Gammaproteobacteria</i> (c)	165	0.83
ASV36	<i>Pseudomonas</i> (g)	516.8	0.82
ASV1230	<i>Gammaproteobacteria</i> (c)	207.2	0.79

ASV25	<i>Microbacteriaceae</i> (f)	693.7	0.72
ASV47	<i>Paenibacillus</i> (g)	231.1	-1.87
ASV61	<i>Paenibacillus</i> (g)	143.9	-2.24

(k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family and (g) denotes genus.

There were 40 bacterial ASVs that had significant differences in abundance between the central location and the outer edge of the tunnels (Table A-9). Only 16 of these ASVs had base means greater than 100. One *Bacillus* ASV was found to be more abundant in the centre of the tunnel, while two *Erwinia* ASVs exhibited higher levels on the outer edge (Table 4-5). Four *Pseudomonas* ASVs had higher abundance in the outer edge; the opposite was true for one other *Pseudomonas* ASV (Table 4-5).

Table 4-5. Bacterial ASVs that significantly differed ($p < 0.05$, BH adjusted) across locations within a polytunnel with a base mean read count of 100 or above. A positive $\log_2$ fold change indicates a higher relative abundance in the centre of the polytunnel vs. the outer edge.

ASV ID	Taxonomy	Base Mean Read Count	$\log_2$ Fold Change
ASV54	<i>Pedobacter</i> (g)	213	1.94
ASV74	<i>Bacillus</i> (g)	205.1	1.47
ASV33	<i>Pseudomonas</i> (g)	314.3	1.46
ASV67	<i>Erwiniaceae</i> (f)	127.1	1.17
ASV18	<i>Luteibacter</i> (g)	662.7	0.96
ASV25	<i>Microbacteriaceae</i> (f)	693.7	-0.89
ASV339	<i>Pseudomonas</i> (g)	129.4	-1.14

ASV800	<i>Pseudomonadaceae</i> (f)	103.9	-1.15
ASV56	<i>Erwiniaceae</i> (f)	332.4	-1.18
ASV2	<i>Pseudomonas</i> (g)	5081	-1.32
ASV20	<i>Pantoea</i> (g)	960.6	-1.38
ASV57	<i>Pantoea</i> (g)	300.6	-1.54
ASV89	<i>Pseudomonas</i> (g)	113.7	-1.62
ASV46	<i>Erwiniaceae</i> (f)	279.3	-1.7
ASV66	<i>Erwiniaceae</i> (f)	116.6	-1.74
ASV44	<i>Pseudomonas</i> (g)	262.4	-2

(k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family and (g) denotes genus.

4.4.2 Variations in Airborne Fungal Inoculum

4.4.2.1 Estimated ITS Copy Numbers and *Cladosporium* Spore Loads During the Daytime and Nighttime

The factor "time" significantly affected the estimated ITS copy number, with higher copy numbers in the nighttime samples ($\chi^2(1) = 4.90$, $p < 0.05$). No significant difference was found between the two rotorod locations ($p = 0.23$; Fig. 4-9). There was a significant interaction between "time" and "location" ($\chi^2(1) = 5.18$, $p < 0.05$), with higher copy numbers in the nighttime than in the daytime for the outside location only (Fig. 4-10).

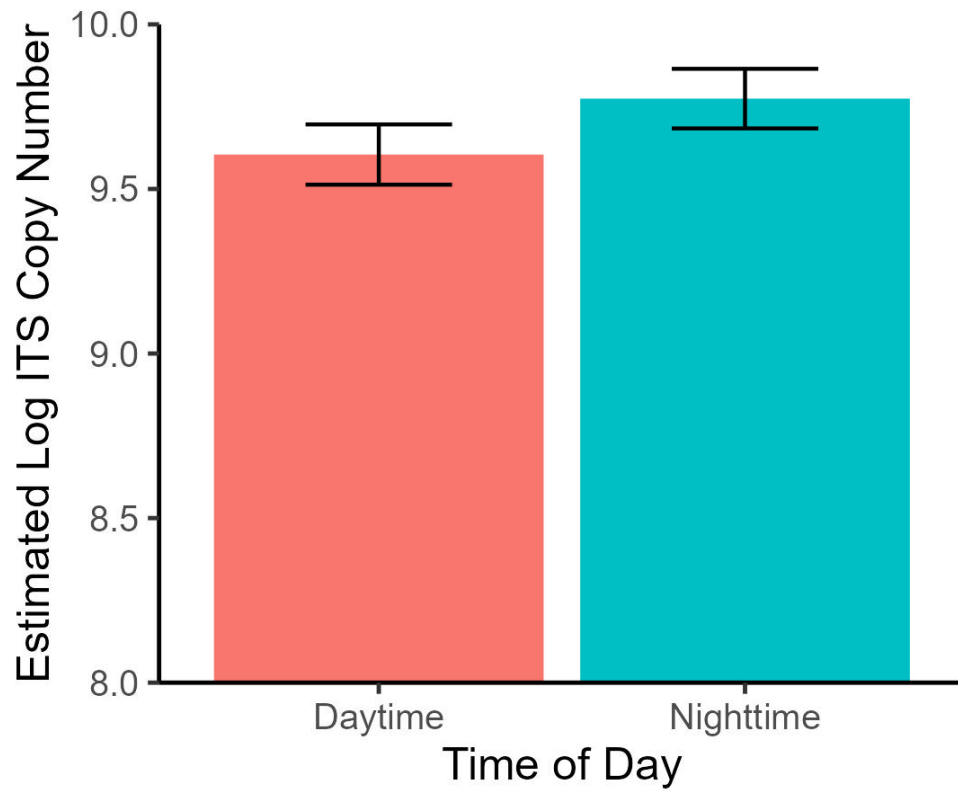


Figure 4-9. The estimated Log ITS copy number from airborne inoculum collected over the day (08:00-20:00) and night (20:00-08:00) across 21 days, averaged over Rotorod locations (outside and in a polytunnel).

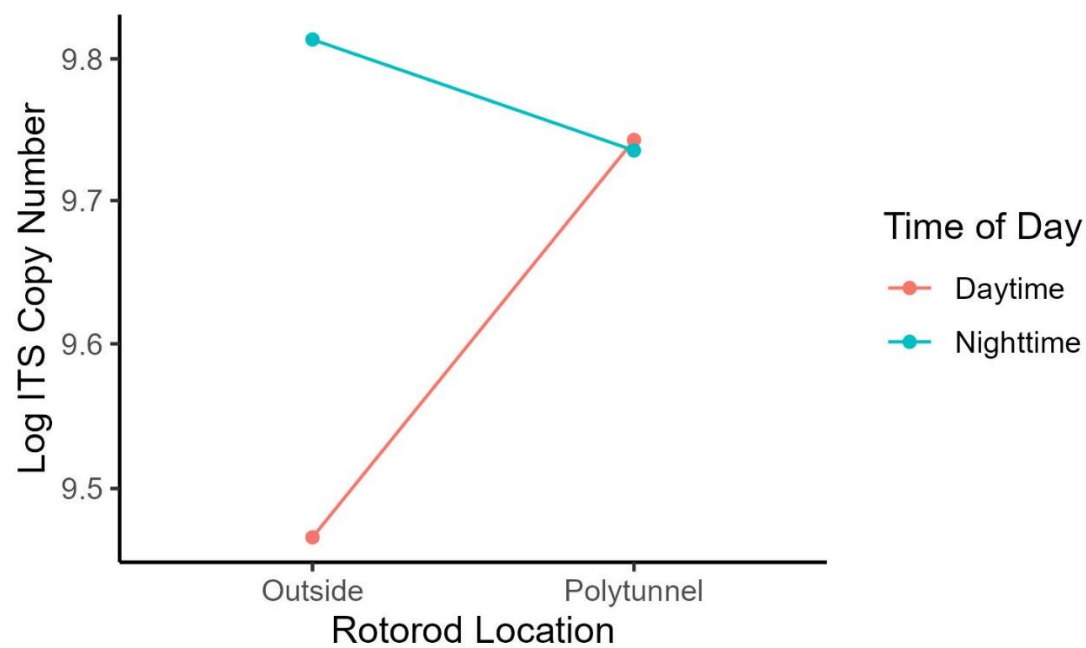


Figure 4-10. An interaction plot of the mean Log ITS copy number from airborne inoculum collected inside a raspberry polytunnel (polytunnel) and from an open field (outside) over the day (08:00-20:00) and night (20:00-08:00) across 21 days.

For *Cladosporium*, both "time" ($\chi^2(1) = 67.12, p < 0.001$) and "location" ($\chi^2(1) = 68.70, p < 0.001$) impacted spore concentrations. In contrast to the ITS copy number, a higher number of *Cladosporium* spores were trapped during the daytime than during the nighttime, and inside the tunnel than in an open field (Fig. 4-11A). There was a significant interaction between "time" and "location" ($\chi^2(1) = 5.17, p < 0.05$), with more *Cladosporium* spores being trapped inside the polytunnel than compared to the open field across the day and night (Fig. 4-11B).

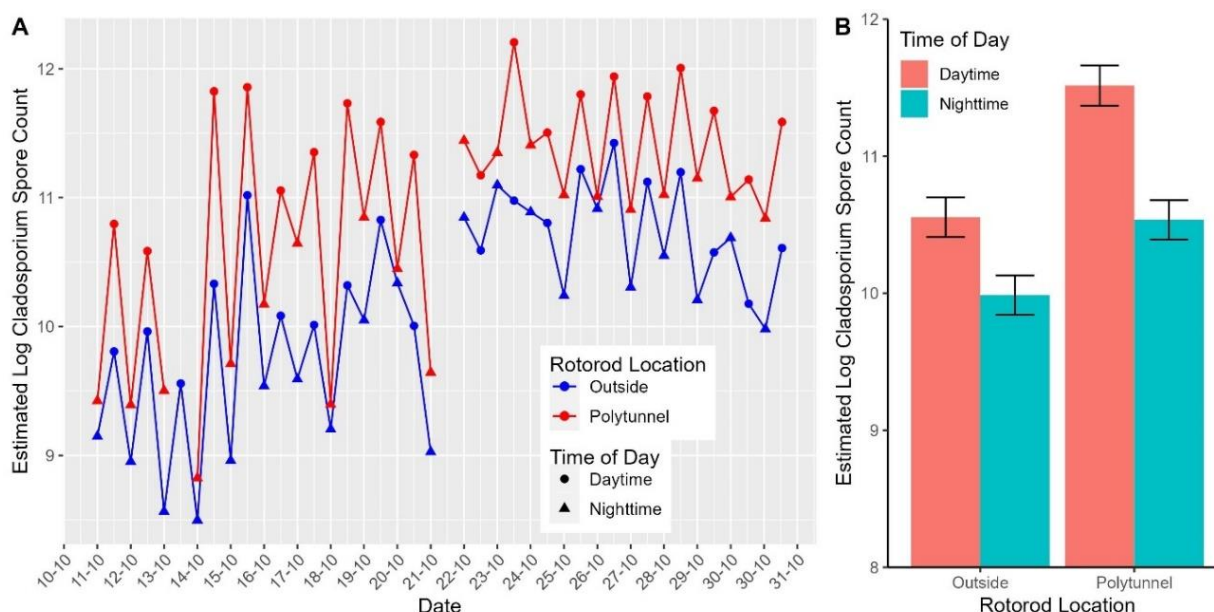


Figure 4-11. A) The estimated Log *Cladosporium* spore count and B) estimated Log *Cladosporium* spore count averaged over location and time of day from spores collected using a rotorod sampler from the centre of a polytunnel and in an open field over the day (08:00-20:00) and night (20:00-08:00) across 21 days in October 2022.

4.4.2.2 Estimated ITS Copy Numbers and *Cladosporium* Spore Loads and Across Time Periods

The estimated ITS copy numbers were significantly affected by the location of the rotorod sampler ($\chi^2(1) = 6.97, p < 0.01$); more ITS copies were found in the inside polytunnel samples (Fig. 4-12). The omnibus test (ANOVA) indicated the interaction between the rotorod location and time of day was close to significant ($\chi^2(2) = 5.87, p = 0.12$); post hoc tests revealed that there were significantly less fungal (ITS) spores outside the tunnel than inside the tunnel in the afternoon ($z = 3.21, p < 0.01$) (Fig. 4-12).

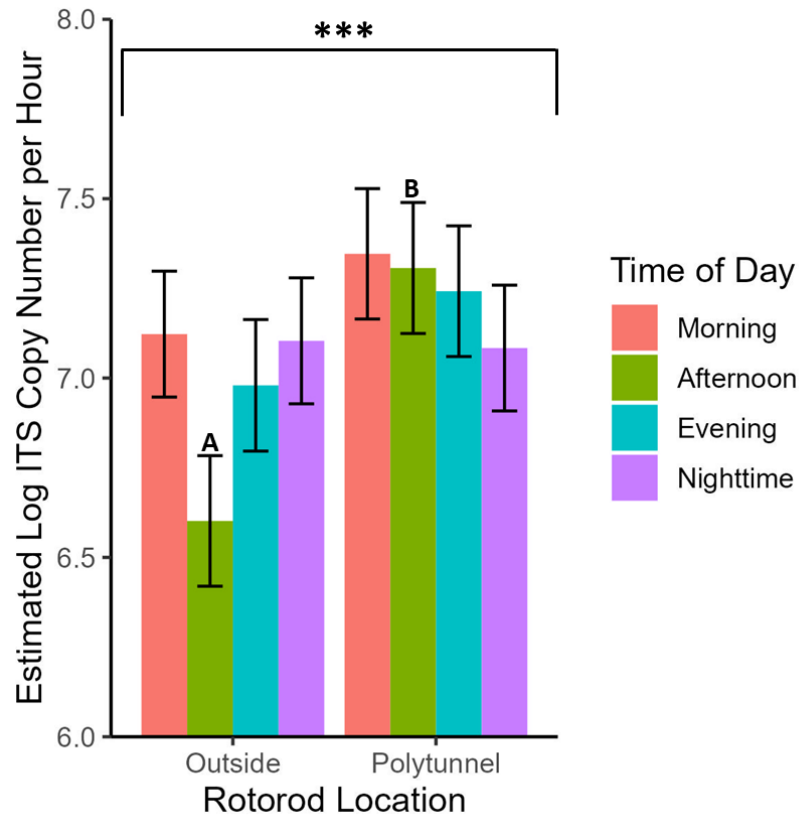


Figure 4-12. The estimated ITS copy number for samples collected from rotorod samplers inside a polytunnel and in an open field across the day (morning 08:00-12:00, afternoon 12:00- 16:00, evening 16:00- 20:00) and the night (20:00-08:00) for ten days. Letters indicate significant differences between factor levels ($p < 0.05$). *** indicates significant differences between factor levels ($p < 0.001$).

Estimated *Cladosporium* spore counts differed among the four sampling periods of a given day ($\chi^2(3) = 83.72$, $p < 0.001$) and the rotorod sampler location ($\chi^2(1) = 77.70$, $p < 0.001$), but no significant interaction was found between the two factors ($p = 0.14$). Similar to the ITS copy numbers, more *Cladosporium* spores were found inside the polytunnel than the outdoor air (Fig. 4-13). The number of *Cladosporium* spores was higher in the morning, afternoon and evening than the nighttime period ((Morning vs. Nighttime, $z = 7.26$, $p < 0.001$), (Afternoon vs. Nighttime, $z = 8.34$, $p < 0.001$), (Evening vs. Nighttime, $z = 5.56$, $p < 0.001$)) and higher in the afternoon than evening ($z = 2.73$, $p < 0.001$; Fig. 4-13).

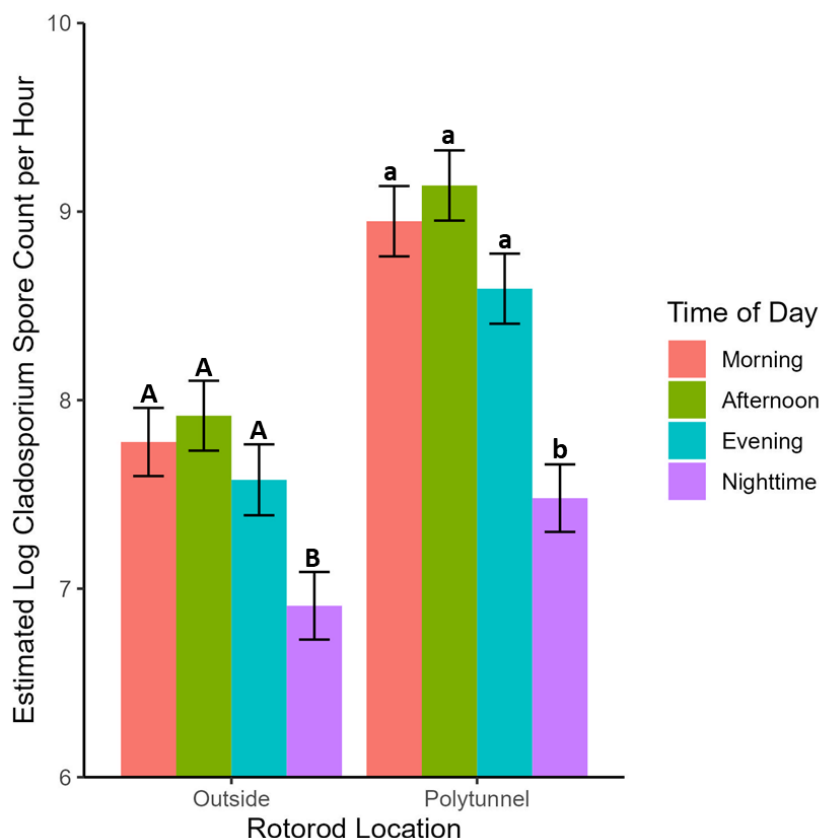


Figure 4-13. The estimated *Cladosporium* spore counts for samples collected from rotorod samplers inside a polytunnel and in an open field across the day (morning 08:00-12:00, afternoon 12:00-16:00, evening 16:00-20:00) and the night (20:00-08:00) for ten days. Letters indicate significant differences between factor levels ($p < 0.05$).

4.5 Discussion

The present results demonstrate that as raspberries ripen, there is a notable shift in microbial communities on the fruit surface, with a decrease in bacterial diversity and an increase in fungal diversity. The presence of microbial genera, such as *Cladosporium*, *Pseudomonas*, *Epiccocum*, *Rouxiella*, and *Bacillus*, varied with fruit development stages. Fruit epiphytes varied with the location of sampled polytunnels and sampling dates but were not much affected by the within-tunnel location (centre vs. the outer edge). Polytunnel cover impacted the airborne *Cladosporium* inoculum concentrations and total fungal community size: higher spore numbers inside the polytunnel than in the open field. There was also a

diurnal pattern of trapped *Cladosporium* spores, with more spores trapped during the daytime.

4.5.1 Raspberry Fruit Epiphytic Microbiome

As fruit ripened, the bacterial diversity on the fruit surface decreased, whereas the fungal diversity increased. Previous research on the raspberry epicarp found fungal phylotypes were more diverse in the middle ripening stages than on ripe fruit (Jones *et al.*, 2022). However, they sampled each ripening stage on different dates; thus, the fruit age effect was confounded with the sampling dates, which was shown to have significant effects on fruit epiphyte communities in the present study. The microbiome from the skin of blueberries was significantly different in composition and species diversity to the fruit pulp (Szymanski *et al.*, 2023), further indicating that more research is required on the epiphytic communities of fruits, where many diseases enter. The contrast in the dynamics of bacterial and fungal alpha diversity indices during fruit ripening may have resulted from multiple causes. On the fruit surface, fungi may have more opportunities to colonise and survive than bacteria due to dispersal via airborne spores. Bacteria may have less opportunity to disperse under polythene as there are no rain splashes to disperse bacteria. It is feasible under polythene, bacteria are more likely to colonise the plant surface from earlier stages of development. This transfer of microorganisms as the plant develops was demonstrated to occur in strawberries (Olimi *et al.*, 2022). It may therefore be necessary to investigate if a comparable pattern arises in other horticultural crops grown under polythene protection. Fungi may also be better adapted to surviving on the exposed fruit surface over time than bacterial species. For instance, many fungal species have thick spore coats to aid in spore survival during dispersal. The nutrient and water availability during ripening or surface abrasions may also provide more niches suitable for fungi than bacteria.

Cladosporium spp. were more abundant on green fruit than on ripening and ripe fruit. As *Cladosporium* can be abundant in airborne inoculum (as demonstrated by the rotorod sampling data), it is perhaps surprising that *Cladosporium* was not more abundant on ripe fruit as more spores could land on the fruit surface over

time. However, *Cladosporium* lesions develop more readily when the fruit surface has been damaged (Swett *et al.*, 2019), hence if fruit surface abrasions are minimal *Cladosporium* may not establish or could be quickly outcompeted. Despite the high inoculum load on the fruit surface, *Cladosporium* development on ripe fruit in the studied tunnels was very limited, indicating the importance of avoiding fruit wounding to reduce *Cladosporium* development. *Drosophila suzukii* can cause wounds on the fruit surface during ovipositioning, providing entry sites and nutritionally facilitating fungal infections such as *Cladosporium* (Swett *et al.*, 2019). At the site sampled, recommended commercial pest and disease management were adopted to control pests (including *D. suzukii*) and diseases. Other organisms may have outcompeted or inhibited *Cladosporium* in the later stages of fruit development when there was limited fruit wounding. Further research is needed to study the survival of *Cladosporium* and its competition with other common fungal groups on the fruit surface.

Many bacterial and fungal epiphytic genera varied with fruit ages in their abundance. Several *Pseudomonas* ASVs were more prevalent earlier in fruit ripening and others later. *Pseudomonas* species have varying roles, with some *P. syringae* strains pathogenic to raspberry (Ivanović *et al.*, 2023) and *P. fluorescens* as a biological control agent against *Didymella applanate* (spur blight) on raspberry (Shternshis *et al.*, 2016). *Epicoccum* and *Rouxiella* spp. were more prevalent at the later fruit development stages, and *Bacillus* more prevalent on green fruit. All these genera contain species with strains that have been tested as biocontrol organisms against raspberry pathogens (Christova & Slavov, 2021; Shternshis *et al.*, 2016). Strains in the *Bacillus* genus have been frequently used in commercial biopesticide development on soft fruits. Of particular interest is the genus *Rouxiella*; an *in vitro* study showed that specific *Rouxiella* strains successfully controlled common pathogens of strawberries (Morales-Cedeño *et al.*, 2021). This genus was more prevalent on the later fruit development stages (when *Cladosporium* was noted to decrease); hence, further investigations into the antagonism of this genus against *Cladosporium* may be warranted. The use of the 16S primers in this study meant bacteria could only be identified to the genus level. Future work with more species-specific primers may allow further

investigations into the abundance of beneficial and pathogenic bacterial species to understand their importance for fruit health.

In addition to fruit age, sampling date and the polytunnel location were also important factors significantly impacting raspberry epiphytic microbial communities. The polytunnel location explained nearly twice as much of the variability in the bacterial microbiome when compared to the fungal microbiome. In contrast, sampling date explained over double the variability in the fungal microbiome when compared to the bacterial microbiome. For both of the factors, we speculate that differences in the micro-climate and the surrounding vegetation (including debris/decaying materials) contributed towards variation in microbes on the fruit surface. A larger effect of sampling time may have been observed had sampling spanned over a period longer than one week. Larger scale distances on farms may facilitate greater climatic and vegetational differences that impact sessile bacteria more than fungi, with fungi potentially being more evenly present due to their airborne spore movement. Geographical location significantly affects the microbiome of multiple fruit crops (Abdelfattah *et al.*, 2021; Mezzasalma *et al.*, 2018; Wicaksono *et al.*, 2023). Therefore, future research on raspberry microbiomes may benefit from including more than one geographical location to understand how large an impact it has on the microbiome composition compared to other factors.

