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| Project leader:          | Dr Aoife O' Driscoll                                  |
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| Key staff:               | Dr Aoife O' Driscoll                                  |
|                          |   |
| Location of project:     | NIAB, Laurence Weaver Road, CB30LE                    |
| Industry Representative: | Claire Donkin, C. Donkin Ltd.                         |
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# **AUTHENTICATION**

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Name : Dr Aoife O' Driscoll

Position: Senior Specialist-Crop Protection and IPM

Organisation: NIAB

Signature: August 2023

### Report authorised by:

[Name]

[Position]

[Organisation]

Signature ..... Date .....



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# **GROWER SUMMARY**

## Background

It is widely recognised that bioprotectants can be more complicated to use than traditional chemistry, as there are many interacting factors, such as relative humidity, temperature, or pest population numbers, that can impact the level of control achieved. Outputs from the AHDB AMBER project provided valuable insights into aspects of using bioprotectants. Information on the use of bioprotectants is also available from manufacturers and distributors. However, the quality and depth of guidance provided can vary substantially. Despite the information available, from their ongoing observations and from more formal trialling, some experienced herb growers assert that even with fine attention to detail and required conditions of use, they achieve negligible levels of pest and disease control with certain bioprotectants. This situation has led herb growers to hypothesise that their lack of success with bioprotectants on herb crops is due not only to grower inexperience in use of these products but more fundamentally, to intrinsic characteristics of the herb species being cultivated. The overall aim of this review was to identify the factors relevant to edible herb species that may be preventing or limiting the efficacy of microbial bioprotectant products in controlling pests and plant pathogens when used on these crops.

## Summary

#### Survival and proliferation of microbial bioprotectants on herb surfaces

Three different forms of microbial protectant were chosen for consideration in the review entomopathogenic fungi for pest management (as *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii*) and bacterial (as *Bacillus subtilis*) and fungal bioprotectants (as Gliocladium spp.) for disease management. No studies or information were identified in available public and published literature addressing the survival and proliferation of these microorganisms on rosemary, basil, or coriander. However, research is available from other horticulture crop species including strawberry, lettuce and cyclamen. Multiple studies show that while both *B. subtilis* and *G. catenulatum* can survive for long periods on the leaf surface, dispersal is very limited particularly under protected conditions where dispersal between leaves is achieved primarily from physical contact between leaves. The efficacy of microbial bioprotectants in practice is thus less likely to be affected by the survival of biocontrol microbes



but more likely by other factors that influence the density of biocontrol cells in the phyllosphere, especially dilution due to rapid host leaf expansion, spray coverage and rain wash-off. It is likely that properties of herb species and their volatiles are limiting the ability of microorganisms to multiply, colonise and where relevant, to produce microbial compounds. The subject area warrants further investigation.

#### Interactions between herb-produced volatiles and microbials used for pest and disease control

Seven studies were identified which directly addressed the interaction between essential oils or associated Volatile Organic Compounds (VOCS) from rosemary, basil, and coriander and the microbial bioprotectants chosen for inclusion in the review. Additional studies were identified which addressed the interaction between relevant VOCs and either B. bassiana or B. subtilis in vitro, or the interaction between essential oils from species with a strong VOC emission profile e.g., lavender, eucalyptus, or coniferous trees. Content et al., 2022 showed that in certain growing practices such as open field production, temperature ranges between 11-20°C, soils high in nutrients, irrigation and fertiliser use can all cause an increase in volatiles abundance for some of the herb species included in this review. This suggests that different growing practices are indirectly manipulating the composition and abundance of volatile compounds that could have a detrimental effect on bioprotectant efficacy. Use of essential oils or VOCs in vitro affected germination and germ tube development, hyphal growth and sporulation of the EPFs B. bassiana, M. anisopliae and L. lecanii. But available evidence was not consistent across organisms or treatments e.g. Nardoni et al., 2018 demonstrated that M. anisopliae showed limited sensitivity against essential oils of eucalyptus, lavender and pelargonium while *B. bassiana* appeared to be sensitive to 1% lavender oil. Liu (2012) showed that cymene had a stimulatory effect on germ tube development of *B. bassiana* at the lowest concentration tested but inhibited germ tube development at the highest concentration (50mM). For *B. subtilis*, the evidence was clearer that VOCs such as  $\beta$ -pinene and q-cymene have antimicrobial properties against intact Bacillus subtilis spores by directly interacting with the hydrophobic regions of membrane proteins in the cell. No evidence was available on the impact of essential oils or VOCs on G. catenulatum, however data is available on fungal pathogens including B. cinerea, F. oxysporum and Verticillium dahliae. It is highly likely that volatiles being produced by herb crops are affecting plant defence responses for individual herb species and the efficacy of microbial bioprotectants.