Of all the factors investigated, the one having the smallest effect on the fruit microbiome was the within-tunnel location, only explaining a small amount of the variability in the fungal microbiome. The architecture of raspberry fruits have many crevices that may allow transpiring water to collect around the fruit surface, perhaps creating a more stable microclimate around the fruits than other plant organs, partially explaining why within-tunnel location is of less importance than other factors. It is, however, important to note that during the sampling periods, there was far less difference in temperature and humidity between the two locations within the tunnel than earlier in the growing season (Fig. A-2 and Fig. A-3). Hence, more samples across the entire season may have resulted in larger differences between locations within a tunnel. Nevertheless, there were

significant differences in the abundances of several putative pathogenic and beneficial genera between the two within-tunnel locations. Three *Cladosporium* ASVs were higher on the outer edge of the tunnels than in the centre, which could be due to more air movement at the open ends of the tunnel, indicating the importance of external sources for these microbes. It is also important to note that one end of the polytunnels faced a wheat field that had been harvested prior to sampling, and the decaying material left over may have provided a source for saprophytic fungi such as *Cladosporium* to thrive. Hedgerows were also present at the end of the tunnels. A meta-analysis found higher numbers of *D. suzukii* taking refuge in these non-cropping habitats (Buck *et al.*, 2023), which may also contribute towards higher wounding of the fruits and thus, the differential abundance of epiphytes. The genus *Bacillus* was in higher abundance at the centre of the tunnel, potentially favouring warmer and more humid conditions.

4.5.2 Airborne Fungal DNA and *Cladosporium* Spores

The number of *Cladosporium* spores was higher within the polytunnel than in the open field conditions. For the total fungal community (estimated by the total ITS copy numbers), a similar trend was found in the hourly numbers across the day, but not in the day vs. night samples. Potentially, the concentration of plant material combined with a polythene covering allowed spores to concentrate inside the polytunnel as opposed to dispersing with air movement. As sampling was performed later in the growing season, dead branches and fruit receptacles were abundant within the raspberry canopy, which may have provided substrate where *Cladosporium* could colonise and sporulate. Previous studies have found a negative correlation between relative humidity and the number of *Cladosporium* airborne spores (Ballero *et al.*, 1992; Grinn-Gofroń & Strzelczak, 2013; Kurkela, 1997). In the present study, the average humidity was 10.7% lower inside the polytunnel than the outdoors (Fig. A-4), and the inside of the polytunnel was warmer by 0.3 °C (Fig. A-5). During sampling, it was noted that dew formed on the grass in the open field, whereas less dew formed inside the polytunnel where the alleyways were mowed. The low humidity may have contributed towards spore release within the polytunnel but would reduce *Cladosporium* germination

and mycelial growth. Future experiments need to investigate if venting practices alter the number of *Cladosporium* spores in the air. Reducing *Cladosporium* inoculum in the air may be important for reducing post-harvest development of *Cladosporium* if the spores are capable of surviving for extended periods of time on the fruit surface. Removing dead material within the canopy and within the polytunnel will reduce the potential material for *Cladosporium* to colonise and sporulate on, which may be another useful management strategy.

The amount of airborne fungal DNA, including *Cladosporium*, varied with sampling time within a 24-hour period. The amount of fungal DNA was higher in the nighttime periods than in the day, but when sampled over the daytime, no difference was detected in the hourly average ITS copy number, which corroborates with previous research investigating the total fungal spore load in outdoor environments in Dublin, Ireland (O’Gorman & Fuller, 2008). In contrast, the average number of airborne *Cladosporium* spores was higher in samples taken during the day than the night, and with a higher number of spores in the morning and afternoon periods than in the nighttime period. This finding agrees with previous studies (Kurkela, 1997; O’Connor *et al.*, 2014; Stephen *et al.*, 1990). The spore number predictions used for allergen warnings may, therefore, also be useful to predict *Cladosporium* within agricultural environments, but need to take into account closed or semi-closed environments such as polytunnels. This experiment only covered the very end of the growing season. It therefore warrants further investigation into the difference in spore numbers across the entire growing season.

Understanding how air movement impacts the risk of disease within polytunnels is complex. If fungal spores are concentrated within a polytunnel due to increased crop debris, air movement may allow for better dispersal of such spores to land and colonise new areas within the crop canopy. Polytunnels, however, are designed to aid air movement to help decrease the humidity within them. Future research should aim to understand how managing air movement within polytunnels affects the spore load within the air, and hence, how this relates to the pathogen load and disease development on the fruit.

4.5.3 Conclusions

The raspberry epiphytic microbiome was significantly affected by fruit age, particularly the fungal microbiome composition. During fruit ripening, fungal diversity increased while bacterial diversity decreased. The polytunnel location and the sampling date were also important, affecting both fungal and bacterial microbiome composition on raspberry fruits surface. Within-tunnel location had a small effect on the composition of the raspberry epiphytic microbiome. *Cladosporium* was the most abundant fungal genera on the surface of raspberries and more prevalent on green fruit and on fruit at the outer edge of tunnels. Poly tunnels had a higher number of *Cladosporium* spores compared to an open field, but numbers still followed a diurnal pattern with higher spore counts in the day than night.

5 Assessing the Efficacy of Biological Control Agents in Plate Assays and Field Applications to Control *Cladosporium* on Raspberry

Authors: L.H. Farwell^{1,2}, A.L. Harris², G. Deakin², N. Magan¹ and X. Xu².

¹ Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, Cranfield, U.K.

² NIAB—New Road, East Malling, West Malling, ME19 6BJ, U.K.

In preparation for submission.

5.1 Abstract

Raspberries are a profitable and delicate fruit that are susceptible to a range of fungal pathogens. In recent years, growers have been experiencing problems with *Cladosporium* on raspberries, potentially due to the movement of plants from open fields to under cover in polythene tunnels. As *Cladosporium* is an understudied pathogen of raspberries, there has been insufficient research on its epidemiology and management. In the present research, five commercially available biocontrol organisms were initially screened in dual-culture plate assays to determine if they could significantly reduce *Cladosporium* mycelial growth. All tested biocontrol organisms significantly inhibited *Cladosporium* mycelial growth in plate assays. The tested strain of *Trichoderma* sp. was the most effective, with the other products having similar effects. In addition to the *Trichoderma* sp., two other *Bacillus*-based biocontrol products were selected for field studies: *Bacillus subtilis* strain QST 713 and *B. amyloliquefaciens* strain FZB24. All three biocontrol organisms led to significant reductions in the proportion of fruit infected with *Cladosporium* skin lesions in field conditions, irrespective of whether used before (preventative) or after (curative) pathogen inoculation. Of the three biocontrol agents tested, the *Trichoderma* sp. was the most effective in reducing *Cladosporium* development on raspberry. Further large-scale experiments are necessary before these biocontrol products are to be used in commercial raspberry production.

5.2 Introduction

Raspberries (*Rubus ideaus*) are a valuable horticultural crop which are susceptible to several fungal pathogens, including *Botrytis*, *Rhizopus*, *Mucor* and *Cladosporium* (Williamson *et al.*, 1991). *Cladosporium* has been increasingly reported as a problem on raspberries (Farwell *et al.*, 2023; Swett *et al.*, 2019), causing both skin lesions pre-harvest (Farwell *et al.*, 2023; Swett *et al.*, 2019) as well as post-harvest fruit decay (O'Neill *et al.*, 2012). *Cladosporium* can cause mass fruit rejection, however, most recommendations for control are generalised

from measures used against other fungal pathogens due to a lack of research on *Cladosporium* management in raspberry.

Until recently, the primary method of controlling fungal pathogens was chemical fungicides. The timing of chemical control applications against *Cladosporium* on raspberry is difficult due to short fruit ripening time and the resulting risk of pesticide residue on the fruit. Strains of *Cladosporium* isolated from raspberries were also resistant to multiple fungicides, including caspofungin, fluconazole and flucytosine (Farian & Wójcik-Fatla, 2022), indicating that more sustainable alternatives need to be investigated.

Due to plant and human pathogen resistance concerns (Bastos *et al.*, 2021; Deising *et al.*, 2008; Hahn, 2014), there are needs to develop and implement alternative control methods against plant diseases. One such alternative is the use of Biological Control Agents (BCAs). BCAs are microorganisms that inhibit pathogen development; for example, by outcompeting pathogens for resources, releasing noxious metabolites and hyper parasitism of pathogen hyphae (Köhl *et al.*, 2019). These BCA-products are primarily applied preventatively to prevent pathogen infections (Moragrega *et al.*, 2021).

Cladosporium cladosporioides was the most prevalent *Cladosporium* species on raspberries collected across the U.K. (Farwell *et al.*, 2023) and prefers colonising raspberries *via* wounds (Swett *et al.*, 2019). *Cladosporium cladosporioides* was also shown to mainly colonise the outer surface of grapes (Briceño & Latorre, 2008). As such, *Cladosporium* species may be outcompeted by other microorganisms on the fruit surface, reducing the development of fruit skin lesions. Several BCAs have been demonstrated to be effective against *Cladosporium* spp. in *in vitro* conditions. For example, *Chaetomium globosum* inhibited the growth of *C. cladosporioides* in plate assays (El-Dawy *et al.*, 2021). However, there is no published evidence yet to show BCAs can control *Cladosporium* on crops under field conditions (studies have investigated *Cladosporium fulvum*, however this species has since been reclassified to *Passalora fulva*).

The objective of the present study was to assess the efficacy of commercially available BCAs for controlling *C. cladosporioides*. Only commercial products were screened as they could be used in the future in commercial raspberry production if they were proven to be effective against *Cladosporium*. Initially, commercial products were tested under *in vitro* conditions. Several products were then taken forward, based on the *in vitro* screening results, to field experiments where they were applied either before (preventatively) or after (curatively) inoculation of raspberry fruit with *Cladosporium* inoculum.

5.3 Materials and Methods

5.3.1 Dual-Culture Plate Screening

The effects of five commercially available BCAs (Table 5-1) were tested *in vitro* for their ability to inhibit *Cladosporium* mycelial growth. In total, there were six treatments (five BCAs – Table 5-1, and an untreated control) with a minimum of seven replicates per treatment. Once the plates were treated, blocks of plates were created by placing one plate of each treatment into a stack with the position of each treatment (plate) being randomly assigned. In the *in vitro* test, BCAs were applied in their product formulation at the maximum dose recommended on the label.

Table 5-1. BCAs tested for their efficacy against *Cladosporium* development in an *in vitro* dual-culture assay and in the field.

Species	Plate Screen Media	Maximum Dose (g/L)	Tested in the field
<i>Bacillus amyloliquefaciens</i> strain FZB24	PDA	0.37	Yes
<i>Bacillus subtilis</i> strain QST 713	PDA	8*	Yes

<i>Bacillus amyloliquefaciens</i> subsp. <i>plantarum</i> D747	PDA	2.5	No
<i>Aureobasidium pullulans</i> strain DSM 14940 and DSM 14941	3% Raspberry	1	No
<i>Trichoderma</i> sp.	3% Raspberry	2	Yes

*Product supplied in liquid form, therefore units were mL/L.

One isolate of *C. cladosporioides* (Isolate #65 as in Farwell *et al.*, 2023) was grown in an incubator set to 25 °C in darkness on PDA plates (Oxoid, Basingstoke, U.K., CM0139) for one week. The plates were then flooded with 20 mL of a 0.5% KCl and 0.1% Tween-20 solution and rubbed with a sterile spreader to release conidia. The inoculum was then poured through sterile muslin to remove large mycelial clumps into a sterile Universal bottle. Spore concentration was estimated with a haemocytometer and adjusted to 10⁷ spores/mL. For the dual culture screening, PDA media was used for bacterial BCAs and 3% raspberry agar was used for fungal BCAs. To make 100 mL of the 3% raspberry agar, 3 g of freeze-dried raspberry powder was added to 50 mL of water in one duran, then 2 g of technical agar (Millipore, Livingston, U.K., 05039) was added in a separate duran containing 50 mL of water. Duran bottles were autoclaved separately (121 °C for 15 mins), then the liquids were combined immediately after autoclaving into one duran, and finally poured into plates. Nine cm Petri dishes were used for plate screens.

For BCA treatments, 20 µL of the *Cladosporium* conidial suspension was placed onto one of the two pre-marked points on the plate (two points with a gap of 30 mm in the centre of the plate), or onto the centre of the plate for the control. The plates were then left inside a laminar flow hood for approximately 20 mins until the liquid had evaporated. The plates were then placed into an incubator in darkness at 25 °C for three days to allow germination and growth of the *C.*

cladosporioides. Then, a 20 µL droplet of the appropriate BCAs was added to the other point (30 mm away from the *C. cladosporioides* colony). The plates were left 20 mins in a laminar flow cabinet to dry as before, then placed back into the incubator in their pre-designated position within each stack.

Diameters of *C. cladosporioides* and BCA colonies in each plate were recorded nine days post-BCA application in one direction (the line where the BCA and *Cladosporium* were inoculated).

5.3.2 Field Applications of BCAs

Two experiments were conducted to examine the effects of preventative and curative treatments of three BCAs (selected on the basis of *in vitro* screening) under field conditions: *Trichoderma*, *B. subtilis* and the *B. amyloliquefaciens* strain FZB24, with two controls, an uninoculated and untreated control (negative control) and a *Cladosporium* inoculated and untreated control (positive control). Experiments were performed on an experimental plot under polythene at NIAB, East Malling (Latitude: 0.454766, Longitude: 51.288350) on primocanes of a popular U.K. proprietor variety, and subjected to industry standard fertigation. The pest *Drosophila suzukii* was managed using industry standard pesticide sprays, but no fungicides were applied. In 2022 and 2023, BCAs were preventatively and curatively applied.

In 2022, a total of 60 plants (in a single row) were arranged into six blocks of ten plants, with five plants randomly assigned to the preventative and five plants to curative BCA applications (Fig. 5-1). In 2023, a total of 80 plants (in a single row) were arranged into eight blocks of ten plants, with five plants in each block randomly assigned to the preventative and five plants to curative BCA applications. Inoculation and BCA treatments were only performed on one side of the plants (the side with heaviest fruit set).

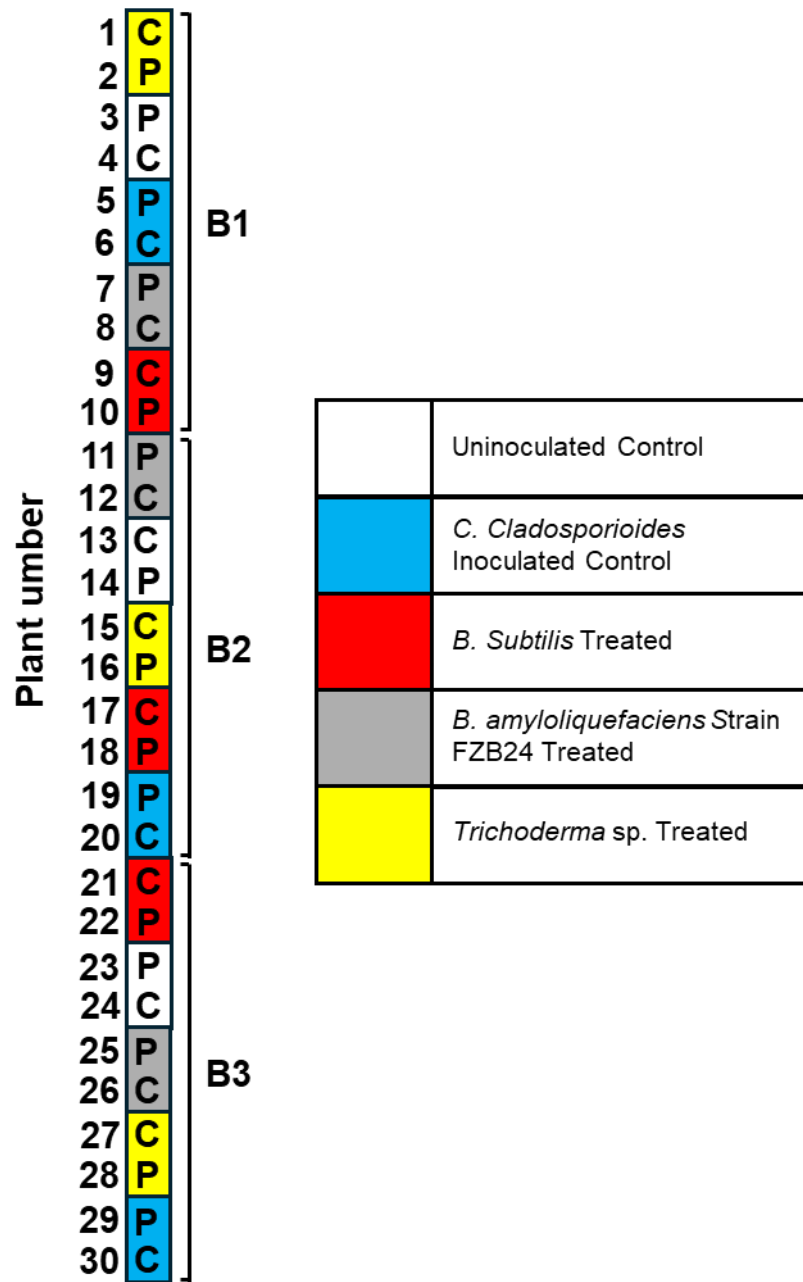


Figure 5-1. A representation of the first three blocks. Different colours represent different BCA treatments/controls as shown above. P = Preventative BCA treatment, C = Curative BCA treatment. While not shown above, between each pair of treatments and blocks is an unused plant to prevent spray drift.

The experiment consisted of: (1) on day one, curative treatments were inoculated with a mixed *C. cladosporioides* inoculum; (2) on day two, applications of the

three BCAs to all plants allocated to both curative (inoculated on day one) and preventative treatments (inoculated on day three); (3) on day three, plants allocated to preventative treatments (those treated with BCAs the day before) were inoculated with a mixed *C. cladosporioides* inoculum, and finally (4) on day four, all experimental fruits (from preventative and curative experiments) were picked for incubation and assessment. BCA inoculation of fruits for preventative and curative treatments was carried out 14/08/2022 and 11/08/2023. The total number of fruits inoculated preventatively and curatively over both years are listed in table 5-2.

Table 5-2. The total number of fruit assessed across each BCA treatment applied preventatively for each year.

Biocontrol Treatment	Preventative or Curative Application	Year	N
Positive Control	Preventative	2022	137
Positive Control	Preventative	2023	528
Negative Control	Preventative	2022	153
Negative Control	Preventative	2023	538
<i>Trichoderma</i> sp.	Preventative	2022	144
<i>Trichoderma</i> sp.	Preventative	2023	540
<i>B. subtilis</i>	Preventative	2022	145
<i>B. subtilis</i>	Preventative	2023	534
<i>B. amyloliquefaciens</i> strain FZB24	Preventative	2022	139
<i>B. amyloliquefaciens</i> strain FZB24	Preventative	2023	553
Positive Control	Curative	2022	141

Positive Control	Curative	2023	650
Negative Control	Curative	2022	159
Negative Control	Curative	2023	574
<i>Trichoderma</i> sp.	Curative	2022	171
<i>Trichoderma</i> sp.	Curative	2023	638
<i>B. subtilis</i>	Curative	2022	129
<i>B. subtilis</i>	Curative	2023	659
<i>B. amyloliquefaciens</i> strain FZB24	Curative	2022	145
<i>B. amyloliquefaciens</i> strain FZB24	Curative	2023	561

A 10^7 spores/mL mixed *C. cladosporioides* conidial suspension was prepared as described previously, but with three isolates of *C. cladosporioides* (Isolate #73, #78 and #80 as in Farwell *et al.*, 2023) combined in equal parts. Inoculum was applied to each individual ripening fruit (just turned pink) with a fine misting hand sprayer. BCAs were applied using handheld pressurised sprayers (one sprayer per treatment) at the maximum dosage as described on the product label (Table 5-1). Each pair of plants (one preventative and one curatively inoculated with the same BCA treatment), received 150 mL of spray on the inoculated side of the plants, equivalent to a spray volume of 1000 L/ha. Spray screens were placed between each plot to prevent drift; the curtains were rinsed with sterile water and 70% ethanol between applications of different BCA products.

All treatment and control fruits were removed by cutting the petiole with a pair of sterile scissors and placed into trays (one tray for each plant) with at least 2 cm between each fruit. Individual trays were then enclosed in a polythene bag and incubated in ambient conditions for four days (average temperature 23 °C) before assessment for the presence or absence of *Cladosporium* skin lesions.

5.3.3 Statistical Analyses

Analyses were performed with R (v. 4.1.2). The dual culture plate assays were analysed using a one-way ANOVA separately for each medium type. Post-hoc *t*-tests were performed using the package emmeans (v. 1.8.8.; Lenth, 2023), with *p*-values adjusted using the Tukey method. For the field applications of BCAs, the preventative and curative applications were analysed separately. The proportions of infected fruit were analysed using the package glmmTMB (Brooks *et al.*, 2017) using a binomial mixed model with the fixed effect of date of BCA application (as a blocking factor) and BCA treatment. The block was then applied as a random effect. Due to overdispersion in the curative model, an additional observation-level random effect was added to the model. To determine if there was a significant effect of the independent variables, a Chi-square analysis of deviance was implemented. To then look at differences between treatments, emmeans (v. 1.8.8.) was utilised to create contrasts, which were adjusted using Sidak.

5.4 Results

5.4.1 Dual-Culture Plate Screens Against *Cladosporium cladosporioides*

There were significant differences in the mycelial growth of *Cladosporium* between biocontrol treatments on raspberry media ($F(2) = 2361.2$, $p < 0.001$) and PDA ($F(3) = 507.1$, $p < 0.001$). For tests on raspberry media, *A. pullulans* ($t(18) = 25.6$, $p < 0.001$) and the *Trichoderma* sp. ($t(18) = 68$, $p < 0.001$) significantly reduced the mycelial growth of *Cladosporium* (Fig. 5-2 and Fig. 5-3), and the *Trichoderma* spp. led to a greater reduction in the growth of *Cladosporium* than *A. pullulans* ($t(18) = 42.4$, $p < 0.001$; Fig. 5-2). On the PDA media, *B. amyloliquefaciens* strain D747 ($t(23) = 32.9$, $p < 0.001$), *B. amyloliquefaciens* strain FZB24 ($t(23) = 30.7$, $p < 0.001$) and *B. subtilis* ($t(23) = 30.7$, $p < 0.001$) all significantly reduced mycelial growth of *Cladosporium* compared to the control (Fig. 5-2 and Fig. 5-4).

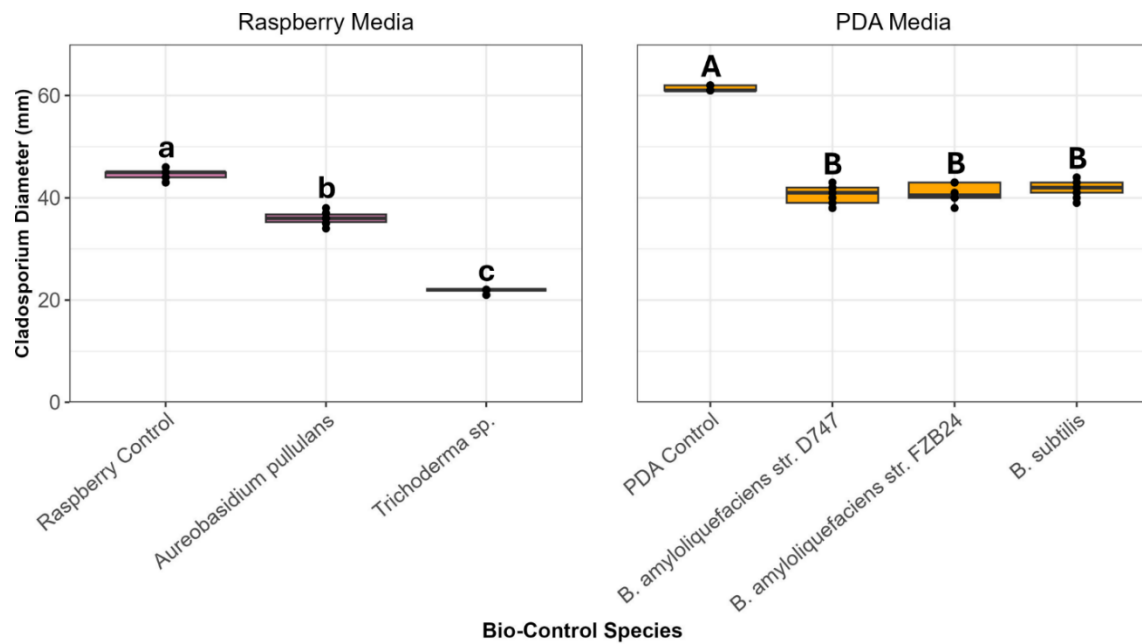


Figure 5-2. Mycelial diameter growth (mm) of *Cladosporium* colonies 9 days post-inoculation on plates treated with a BCA or a control. Bacterial BCAs were tested on PDA and fungal BCAs were tested on raspberry media. Letters denote significant differences ($p < 0.05$) between treatments in Tukey corrected post-hoc t tests.