Repellent or attractant effects of herb volatiles on pest species and/or their natural enemies

Three studies were identified that specifically addressed the interaction between essential oils and VOCs from culinary herbs and their associated pests or natural enemies. Additional studies were identified which addressed the interaction between crop species containing relevant VOCs- specifically sweet pepper and melon cotton aphids, whiteflies, and their natural enemies, either *in vitro* or *in planta*. These studies showed that endophytic colonisation by EPFs including *B. bassiana* and *M. brunneum* have the potential to induce characteristic blends of volatiles which can influence olfactory behaviour and performance of aphids and whitefly, but the effects are small and dependant on the EPF strain used. It was also clear that emission of individual volatiles was not likely sufficient to elicit repellent or attractant effects, and that any effects were a consequence of individual VOCs acting in a blend with other VOCs. Several studies demonstrated that whitefly preference on rosemary is repellency based, with preference recorded as a continuum of repellencies. Based on the evidence it is highly likely that volatiles being produced by adjacent herb crops are interfering with the attraction and distraction of pests affecting herb species and their natural enemies.

# The role of bioprotectants, specifically elicitors in activating specific plant defence pathways and/or induced systemic resistance (ISR) mechanisms in herbs

Limited research to date does indicate that elicitors can trigger VOC emission in plants but comparative studies between different elicitors are rare and no publicly available research was identified which addressed the role of bioprotectants in ISR mechanisms in herbs. The available research suggests that it is highly unlikely that plant defence responses are limited or absent in herbs such that there is no induced systemic resistance. Available research from other pathosystems suggests that elicitor based bioprotectants such as COS-OGA, cerevisiane and laminarin based products which are commercially available and used in herb production in the UK may induce changes in VOC emission as part of their defence response. However, the effect is likely to be dependent on a myriad of variables including herb species, variety, crop phenological stage and environmental parameters. The strength and blend of VOC emissions are also highly likely to vary according to the elicitor being used. This area of research warrants further investigation in herbs.



# **Action Points**

- Growers' hypotheses that lack of success with bioprotectants on herb crops may be due to intrinsic characteristics of the herb species being cultivated has been partly confirmed through limited evidence identified through completion of this review. But the effects are likely to be small and dependent on a myriad of compounding, interacting factors including the bioprotectant, the growing environment and crop species.
- The efficacy and survival of microbial bioprotectants in practice is less likely affected due to biochemical characteristics of herbs, and more likely by other factors that influence the density and further dilution of these microorganisms on foliar surfaces. This dilution in concentration of the microbial protectant will be compounded by crop growth and abiotic factors including slow or fast host leaf expansion, spray coverage and rain wash-off.
- Outcomes from this review on the biochemical and physical characteristics of herbs which affect bioprotectant efficacy should form the basis for more detailed research through other methods of funding, e.g., through a BBSRC-CASE Studentship or through IUK.



# **SCIENCE SECTION**

## Introduction

It is widely recognised that bioprotectants can be more complicated to use than traditional chemistry, as there are many interacting factors, such as relative humidity, temperature, or pest population numbers, that can impact the level of control achieved. Outputs from the AHDB AMBER project provided valuable insights into aspects of using bioprotectants. Information on the use of bioprotectants is also available from manufacturers and distributors. However, the quality and depth of guidance provided can vary substantially. Despite the information available, from their ongoing observations and from more formal trialling, some experienced herb growers assert that even with fine attention to detail and required conditions of use, they achieve negligible levels of pest of disease control with certain bioprotectants. This situation has led herb growers to hypothesise that their lack of success with bioprotectants on herb crops is due not only to grower inexperience in use of these products but more fundamentally, to intrinsic characteristics of the herb species being cultivated. The overall aim of this review was to identify the factors relevant to edible herb species that may be preventing or limiting the efficacy of microbial bioprotectant products in controlling pests and plant pathogens when used on these crops.

## Materials and methods

Literature searches were carried out principally using Google Scholar, Science Direct, NCBI PubMed and the AHDB Horticulture website. In addition to formally published scientific literature, conference proceedings, PhD theses and project reports were consulted. Recent papers from 2000 onwards have been the primary focus. However, where deemed necessary and relevant, older literature has been explored, Relevant AHDB Horticulture reports were assessed to highlight relevant information within the context of this review.

## Results

Phytochemical compounds are divided into two, primary metabolites and secondary metabolites. Primary metabolites are compounds used in plant growth, such as carbohydrates and amino acids. Secondary metabolites are produced, either constitutively or inductively, to protect plants from environmental stresses. Secondary metabolites are further grouped into several categories; this review focuses on two functional groups of secondary metabolites-



phenolic compounds and volatile organic compounds (VOCs). Individual compounds and their potential role, directly or indirectly, in plant/insect or microorganism interactions are further discussed.

There are over 8000 polyphenolic compounds, all of which are derived from a common precursor, phenylalanine (PAL), which is produced via the shikimic acid pathway and then chemically modified for specific functions (Eseberri et al., 2022). The major groups of polyphenols include phenolic acids, flavonoids and isoflavonoids, lignans, and stilbenes. After synthesis, polyphenols are translocated to specific plant tissues where they accumulate and help alleviate specific stress-induced effects. Many phenolic compounds show antimicrobial properties by altering the microbe cell wall permeability, or by directly inhibiting fungal growth in the apoplast (reviewed in Zaynab et al., 2018). Volatile organic compounds are a large group of carbon-based chemicals with low molecular weights and high vapour pressure. VOCs fall into several major classes based on their biosynthetic origin, which include terpenoids, phenylpropanoids/benzenoids, fatty acid derivatives and amino acid derivatives (Dudareva et al., 2013).

Plants produce VOCs constitutively but are also induced in response to herbivore infestation, referred to as herbivore induced plant volatiles (HIPVs) (Brilli et al., 2019). HIPVs mainly consist of terpenoids, green leaf volatiles (GLVs), aromatic compounds and amino acid derivatives (Clavijo McCormick et al., 2012). HIPVs can serve as attractants to beneficial insects or alert neighbouring plants to the presence of biotic stressors, causing these receiver plants to induce their own defence mechanisms (Erb, 2018), otherwise known as defence priming. Defence priming is an adaptive, low-cost defensive measure because a response is only transiently activated by a priming stimulus (Martinez-Medina et al., 2016). Primed plants will then produce a more rapid and effective defence response upon subsequent challenge from the triggering stress. There is little to no energy cost to the plant incurred during this primed state, meaning yield will not be impacted if the pest never successfully infests the crop (Conboy et al., 2020). VOCs have been successfully found to prime plants as either mixtures (Hu and Erb, 2018) or individual compounds (Song and Ryu, 2018). GLVs are another class of plant VOCs which comprise of fatty acid derivatives such as 1-hexanal, cis-3-hexenol, nonanal and methyl jasmonate. Green leaf volatiles are generated through the oxylipin pathway from  $\alpha$ -linolenic acid (ALA) and linoleic acid (Matsui, 2006). The production of GLVs has been reported in studies investigating the headspace of plants after wounding or herbivore



attack (Shiojiri et al., 2006), however it has been shown that pathogens can also induce the release of GLVs (Piesik et al., 2011; Scala et al., 2013).

#### Phytochemical composition of rosemary, basil, and coriander

Contente et al., 2022 detected 125 compounds in the headspace of rosemary samples: including 45 monoterpenes, eight sesquiterpenes, three alcohols and three aldehydes. Available literature has described higher VOC content and compounds such as eucalyptol and camphor when rosemary was produced at higher temperatures during the summer season (Lakušić et al., 2012; Salido et al., 2003). Bellumori et al., 2021 showed that the main phenols identified in rosemary tissues, rosmarinic acid, carnosic acid and its derivatives, were not significantly different between leaf tissues of different ages, however there were differences in terpene profiles among the three tissue sources. Terpene profiles of the leaves were characterised by high relative contents of  $\alpha$ -pinene and 1,8-cineole. High relative contents of verbenone and  $\beta$ -caryophyllene were also detected in mature and young leaves, respectively. Cortical terpene profiles had high relative contents of  $\alpha$ -pinene, camphene,  $\beta$ -pinene, camphor and caryophyllene oxide in mature bark, while xylem tissue was characterised by the presence of  $\beta$ -pinene. Both the Contente study and the Bellumori study showed that  $\alpha$ -pinene was overall the predominant enantiomer released from rosemary tissues, in agreement with data in the literature e.g., Pintore et al., 2002 and Socaci et al., 2008. Contente et al., 2022 detected 109 compounds from basil samples, including 23 monoterpenes, 15 sesquiterpenes, four phenylpropanoids and nine aldehydes. Al-Maskri et al., 2011 demonstrated changes in some compounds of basil essential oil between winter and summer harvests. In the summer harvested essential oil, there was significantly more linalool, p-allylanisole and  $\beta$ -farnesene, and much less content of limonene and 1,8-cineole. Skagel (2012) found that the availability of phosphorous or inoculation with the arbuscular mycorrhizal fungus (AMF), Rhizophagus influenced the phenolic composition of basil with significant differences in rosmarinic acid content between treatments, which impacted plant growth and nutrient uptake. Contente et al., 2022 detected 92 compounds from coriander samples including 16 aldehydes, seven alcohol and six alkanes. A summary of findings from Content et al., 2022 for rosemary, basil and coriander, detailing specific crop growth parameters ('variable') and the main volatile compounds identified which associated with each specific growth parameter is presented in Tables 1-3.



**Table 1.** A summary of findings from Content et al., 2022 for rosemary, detailing specific crop growth parameters ('variable') and the main volatile compounds identified which associated with each specific growth parameter.

| Variable                      | Main volatile components associated with the variable   |
|-------------------------------|---|
| Variety                       | Varieties Barbeque and Perigord; linalool. Miss Jessops; gamma-<br>terpineol and neral  |
| Sample location               | Reading and Norwich samples; eucalyptol, (E)-2-heptenal and<br>geraniol. West Sussex and Worcester samples; linalool and<br>thymol. York samples; gamma-terpineol and fenchol |
| Production system             | Rosemary produced in pots; linalool and isoborneol. Production<br>in field under protected conditions; neral. Open field production;<br>eucalyptol and geraniol               |
| Age of material at<br>harvest | Samples harvested when 'fully matured' or at first cut; borneol, α-<br>pinene, camphene and camphor Second cut crops; isoborneol,<br>linalool and thymol                      |
| Soil type                     | Loamy soil; eucalyptol. Production in peat; linalool. Loamy/clay soil; camphor and borneol  |
| Fertiliser use                | Gamma-terpineol and neral.  |
| Light source                  | Sunlight alone associated with camphor, camphene and $\alpha$ -pinene. The combination of sunlight and additional HPS associated with linalool                                |
| Temperature                   | Rosemary grown at 11-15°C and 16-20°C; borneol  |