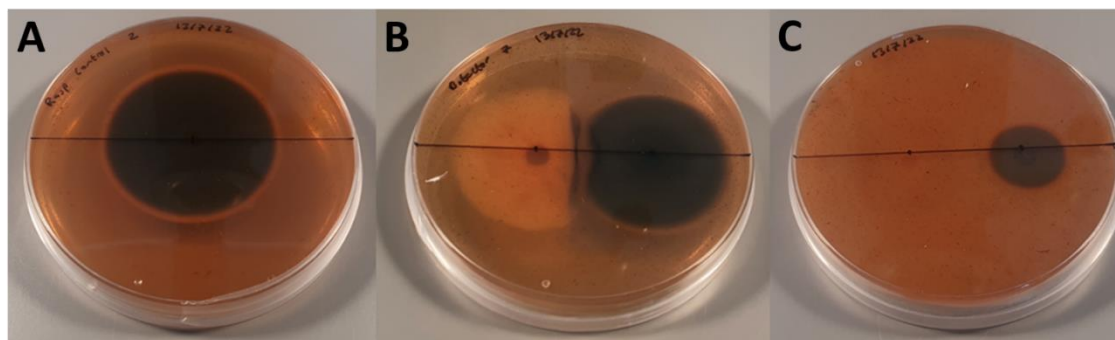


Figure 5-3. Dual plate screening images of BCAs against *Cladosporium* on 2% raspberry media. A: *Cladosporium* control, B: *Aureobasidium pullulans* and C: *Trichoderma sp.*

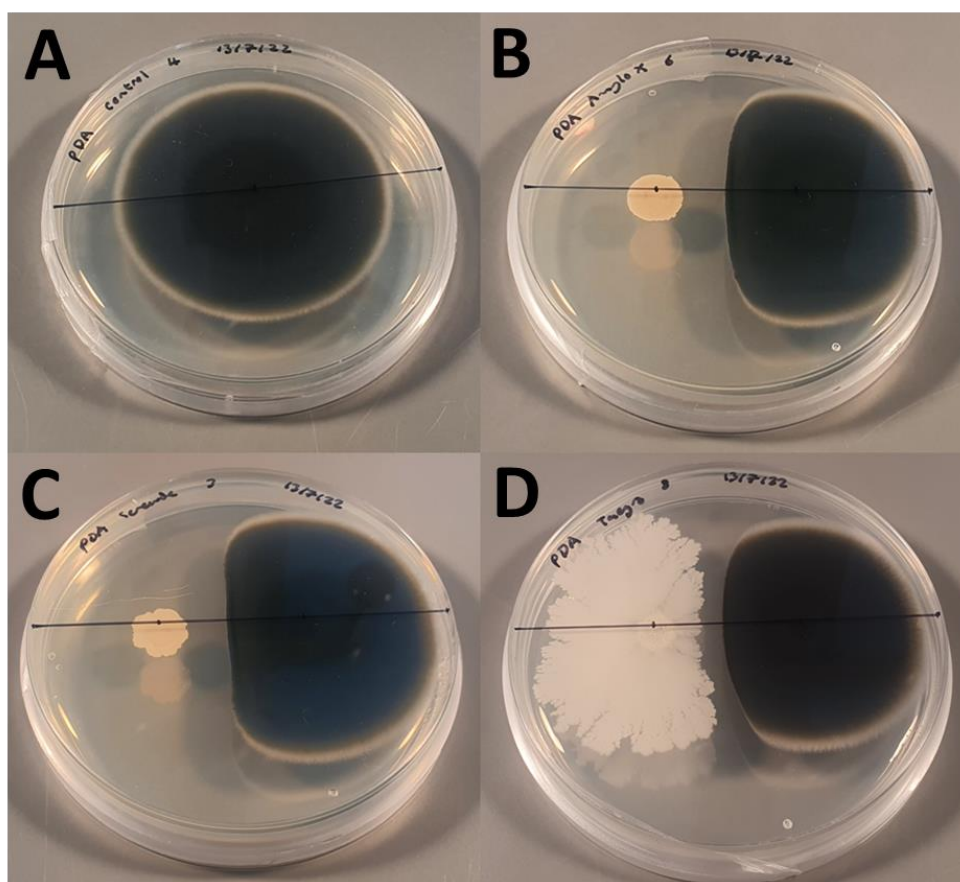


Figure 5-4. Dual plate screening images of BCAs against *Cladosporium* on potato dextrose agar. A: *Cladosporium* control, B: *Bacillus amyloliquefaciens* strain D747, C: *B. subtilis* and D: *B. amyloliquefaciens* strain FZB24.

5.4.2 Field Applications of BCAs Against *Cladosporium cladosporioides*

The total number of fruits inoculated each year for preventative and curative treatments is shown in Table 5-2. In both years, BCAs were applied when temperatures were around 25 °C on overcast days (Fig. A-6 and Fig. A-7) with low wind speeds.

Preventative applications of BCAs led to significant reductions in the proportion of infected fruit ($\chi^2(4) = 161.91, p < 0.001$). On the positive control fruit, inoculation with *Cladosporium* led to a 26% increase in the incidence of infected fruits ($z = 10.03, p < 0.001$; Fig. 5-5). All three BCA treatments significantly reduced fruit

infection when compared to the positive *Cladosporium* inoculated control, with *B. amyloliquefaciens* strain FZB24 reducing the incidence of infected fruit by 19%, *B. subtilis* by 15% and the *Trichoderma* sp. by 28% {(*B. amyloliquefaciens* strain FZB24, $z = -7.26$, $p < 0.001$), (*B. subtilis*, $z = -5.33$, $p < 0.001$), (*Trichoderma* sp., $z = -10.89$, $p < 0.001$); Fig. 5-5}.

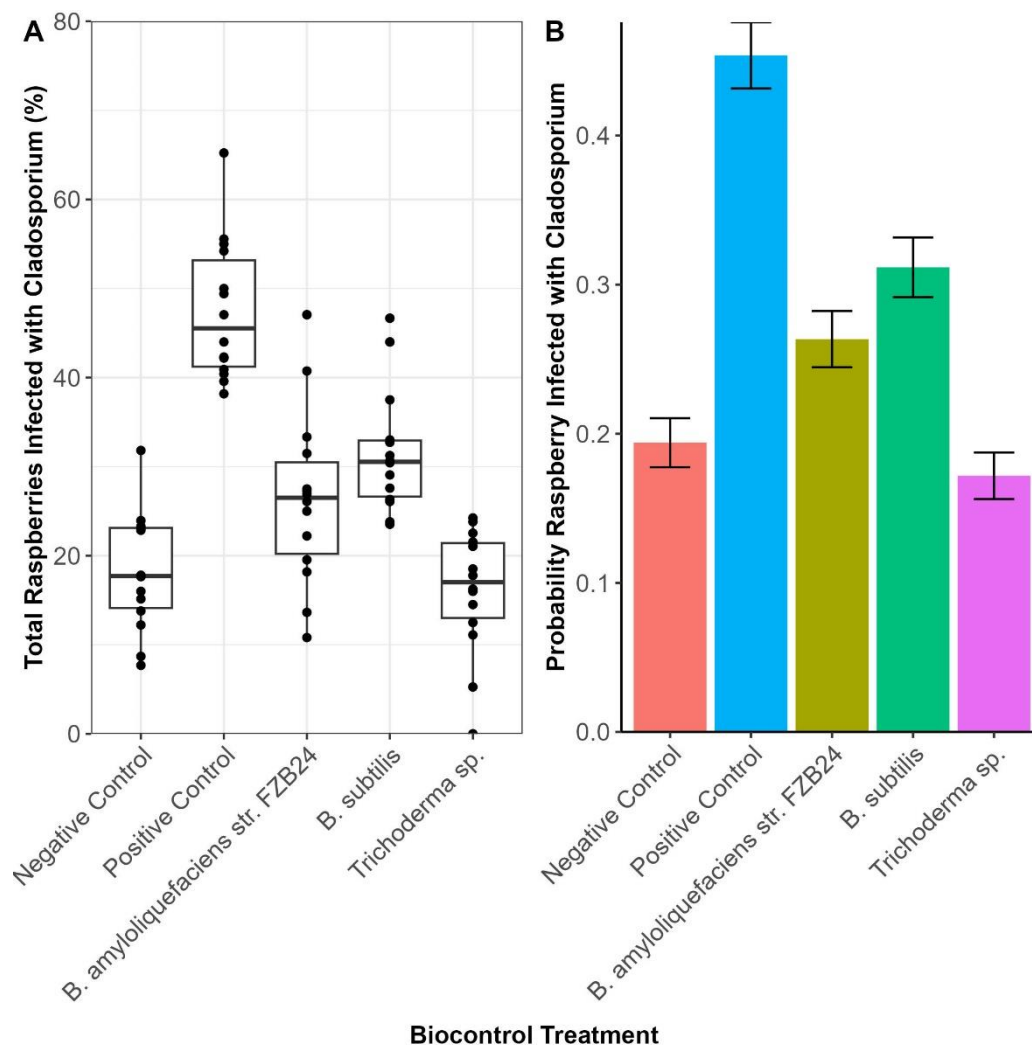


Figure 5-5. The A) percentage of raspberries infected with *Cladosporium* skin lesions and B) probability that raspberry fruits are infected with *Cladosporium* skin lesions after being preventatively treated with a biocontrol treatment compared to raspberries inoculated with only *Cladosporium* (positive control), and to raspberries that have undergone no inoculation (negative control). BCAs were applied on 14/08/2022 and 11/08/2023.

Curative applications of BCAs also significantly reduced the proportion of fruit infected with *Cladosporium* ($\chi^2(4) = 124.04$, $p < 0.001$). As before on positive control fruits, inoculations successfully increased the proportion of infected fruits, with a 24% increase in the incidence of *Cladosporium* ($z = 8.60$, $p < 0.001$; Fig. 5-6). When applied curatively, all BCAs successfully reduced the number of fruits infected with *Cladosporium* when compared to the positive *Cladosporium* inoculated controls, with *B. amyloliquefaciens* strain FZB24 reducing the incidence of *Cladosporium* by 13%, *B. subtilis* by 14% and the *Trichoderma* sp. by 27% {(*B. amyloliquefaciens* strain FZB24, $z = -4.37$, $p < 0.001$), (*B. subtilis*, $z = -4.82$, $p < 0.001$), (*Trichoderma* sp., $z = -9.88$, $p < 0.001$); Fig. 5-6}.

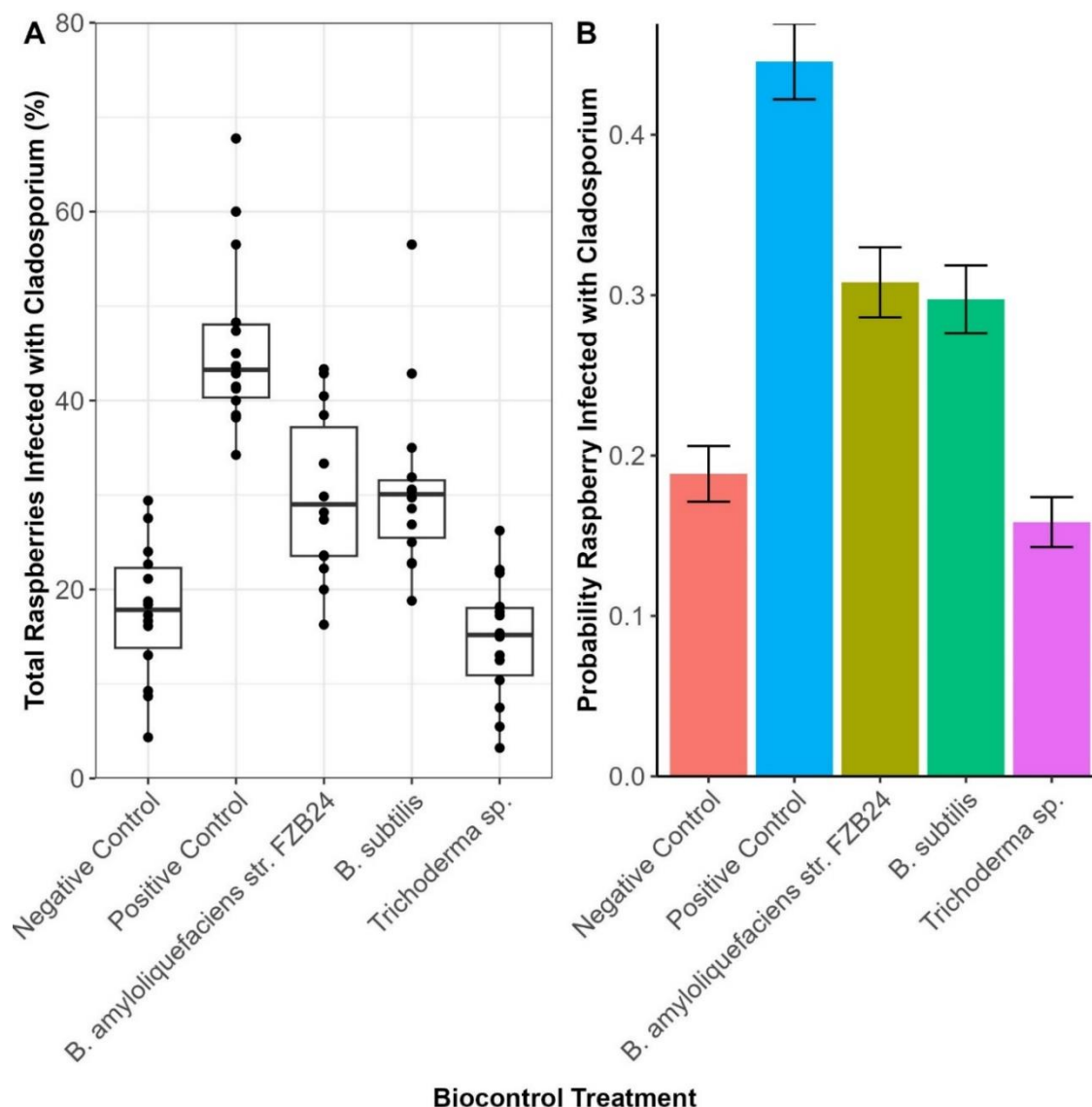


Figure 5-6. The A) percentage of raspberries infected with *Cladosporium* skin lesions and B) probability that raspberry fruits are infected with *Cladosporium* skin lesions after being curatively treated with a biocontrol treatment compared to raspberries inoculated with only *Cladosporium* (treated control), and to raspberries that have undergone no inoculation (untreated control). BCAs were applied on 14/08/2022 and 11/08/2023.

5.5 Discussion

In plate assays, all BCAs tested significantly reduced *C. cladosporioides* growth, with the *Trichoderma* sp. being the most effective. BCAs applied in the field were also effective at reducing the incidence of fruit with *Cladosporium* skin lesions,

with the *Trichoderma* sp. once again being the most effective in both preventative and curative applications. *Bacillus amyloliquefaciens* strain FZB24 and *B. subtilis* had similar efficacy in both curative and preventative treatments.

In vitro testing showed that all five BCAs tested were effective at reducing mycelial growth of a single *C. cladosporioides* strain. Hence, it may be warranted to test the BCAs on more strains of *C. cladosporioides* to determine if there is intra-species variation, and if BCAs have a uniform effect.

The tested bacterial BCAs grew poorly on the 3% raspberry agar in preliminary tests (data not shown). The high acidity of the raspberry media may have interfered with bacterial growth more than fungi, perhaps due to fungi having thick spore coats. The surface of raspberry fruits are unlikely to be as acidic as the raspberry media, unless sizeable lesions leading to leakage, are present on the fruit surface. As fruit wounding caused by *Drosophila suzukii* is shown to facilitate *Cladosporium* infections of raspberry (Swett *et al.*, 2019), this may mean that *Cladosporium* is well adapted at surviving in acidic conditions. Therefore, testing to see if bacterial BCAs are still effective when raspberries are wounded by *D. suzukii* is needed. The temperature of incubation was maintained at an optimum for *Cladosporium* growth in this assay, whereas true field conditions vary greatly both diurnally and throughout the growing season. The inoculated control fruit in the curative treatments in 2022 had a higher probability of *Cladosporium* lesions than 2023, perhaps due to extreme weather conditions earlier in the season negatively impacting plant health (Fig. A-6), thus making plants more susceptible to infection. Thus, future screening of BCAs should be conducted across the entire fruiting season, and therefore under a range of conditions.

Applications of BCAs were effective in the present study when applied preventatively and curatively. Out of all the BCAs tested, the most effective was the *Trichoderma* product, which reduced *Cladosporium* incidence to the same level as the un-inoculated control. This agrees with previous findings that multiple *Trichoderma* spp. were effective at controlling a range of *Cladosporium* spp. in plate assays (Barbosa *et al.*, 2001; Rolim *et al.*, 2019). While *Bacillus* and *Trichoderma* spp. are known to have several biocontrol mechanisms such as

volatile release and competition with pathogens, *Trichoderma* spp. are also capable of mycoparasitism, whereby the hyphae release digesting enzymes as they coil around their host (Asad, 2022). This mode of action may have explained why the *Trichoderma* sp. was more effective at reducing the incidence of fruit skin lesions than the *Bacillus* spp. *Trichoderma* spp. can also induce plant defences for resistance to pathogens and abiotic stressors (Pacheco-Trejo *et al.*, 2022). The *Bacillus* spp. tested have similar modes of action, being able to produce antifungal metabolites (Siahmoshteh *et al.*, 2018) and promote the expression of plant defence genes (Liu *et al.*, 2019). Future investigations may be worthwhile combining *Trichoderma* spp. with *Bacillus* spp. due to the differences in their modes of action. It should be noted that BCAs were applied at the maximum dose in the present study, which may not be cost-effective for growers. Furthermore, the extent of pathogen-BCA contact achieved in the present artificial control study is unlikely to be achieved in commercial spray applications. Post-harvest cool-chain management can effectively delay fruit *Botrytis* rot development in raspberry (O'Neill *et al.*, 2012) and strawberry (Berrie, Passey & Xu, 2022). Thus, it may be worthwhile investigating the effectiveness of combining BCAs with cool-chain management in managing *Cladosporium* skin lesions.

Cladosporium is ubiquitous (Harvey, 1967), therefore using an inoculum threshold to predict disease risk and to inform when to apply BCAs may not be practical. Investigations linking the *Cladosporium* spore load inside polytunnels to the skin lesions on fruit may be warranted. Further research into the conditions conducive for *Cladosporium* development is required to develop disease risk models. It may also be warranted to investigate the efficacy of BCAs applied curatively after 24 hours. BCAs are likely to be incorporated into a larger spray programme and work is needed to see how best they can be combined with consideration of timing, product selection, and climatic conditions. In addition to prediction of disease risks, equally we need to understand BCA survival on plant tissue surfaces following their application under natural conditions (Tut *et al.*, 2023). This will provide further information on the ecology of BCAs to maximise their effectiveness when applied in the field.

6 Determining if Raspberry Varieties are Resistant to *Cladosporium* Skin Lesions and Stigmata Infections

6.1 Abstract

Raspberries are delicate fruits susceptible to a variety of fungal pathogens, including *Cladosporium*. Current management practices for *Cladosporium* are derived from other pathogen programmes, due to the limited research on this disease. As raspberries show variation in their firmness, we postulate that there will be differences in the susceptibility to *Cladosporium* skin lesions across raspberry varieties. We conducted field trials to screen five commercial varieties of raspberries for their susceptibility to *Cladosporium* skin lesions and stigmata infections across two years. Omnibus statistics (Chi-square test) showed *Cladosporium* skin lesions were significantly affected by variety ($p < 0.05$); however, post-hoc testing could not differentiate which varieties were more susceptible. Notably, Malling Charm was significantly more susceptible to stigmata infections than compared with Malling Bella and Cascade Gem ($p < 0.001$).

6.2 Introduction

Raspberries (*Rubus ideaus*) are a valuable horticultural crop, bringing £128.6 M to the U.K. economy in 2022 (DEFRA, 2023). However, the delicate fruits are susceptible to a range of fungal pathogens. *Cladosporium* is a growing problem that has been reported on raspberries in both the U.S.A. (Swett *et al.*, 2019) and the U.K. (Farwell *et al.*, 2023), causing dark mycelial lesions that render the fruit unmarketable. *Cladosporium* was traditionally considered a post-harvest pathogen, but recent studies indicate that under certain conditions, it can behave as a close-to-harvest pathogen (Swett *et al.*, 2019). The species *C. cladosporioides* was found to be most prevalent on raspberries collected across the U.K. (Farwell *et al.*, 2023), and is therefore considered the species of largest threat to U.K. raspberry production.

Methods of control often centre on chemical control and biological control agents; one alternative approach is breeding resistant varieties (Graham *et al.*, 2006).

Multiple phenological factors may impact the susceptibility of plant tissues to pathogens. For soft fruits, such as raspberry, one significant factor that contributes to this is fruit firmness which is highly variable between varieties (Giongo *et al.*, 2019). This impacts fruit storage and susceptibility to post-harvest rots. Raspberries often have dense canopies with fruit likely to be moving against plant material (e.g. leaves and branches). Therefore, the thickness of the fruit's skin impacts susceptibility to abrasions. If skin thickness variations exist, abrasions on the fruit surface may provide entry points for pathogens to colonise, thus helping with pathogen development. However, no such studies have investigated the variance of thickness in raspberry fruit skins. Furthermore, other fruit and plant architecture traits could impact susceptibility; high numbers of lateral branches may reduce air movement, increasing humidity around the fruits. An increased number of drupelets may provide more crevices for air to collect and increase humidity on the fruit surface. *Cladosporium* is often referred to as a saprophyte, colonising decaying plant material (Bensch *et al.*, 2012). As raspberries are aggregate fruits with a single stigma on each drupelet, these sources of dead material near the fruit surface that may allow *Cladosporium* to colonise and cause subsequent infections on the skin. If varieties vary in the rate of stigmata decay once pollinated, there may be variation in stigmata infection by *Cladosporium*, and may be another trait for breeders to monitor. Previous research has investigated the susceptibility of raspberry varieties to post-harvest *Cladosporium* prevalence, with differences in susceptibility being reported (Knight, 1980); these results could not however be analysed statistically. It is unknown if modern varieties of raspberry vary in their susceptibility to *Cladosporium* infections. If, for example, modern varieties have been developed to produce more fruits by increasing the number of lateral branches, the humidity around the fruit may be higher, resulting in more fungal pathogen development. Multiple modern traits such as this may be impacting susceptibility to pathogens, such as *Cladosporium*.

The overall aim of this study was to assess if modern varieties of raspberry vary in susceptibility to *Cladosporium* infections. The objectives of this study were to:

1) screen varieties of raspberry for resistance to *C. cladosporioides* a) skin lesions and b) stigmata infections.