**Table 2.** A summary of findings from Content et al., 2022 for basil, detailing specific crop growth parameters ('variable') and the main volatile compounds identified which associated with each specific growth parameter.

| Variable        | Key volatile components identified that associated with the variable   |  |
|-----------------|--|--|
| Sample location | Lincolnshire samples; nerol and sesquithujene. Worcester samples; sabinene hydrate and (E)-2-nonenal   |  |
| Soil type       | Loamy soil or peat; isoeugenol, sabinene hydrate, (E)-2-nonenal .<br>Hydroponic production; eugenol  |  |
| Fertiliser use  | Nerol, 2-methylpyridine and furaneol   |  |
| Light source    | Sun lighting or its combination with HPS lighting; eucalyptol, linalool, a eugenol   |  |
| Temperature     | Average growth temperature of 16-20°C; eucalyptol, sesquithujene. Average growth temperatures of 6-10°C, 16-20°C and 20-25°C; negatively correlated with eugenol, linalool, and eucalyptol and highly correlated with gamma-terpinene, sesquithujene and isoeugenol. |  |

**Table 3.** A summary of findings from Content et al., 2022 for coriander detailing specific crop growth parameters ('variable') and the main volatile compounds identified which associated with each specific growth parameter.

| Variable        | Key volatile components associated with the variable  |
|-----------------|---|
| Sample location | York samples (E)-2-dodecenal) and decanal. West Sussex,<br>Worcester, and Lincolnshire samples; low correlation with most<br>compounds apart from dodecanal, nonane and (E)-2-undecenal |
| Fertiliser use  | (E)-2-decenal, tetradecanal and nonanal   |
| Light source    | HPS lighting plus sunlight; dodecanal and (E)-2-heptenal.<br>Sunlight only in open field samples; (E)-2-decenal   |
| Temperature     | Average growth temperature of 11-15°C; (E)-2-dodecenal, undecanal and (Z)-2-nonenal   |

#### Survival and proliferation of microbial bioprotectants on herb surfaces

Three different forms of microbial protectant were considered for this part of the review-Entomopathogenic fungi for pest management (as *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii*), bacteria (as Bacillus subtilis) and fungal bioprotectants (as Gliocladium spp.) for disease management. No studies or information were identified in the available public literature addressing the survival and proliferation of these organisms on rosemary, basil, or coriander. Survival and proliferation of root colonising bioprotectants such as Trichoderma spp. were considered but not included as part of the review due to the available project budget and time constraints.

Biocontrol outcomes critically depend on the rate of BCA colonizing healthy and infected or diseased host tissues, as well as BCA mortality. Two aspects of the rate of BCA colonising healthy tissues need to be considered: the colonisation of immediate neighbouring tissues in the same host tissue unit and the ability to disperse or spread to new tissue. The leaf harbours diverse microorganisms that inhabit the surface and interior of leaf surfaces, known as epiphytes and endophytes, respectively (Beattie and Lindow, 1999; Lindow and Brandl, 2003). Stressed and nutrient-poor conditions on the leaf surface makes this environment selective to certain microorganisms. Hence, different microbial mechanisms such as the ability to extract nutrients, produce hormones, toxins, and surfactants, as well as motility and biofilm formation can be key to colonization success (Oso et al., 2019; Streletskii et al., 2019).

Entomopathogenic fungi have a worldwide distribution, with the most important genera being Beauveria, Verticillium, Metarhizium, Nomuraea, Paecilomyces, and Hirsutella. Metarhizium is the most studied genus with three entomopathogenic species; M. anisopliae, M. album, and M. flavoviride, with 15 species from the genus Beauveria currently recognised (Agrawal et al., 2014; Imoulan et al., 2016). Entomopathogenic fungi can be an effective form of pest control, but uptake is hindered due to the time between infection and death of the host, during which the pest can continue to feed and reproduce. Beauveria bassiana (the EPF contained in the commercially available bioprotectants Botanigard WP and Naturalis-L) colonises insects with the aid of specific pigments or mycotoxins, such as beauvericin and oosporein, then continues to grow out of the cadaver, forms conidiophores and releases conidia for dispersal (Boucias and Pendland, 1998). Ferron (1985) argued that chitin and certain fatty acids in the cuticle were required to meet the carbon needs of germinating conidia on the insect cuticle, however some cuticular components, such as medium chain and short-chain fatty acids, are inhibitory to conidial germination. Host plant characteristics can affect B. bassiana performance on plants with e.g., hairy or waxy leaves where searching by the EPF is slowed down. Crop architecture can also affect *B. bassiana* host-finding; on herbs there are many small leaves which need to be searched. On pot herbs which can be turned around in 5-6 weeks during the summer, there may not be enough time for *B. bassiana* to emerge from infected insects before the plants are sold.

Gliocladium catenulatum strain J1446 (syn. Clonostachys rosea), is known for its ability to control soil-borne fungal pathogens and has also shown efficacy against certain foliar diseases like Botrytis cinerea. Strain J1446, originally isolated from Finnish field soil, was developed into a commercial bioprotectant, Prestop. It is hypothesised to have multiple modes of action including production of cell wall-degrading enzymes, mycoparasitism, competition for nutrients or space antibiotic production and the induction of defence responses. The genus Bacillus are rod-shaped, gram-positive bacterial microorganisms, of which the biocontrol product Serenade ASO, formulated of a single *B. subtilis* isolate QST713, is probably the most widely used biocontrol product of plant diseases worldwide. Lipopeptides of Bacillus facilitate surface attachment of bacterial cells to host tissues, as well as biofilm formation, which protect microorganisms from adverse environmental stresses. A role for the biofilm in colonisation and biocontrol activity was established by Salvatierra-Martinez et al., 2018 but the precise mechanism(s) by which plants regulate biofilm-associated communities remains unclear. Some lipopeptides also support the mobility of bacteria, most likely via changing the viscosity of the colonized surfaces, allowing bacteria to move to nutrient rich locations and change the water dynamics on leaf surfaces which indirectly affects pathogen development.

Early studies by Köhl et al., 1998 described the colonisation of Ulocladium atrum and Gliocladium roseum on cyclamen, inoculated with Botrytis cinerea. Both U. atrum and G. roseum colonised senesced leaves and reduced B. cinerea development on these leaves, thus reducing the inoculum potential on petioles adjacent to necrotic leaf tissues. The fate of U. atrum conidia on surfaces of healthy cyclamen leaves during a 70- day period after application was studied. The number of conidia per square centimetre of leaf surface remained relatively constant during the entire experiment with 60% of the conidia sampled during the experiments retaining the ability to germinate. Kessel et al., 2005 developed a spatially explicit model describing colonization of dead cyclamen leaf tissue by B. cinerea and U. atrum. The model found that both fungi explore the leaf and utilise the resources it provides, but at temperatures from 5°C to 25°C, B. cinerea colonies expanded twice as rapidly as U. atrum colonies. In practical biological control, the slower colonization of space by U. atrum thus needs to be compensated by a sufficiently dense and even distribution of conidia on the leaf. Wei et al., 2016 conducted studies to specifically assess the extent of dispersal of B. subtilis among strawberry leaves in both open field and protected conditions. Relative population sizes of phyllosphere microbiota, including B. subtilis, were quantified and differences in the phyllosphere microbiota between open field and under protection to

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determined which microbes were affected by the introduced *B. subtilis* was also assessed. The dispersal of *B. subtilis* was shown to be very limited, particularly under protected conditions, however the reduction in its population size was relatively small within 8 days of application, and no overall reduction in its abundance was observed. These results suggested that limited dispersal is probably the main reason for its variable control efficacy under field conditions. In contrast to the findings under protected conditions, there was a loss of 50% B. subtilis under open field conditions 8 days after leaves received the application. This difference is most likely from the wash-off of bacteria by rainfall which can dislodge and disperse conidia from substrates as well as aid in the dispersion of propagules. In this study, dispersal of *B. subtilis* between leaves under protection was achieved primarily from physical contact between leaves. Tut et al., 2023 studied the impact of abiotic conditions on temporal population dynamics of *B. subtilis* QST 713 and *G. catenulatum* J1446 on the phyllosphere of lettuce and strawberry. On fully expanded leaves of both crops, under a range of temperature and relative humidity (RH) combinations there was a small, non-significant decline in the population size of viable cells for both organisms within 10 days of application. Under optimal temperature conditions for both organisms, the viable populations increased significantly with high RH. Only ambient temperature and dew point significantly affected the rate of temporal changes in the viable populations of each organism. The authors' conclusion again was that the efficacy of these bioprotectants in practice is less likely to be affected by the survival of microbes but more likely by abiotic factors and factors that influence the density of biocontrol organisms in the phyllosphere, especially dilution due to rapid host leaf expansion, spray coverage and rain wash-off.

# Interactions between herb-produced volatiles and microbials used for pest and disease control

Seven studies were identified which directed addressed the interaction between essential oils or associated VOCS from rosemary, basil, and coriander and the microbial bioprotectants chosen for inclusion in the review; these were Cho et al., 2015, Pavic et al., 2019, Turker Saricaoglu and Turhan, 2018, Zheng et al., 2013, Bellumori et al., 2021, Lukošiūtė et al., 2020 and Karpinski (2020). Additional studies were identified which addressed the interaction between relevant VOCs and either *B. bassiana* or *B. subtilis* in vitro, or the interaction between essential oils from species with a strong VOC emission profile e.g., lavender, eucalyptus, or coniferous trees.