6.3 Materials and Methods

6.3.1 Experimental Design

Five raspberry varieties (a popular U.K. proprietor variety, Cascade Gem, Paragon, Malling Bella, and Malling Charm) were screened for their susceptibility to *Cladosporium* skin lesions and stigmata infections. For each plot (plant), a minimum of one branch was inoculated with a mixed *C. cladosporioides* inoculum, and one branch was left untreated as a control for comparison. Inoculations were carried out with handheld sprayers towards the ends of the growing season in 2021 and 2022 (Table A-10) in an experimental polytunnel at NIAB, East Malling (Longitude: 51.288350, Latitude: 0.454766), where plants were subjected to industry used pesticide sprays to prevent the pest *Drosophila suzukii*, however, no fungicide applications were made. All varieties of raspberries were screened in 2021; due to losses of plants from pest and disease outbreaks, only the popular U.K. proprietor variety, Paragon and Malling Bella were screened in 2022 inoculations (Table A-11).

Plots (2 canes in one pot) were organised into an incomplete randomised block design of six blocks. Each plant had one to two new branches selected (based on availability of fruit) for each inoculation treatment (*Cladosporium* inoculated and uninoculated control) at each inoculation date, with the same plants being used across inoculation dates (but new branches used for each date; Fig. 6-1). Due to each of the varieties fruiting at different time points throughout the experiment, all treated branches were covered with bags to minimise the variation in relative humidity across inoculation dates. Raspberries were inoculated when fruit were just under-ripe (red-skinned but still firm to touch). Nine inoculations were performed in total in 2021 and three in 2022 (Table A-10) due to severe temperatures limiting appropriate conditions for field inoculations. The total number of fruits inoculated for each treatment are displayed in table A-11.

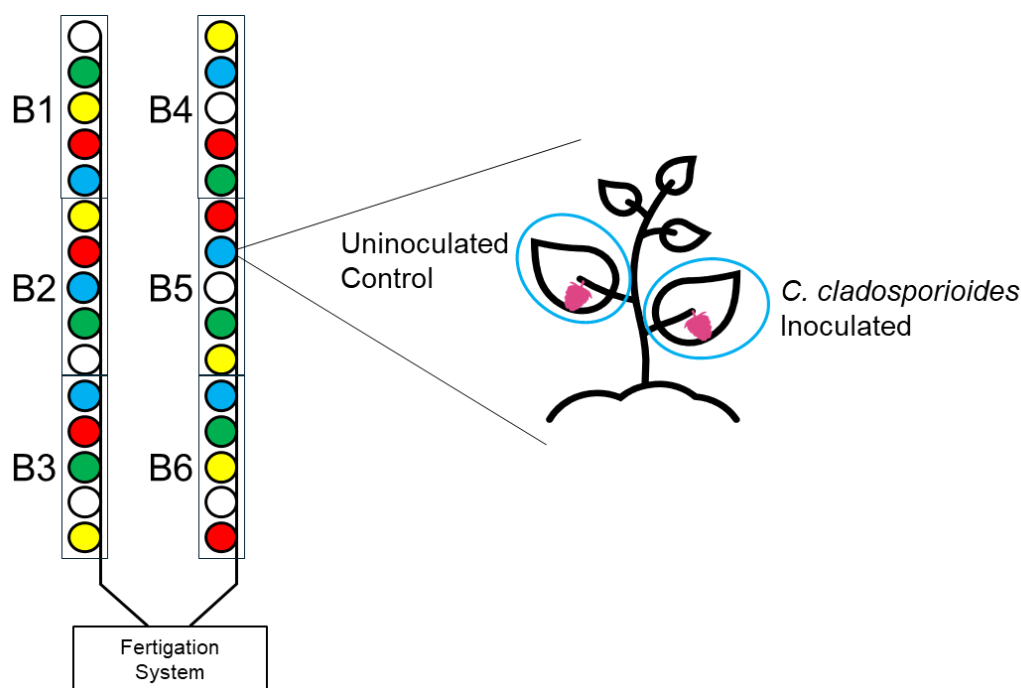


Figure 6-1. A representation of the six blocks originally set up for the field genetic screening assays. Note that during the experiments, plants were lost to disease and pest outbreaks, leading to an incomplete block design. Different colours represent different raspberry varieties: White = Malling Bella, Green = U.K. Proprietor Variety, Yellow = Paragon, Red = Malling Charm and Blue = Cascade Gem. Each plant received a *C. cladosporioides* inoculation and had an uninoculated control on separate branches, with each branch covered by an individual polythene bag (represented as a blue circle). Branches were labelled across inoculations to ensure they were not re-used.

6.3.2 Inoculum Preparation, Field Inoculations and Post-Harvest Processing

Three isolates of *C. cladosporioides* (identified *via* DNA barcoding, #73, #78 and #80; Farwell *et al.*, 2023) were grown separately on PDA (Oxoid, Basingstoke, U.K., CM0139) petri dishes for one week to ensure that suitable sporulation occurred. Plates were then flooded with 20 mL of a 0.5% KCl and 0.1% Tween-20 solution and rubbed with a sterile spreader to release conidial spores. Inoculum was passed through sterile muslin to remove large mycelial clumps and

collected in a sterile Universal bottle. The spores of each isolate were counted using a haemocytometer and adjusted to 10^7 spores/mL. A 60 mL aliquot of each isolate was combined to create the final inoculum.

Branches with the largest number of ripe fruits were selected for inoculation and labelled to avoid re-inoculation of the same branches across dates. Diseased or damaged fruit were removed prior to inoculation. The individual fruits were thoroughly wetted with inoculum using a fine-misting handheld sprayer (one spray on each side of the fruit). Directly after inoculation, branches were covered with a polythene bag, and the bag opening plugged with a damp cotton ball to retain humidity to encourage disease development. Branches assigned to the uninoculated treatment were covered prior to *Cladosporium* inoculations to prevent inoculum drift. Branches were left for 24 hrs; the bags were then removed and fruit collected from each branch into individual punnets. Fruits were immediately taken back to the lab for processing.

Fruits were grouped by their designated branch and washed twice with 0.1% Tween-20 for one min, and finally with sterile water for one min. Fruits were separated into clean punnets (2 cm between fruits) and dried in a laminar flow hood for approximately 20 mins, then covered with a polythene bag. They were then incubated in ambient room conditions with 24 hrs of light for four days. The presence/absence of *Cladosporium* skin lesions was recorded. Additionally, ten stigmata on one shoulder of the fruit and the apex were assessed for *Cladosporium* infection under a magnifying lens and the number infected recorded (the first 10 seen in the areas were selected for assessment).

6.3.3 Statistical Analyses

Analyses were performed in R (v. 4.1.2.). For both skin lesions and stigmata infections, a binomial mixed model was fitted to the data using the package glmmTMB (Brooks *et al.*, 2017) with the fixed effects of block, *Cladosporium* inoculation (inoculated or uninoculated) and variety. The following random factors were applied: 1) Inoculation Date : Block : Plot : *Cladosporium* Inoculation to account for pseudo-replication, 2) Plot : *Cladosporium* Inoculation to account for applying both treatments to the same plots and, 3) Inoculation date to account for

the random variation in environmental conditions across time. For stigmata infections, the proportion of stigmata infected out of the total 20 surveyed was used for analysis. Statistical significance of the fixed effects was determined by analysis of deviance *via* a Chi-square test. Post-hoc testing to determine which varieties were significantly different was also tested with analysis of deviance *via* a Chi-square test, with contrasts then corrected with the Benjamini-Hochberg (Benjamini & Hochberg, 1995) procedure.

6.4 Results

6.4.1 Susceptibility to *Cladosporium* Skin Lesions

Inoculating plants significantly increased the incidence of *Cladosporium* skin lesions ($\chi^2(1) = 172.60$, $p < 0.001$; Fig. 6-2), independent of variety.

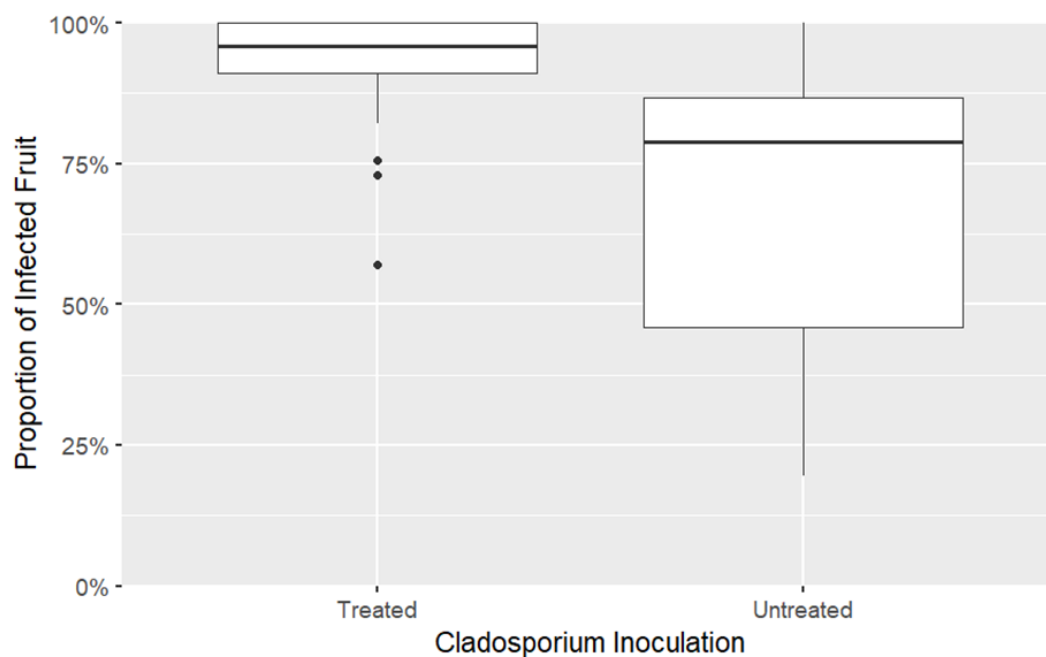


Figure 6-2. The proportion of infected fruit with *Cladosporium* skin lesions when inoculated with a mixed *C. cladosporioides* inoculum compared to the untreated control averaged over two years.

The omnibus statistical test found significant differences in the incidence of skin lesions between varieties ($\chi^2(4) = 9.64$, $p < 0.05$; Fig. 6-3); however, post-hoc pairwise analysis of deviance showed no significant differences in the incidence of *Cladosporium* skin lesions between varieties ($p > 0.05$). No significant interaction was found between varieties and *Cladosporium* inoculation treatment on skin lesions ($p > 0.05$).

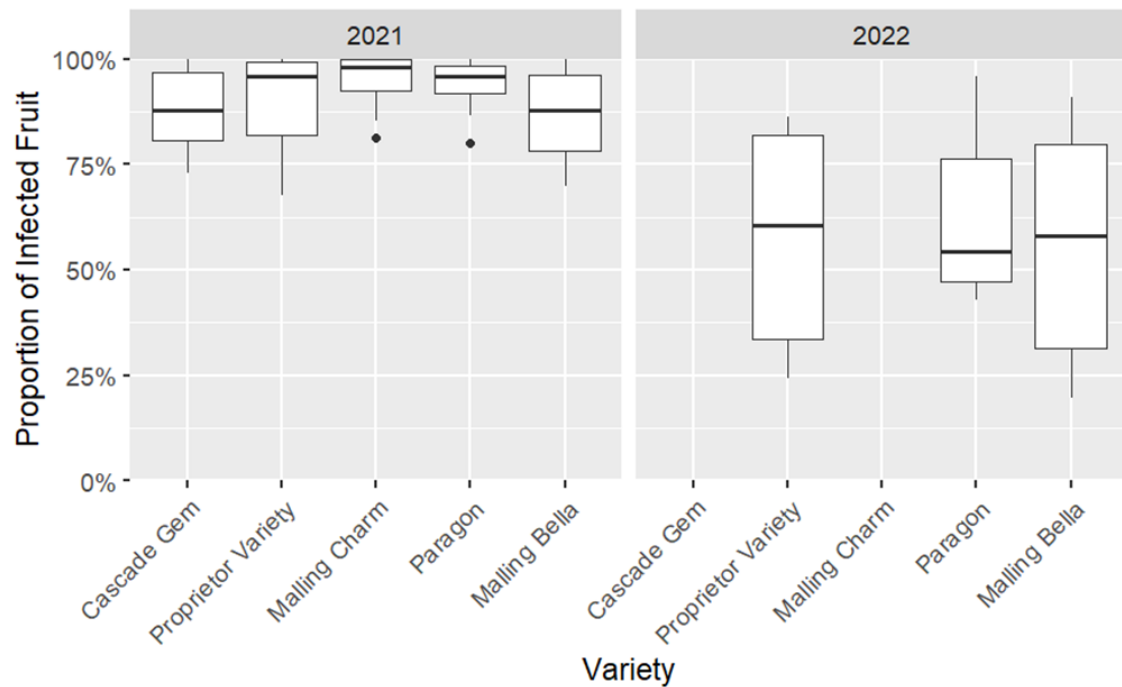


Figure 6-3. The proportion of fruit infected with *Cladosporium* skin lesions across five varieties (Cascade Gem, a Proprietor Variety, Malling Charm, Paragon and Malling Bella). Cascade Gem and Malling Charm are absent in 2022 due to plant losses.

6.4.2 Susceptibility to *Cladosporium* Stigmata Infections

The proportion of stigmata infected with *Cladosporium* also significantly increased with inoculation ($\chi^2(1) = 89.36$, $p < 0.001$; Fig. 6-4), independent of variety.

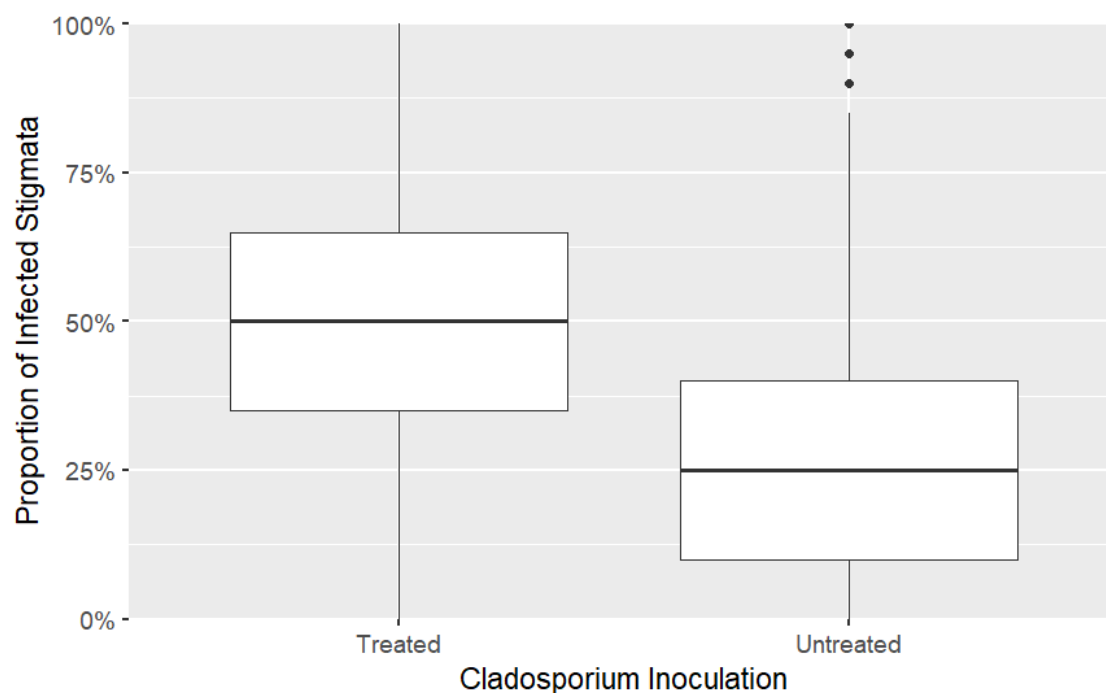


Figure 6-4. The proportion of infected stigmata when inoculated with a mixed *C. cladosporioides* inoculum compared to the untreated control averaged over two years.

Significant differences were found between the proportion of infected stigmata across varieties ($\chi^2(4) = 33.09$, $p < 0.001$; Fig. 6-5). No significant interaction was found between varieties and *Cladosporium* inoculation treatment on stigmata infections ($p > 0.05$). Post-hoc analysis of deviance revealed significant differences in the proportion of infected stigmata between varieties, with Malling Charm having significantly more infected stigmata than Cascade Gem ($\chi^2(2) = 7.08$, $p < 0.001$) and Malling Bella ($\chi^2(2) = 17.0864$, $p < 0.001$; Fig. 6-5) across two years.

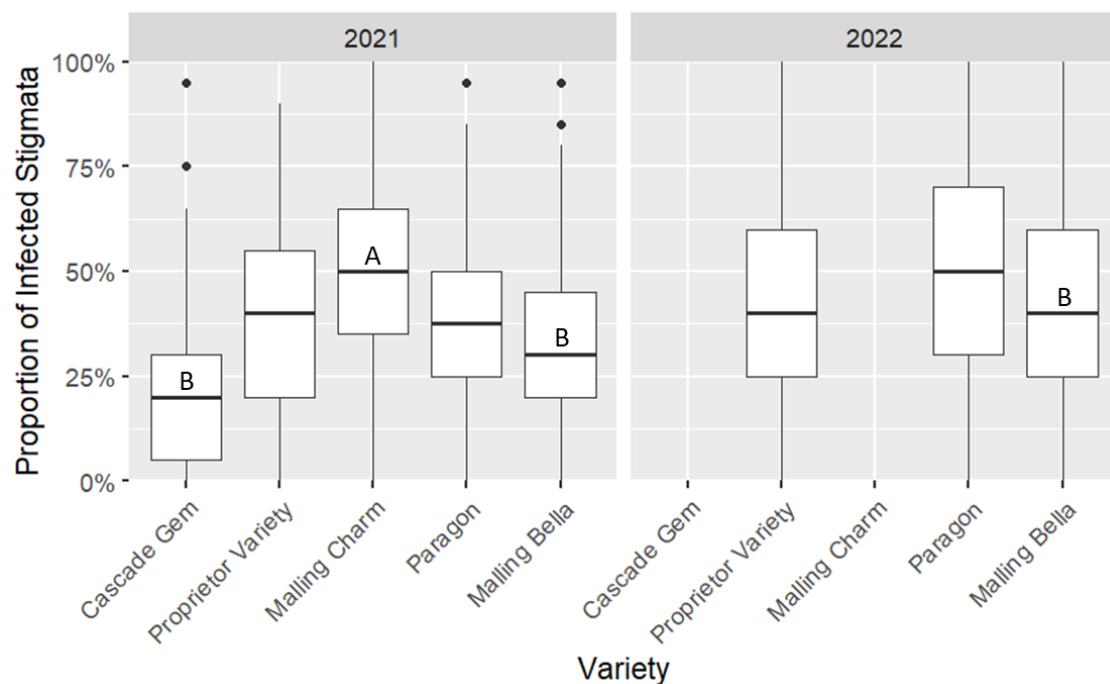


Figure 6-5. The proportion of stigmata infected with *Cladosporium* across five varieties (Cascade Gem, a Proprietor Variety, Malling Charm, Paragon and Malling Bella). Cascade Gem and Malling Charm are absent in 2022 due to plant losses. Letters denote varieties with a significant difference ($p < 0.001$) in Benjamini-Hochberg corrected analysis of deviance which was averaged over the two years .

6.5 Discussion

There were significant differences in *Cladosporium* skin lesions and stigmata infections on different varieties of raspberries; however, for skin lesions, post-hoc testing could not differentiate varieties. For stigmata infections, Malling Charm appeared more susceptible to infection than compared to Cascade Gem and Malling Bella.

Raspberry varieties had significant differences in their susceptibility to skin lesions, however, post-hoc testing could not reveal which varieties differed. Malling Charm had a median skin lesion incidence closer to 100% when compared to other varieties such as Cascade Gem and Malling Bella in 2021, but this was not significantly different. To enhance the robustness of future experiments, increasing the number of plants inoculated and sampled over time

is required, as there is a clear indication that varieties do differ in their susceptibility. Malling Charm also had a significantly higher proportion of infected stigmata compared to Cascade Gem and Malling Bella. In 2022, Malling Charm was severely affected by the root pathogen *Phytophthora*, which can remain asymptomatic until external stressors are present. The 2022 growing season had extreme temperatures in excess of 30 °C in August and September, where the 10-year average maximum temperatures (from 1991 – 2020) were 22.64 °C and 19.49 °C, respectively (Met Office, 2023; Fig. A-8), exacerbating symptoms of *Phytophthora* in any infected plants. Potential asymptomatic infections from the 2021 season may have altered fruit morphology, increasing susceptibility to *Cladosporium*. For instance, stigmata may have been more susceptible if less water was available for plant uptake, causing the accelerated senescence of some plant organs (for instance, leading to the increased proportion of stigmata infected in Malling Charm). This may indicate that overall plant health is an important factor in the susceptibility of raspberries to *Cladosporium* infection, highlighting the need for healthy and disease-free plant material for growers. It is important to note that *Cladosporium* has an optimal growth range between 18 – 28 °C, with mycelial growth dropping at 30 °C (Briceño & Latorre, 2008; Magan & Lacey, 1984a). *Cladosporium* spp. could potentially survive better in these warmer conditions than other more susceptible fungi, e.g. *Botrytis cinerea* (Ciliberti *et al.*, 2015), however growth would still be stunted, hence very warm conditions likely see a reduction in *Cladosporium* infection, as seen across the years of the experiment.

The other varieties did not exhibit significant differences in stigmata infections or skin lesions, suggesting that other risk factors may be more important in controlling *Cladosporium* than variety alone. The presence of decaying stigmata may provide a nutritional source for *Cladosporium* to colonise and sporulate on if the inoculum load is high. Breeders may consider selecting varieties where stigma decay at a slower rate to reduce dead tissue available for saprophytic fungi to colonise. When looking at the incidence of skin lesions and proportions of stigmata infected across 2021 and 2022, there is a clear decrease in infections in 2022. The 2021 growing season had frequent rain causing relative humidity to

rarely dip below 50% (Fig. A-9); conversely, the 2022 growing season was remarkably dry and hot (Fig. A-8 and A-9), and hence unfavourable for fruit fungal pathogens. Due to the effects of climate change in the U.K. causing frequent extreme weather events, it could be hypothesised that the severity of *Cladosporium* outbreaks are going to change according to the variable climate of each growing season. Consequently, to prevent future outbreaks of *Cladosporium*, producing models capable of assessing the risk of skin lesions will also help to create a cohesive strategy for *Cladosporium* management.

It is important to note that there are various traits across the entire plant that may impact susceptibility to *Cladosporium* infections. While traits such as differences in fruit firmness has been recorded in some studies (Giongo *et al.*, 2019), there are little reports of how these traits quantitatively vary across the varieties studied here. However, throughout the experiment, there were some clear differences in the phenotypes of the studied plants. For example, Paragon produced shorter canes with much heavier lateral branching, likely reducing airflow around the fruit due to the dense crop canopy. This was vastly different to Cascade Gem, which produced long canes with sparser laterals, perhaps allowing better air movement in the canopy. Differences due to the canopy density may not be found in this study due to the use of polythene bags to retain high humidity, but may be a trait of interest to breeders in the future. Denser canopies may also mean more abrasion from leaves and spikes from the stems on the fruit surface, facilitating *Cladosporium* and other fungal infections. Fruits also clearly varied in size and drupelet number across varieties; Cascade Gem produced longer berries with a high drupelet count, Malling Bella and the U.K. Proprietor Variety having less drupelets, but much larger in size. Paragon fruits were smaller in comparison to all the other fruits tested. All of these fruit variations may cause differences that affect susceptibility, for example higher drupelets meaning more crevices for humid air to collect around the fruit, aiding fungal germination.