Liu (2012) investigated the effect of cymene, carvacrol, thymol, borneol and geraniol on germination of *B. bassiana in vitro*. Five concentrations of cymene, carvacrol, or thymol and six concentrations of borneol or geraniol ranging from 0 to 500mM were tested, with ethanol used as the control. Spore suspensions of *B. bassiana* were placed on water agar in the

presence or absence of the essential oil with spore germination and germ tube development observed up to 24 hours after application. Cymene had a stimulatory effect on germ tube development of *B. bassiana* at the lowest concentration tested (0.5 mM) but inhibited germ tube development at the high concentration (50mM). No spores germinated in treatments with borneol (50 mM), carvacrol (50 mM and 500  $\mu$ M) or cymene (500 mM). Thymol was the least inhibitory of all tested oils; exposure slowed germ tube development but did not kill spores at any of the concentrations tested. Nardoni et al., 2018 evaluated the in vitro sensitivity of B. bassiana and M. anisopliae, to essential oils of eucalyptus (Eucalyptus globulus), lavender (Lavandula hybrida), pelargonium (Pelargonium graveolents) and to their main constituents; linalool, linalyl acetate, geraniol, citronellol and 1,8 cineole. M. anisopliae showed limited sensitivity against the selected essential oils, while *B. bassiana* appeared to be sensitive to 1% of *L. hybrida*. 1,8 cineole and geraniol were the most active against *B. bassiana*; linalool and citronellol were moderately active and linalyl acetate had no activity. In a study to evaluate the compatibility of conidial suspensions of *B. bassiana* with  $\alpha$ -pinene, Hussain (2021) showed that all the tested concentrations of  $\alpha$ -pinene affected the studied parameters of *B*. bassiana strain ARSEF 8465 with a directly proportional concentration-dependent response. There were significant differences in germination of *B. bassiana* strain ARSEF 8465 conidia recorded 12 h post-inoculation in the presence of all tested concentrations of  $\alpha$ -pinene, but vegetative growth and conidial viability were not adversely affected. Lin et al., 2017 investigated whether methyl salicylate and menthol affected growth and pathogenicity of the EPF Lecanicillium lecanii strain V3450. Decan-3-ol, benzaldehyde, phenylacetaldehyde, salicylate acid and menthol were considered key synergetic compounds that enhanced conidial performance and improved the pathogenicity and virulence of *L. lecanii* strain V3450. Methyl salicylate and menthol induced appressorium formation, promoted hyphal growth and promoted sporulation of L. lecanii while decan-3-ol, benzaldehyde, phenylacetaldehyde inhibited conidial germination. Strains of different EPF, including *B. bassiana* have been used to control bark beetle populations in vulnerable conifer trees of temperate forest landscapes (Chiu et al., 2017; Erbilgin, 2019) with pine monoterpenes released by the affected trees found to play a role in negating the survival, proliferation, and invasion of forests by this pest. Mann and Davis (2020) reviewed how environmental conditions unique to bark beetle habitats may have limited the success of previous *B. bassiana* applications, including variable temperatures. ultraviolet light, bark beetle symbiotic microorganisms and tree phytochemicals. Since EPF take multiple days to parasitise their hosts, the fungi inevitably come into contact with tree secondary metabolites regardless of the biocontrol application method. Conifer monoterpenes can have detrimental effects on B. bassiana efficacy, and some isolates appear to be inhibited by even low amounts of conifer phytochemicals. The authors speculated that adult beetle colonisation of conifer phloem results in the beetle being

'sanitised' from certain *B. bassiana* isolates. Additionally, both Davis et al., 2018 and Remus et al., 2021 showed under field conditions that *B. bassiana* genotypes had variable tolerance to pine monoterpenes, with in vitro tests revealing that a combination of temperatures below 15°C and exposure to spruce monoterpenes  $\alpha$ -pinene or 3-carene were likely factors limiting field performance of *B. bassiana*.

Studies by Cho and Chung 2017 have shown that surfactants with hydrophilic and hydrophobic properties, such as  $\beta$ -pinene and q-cymene, have antimicrobial properties against intact Bacillus subtilis spores by directly interacting with the hydrophobic regions of membrane proteins in the cell. Cho et al., 2015 investigated the sporicidal activities of a range of compounds extracted from coriander against B. subtilis spores: linalool,  $\alpha$ -pinene,  $\beta$ pinene, p-cymene, (2)-bornyl acetate, geranyl acetate, camphene, and c-terpinene. Compounds with more hydrophobic groups e.g., p -cymene and bornyl acetate were significantly more effective in sterilisation of *B. subtilis* spores than those with hydrophilic groups, such as b-pinene. Pavic et al., 2019 invested the effects of a number of compounds responsible for the biological activity of sage; namely carnosic and rosmarinic acid, carnosol, rosmanol, and ferruginol on *B. subtilis* isolate ATCC 6633. The extract with a high carnosic acid and carnosol content showed good antibacterial activity against a number of bacterial pathogens, in particular B. subtilis. Saricaoglu and Turhan, 2018 evaluated the antimicrobial properties of thyme, rosemary and clove essential oils and their mixtures in a 1/1 ratio against B. subtilis. All of the essential oils displayed an inhibitory effect with thyme essential oil displaying the highest inhibition zones against B. subtilis and rosemary essential oil having the lowest antimicrobial activity. Porras et al., 2021 also details the minimum inhibitory concentration for compounds targeting *B. subtilis* including curcumin, a-terpineol and linalool. Biofilms produced by *B. subtilis* and related species are well known to have a direct impact on the control efficacy of plant pathogens and Duarte et al. 2013 found at least 85% inhibition of biofilm formation in experiments with coriander essential oils.

Shirazi et al., 2022 studied the antifungal activity of several VOCs, including 3-octanone, 1octen-3-ol, and isovaleric acid on a number of fungal pathogens isolated from rosemary plants by measuring their mycelial growth inhibition and efficacy percentage. The mycelial growth of four isolated fungi declined after treatment with different concentrations of VOCs with the most significant inhibition of fungal growth observed following treatment with 1-octen-3-ol. The growth rate of *Rhizoctonia solani* and *Fusarium oxysporum* declined by 98% in treatment with 1-octen-3-ol compared to the control and similar to the *in vitro* results, treatment with 1octen-3-ol in planta reduce disease incidence by ca. 90%. Quintana-Rodriguez et al. 2015 performed a screen on the efficacy of 22 VOCs known to be volatilised from infected plant leaves of faba bean on *Colletotrichum lindemuthianum*, *Fusarium oxysporum*, and *Botrytis*  *cinerea*. The work results showed that nonanal, (+)-carvone, citral, trans-2-decenal, L-linalool, nerolidol, and eugenol significantly inhibited the growth of the three fungal species, and eugenol had the most active among them. Sekine et al., 2007 reported that other VOCs such as cumin aldehyde and p-cymene possess antifungal activity against *B. cinerea*, *F. oxysporum*, *Verticillium dahliae* and *Alternaria mali*.

Bellumori et al., 2021 evaluated the antimicrobial activity of selected terpenic compounds and rosmarinic acid, against Alternaria alternata and the bacterium Pseudomonas viridiflava. The growth of A. alternata was affected by eight out of the ten compounds tested, with  $\beta$ caryophyllene and β-pinene being totally ineffective, camphor the most effective and borneol also demonstrating strong inhibition of fungal growth. Rosmarinic acid showed a partial inhibiting activity against A. alternata. Only  $\alpha$ -pinene enantiomers, verbenone and rosmarinic acid inhibited the growth P. viridiflava. It is thought that gram-negative bacteria (including Pseudomonas and Bacillus species) show low sensitivity to the antimicrobial activity of terpenic compounds due to the hydrophilic polysaccharides contained in their outer membrane which create a barrier against hydrophobic compounds (Murínová and Dercová, 2014). Lukošiūtė et al., 2020 evaluated the ability of essential oils from lavender and thyme to suppress the growth of Alternaria spp., Botrytis spp., and Colletotrichum spp. in vitro. Thyme essential oil significantly suppressed the growth of all three fungal pathogens up to 7 days after treatment while lavender essential oil had low to moderate antifungal activity against the three pathogens. Finally, a study by Karpinski (2020) provides a useful review of the antifungal activities of essential oils from 72 Lamiaceae plant including rosemary.

# Repellent or attractant effects of herb volatiles on pest species and/or their natural enemies

Three studies were identified that specifically addressed the interaction between essential oils and VOCs from culinary herbs and their associated pests or natural enemies. These were Dardouri et al., 2019; Sadeh et al., 2017 and Yang et al., 2010. Additional studies were identified which addressed the interaction between crop species containing relevant VOCs-specifically sweet pepper and melon cotton aphids, whiteflies, and their natural enemies, either *in vitro* or *in planta*. Bioprotectant-induced plant VOCs attract or deter arthropod colonisation, potentially enabling herbivore and nonhost signalling. VOCs could also result in insect deterrence and priming of antiherbivore responses in noncolonised plants via plant-to-plant communication via airborne or soilborne VOCs.

#### Aphids

Wilberts et al., 2022 investigated whether endophytic colonisation of the roots of sweet pepper by the EPFs *Akanthomyces muscarius* and *B. bassiana* increases plant resistance

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against the tobacco peach aphid Myzus persicae var. nicotianae. Dual-choice experiments tested whether the fungi deter aphids via modifying plant volatile profiles and then whether EPF colonisation negatively affects aphid life history traits, such as fecundity, development, and mortality rate. Aphids were significantly attracted to the odour of plants inoculated with A. muscarius over non-inoculated plants. Plants inoculated with A. muscarius emitted significantly higher amounts of indole than *B. bassiana*-inoculated and non-inoculated plants. Inoculation with both fungal strains also caused significantly higher emissions of terpinolene. Sweet pepper plants inoculated with B. bassiana ARSEF 3097 did not elicit a significant behavioural response nor affect the investigated life history traits. The study showed that endophytic colonisation by EPF has the potential to alter olfactory behaviour and performance of *M. persicae* var. *nicotianae*, but the effects are small and dependant on the EPF strain used. González-Mas et al., 2019 showed that when melon plants inoculated with different strains of *B. bassiana* or *M. brunneum* were subsequently infested by *Aphis gossypii*, they showed gualitative and guantitative differences in aphid behaviour depending on the EPF that had colonised the leaves. When aphids fed on leaves that were only endophytically colonised there was a significant difference in aphid mortality on leaves colonised with the B. bassiana isolate EABb 01/33-Su compared with the control. GC-MS analysis revealed the presence in both treatments of a characteristic blend of melon plant volatiles derived from aldehydes, alcohols, ketones, terpenoids and phenolic compounds. In aphid species, perception of some of these plant-specific volatile components may assist olfactory discrimination between host and non-host plants. Dardouri et al., 2019 investigated which of five rosemary varieties may have a repellent effect on *M. persicae* and aimed to determine the possible VOCs involved. Fifteen main VOCs were identified, with each variety characterised by a specific volatile profile. By testing the identified VOCs individually, using a dual-choice olfactometer, the authors observed that five volatiles had a significant repulsive effect on *M. persicae*: bornyl acetate, camphor,  $\alpha$ -terpineol, terpinene-4-ol and geranyl acetone, but only one variety elicited a significant repulsive action. The authors concluded that emission of individual volatiles was not likely sufficient to elicit a repellent effect, and that any effects were a consequence of individual VOCs acting in a blend with other VOCs.