Limitations of this study include the differences of climate and the environment during inoculation at different time points. While bagging individual branches minimises variations in humidity across inoculations, the temperatures were still

highly variable (Fig. A-8). Another problem are the differences in pest pressures throughout the season, in particular *Drosophila suzukii*, which oviposits in ripening fruit leaving wounds. Multiple studies have shown a relationship between *D. suzukii* and *Cladosporium*, whereby the oviposition damage can provide *Cladosporium* with nutrition conducive to disease development (Lewis *et al.*, 2019; Swett *et al.*, 2019). *Drosophila suzukii* shows preferences for certain varieties of raspberry in both laboratory and field conditions (Burrack *et al.*, 2013); if the two factors are linked, then this may contribute towards the susceptibility to *Cladosporium* in these varieties. Throughout this study, pesticides were used to control *D. suzukii*, and infested fruit were discarded at the assessment stage.

Future experiments should source more plants and enough varieties that are fruiting simultaneously so comparisons can be made for each section of the growing season (e.g. early vs. late fruiting), which will have different pest pressures and climatic conditions affecting susceptibility to plant pathogens. In this study, it was not possible to source enough plant material to separate varieties into early and late cropping due to the impact of both COVID-19 and Britain leaving the E.U.

For growers, ensuring plant material is healthy will aid in preventing poor plant health exacerbating infections from fungi such as *Cladosporium*. When polytunnel humidity is very high (as seen in 2022), timely venting to reduce conditions conducive to germination and growth of *Cladosporium* is vital. For breeders, considering characteristics beyond the fruit to aid overall plant health, such as reduced decaying material (e.g. petals and stigmata), thicker skin to decrease *Drosophila suzukii* damage, or decreased lateral branching to aid air movement may help future proof raspberry production to create plants that are resistant as possible to fungal pathogens.

7 General Discussion and Conclusions

Cladosporium is an emerging pathogen that can be prevalent both pre- and post-harvest when conditions are conducive to its development (O'Neill *et al.*, 2012; Swett *et al.*, 2019). In the present research, we used controlled inoculations, metabarcoding and qPCRs to study the ecology and epidemiology of *Cladosporium* on raspberries. Based on our new knowledge on its epidemiology, we then tested two management approaches (BCAs and resistant varieties) to determine if they were capable of reducing the incidence of *Cladosporium* on raspberries. This is the first work targeting *Cladosporium* on raspberry to find management strategies for growers.

The first experiment of this work helped to identify the most common species of *Cladosporium* found on U.K. fruit to tailor future work to the most prevalent species. Then, the most susceptible stages of fruit development to *Cladosporium* infection were determined to target future management strategies and experiments to these stages. To further the understanding of how *Cladosporium* inoculum behaves, the fungal and bacterial profiles of raspberry epiphytes were investigated, so that the changes in the amount of *Cladosporium* and other microorganisms it interacts with on the fruit surface could be determined during fruit ripening. The airborne inoculum was also investigated to determine if polytunnels affected the inoculum load, which could indicate if venting can aid in reducing the airborne inoculum. This also included studying if the diurnal patterns in spore numbers were affected by the presence of polythene, so existing spore predictions for *Cladosporium* may be modified for horticultural settings in the future. Finally, the efficacy of BCAs and resistant varieties of raspberries were tested against *Cladosporium* on ripe raspberries. This will provide growers with strategies to combat or prevent outbreaks of *Cladosporium* if they frequently experience problems with this pathogen.

Identification of *Cladosporium* isolates from U.K. and Spanish raspberries showed that *C. cladosporioides* was the most frequently found species, and hence the focus of our work. This finding suggests that *C. cladosporioides* primary inoculum is likely coming from airborne spores, as this species is

prevalent in the air (Harvey, 1967). *Cladosporium fusiforme* was also isolated from raspberries, which is the first time in the literature that this species was isolated from plant material, therefore identifying if it can grow on other plant species is warranted. Raspberry fruits were then inoculated with *C. cladosporioides* to determine which stages were susceptible, showing that only the ripening and ripe fruit are susceptible to skin lesions; all fruit stages were susceptible to stigmata infections. It suggests that as fruits ripen, they become softer and more prone to damage and abrasions that may allow *Cladosporium* to colonise. Determining if *Cladosporium* can survive for long periods when colonising stigmata is needed as they may act as a reservoir for subsequent colonisation of the skin and cause lesions. This is particularly important in the context of post-harvest rots when fruits are placed into punnets.

The microbiome on the surface of raspberries is dynamic, with fungal diversity increasing during ripening and bacterial diversity decreasing. As this research built upon the existing work from Jones *et al.* (2022), it demonstrated that sampling different fruit ripening stages over different time periods may introduce differences in the microbial communities. In contrast to their study, we found that ripe fruit had the highest fungal diversity, this difference may be the result of the differences in sampling time. We originally hypothesised that *Cladosporium* would be more present on ripe fruit as spores would have a longer amount of time to land on the fruit surface, but our results showed the contrary. Further studies to understand potential reasons for this may be warranted, for example the genus *Rouxiella* was more prevalent on ripe fruit; investigating these competing microorganisms may provide potential future sources of BCAs. Investigating if these results occur across other geographical locations are needed, in case these findings are unique to the area sampled from. We also found that more *Cladosporium* was abundant at the outer edge of polytunnels than the centre, with further investigation warranted on why this occurred. Potentially, increased airflow at the outer edge may have allowed more inoculum to disperse and land onto the fruit. One end of the polytunnels sampled faced a wheat field that had been harvested in the summer, which may indicate the importance of managing inoculum sources from decaying debris around raspberry crops.

A higher amount of airborne *Cladosporium* inoculum was collected inside a raspberry polytunnel than an open field, indicating that venting of the tunnels may aid in reducing the airborne inoculum. This study was the first in the literature to focus on how polytunnel infrastructure impacts the airborne inoculum load. If previous models of disease risk were collected from before the widespread use of polytunnels, then further research may be needed on other pathogens and crops. Furthermore, this research was conducted at the end of the growing season when plants have the most decayed material that *Cladosporium* can colonise; further research across the entire growing season will provide knowledge on how the inoculum and therefore disease risk changes throughout the growing season. There are multiple factors that may impact the level of air flow within polytunnels, such as the type of tunnel, netting on tunnel ends, venting, and tunnel location within a field that may also impact airflow and therefore inoculum dispersal. All of these factors may impact spore dispersal and deposition, and require further investigation. It is currently unclear if venting may aid in reducing the spore load in the air by allowing air movement to take spores out of the tunnel, or if the increased air flow may aid in spore dispersal within the tunnel, facilitating further spread of disease outbreaks. *Cladosporium* spores are dry air borne spores, meaning higher spore loads are found at low humidities, and therefore the highest spore counts are found around noon (Olsen *et al.*, 2020). Venting at noon may decrease the number of spores within the tunnel, however as previously mentioned, it may aid dispersal into the canopy. As *Cladosporium* is a prevalent airborne spore, trying to decrease the inoculum load in the tunnels may be difficult, and it may instead be better to invest in venting the tunnels when humidity is high and conditions are conducive to germination. However, if the humidity is both high inside and outside of a polytunnel, venting may not have a large impact at reducing the relative humidity inside the tunnel.

The commercial BCAs tested in this study worked well in both *in vitro* and field conditions to reduce the mycelial growth and incidence of *Cladosporium* on raspberries, with the *Trichoderma* sp. being the most effective in both assays. This study was the first in the literature to apply BCAs to control *Cladosporium* on plants in field conditions; previous studies have focused on *in vitro* assays

(Barbosa *et al.*, 2001; El-Dawy *et al.*, 2021). While this study provides a starting point for managing *Cladosporium* on raspberries, further experiments adapting these applications closer to the industry standard are needed. Using a commercial sprayer to deliver realistic spray coverage and comparing various doses of the BCAs used are needed to show efficacy can still be achieved with realistic applications. The preventative and curative applications within this study only differed by 24 hours between their respective BCA applications and *C. cladosporioides* inoculations. Investigating if BCAs still maintain efficacy when applied curatively beyond 24 hours would be of use to growers, particularly if visible mycelial matting is present, which is likely a first indicator to growers that a problem is occurring. While research has not focused on the timing of BCA application on raspberries, studies on other crops appear to vary depending on the targeted pathogen and BCA species applied in regards to optimal application times. For instance, *Bacillus subtilis* spp. were shown to be better applied preventatively, whereas *Trichoderma* spp. had better efficacy when applied curatively (Moragrega, Carmona & Llorente, 2021). Arguably, *Trichoderma* spp. are more promising for curative applications due to their aggressive myco-parasitism allowing them to attack well established pathogens in the correct conditions (Mukherjee *et al.*, 2021). *Botrytis cinerea* was best applied 96 hours post fruit picking, which authors speculated was due to host responses not differentiating the yeast BCAs from the pathogens present (Cook, Long & Ganesh, 1999). Therefore, to find the best BCAs for specific pathogens, there is a complex network of interactions that needs to be studied between the host, pathogen and BCA to optimise these products. Additionally, including cool-chain management in these applications will provide insight on how effective BCAs can be when combined into an integrated programme to manage *Cladosporium*. While our research investigated BCAs individually, further research is needed to study management efficacies when multiple management strategies are combined. It may be that combinations of BCAs have better efficacy than when applied on their own, particularly if using BCAs with different modes of action to target multiple areas of weakness (Xu & Jeger, 2013). Finding BCAs that exist naturally within commercial raspberry plantations may aid in finding organisms

that are adapted to this environment for better survival and hence better efficacy. From the microbiome investigations, attempting to isolate *Rouixella* from raspberries may be one avenue of investigation worth continuing as a species from this genus was previously shown to reduce pathogen development on strawberries (Morales-Cedeño *et al.*, 2021).

While BCAs are just a small part of the agronomic toolbox that can be used to combat *Cladosporium* on raspberries, it is important to consider the other measures of control that may also need further investigation in the future. Cool-chain of fruit is frequently used to combat post-harvest diseases such as *Botrytis cinerea* on soft fruits (O'Neill *et al.*, 2012; Berrie, Passey & Xu, 2022). However, *Cladosporium* was found to be unaffected by cool-chain on raspberries in the U.K. (O'Neill *et al.*, 2012). Cool-chain will likely still decrease the rate of growth of *Cladosporium* present on fruit, and should therefore still be implemented by growers. Modified atmosphere may be a useful strategy for the duration of cool-chain, however realistically due to the short time interval from picking to getting fruits to supermarkets, it is unlikely to be useful beyond this stage. Most raspberry packaging has air holes to prevent humidity build up and would not be suitable for modified atmosphere. As discussed in chapter five, chemical fungicides are not suitable for application on raspberries due to their corresponding harvest intervals and raspberries needing frequent picking. Biological control agents may produce secondary metabolites that could be used as a basis for synthetic fungicides, but the same concerns would stand with testing required to confirm their safety. Currently within soft fruit production, robotics are being utilised to emit high UV-C radiation to control pathogens such as powdery mildew (Onofre *et al.*, 2021). However, previous testing of UV light to control blueberry fruit pathogens found that UV light had little effect on *Cladosporium* on the fruit surface (Latorre *et al.*, 2012). This may be due to the high melanisation of *Cladosporium* protecting the spores and mycelium from radiation damage (Wong *et al.*, 2019), and hence may be ineffective as a control measure. Finally, utilising models to predict when conditions are conducive for *Cladosporium* germination may be a useful tool for when to apply control measures such as venting or BCA applications. Currently, to the author's knowledge, only one company has a

model to predict high risk of *Cladosporium* outbreaks in raspberries (Agro Smart Lab, 2024), and it may be warranted for similar models to be used across the world where outbreaks are common.

Screening of raspberry varieties indicated that there is variability in their susceptibility to skin lesions, however further research with more replicates are needed. For a more robust assessment, we suggest separating plants into their respective growing season (e.g. early fruiting, mid-season fruiting, late fruiting) in addition to increasing replication. This is due to both environmental differences across inoculations and differences in pest pressure such as SWD which may impact susceptibility to *Cladosporium*. Current raspberry breeding programmes at NIAB East Malling are assessing the variation in susceptibility to SWD ovipositioning, which may have an indirect impact on susceptibility to *Cladosporium* infection (Fernández-Fernández, *pers. comm.*, 2024). Previous research on other varieties confirmed differences in fruit firmness between raspberry varieties that may also confer differences in the varieties tested in these screens (Giongo *et al.* 2019). To the author's knowledge, no studies have focused on differences in the thickness of raspberry skins, which may be an important genomic resource that breeders could use to improve fruit defenses to various fungal diseases and provide protection to SWD. Therefore, histology comparisons of modern fruiting raspberry varieties used within breeding programmes may be a useful avenue of investigation in the future; this procedure was used successfully to assess differences in skin thickness across apple varieties (Homutová & Blažek, 2006).

Differences were noted in the susceptibility to stigmata infections; however, this may have been due to plants having asymptomatic infections of *Phytophthora* causing increased senescence of tissues that would naturally decay, providing increased media for *Cladosporium* to colonise. It indicates that overall plant health may also be an important tool for managing *Cladosporium* outbreaks, and consequently, growers need to source plant material with high hygiene standards. The positioning of stigmata on raspberry flowers have also been studied as growers observed more open flowers to have less *Cladosporium*

infections on subsequent fruit. Flower morphology may impact the air flow and humidity around these plant organs to affect fungal germination, but may also impact pollinating insects' ability to access the nectar in flowers, with excess nectar providing a food source for saprophytic fungi. The morphology of flowers in a breeding programme were shown to have considerable variation, and may therefore be another trait that breeders could monitor to reduce the severity of *Cladosporium* (Luz *et al.* 2023). It is also important to consider other phenotypic traits, such as lateral branching, which could impact the air flow around the fruit surface and the amount of potential abrasions to the fruit surface. If robotic pickers are commercially developed and produced extensively in the future, it is likely that less dense lateral branching will be selected for to allow better fruit presentation, and therefore decrease canopy density and potentially the humidity around the fruit. This is just one of many potential traits that could be investigated further for their impact on raspberry susceptibility to various fungal pathogens.

The limitations of the work described include a lack of taking samples throughout the growing season, if more funding could be secured in the future for more replicates over time, it would provide insight on how fruit microbiomes, airborne inoculum and BCA efficacy changes over the season. The BCAs tested in this study were commercially available, but to optimise BCAs for raspberries, it may be beneficial to isolate microorganisms directly from the raspberry epicarp that are adapted to survive in that environmental niche. All of the BCA and variety susceptibility screens conducted on fruits did not include cool-chain management, hence further repeats including this will provide results more directly applicable to growers.

As *Cladosporium* appears to be more of a problem on raspberries than other soft fruit crops, despite being highly abundant on other fruit surfaces (Jones *et al.*, 2022), it indicates that raspberries have a unique niche that *Cladosporium* can exploit. Multiple factors may contribute towards this, such as raspberries being one of the softest and thin-skinned fruits available. Their fruit and plant architecture may allow for more humid air to collect around the fruits to make them susceptible to *Cladosporium* infection. Raspberries are also highly

susceptible to SWD which facilitates damage to the fruit surface that *Cladosporium* can subsequently colonise (Swett. *et al.*, 2019). When growers experience severe problems with *Cladosporium* on raspberries, it is likely an indicator of issues within the overall management of the crop; for instance, an outbreak of SWD or aphids, not venting the tunnels in cooler years (allowing humidity to rise), *etc.* As climate change is making extreme weather events more common, there may be more years where *Cladosporium* can become a problem. To future proof sustainable soft fruit production in the U.K., more work is required to understand how the changing climate will affect plant susceptibility to diseases, and how pathogen presence and viability will be affected.

In order to provide sustainable management strategies for plant pathogens, we need to consider how their ecology will change with the changing climate. The U.K. is predicted to become both warmer in the summer and winter, and to become wetter over the winter (Met office, 2022). The drier weather seen during the days in summer is predicted to cause an increase in dry airborne spores in the U.K. air (Lam *et al.*, 2024). This increase in primary inoculum may increase disease risk if the humidity is high enough in the nighttime period to allow for spore germination. In Poland, they found that the warmer temperatures recorded in the time period of their study coincided with higher numbers of *Cladosporium* spores and an extended period of spores being present in the air (Kasprzyk *et al.*, 2016), further raising concerns of how the future climate may contribute to a higher inoculum load in the air. In contrast, a more complex pattern of airborne *Cladosporium* spores and changes in climate were found in France, with different locations showing different patterns in spore numbers (Sindt, Besancenot & Thibaudon, 2016). Spore loads typically decrease in the winter months (Olsen *et al.*, 2020), however the warmer winter months may impact this pattern in future years. If *Cladosporium* spores can survive over the winter months, plants may be at a higher risk earlier in the year if there is high rainfall increasing humidity in early cropping varieties. It is also important to consider that with trying to utilise more sustainable control measures for *Cladosporium* is desirable, most biological control agents used in the U.K. are currently not optimised for application in excessively warm and dry conditions. It may be important to begin screening

BCAs that can withstand these conditions to future-proof control measures, or look at alternatives such as chemical fungicides derived from BCAs that do not rely on organisms that need to survive in an adverse environment.

Based on the findings of this thesis, there are some preliminary recommendations that can be given to raspberry growers. As *Cladosporium* species are saprophytic and will colonise most available dead plant material, growers should keep plant hygiene standards high within their tunnels. This includes removing dead material such as pruning waste from their tunnels and within the crop canopy, so that less local inoculum is produced inside of the tunnels. It is also important to consider how multiple insects may interact with raspberry plants to exacerbate *Cladosporium* outbreaks. The pest SWD should always be well managed within raspberry tunnels due to the damage they cause during ovipositing which can allow readily available food sources for fungi (Swett *et al.* 2019). The same reasoning also applies to outbreaks of aphids, where the honeydew they excrete will provide *Cladosporium* a readily accessible food resource (Gordon *et al.*, 1997). It is also important to consider the role of pollinating insects which can feed on excess nectar produced within flowers to prevent nectar building up on the raspberry fruit shoulder, allowing subsequent rots to develop. This is particularly important in areas such as glasshouses which will need to provide enough pollinators present for the number of flowers within. While venting polytunnels may lead to higher dispersal of *Cladosporium* spores by either allowing spores in from the outside or by moving the crop canopy, the subsequent reduction in humidity will prevent the germination of spores on plant material. As shown by the fruit microbiome experiment, *Cladosporium* can be highly abundant and not cause visible fruit rotting symptoms. Based on the fact that *Cladosporium* is also a highly abundant airborne spore (Kurkela, 1997), it is likely that basing disease risk on spore numbers in the air will be a less effective model than using the environmental conditions conducive to germination and growth. Finally, while the results from the biological control agent screens were promising, we advise

growers to discuss with a BASIS certified agronomist before considering their applications. Some of the products tested were not licensed for use on raspberries (but have subsequently been approved since testing), and all products were applied at their maximum recommended label dose and sprayed with a large water rate. The efficacy of these products may therefore vary greatly when used by growers. Further testing of these products are therefore needed closer to industry applications to provide better advice to growers and agronomists.

This work has built a broader understanding of the epidemiology of *Cladosporium* on raspberry and indicated management strategies that need further investigation (Fig. 7-1). Based on the results of these experiments, further work is needed to understand how cultural, husbandry and management practices can be combined to form a cohesive IPM strategy against *Cladosporium* on raspberries. It highlights that reduced chemical pesticide inputs, changing production systems and climate change may inadvertently lead to new emerging pathogens that growers will need to combat in the future.

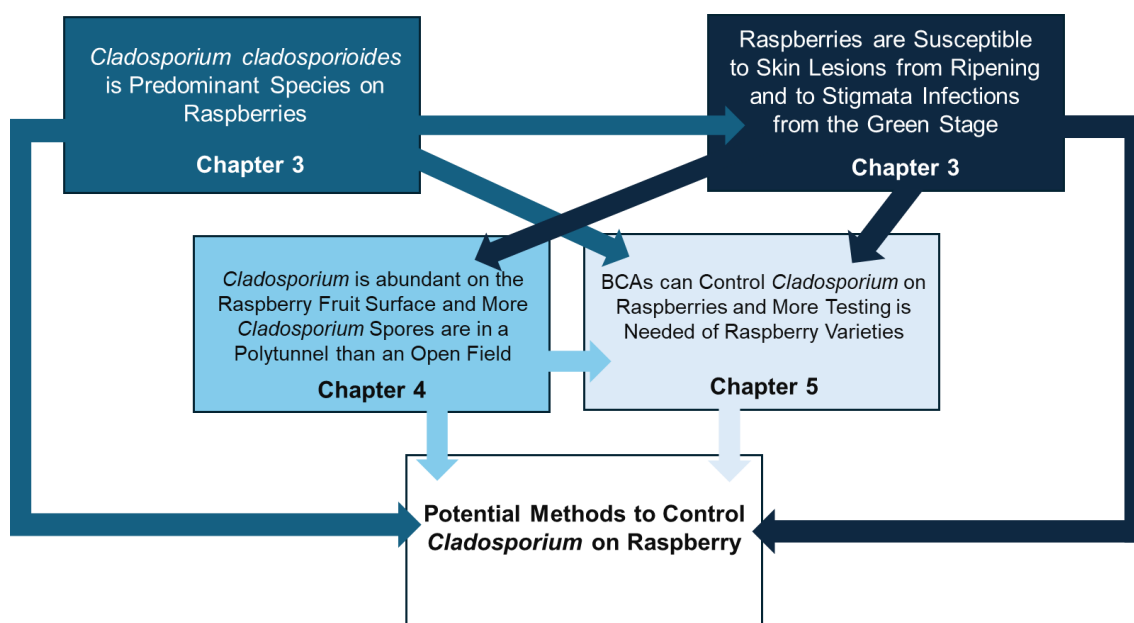


Figure 7-1. A schematical representation of the results from each of the studies conducted to show how they link to inform potential methods to control *Cladosporium* on raspberry.

8 Suggestions for Future Work

- This study found multiple species of *Cladosporium* that were present on raspberries. Koch's postulates were only confirmed for the most prevalent species (*C. cladosporioides*), hence confirming Koch's postulates for the other species isolated from raspberry is needed.
- The stages of fruit development susceptible to *Cladosporium* fruit rot were identified, however there is currently no knowledge of how long *Cladosporium* spores can survive and remain viable. This will provide insight into how long *Cladosporium* spores can remain latent on fruit to cause subsequent infection (pre- or post-harvest).
- Understanding how microbial communities behave on the fruit surface provides further insight into how pathogens interact with the complex communities they reside within. Investigating these communities over the growing season (which is long for raspberries; that can fruit from May to November in the U.K.) will provide a wider breadth of how abiotic factors impact the epiphytic microbiome. It would also benefit growers to see if similar trends occur across multiple varieties of raspberries to understand if disease risk changes. As *Rouxiella* is a genus that was previously shown to have biocontrol agent potential, it may be beneficial to attempt isolation of *Rouxiella* strains from raspberries for further testing.
- As *Cladosporium* spores were higher inside polytunnels, investigating if venting tunnels can decrease the airborne spore load would aid in providing suggestions of cultural management for growers. It would also benefit to understand how *Cladosporium* on the fruit is impacted by such management practices to give informed advice to growers and agronomists. This was a relatively short time frame of investigating

Cladosporium towards the end of the growing season, hence further repeats throughout the growing season will provide more information on *Cladosporium* primary inoculum. As this was the first study investigating polytunnel infrastructures impact on pathogen inoculum, further work on other pathogens will aid in understanding the aetiology of more soft fruit pathogens.

- Biological Control Agents were effective at controlling *Cladosporium* in field applications, however, repeats of these experiments using industry standard sprayers with realistic spray coverage and testing doses of the BCAs are needed. It would also benefit to include cool-chain management within these experiments to see if the incidence of skin lesions can be further reduced.
- Further screening of modern raspberry varieties is needed to determine which vary in their susceptibility to *Cladosporium* skin lesions and stigmata infections. Using a larger number of plants that are split into periods within the season they are fruiting will provide information more relevant for growers.
- Future work surveying the conditions within different growing systems (e.g. temperature, RH, crop density, windspeed, etc.) and then assessing for the prevalence of *Cladosporium* would provide further insight on how cultural practices impact the susceptibility of fruit to *Cladosporium*.

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APPENDICES

Appendix A – Supplementary Material

A.1 *Cladosporium* Species: The Predominant Species Present on Raspberries from the U.K. and Spain and Their Ability to Cause Skin and Stigmata Infections

Table A-1. The primer sequences used for the amplifications of the ACT and TEF1 α regions.

Primer Name	Sequence (5'-3')
EF1-728F	ATCGAGAAGTTCGAGAAGG
EF-2R	GGARGTACCAGTSATCATGTT
ACT-512F	ATGTGCAAGGCCGGTTTCGC
ACT-783R	TACGAGTCCTTCTGGCCCAT

Table A-2. The reference sequences used for the ACT and TEF1 α phylogenetic trees.

Species	Culture Accession Number	ACT	TEF1 α
<i>Cladosporium cladosporioides</i>	CBS 112388	HM148490.1	HM148244.1
<i>Cladosporium cladosporioides</i>	CPC 11120	HM148504.1	HM148258.1
<i>Cladosporium cladosporioides</i>	CPC 11121	HM148505.1	HM148259.1
<i>Cladosporium cladosporioides</i>	CPC 13669	HM148522.1	HM148276.1
<i>Cladosporium</i>	CPC 14238	HM148542.1	HM148297.1

<i>Europaeum</i>			
<i>Cladosporium europaeum</i>	CPC 14296	HM148543.1	HM148298.1
<i>Cladosporium fusiforme</i>	CBS 452.71	EF101371.1	–
<i>Cladosporium fusiforme</i>	CBS 119414	KJ596640.1	–
<i>Cladosporium limoniforme</i>	CPC 22394	MF473985.1	MF473564.1
<i>Cladosporium ramotenellum</i>	CBS 121628	EF679538.1	EF679462.1
<i>Cladosporium ramotenellum</i>	CPC 19119	MF474080.1	MF473653.1
<i>Cladosporium sphaerospermum</i>	CBS 193.54	EU570269.1	EU570261.1
<i>Cladosporium sphaerospermum</i>	CPC 12476	EU570271.1	EU570264.1
<i>Cladosporium sphaerospermum</i>	CPC 13368	EU570272.1	EU570265.1
<i>Cercospora beticola</i>	CBS 116456	AY840458.1	AY840494.1
<i>Cladosporium fusiforme</i>	EXF 449	EF101372.1	JN906988.1
<i>Cladosporium aciculare</i>	CBS 140488	KT600607.1	KT600509.1
<i>Cladosporium velox</i>	CPC 22359	MF474158.1	MF473731.1
<i>Cladosporium coloradense</i>	CPC 22238	MF473795.1	MF473372.1

Table A-3. The number of raspberries inoculated for the 2020 stigmata infection study at each inoculation date.

Inoculation Date	Sterilised	Fruit Stage	N
09/09/2020	Sterile	Green	379
09/09/2020	Unsterile	Green	172
09/09/2020	Sterile	Ripening	57
09/09/2020	Unsterile	Ripening	55
09/09/2020	Sterile	Ripe	34
09/09/2020	Unsterile	Ripe	94
16/09/2020	Sterile	Green	277
16/09/2020	Unsterile	Green	89
16/09/2020	Sterile	Ripening	73
16/09/2020	Unsterile	Ripening	45
16/09/2020	Sterile	Ripe	45
16/09/2020	Unsterile	Ripe	40
23/09/2020	Sterile	Green	201

A.2 Investigating the Inoculum Dynamics of *Cladosporium* on the Surface of Raspberry Fruits and in the Air

Table A-4. The primer sequences used in Amplicon Metagenomic Sequencing (sourced from Novogene U.K.) and *Cladosporium* genus qPCRs.

Types	Region	Fragment Length	Primer	Primer Sequence 5'-3'
Bacterial 16S	V3-V4	466bp	341F	CCTAYGGGRBGCASCAG
			806R	GGACTACNNGGGTATCTAAT
Fungal ITS	ITS1-1F	321bp	ITS1-1F-F	CTTGGTCATTTAGAGGAAGTAA
			ITS1-1F-R	GCTGCGTTCTTCATCGATGC
<i>Cladosporium</i> Genus Primer	mt SSU rDNA	87bp	Clado-SYBRG-PF	TACTCCAATGGTTCTAATATTTTCCTCTC
			Clado-SYBRG-PR	GGGTACCTAGACAGTATTTCTAGCCT

Table A-5. The most abundant ASVs present in the samples for the 16S and ITS regions used to create synthetic genes (gBlocks) for use as a qPCR standard for estimating microbial community mass.

Metabarcoding Region	Sequence
16S	CCTACGGGGTGCAGCAGCAGCAGTGGGGAATATTGCACAATGGGCG CAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGT TGTAAGTACTTTACGCGGGGAGGAAGGCGATAAGGTTAATAACCTT GTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCC AGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACT GGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCAGATGTGAAATC CCCGGGCTTAACCTGGGAACTGCATTTGAACTGGCAGGCTAGAGT CTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAG AGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGA CTGACGCTCAGGTGCGAAAGCGTG GGGAGCAAACAGGATTAGAATT AGATACCCGAGTAGTCC
ITS	CTTGGTCATTTAGAGGAAGTAAAAGTAAAAGTCGTAACAAGGTCTCC GTAGGTGAACCTGCGGAGGGATCATTACAAGTGACCCCGGTCTAAC CACCGGGATGTTTCATAACCCTTTGTTGTCCGACTCTGTTGCCTCCGG GGCGACCCTGCCTTCGGGCGGGGGCTCCGGGTGGACACTTCAAAC TCTTGCGTAACTTTGCAGTCTGAGTAACTTAATTAATAAATTA AAACT TTTAACAACGGATCTCTTGTTCTGGCATCGGCATCGATGAAGAACG CAGC

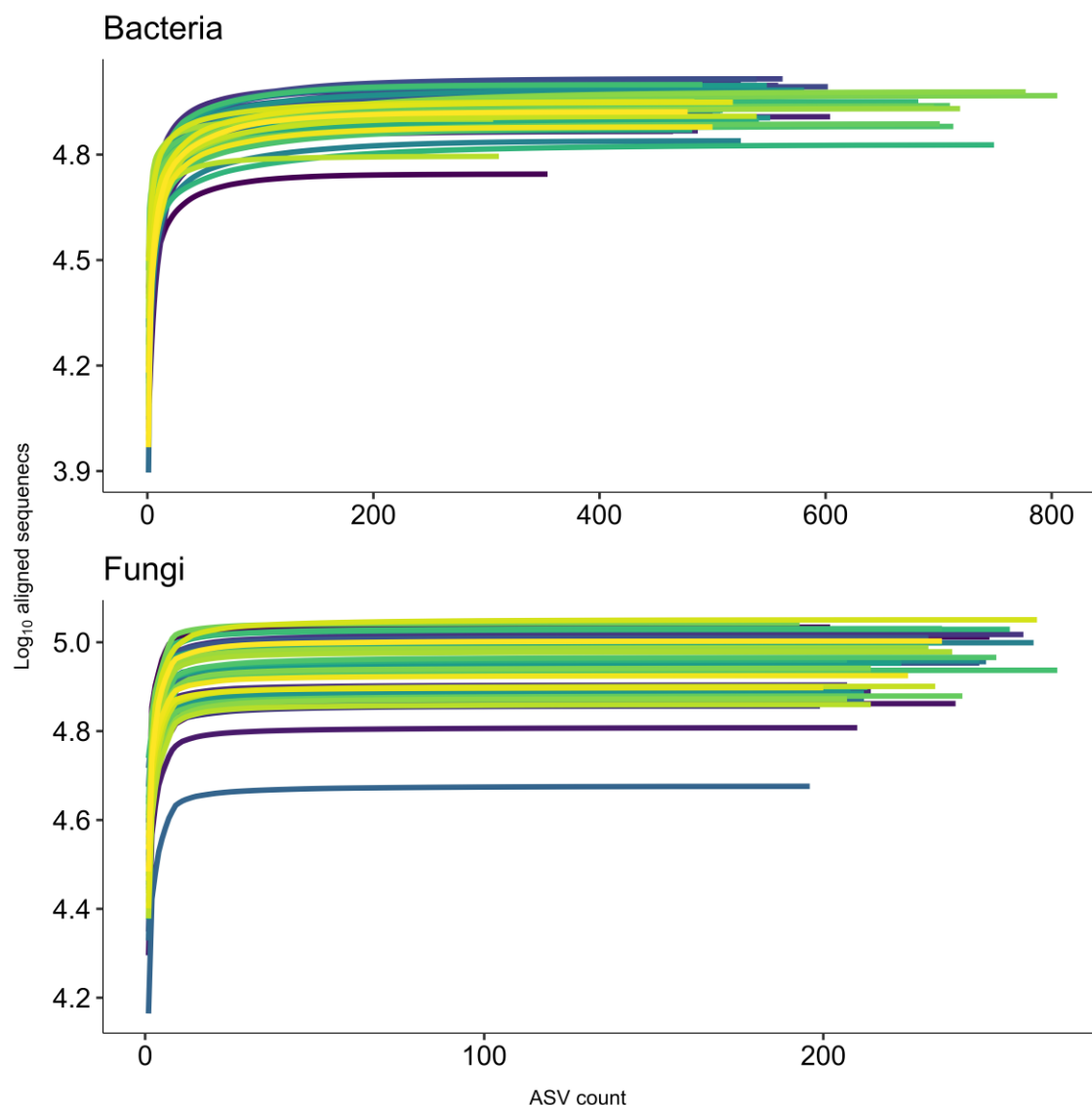


Figure A-1. Rarefaction curves of $\log_{10}$ ASV counts of fungal and bacterial ASVs.

Table A-6. Fungal ASVs that significantly differ ($p < 0.05$, Benjamini-Hochberg (BH) adjusted) across raspberry fruit ages. A positive $\log_2$ fold change indicates a higher relative abundance in the factor listed first.

Factor Comparison	ASV ID	Taxonomy	Base Mean	$\log_2$ Fold Change
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Green vs. Ripening	ASV121	<i>Basidiomycota</i> (p)	5.68	2.26
	ASV166	<i>Ascomycota</i> (p)	3.27	1.52
	ASV57	<i>Ascomycota</i> (p)	27.56	0.91
	ASV1	<i>Cladosporium</i> (g)	29411	0.56
	ASV2	<i>Ascomycota</i> (p)	23509	-0.51
	ASV3	<i>Ascomycota</i> (p)	6959	-0.57
	ASV5	<i>Ascomycota</i> (p)	3877	-0.57
	ASV51	<i>Ascomycota</i> (p)	19.63	-0.6
	ASV17	<i>Epicoccum nigrum</i> (s)	269.3	-0.66
	ASV56	<i>Ascomycota</i> (p)	17.78	-0.66
	ASV78	<i>Ascomycota</i> (p)	17.24	-0.75
	ASV32	<i>Ascomycota</i> (p)	42.66	-0.8
	ASV39	<i>Hypocreales</i> (o)	33.68	-0.8
	ASV40	<i>Hypocreales</i> (o)	31.1	-0.83
	ASV55	<i>Pithomyces chartarum</i> (s)	27.49	-0.84
	ASV46	<i>Vishniacozyma carnescens</i> (s)	27.67	-0.87
	ASV54	<i>Epicoccum nigrum</i> (s)	28.97	-0.91
	ASV58	<i>Sarocladium strictum</i> (s)	20.57	-0.93
	ASV37	<i>Vishniacozyma victoriae</i> (s)	42.9	-0.95
	ASV20	<i>Fungi</i> (k)	199.86	-1.02
	ASV18	<i>Fungi</i> (k)	246.23	-1.08
	ASV22	<i>Wallemia sebi</i> (s)	99.72	-1.12
	ASV24	<i>Fungi</i> (k)	84.37	-1.14

	ASV131	<i>Peniophora</i> (g)	4.44	-1.25
	ASV112	<i>Vishniacozyma victoriae</i> (s)	4.76	-1.37
	ASV48	<i>Podosphaera</i> (g)	28.47	-1.4
	ASV61	<i>Tremellales</i> (o)	15.04	-1.4
	ASV83	<i>Tremellomycetes</i> (c)	7.22	-1.56
	ASV153	<i>Nectriaceae</i> (f)	2.2	-1.65
	ASV110	<i>Vishniacozyma victoriae</i> (s)	5.12	-1.67
	ASV77	<i>Saccharomycetes</i> (c)	6.55	-1.68
	ASV143	<i>Dioszegia hungarica</i> (s)	1.69	-1.7
	ASV111	<i>Vishniacozyma tephrensensis</i> (s)	4.56	-1.75
	ASV81	<i>Plectosphaerellaceae</i> (f)	8.45	-2.24
	ASV104	<i>Mucor mucedo</i> (s)	11.02	-2.87
Green vs. Ripe	ASV168	<i>Ascomycota</i> (p)	1.96	2.78
	ASV102	<i>Basidiomycota</i> (p)	8.84	2.44
	ASV121	<i>Basidiomycota</i> (p)	5.68	2.2
	ASV57	<i>Ascomycota</i> (p)	27.56	1.1
	ASV1	<i>Cladosporium</i> (g)	29411.2	0.5
	ASV40	<i>Hypocreales</i> (o)	31.1	-0.65
	ASV39	<i>Hypocreales</i> (o)	33.68	-0.82
	ASV58	<i>Sarocladium strictum</i> (s)	20.57	-1.12
	ASV72	<i>Plectosphaerella</i> (g)	11.54	-1.26
	ASV48	<i>Podosphaera</i> (g)	28.47	-1.4
	ASV81	<i>Plectosphaerellaceae</i> (f)	8.45	-1.85

	ASV77	<i>Saccharomycetes</i> (c)	6.55	-2.1
	ASV119	<i>Plectosphaerella</i> (g)	3.52	-2.2
	ASV182	<i>Leotiomyces</i> (c)	2.27	-3.77
Ripening vs. Ripe	ASV104	<i>Mucor mucedo</i> (s)	11.02	2.81
	ASV54	<i>Epicoccum nigrum</i> (s)	28.97	0.95
	ASV22	<i>Wallemia sebi</i> (s)	99.72	0.93
	ASV24	<i>Fungi</i> (k)	84.37	0.91
	ASV20	<i>Fungi</i> (k)	199.86	0.82
	ASV51	<i>Ascomycota</i> (p)	19.63	0.81
	ASV18	<i>Fungi</i> (k)	246.2	0.71
	ASV31	<i>Entyloma dahlia</i> (s)	79.64	-1.42
	ASV119	<i>Plectosphaerella</i> (g)	3.52	-2.8

(k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family, (g) denotes genus and (s) denotes species.

Table A-7. Fungal ASVs that significantly differ ($p < 0.05$, Benjamini-Hochberg (BH) adjusted) across locations within a polytunnel. A positive $\log_2$ fold change indicates a higher relative abundance in the inside of polytunnel vs. the outer edge.

ASV ID	Taxonomy	Base Mean	Log ₂ Fold Change
ASV197	<i>Podosphaera</i> (g)	1.61	1.52
ASV63	<i>Bullera</i> (g)	14.35	1.5
ASV48	<i>Podosphaera</i> (g)	28.47	1.31
ASV53	<i>Neovysiphe galeopsidis</i> (s)	30.95	1.3

ASV74	<i>Corynespora cassiicola</i> (s)	27.79	1.24
ASV80	<i>Didymellaceae</i> (f)	25.11	1.08
ASV97	<i>Penicillium adametzioides</i> (s)	11.84	0.68
ASV24	<i>Fungi</i> (k)	84.37	0.52
ASV20	<i>Fungi</i> (k)	199.9	0.33
ASV13	<i>Botrytis</i> (g)	466.8	-0.39
ASV7	<i>Cladosporium ramotenellum</i> (s)	2746	-0.45
ASV60	<i>Cladosporium</i> (g)	14.63	-0.54
ASV15	<i>Cladosporium</i> (g)	255.1	-0.55
ASV44	<i>Mycosphaerellaceae</i> (f)	30.24	-0.55
ASV58	<i>Sarocladium strictum</i> (s)	20.57	-0.58
ASV29	<i>Golovinomyces orontii</i> (s)	78.16	-0.6
ASV67	<i>Saccharomycetes</i> (c)	16.52	-0.69
ASV42	<i>Sarocladium kiliense</i> (s)	39.68	-0.89
ASV117	<i>Hypocreales</i> (o)	4.89	-0.89
ASV251	<i>Dissoconium aciculare</i> (s)	5.6	-0.9
ASV92	<i>Vishniacozyma victoriae</i> (s)	7.08	-0.91
ASV11	<i>Cladosporium iridis</i> (s)	610.8	-1
ASV99	<i>Ramularia collo-cygni</i> (s)	5.61	-1.02
ASV37	<i>Vishniacozyma victoriae</i> (s)	42.9	-1.04
ASV166	<i>Ascomycota</i> (p)	3.27	-1.06
ASV118	<i>Ascomycota</i> (p)	3.38	-1.13
ASV35	<i>Pichia terricola</i> (s)	30	-1.16

ASV114	<i>Sordariomycetes</i> (c)	3.59	-1.18
ASV132	<i>Podosphaera fuliginea</i> (s)	3.84	-1.18
ASV75	<i>Ramularia</i> (g)	10.28	-1.19
ASV111	<i>Vishniacozyma tephrensensis</i> (s)	4.56	-1.19
ASV112	<i>Vishniacozyma victoriae</i> (s)	4.76	-1.21
ASV46	<i>Vishniacozyma carnescens</i> (s)	27.67	-1.23
ASV198	<i>Mycosphaerellaceae</i> (f)	2.66	-1.23
ASV28	<i>Pichia terricola</i> (s)	44.68	-1.27
ASV45	<i>Sporidiobolus</i> (g)	29.79	-1.27
ASV145	<i>Blumeria graminis</i> (s)	3.35	-1.27
ASV69	<i>Alternaria</i> (g)	23.01	-1.41
ASV17	<i>Epicoccum nigrum</i> (s)	269.3	-1.46
ASV124	<i>Golovinomyces</i> (g)	4.23	-1.48
ASV126	<i>Hypocreales</i> (o)	3.3	-1.48
ASV175	<i>Tremellomycetes</i> (c)	1.44	-1.51
ASV54	<i>Epicoccum nigrum</i> (s)	28.97	-1.52
ASV169	<i>Itersonilia perplexans</i> (s)	2.13	-1.54
ASV173	<i>Didymella</i> (g)	3.86	-1.56
ASV83	<i>Tremellomycetes</i> (c)	7.22	-1.57
ASV177	<i>Vishniacozyma victoriae</i> (s)	1.35	-1.63
ASV61	<i>Tremellales</i> (o)	15.04	-1.66
ASV85	<i>Vishniacozyma victoriae</i> (s)	8.89	-1.66
ASV190	<i>Sarocladium implicatum</i> (s)	1.77	-1.66

ASV36	<i>Alternaria</i> (g)	96.73	-1.67
ASV244	<i>Ramularia</i> (g)	3.25	-1.67
ASV259	<i>Peniophora</i> (g)	2.62	-1.69
ASV91	<i>Ascomycota</i> (p)	4.32	-1.74
ASV141	<i>Stemphylium vesicarium</i> (s)	3.03	-1.8
ASV161	<i>Mycosphaerellaceae</i> (f)	2.36	-1.8
ASV130	<i>Tremellomycetes</i> (c)	2.67	-1.83
ASV98	<i>Sporobolomyces ruberrimus</i> (s)	5.64	-1.89
ASV167	<i>Vishniacozyma victoriae</i> (s)	2.26	-1.91
ASV86	<i>Vishniacozyma victoriae</i> (s)	7.74	-1.93
ASV93	<i>Alternaria metachromatica</i> (s)	11.94	-1.93
ASV55	<i>Pithomyces chartarum</i> (s)	27.49	-1.95
ASV100	<i>Tremellales</i> (o)	4.32	-1.99
ASV157	<i>Saccharomycetales</i> (o)	2.1	-2.02
ASV184	<i>Fusarium cerealis</i> (s)	1.5	-2.04
ASV170	<i>Cystofilobasidium macerans</i> (s)	1.64	-2.13
ASV189	<i>Rhodotorula babjevae</i> (s)	1.98	-2.13
ASV108	<i>Vishniacozyma carnescens</i> (s)	4.89	-2.15
ASV115	<i>Tremellales</i> (o)	3.16	-2.27
ASV140	<i>Ascomycota</i> (p)	2.1	-2.28
ASV195	<i>Monilinia fructigena</i> (s)	1.19	-2.34
ASV201	<i>Vishniacozyma victoriae</i> (s)	1.37	-2.4
ASV104	<i>Mucor mucedo</i> (s)	11.02	-2.61

ASV136	<i>Dothideomycetes</i> (c)	2.22	-2.64
ASV77	<i>Saccharomycetes</i> (c)	6.55	-2.8

(k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family, (g) denotes genus and (s) denotes species.

Table A-8. Bacterial ASVs that significantly differ ($p < 0.05$, Benjamini-Hochberg (BH) adjusted) across raspberry fruit ages. A positive $\log_2$ fold change indicates a higher relative abundance in the factor listed first.