#### Whiteflies

*Bemisia tabaci* is a polyphagous insect and feeds on hundreds of plant species. This diversity of plant hosts suggests that *B. tabaci* may be familiar with a wide diversity of plant VOCs and that there may be only minor differences among the host plants. Liu et al., 2010 observed the pathogenesis of an isolate of *B. bassiana* in *B. tabaci* using scanning electron and light microscopy. The infection process was associated with specific regions of the whitefly nymph surface. Fungal blastospores were observed under the cuticle 36 and 48 h after inoculation

and eventually filled the body of the nymph. The nymphs died by the time a red pigment appeared in their bodies ca. 96 h post inoculation. By 120 h, large quantities of conidia were found on the surfaces of the whitefly nymphs. Numerous case studies support the finding that individual major constituents in rosemary oil repel whiteflies at various concentrations. Sadeh et al., 2017 reported a differential preference (attractiveness and repellency) of rosemary by B. tabaci with the whiteflies observed to colonise two different rosemary varieties. D-limonene and  $\beta$ -caryophyllene, were suggested as triggers of preference with D-limonene attracting B. *tabaci* at rates that were 10-fold lesser than the rates of  $\beta$ -caryophyllene. Preference was solely due to variation in rosemary's ability to repel B. tabaci. 'Choice' bioassays using essential oil blends obtained through GC-MS of the two extreme varieties indicated that whitefly preference for 1,8-cineole, verbenone, borneol, bornyl acetate, linalool, methyl chavicol, and camphor were negatively associated with preference. Several other studies have demonstrated that whitefly preference to rosemary is repellency based, with preference recorded as a continuum of repellencies. Yang et al., 2010, reported that the survival rate of B. tabaci offspring reduced up to 79% following exposure to essential oils extracted from thyme containing, among other VOCs, linalool, and 1,8-cineole. Çalmasur et al., 2006 also found linalool eradicate adult *B. tabaci* after 120 h of exposure.

#### Natural enemies with a focus on parasitoid wasps

Caccavo, 2023 showed that root colonisation of tomato plants by the fungal bioprotectant Trichoderma longibrachiatum can modulate the release of VOCs emitted, which influences performance of the aphid Macrolophus pygmaeus and its natural antagonist; the parasitoid wasp Aphidius ervi. Indirect defence responses in tomato, activated by T. harzianum against the aphid Macrosiphum euphorbiae, were found to include attraction of females of A. ervi, as a consequence of the fungal induction of terpenoid biosynthesis. It has been hypothesised by Holopainen et al., 2013 that VOC emissions induced by elicitors or plant activators could be similar to particular HIPVs. Sobhy et al., 2012 investigated whether treatment of maize with benzo (1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) and laminarin before plants were damaged by the chewing larvae of Spodoptera littoralis, increased the attractiveness of maize to three important parasitic wasps. Plants treated with the plant strengtheners did not show any consistent increase in volatile emissions but instead resulted in decreased amounts of certain volatiles emitted, most notably indole, which has been reported to interfere with parasitoid attraction by masking other attractive volatiles. Emission of the sesquiterpenes (E)- $\beta$ -caryophyllene,  $\beta$ -bergamotene, and (E)- $\beta$ -farnesene was similarly reduced by the treatment. de la Fuente, 2021 evaluated the blend of volatiles emitted by melon treated with BTH at different periods, and their effect in terms of physiological responses on aphid. BTH-treated melon plants did not emit GLV, but did release limonene

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and tetradecane, both are compounds known to attract aphid parasitoid species such as *Aphidius colemani*. Aside from information on maize, melon, and cotton (Rostas and Turlings, 2008), there is a lack of information about the effect of specific elicitors on the emission of HIPVs, and how blends of VOCs could attract or deter parasitoids.

# The role of bioprotectants, specifically elicitors in activating specific plant defence pathways and/or induced systemic resistance (ISR) mechanisms in herbs

Limited research to date has reported that elicitors can trigger VOC emission in plants e.g., Yi et al. (2009) and Zhang and Chen (2009); comparative studies between different elicitors are rare and no publicly available research was identified which addressed the role of bioprotectants in ISR mechanisms in herbs. Lemaitre-Guillier et al., 2021 analysed and compared VOC emission by grapevine leaves treated by different elicitors, focusing on three commercial products: Bastid®, Redeli® and Romeo®. Bastid® is a mixture of chitooligosaccharides (COS) and oligogalacturonides (OGA); this complex, known as COS-GA exhibits higher eliciting activity and triggers plant defence more than the individual components do. Redeli® is a phosphonate-based product with anti-oomycete and elicitor activities, used to control grapevine downy mildew (*Plasmopara viticola*, Burdziej et al., 2021). Romeo® is made up of cerevisiane extracted from yeast cell walls (De