Factor Comparison	ASV ID	Taxonomy	Base Mean	Log₂ Fold Change
Green vs. Ripening	ASV62	<i>Janthinobacterium</i> (g)	145.7	5.06
	ASV208	<i>Sphingobacterium</i> (g)	18.33	4.68
	ASV80	<i>Pedobacter</i> (g)	108.5	4.6
	ASV141	<i>Paenibacillus</i> (g)	40.31	4.17
	ASV158	<i>Janthinobacterium</i> (g)	25.47	3.98
	ASV409	<i>Sphingobacterium</i> (g)	8.36	3.95
	ASV368	<i>Janthinobacterium</i> (g)	6.52	3.8
	ASV213	<i>Paenibacillus</i> (g)	23.26	3.75
	ASV321	<i>Carnobacterium</i> (g)	9.03	3.59
	ASV258	<i>Janthinobacterium</i> (g)	12.75	3.55
	ASV40	<i>Janthinobacterium</i> (g)	315.1	3.49
	ASV306	<i>Sphingobacteriaceae</i> (f)	16.79	3.48
	ASV96	<i>Listeria</i> (g)	58.76	3.35
	ASV103	<i>Janthinobacterium</i> (g)	50.62	3.35

ASV95	<i>Sphingobacterium</i> (g)	100.5	3.31
ASV61	<i>Paenibacillus</i> (g)	143.9	3.23
ASV47	<i>Paenibacillus</i> (g)	231.1	3.22
ASV49	<i>Pseudomonas</i> (g)	199	3.04
ASV301	<i>Pedobacter</i> (g)	13.06	3
ASV352	<i>Pedobacter</i> (g)	7.71	3
ASV319	<i>Pedobacter</i> (g)	8.78	2.96
ASV452	<i>Janthinobacterium</i> (g)	6.1	2.91
ASV345	<i>Sphingobacteriaceae</i> (f)	8.88	2.9
ASV54	<i>Pedobacter</i> (g)	212.98	2.83
ASV166	<i>Erwinia</i> (g)	27.34	2.77
ASV322	<i>Chryseobacterium</i> (g)	9.87	2.76
ASV125	<i>Pseudomonas</i> (g)	80.9	2.7
ASV313	<i>Massilia</i> (g)	13.05	2.65
ASV153	<i>Janthinobacterium</i> (g)	26.53	2.54
ASV184	<i>Pedobacter</i> (g)	28.85	2.34
ASV43	<i>Pseudomonas</i> (g)	202.8	2.32
ASV76	<i>Pedobacter</i> (g)	77.41	2.25
ASV74	<i>Bacillus</i> (g)	205.1	2.24
ASV200	<i>Rhizobiales</i> (o)	12.43	2.19
ASV259	<i>Comamonadaceae</i> (f)	9.46	2.19
ASV239	<i>Bacillus</i> (g)	44.65	2.15
ASV132	<i>Chryseobacterium</i> (g)	39.27	2.1

ASV217	<i>Duganella</i> (g)	14.88	2.1
ASV157	<i>Pedobacter</i> (g)	39.34	2
ASV106	<i>Pedobacter</i> (g)	56.21	1.78
ASV115	<i>Devosia</i> (g)	18.49	1.78
ASV122	<i>Comamonadaceae</i> (f)	46.48	1.76
ASV38	<i>Enterobacterales</i> (o)	258.2	1.72
ASV32	<i>Ewingella</i> (g)	421.7	1.67
ASV422	<i>Pseudomonas</i> (g)	19.45	1.55
ASV150	<i>Duganella</i> (g)	30.42	1.42
ASV924	<i>Pseudomonas</i> (g)	22	1.39
ASV110	<i>Duganella</i> (g)	60.44	1.33
ASV136	<i>Duganella</i> (g)	43.95	1.3
ASV787	<i>Erwiniaceae</i> (f)	42.56	1.04
ASV1134	<i>Pseudomonas</i> (g)	24.15	1.03
ASV36	<i>Pseudomonas</i> (g)	516.8	-0.79
ASV8	<i>Pseudomonas</i> (g)	3286	-0.83
ASV1081	<i>Pseudomonas</i> (g)	70.19	-0.87
ASV365	<i>Pseudomonas</i> (g)	15.63	-1.08
ASV29	<i>Pseudomonas</i> (g)	493.1	-1.11
ASV108	<i>Pseudomonas</i> (g)	114.5	-1.12
ASV800	<i>Pseudomonadaceae</i> (f)	103.9	-1.31
ASV1115	<i>Yersiniaceae</i> (f)	84.51	-1.33
ASV31	<i>Pseudomonas</i> (g)	562.1	-1.46

	ASV806	<i>Gammaproteobacteria</i> (c)	104.8	-1.49
	ASV2	<i>Pseudomonas</i> (g)	5081	-1.56
	ASV21	<i>Rouxiella</i> (g)	655.1	-1.71
	ASV165	<i>Phyllobacteriaceae</i> (f)	17.84	-1.9
	ASV1205	<i>Enterobacterales</i> (o)	47.15	-2.06
	ASV24	<i>Rouxiella</i> (g)	686.3	-2.13
	ASV130	<i>Pantoea</i> (g)	52.99	-2.15
	ASV195	<i>Yersiniaceae</i> (f)	26.55	-2.65
	ASV503	<i>Rickettsia</i> (g)	4.63	-3.2
	ASV48	<i>Rouxiella</i> (g)	279	-3.24
	ASV140	<i>Bacteria</i> (k)	11.01	-3.91
Green vs. Ripe	ASV280	<i>Bacteria</i> (k)	3.59	13.83
	ASV1045	<i>Pedobacter</i> (g)	2.21	10.55
	ASV80	<i>Pedobacter</i> (g)	108.5	6.75
	ASV141	<i>Paenibacillus</i> (g)	40.31	5.45
	ASV54	<i>Pedobacter</i> (g)	213	4.92
	ASV166	<i>Erwinia</i> (g)	27.34	4.87
	ASV96	<i>Listeria</i> (g)	58.76	4.8
	ASV62	<i>Janthinobacterium</i> (g)	145.7	4.72
	ASV306	<i>Sphingobacteriaceae</i> (f)	16.79	4.67
	ASV68	<i>Sphingobacterium</i> (g)	63.58	4.66
	ASV208	<i>Sphingobacterium</i> (g)	18.33	4.6
	ASV95	<i>Sphingobacterium</i> (g)	100.5	4.58

ASV76	<i>Pedobacter</i> (g)	77.41	4.53
ASV322	<i>Chryseobacterium</i> (g)	9.87	4.28
ASV821	<i>Alkalihalobacillus</i> (g)	7.56	4.06
ASV409	<i>Sphingobacterium</i> (g)	8.36	4.05
ASV239	<i>Bacillus</i> (g)	44.65	4.03
ASV189	<i>Buttiauxella</i> (g)	18.05	4.01
ASV559	<i>Carnobacterium</i> (g)	4.6	3.99
ASV158	<i>Janthinobacterium</i> (g)	25.47	3.94
ASV258	<i>Janthinobacterium</i> (g)	12.75	3.93
ASV103	<i>Janthinobacterium</i> (g)	50.62	3.92
ASV77	<i>Pseudomonas</i> (g)	110.9	3.9
ASV321	<i>Carnobacterium</i> (g)	9.03	3.85
ASV171	<i>Sphingobacterium</i> (g)	20.33	3.79
ASV125	<i>Pseudomonas</i> (g)	80.9	3.56
ASV232	<i>Erwinia</i> (g)	15.14	3.55
ASV192	<i>Pedobacter</i> (g)	9.43	3.46
ASV150	<i>Duganella</i> (g)	30.42	3.43
ASV313	<i>Massilia</i> (g)	13.05	3.42
ASV592	<i>Mucilaginibacter</i> (g)	2.61	3.39
ASV184	<i>Pedobacter</i> (g)	28.85	3.33
ASV555	<i>Massilia</i> (g)	4.42	3.31
ASV666	<i>Pedobacter</i> (g)	8.17	3.31
ASV301	<i>Pedobacter</i> (g)	13.06	3.29

ASV74	<i>Bacillus</i> (g)	205.1	3.24
ASV505	<i>Massilia</i> (g)	3.35	3.16
ASV259	<i>Comamonadaceae</i> (f)	9.46	3.15
ASV145	<i>Pedobacter</i> (g)	7.21	3.07
ASV284	<i>Pedobacter</i> (g)	13.97	3.03
ASV122	<i>Comamonadaceae</i> (f)	46.48	3
ASV615	<i>Microbacteriaceae</i> (f)	2.54	2.96
ASV620	<i>Microbacteriaceae</i> (f)	2.91	2.9
ASV514	<i>Bacillaceae</i> (f)	5.46	2.89
ASV452	<i>Janthinobacterium</i> (g)	6.1	2.88
ASV722	<i>Pseudomonas</i> (g)	21.94	2.88
ASV586	<i>Pedobacter</i> (g)	8.17	2.87
ASV684	<i>Alkalihalobacillus</i> (g)	4.22	2.84
ASV40	<i>Janthinobacterium</i> (g)	315.1	2.8
ASV319	<i>Pedobacter</i> (g)	8.78	2.76
ASV667	<i>Massilia</i> (g)	2.61	2.63
ASV43	<i>Pseudomonas</i> (g)	202.8	2.56
ASV157	<i>Pedobacter</i> (g)	39.34	2.39
ASV950	<i>Stenotrophomonas</i> (g)	49.2	2.37
ASV49	<i>Pseudomonas</i> (g)	199	2.34
ASV41	<i>Pseudomonas</i> (g)	293.7	2.27
ASV115	<i>Devosia</i> (g)	18.49	2.27
ASV686	<i>Pseudomonas</i> (g)	8.73	2.21

ASV193	<i>Enterobacterales</i> (o)	22.71	2.2
ASV491	<i>Pseudomonas</i> (g)	7.45	2.19
ASV179	<i>Pedobacter</i> (g)	12.83	2.18
ASV760	<i>Erwinia</i> (g)	7.81	2.16
ASV217	<i>Duganella</i> (g)	14.88	2.13
ASV250	<i>Enterobacteriaceae</i> (f)	16.88	2.08
ASV1214	<i>Enterobacterales</i> (o)	12.23	2.08
ASV42	<i>Brucella</i> (g)	151.8	2.06
ASV902	<i>Pseudomonas</i> (g)	3.17	2.06
ASV71	<i>Pseudomonas</i> (g)	111.5	1.98
ASV15	<i>Stenotrophomonas</i> (g)	1055	1.96
ASV1134	<i>Pseudomonas</i> (g)	24.15	1.96
ASV898	<i>Pseudomonadaceae</i> (f)	4.65	1.94
ASV376	<i>Massilia</i> (g)	6.24	1.87
ASV136	<i>Duganella</i> (g)	43.95	1.85
ASV52	<i>Pseudomonas</i> (g)	298.7	1.84
ASV127	<i>Enterobacteriaceae</i> (f)	34.94	1.84
ASV924	<i>Pseudomonas</i> (g)	22	1.84
ASV93	<i>Pedobacter</i> (g)	59.94	1.83
ASV456	<i>Pedobacter</i> (g)	9.11	1.82
ASV780	<i>Stenotrophomonas</i> (g)	20.29	1.82
ASV106	<i>Pedobacter</i> (g)	56.21	1.8
ASV225	<i>Pigmentiphaga</i> (g)	12.68	1.77

ASV1100	<i>Betaproteobacteria</i> (c)	45.62	1.75
ASV12	<i>Pseudomonas</i> (g)	2272	1.7
ASV38	<i>Enterobacterales</i> (o)	258.2	1.7
ASV1070	<i>Pseudomonas</i> (g)	17.53	1.69
ASV64	<i>Pseudomonas</i> (g)	195.5	1.66
ASV422	<i>Pseudomonas</i> (g)	19.45	1.66
ASV1063	<i>Pseudomonas</i> (g)	50.32	1.66
ASV110	<i>Duganella</i> (g)	60.44	1.64
ASV116	<i>Sanguibacter</i> (g)	49.5	1.63
ASV275	<i>Sanguibacter</i> (g)	17.5	1.63
ASV552	<i>Enterobacteriaceae</i> (f)	9.6	1.6
ASV32	<i>Ewingella</i> (g)	421.7	1.56
ASV773	<i>Gammaproteobacteria</i> (c)	20.4	1.49
ASV743	<i>Pseudomonas</i> (g)	106.9	1.48
ASV629	<i>Pseudomonas</i> (g)	124.5	1.47
ASV18	<i>Luteibacter</i> (g)	662.7	1.45
ASV526	<i>Pseudomonas</i> (g)	157.1	1.45
ASV938	<i>Gammaproteobacteria</i> (c)	76.17	1.44
ASV128	<i>Pseudomonas</i> (g)	42.6	1.4
ASV873	<i>Gammaproteobacteria</i> (c)	27.99	1.33
ASV744	<i>Pseudomonas</i> (g)	72.59	1.31
ASV1033	<i>Xanthomonadales</i> (o)	83.13	1.3
ASV619	<i>Pseudomonadales</i> (o)	114.2	1.26

ASV1225	<i>Pseudomonas</i> (g)	49.01	1.23
ASV63	<i>Pseudomonas</i> (g)	524.2	1.22
ASV3	<i>Pseudomonas</i> (g)	3831	1.21
ASV859	<i>Pseudomonas</i> (g)	21.16	1.2
ASV830	<i>Pseudomonas</i> (g)	14.59	1.18
ASV113	<i>Pseudomonas</i> (g)	278.6	1.15
ASV727	<i>Pseudomonadales</i> (o)	72.6	1.13
ASV750	<i>Xanthomonadales</i> (o)	47.94	1.13
ASV1200	<i>Yersiniaceae</i> (f)	74.1	1.06
ASV1193	<i>Pseudomonas</i> (g)	24.43	1
ASV146	<i>Microbacteriaceae</i> (f)	34.96	0.97
ASV892	<i>Pseudomonas</i> (g)	50.94	0.96
ASV1207	<i>Gammaproteobacteria</i> (c)	165	0.9
ASV787	<i>Erwiniaceae</i> (f)	42.56	0.87
ASV55	<i>Pseudomonas</i> (g)	211.6	0.86
ASV1084	<i>Yersiniaceae</i> (f)	85.98	0.85
ASV1230	<i>Gammaproteobacteria</i> (c)	207.2	0.85
ASV1162	<i>Yersiniaceae</i> (f)	37.79	0.82
ASV22	<i>Pseudomonas</i> (g)	1015	-1.12
ASV800	<i>Pseudomonadaceae</i> (f)	103.9	-1.15
ASV286	<i>Pseudomonas</i> (g)	24.7	-1.31
ASV2	<i>Pseudomonas</i> (g)	5081	-1.38
ASV70	<i>Rhizobium</i> (g)	67.71	-1.63

	ASV39	<i>Rhizobiaceae</i> (f)	175.1	-1.69
	ASV126	<i>Gluconobacter</i> (g)	26.33	-1.89
	ASV297	<i>Alphaproteobacteria</i> (c)	6.26	-2.1
	ASV87	<i>Pseudomonas</i> (g)	68.39	-2.22
	ASV263	<i>Enterobacterales</i> (o)	14.68	-2.3
	ASV214	<i>Bosea</i> (g)	11.78	-2.52
	ASV51	<i>Yersiniaceae</i> (f)	41.47	-3.51
Ripening vs. Ripe	ASV171	<i>Sphingobacterium</i> (g)	20.33	4.59
	ASV232	<i>Erwinia</i> (g)	15.14	4.38
	ASV48	<i>Rouxiella</i> (g)	279	3.4
	ASV68	<i>Sphingobacterium</i> (g)	63.58	3.3
	ASV102	<i>Erwinia</i> (g)	67.51	3.24
	ASV237	<i>Erwiniaceae</i> (f)	14.92	3.13
	ASV950	<i>Stenotrophomonas</i> (g)	49.2	3.1
	ASV780	<i>Stenotrophomonas</i> (g)	20.29	2.87
	ASV149	<i>Stenotrophomonas</i> (g)	19.03	2.65
	ASV195	<i>Yersiniaceae</i> (f)	26.55	2.4
	ASV666	<i>Pedobacter</i> (g)	8.17	2.34
	ASV192	<i>Pedobacter</i> (g)	9.43	2.33
	ASV76	<i>Pedobacter</i> (g)	77.41	2.28
	ASV773	<i>Gammaproteobacteria</i> (c)	20.4	2.25
	ASV89	<i>Pseudomonas</i> (g)	113.7	2.24
	ASV15	<i>Stenotrophomonas</i> (g)	1055	2.12

ASV54	<i>Pedobacter</i> (g)	213	2.09
ASV77	<i>Pseudomonas</i> (g)	110.9	2.08
ASV1214	<i>Enterobacterales</i> (o)	12.23	2.08
ASV150	<i>Duganella</i> (g)	30.42	2.01
ASV128	<i>Pseudomonas</i> (g)	42.6	2
ASV130	<i>Pantoea</i> (g)	52.99	1.97
ASV1115	<i>Yersiniaceae</i> (f)	84.51	1.91
ASV938	<i>Gammaproteobacteria</i> (c)	76.17	1.9
ASV239	<i>Bacillus</i> (g)	44.65	1.88
ASV830	<i>Pseudomonas</i> (g)	14.59	1.82
ASV1225	<i>Pseudomonas</i> (g)	49.01	1.81
ASV456	<i>Pedobacter</i> (g)	9.11	1.8
ASV226	<i>Pseudomonas</i> (g)	68.41	1.79
ASV927	<i>Gammaproteobacteria</i> (c)	129.3	1.77
ASV548	<i>Pseudomonas</i> (g)	32.78	1.75
ASV744	<i>Pseudomonas</i> (g)	72.59	1.72
ASV743	<i>Pseudomonas</i> (g)	106.9	1.68
ASV123	<i>Stenotrophomonas</i> (g)	69.84	1.64
ASV1159	<i>Pseudomonas</i> (g)	90.05	1.62
ASV873	<i>Gammaproteobacteria</i> (c)	27.99	1.57
ASV1231	<i>Pseudomonas</i> (g)	14.65	1.56
ASV113	<i>Pseudomonas</i> (g)	278.6	1.55
ASV1153	<i>Xanthomonadaceae</i> (f)	52.03	1.51

ASV985	<i>Pseudomonadales</i> (o)	42.63	1.48
ASV262	<i>Microbacteriaceae</i> (f)	14.32	1.45
ASV1200	<i>Yersiniaceae</i> (f)	74.1	1.45
ASV42	<i>Brucella</i> (g)	151.8	1.4
ASV577	<i>Yersiniaceae</i> (f)	16.06	1.4
ASV1059	<i>Pseudomonas</i> (g)	49.79	1.38
ASV1033	<i>Xanthomonadales</i> (o)	83.13	1.36
ASV17	<i>Yersiniaceae</i> (f)	1088	1.35
ASV5	<i>Yersiniaceae</i> (f)	3013	1.34
ASV1205	<i>Enterobacterales</i> (o)	47.15	1.33
ASV699	<i>Gammaproteobacteria</i> (c)	188.1	1.29
ASV275	<i>Sanguibacter</i> (g)	17.5	1.28
ASV6	<i>Pseudomonas</i> (g)	2987	1.27
ASV747	<i>Gammaproteobacteria</i> (c)	300.9	1.23
ASV1144	<i>Xanthomonadaceae</i> (f)	32.12	1.23
ASV63	<i>Pseudomonas</i> (g)	524.2	1.21
ASV8	<i>Pseudomonas</i> (g)	3286	1.14
ASV619	<i>Pseudomonadales</i> (o)	114.2	1.14
ASV593	<i>Pseudomonas</i> (g)	143.9	1.1
ASV772	<i>Gammaproteobacteria</i> (c)	144.8	1.08
ASV29	<i>Pseudomonas</i> (g)	493.1	1.06
ASV736	<i>Pseudomonadaceae</i> (f)	53.23	1.06
ASV55	<i>Pseudomonas</i> (g)	211.6	1.05

ASV108	<i>Pseudomonas</i> (g)	114.5	1.05
ASV895	<i>Xanthomonadales</i> (o)	98.63	1.04
ASV762	<i>Gammaproteobacteria</i> (c)	173.4	1.03
ASV879	<i>Gammaproteobacteria</i> (c)	229.5	1
ASV727	<i>Pseudomonadales</i> (o)	72.6	0.97
ASV1193	<i>Pseudomonas</i> (g)	24.43	0.97
ASV146	<i>Microbacteriaceae</i> (f)	34.96	0.96
ASV1178	<i>Enterobacterales</i> (o)	27.79	0.96
ASV23	<i>Rahnella</i> (g)	735.4	0.92
ASV633	<i>Pseudomonas</i> (g)	55.85	0.9
ASV1055	<i>Pseudomonas</i> (g)	58.18	0.88
ASV1207	<i>Gammaproteobacteria</i> (c)	165	0.83
ASV36	<i>Pseudomonas</i> (g)	516.8	0.82
ASV1230	<i>Gammaproteobacteria</i> (c)	207.2	0.79
ASV1162	<i>Yersiniaceae</i> (f)	37.79	0.77
ASV25	<i>Microbacteriaceae</i> (f)	693.7	0.72
ASV107	<i>Chryseobacterium</i> (g)	69.8	-1.78
ASV47	<i>Paenibacillus</i> (g)	231.1	-1.87
ASV61	<i>Paenibacillus</i> (g)	143.9	-2.24
ASV78	<i>Acetobacteraceae</i> (f)	65.31	-2.97

(k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family and (g) denotes genus.

Table A-9. Bacterial ASVs that significantly differ ($p < 0.05$, Benjamini-Hochberg adjusted) across locations within a polytunnel. A positive $\log_2$ fold change indicates a higher relative abundance in the inside of polytunnel vs. the outer edge.

ASV ID	Taxonomy	Base Mean	$\log_2$ Fold Change
ASV142	<i>Chryseobacterium</i> (g)	48.6	2.33
ASV117	<i>Paenibacillus</i> (g)	43.29	2.31
ASV179	<i>Pedobacter</i> (g)	12.83	2.17
ASV222	<i>Pedobacter</i> (g)	25.66	1.98
ASV54	<i>Pedobacter</i> (g)	213	1.94
ASV90	<i>Enterobacterales</i> (o)	72.97	1.74
ASV76	<i>Pedobacter</i> (g)	77.41	1.64
ASV74	<i>Bacillus</i> (g)	205.1	1.47
ASV33	<i>Pseudomonas</i> (g)	314.3	1.46
ASV251	<i>Rathayibacter</i> (g)	13.44	1.46
ASV239	<i>Bacillus</i> (g)	44.65	1.44
ASV67	<i>Erwiniaceae</i> (f)	127.1	1.17
ASV18	<i>Luteibacter</i> (g)	662.7	0.96
ASV340	<i>Erwiniaceae</i> (f)	19.49	0.94
ASV136	<i>Duganella</i> (g)	43.95	0.89
ASV25	<i>Microbacteriaceae</i> (f)	693.7	-0.89
ASV365	<i>Pseudomonas</i> (g)	15.63	-1
ASV534	<i>Pseudomonas</i> (g)	9.68	-1.05
ASV339	<i>Pseudomonas</i> (g)	129.4	-1.14
ASV800	<i>Pseudomonadaceae</i> (f)	103.9	-1.15

ASV56	<i>Erwiniaceae</i> (f)	332.4	-1.18
ASV118	<i>Methylobacterium</i> (g)	35.92	-1.3
ASV2	<i>Pseudomonas</i> (g)	5081	-1.32
ASV116	<i>Sanguibacter</i> (g)	49.5	-1.32
ASV20	<i>Pantoea</i> (g)	960.6	-1.38
ASV242	<i>Bacteria</i> (k)	9.91	-1.43
ASV57	<i>Pantoea</i> (g)	300.6	-1.54
ASV89	<i>Pseudomonas</i> (g)	113.7	-1.62
ASV46	<i>Erwiniaceae</i> (f)	279.3	-1.7
ASV66	<i>Erwiniaceae</i> (f)	116.6	-1.74
ASV273	<i>Erwiniaceae</i> (f)	14.25	-1.8
ASV102	<i>Erwinia</i> (g)	67.51	-1.9
ASV44	<i>Pseudomonas</i> (g)	262.4	-2
ASV351	<i>Erwiniaceae</i> (f)	12.2	-2.13
ASV623	<i>Frondihabitans</i> (g)	3.5	-2.22
ASV87	<i>Pseudomonas</i> (g)	68.39	-2.31
ASV420	<i>Acetobacteraceae</i> (f)	5.35	-2.31
ASV215	<i>Erwiniaceae</i> (f)	19.1	-2.75
ASV92	<i>Erwinia</i> (g)	81.37	-2.8
ASV324	<i>Brachybacterium</i> (g)	9.96	-2.85

(k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family and (g) denotes genus.

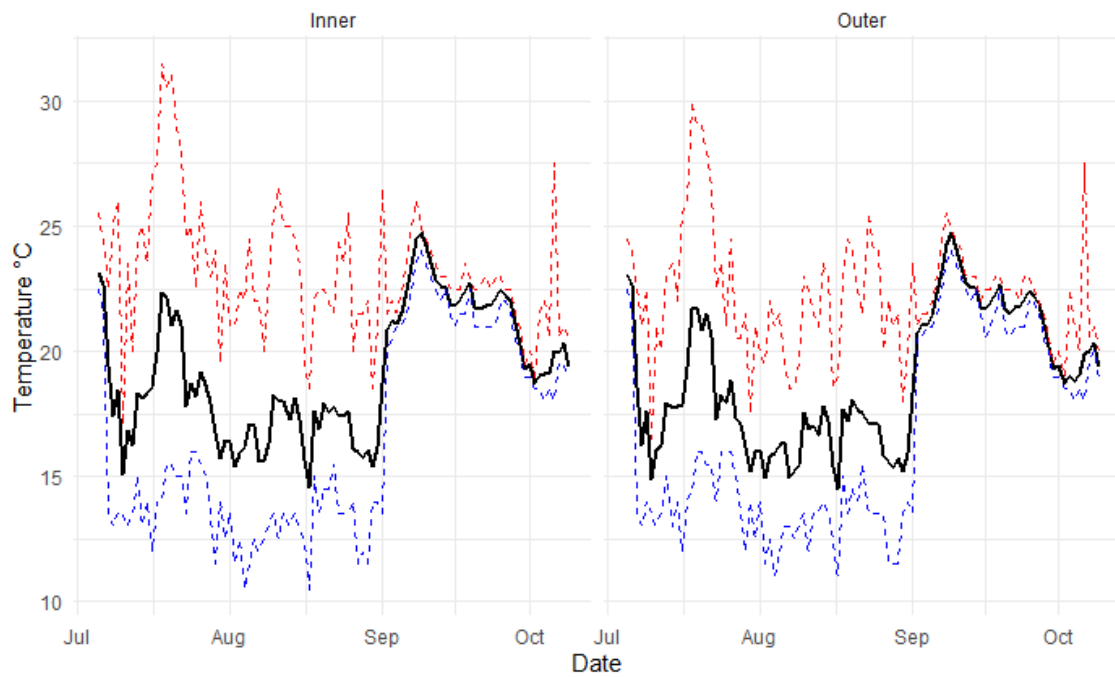


Figure A-2. The average weekly temperature from the outer edge and centre of a polytunnel, the first samples were taken on 02/10/2021 and the second set of samples were taken on 10/10/2021.

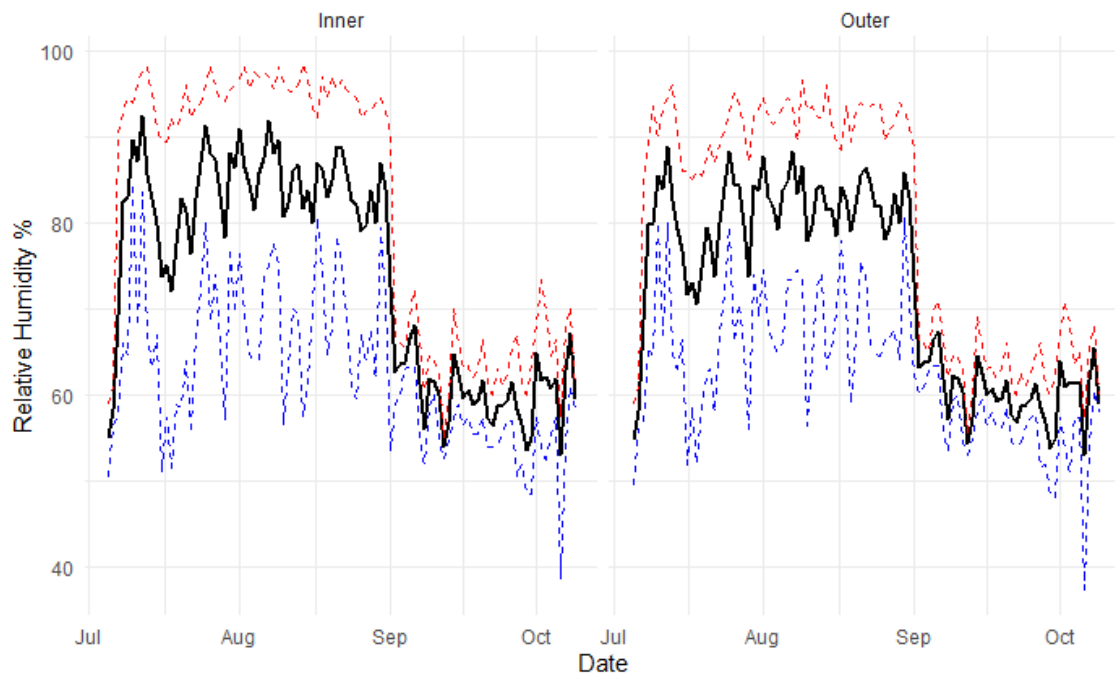


Figure A-3. The average weekly humidity from the outer edge and centre of a polytunnel, the first samples were taken on 02/10/2021 and the second set of samples were taken on 10/10/2021.

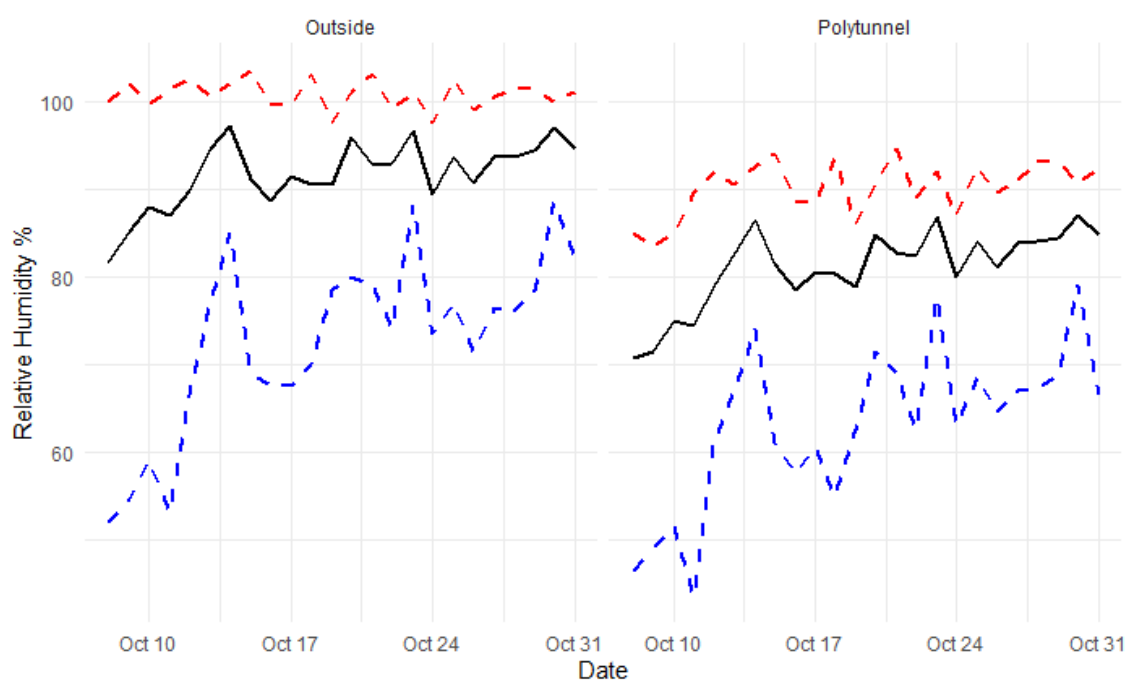


Figure A-4. The average (black line), maximum (red line) and minimum (blue line) relative humidity from inside a polytunnel and in the outdoor air collected from dataloggers near to the rotorod samplers.

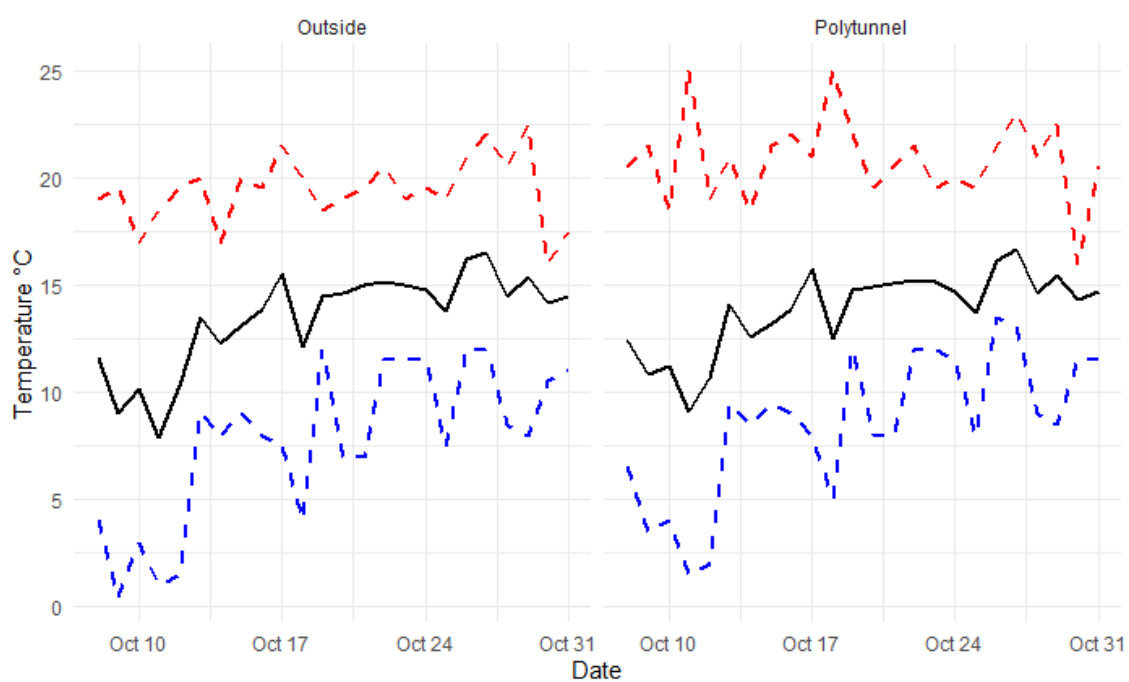


Figure A-5. The average (black line), maximum (red line) and minimum (blue line) temperatures (°C) from inside a polytunnel and in the outdoor air collected from dataloggers near to the rotorod samplers.

A.3 Assessing the Efficacy of Biological Control Agents in Plate Assays and Field Applications to Control *Cladosporium* on Raspberry

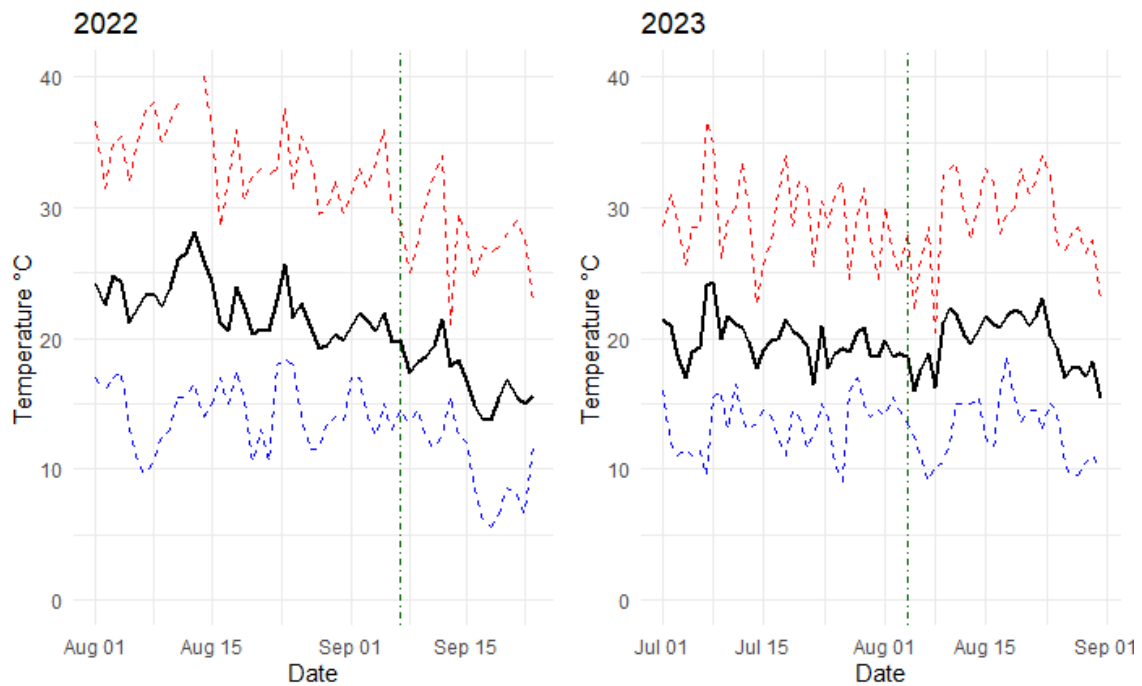


Figure A-6. The average (black line), maximum (red line) and minimum (blue line) temperatures (°C) from the polytunnel where Biological Control Agents were applied (the green vertical line indicates date of BCA inoculation for both the preventative and curative plots).

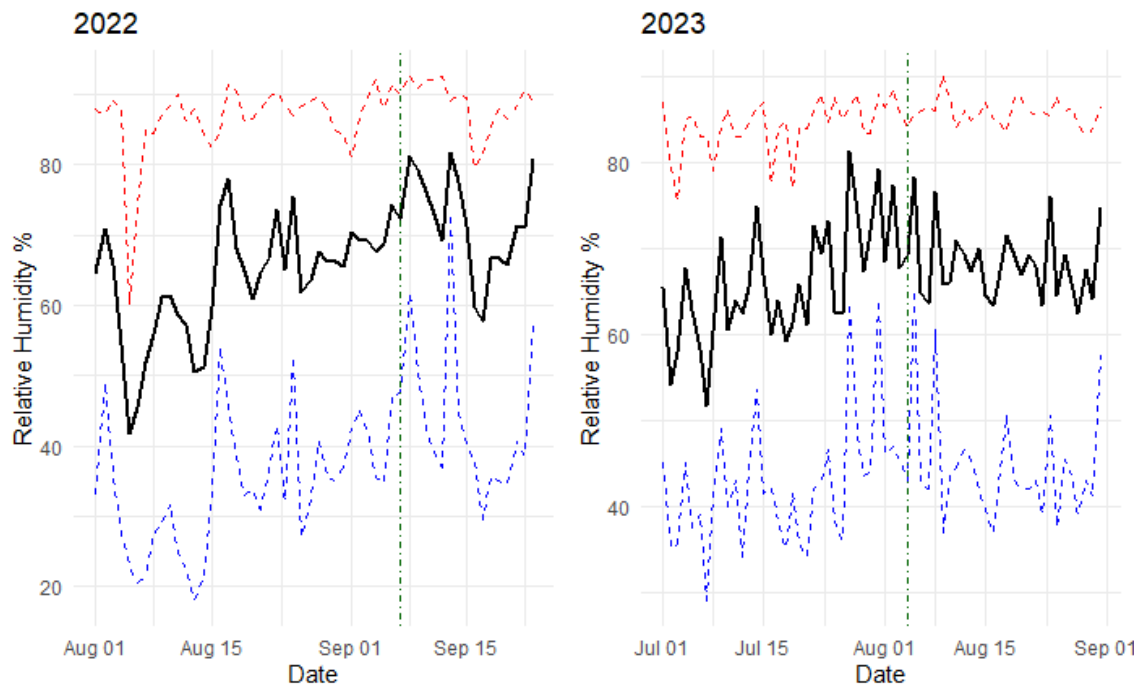


Figure A-7. The average (black line), maximum (red line) and minimum (blue line) relative humidities (%) from the polytunnel where Biological Control Agents were applied (the green vertical line indicates date of BCA inoculation for both the preventative and curative plots).

A.4 Determining if Raspberry Varieties are Resistant to *Cladosporium* Skin Lesions and Stigmata Infections

Table A-10. The dates of the variety screening inoculations across 2021 and 2022, and which varieties were inoculated at each date.

Inoculation Date	Varieties Inoculated
05/08/2021	Cascade Gem and Malling Charm.
12/08/2021	Cascade Gem and Malling Charm.
19/08/2021	Cascade Gem and Malling Charm.
02/09/2021	Malling Charm and Paragon.
08/09/2021	Malling Charm and Paragon.
16/09/2021	Malling Charm, Paragon and Malling Bella.
23/09/2021	Paragon, Malling Bella and a Proprietor Variety.
30/09/2021	Malling Bella and a Proprietor Variety.
07/10/2021	Malling Bella and a Proprietor Variety.
07/09/2022	Paragon, Malling Bella and a Proprietor Variety.
15/09/2022	Paragon, Malling Bella and a Proprietor Variety.
22/09/2022	Paragon, Malling Bella and a Proprietor Variety.

Table A-11. The number of raspberry fruits inoculated across each treatment when screening for varietal susceptibility.

Variety	<i>Cladosporium</i> Treatment	Year	N
Malling Bella	Treated	2021	154
Malling Bella	Untreated	2021	155

Malling Bella	Treated	2022	182
Malling Bella	Untreated	2022	197
Malling Charm	Treated	2021	151
Malling Charm	Untreated	2021	143
Malling Charm	Treated	2022	0
Malling Charm	Untreated	2022	0
Cascade Gem	Treated	2021	123
Cascade Gem	Untreated	2021	115
Cascade Gem	Treated	2022	0
Cascade Gem	Untreated	2022	0
Paragon	Treated	2021	122
Paragon	Untreated	2021	132
Paragon	Treated	2022	105
Paragon	Untreated	2022	86
Proprietor Variety	Treated	2021	137
Proprietor Variety	Untreated	2021	115
Proprietor Variety	Treated	2022	95
Proprietor Variety	Untreated	2022	91

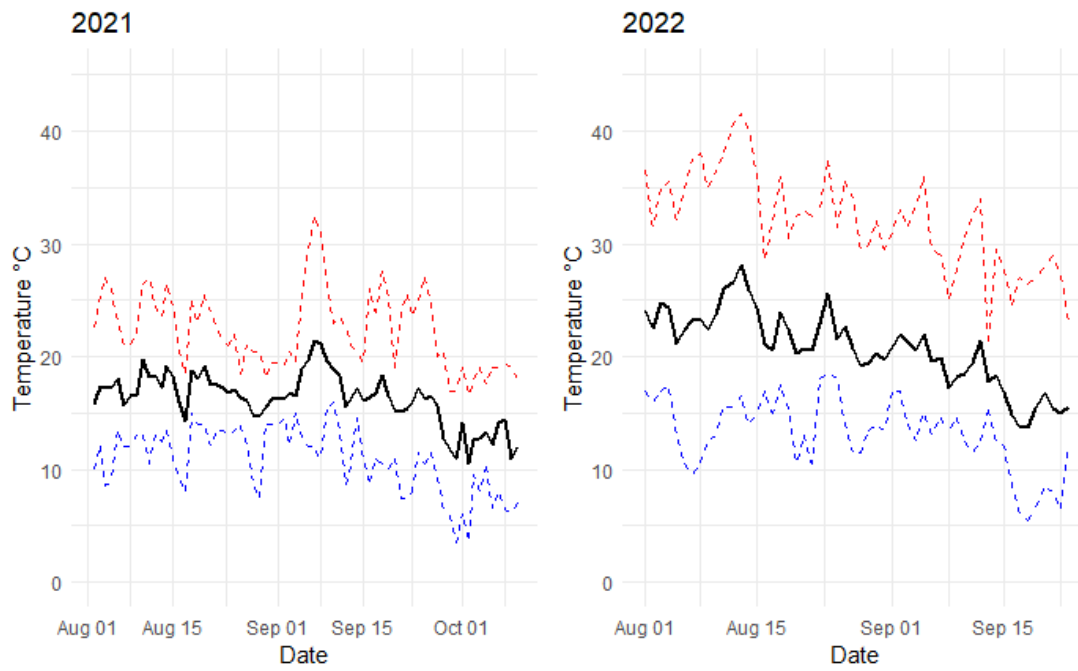


Figure A-8. The average (black line), maximum (red line) and minimum (blue line) temperatures (°C) from the variety screening polytunnel across both years of sampling.

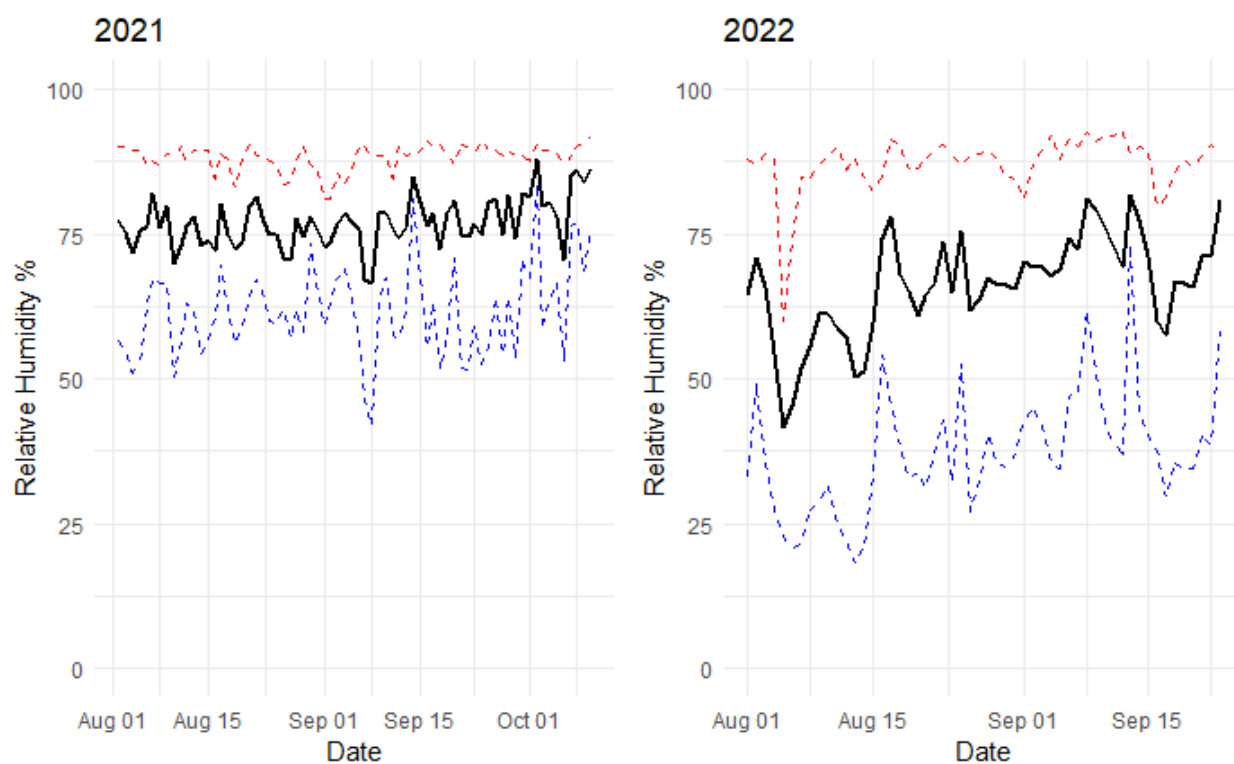


Figure A-9. The average (black line), maximum (red line) and minimum (blue line) relative humidities (%) from the variety screening polytunnel across both years of sampling.

Appendix B – Awards

“*Cladosporium*: The Unknown Pathogen Rotting Raspberries”

L. Farwell, N. Magan and X. Xu.

AHDB Soft Fruit Technical Day

NIAB, East Malling, Kent, 2019

Best 1st Year Poster

“Prevalence and Diversity of *Cladosporium* Species on UK and Spanish Raspberries and Determining When Fruit are Susceptible to *Cladosporium* Skin Lesions”

L. Farwell, N. Magan & X. Xu.

July, 2022

Worshipful Company of Fruiterers Student Prize

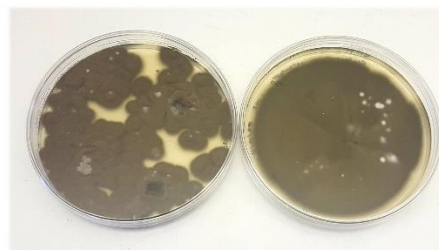
Cladosporium: The Unknown Pathogen Rotting Raspberries

Lauren Farwell^{1,2}, Prof. Naresh Magan² & Prof. Xiangming Xu¹

1. NIAB EMR 2. Cranfield University

The Problem:

In recent years, growers in the industry have lost raspberries due to *Cladosporium* rot¹, which was previously considered to be a post-harvest opportunistic fungus². Recent research has suggested that *Cladosporium* is a pre-harvest pathogen¹. Therefore, further studies are needed to understand its epidemiology for developing management strategies.



What are we doing?:

To obtain *Cladosporium* isolates from raspberry fruit and conduct inoculation studies

To understand the relationships among *Cladosporium* spp. from different fruit crops and conduct cross inoculation work

To understand how fruit infection is affected by (a) fruit age and cultivars, and (b) climatic conditions

To study seasonal inoculum dynamics in relation to crop management practices

To study methods of controlling *Cladosporium*, including on bio-control, UV, cool-chain management and fungicide treatments.

Why are we doing this?:

To find which species are capable of infecting raspberries.

To determine the inoculum source

To predict disease risks for effective management

To inform growers best crop management practice(s) to suppress *Cladosporium* inoculum production

To provide growers with information on methods for effective management of *Cladosporium* on raspberry

1. Swett, C.L. et al. 2019. *Phytoparasitica* **47**: 415-428.

2. Richards, M. 1953. *Nature* **171**: 615-616.

The Worshipful Company of Fruiterers



The is to certify that

Lauren Farwell

was awarded

The Berry Gardens Student Prize 2022

Chairman of the Awards Council

Clerk

Dated this Tenth day of July 2022

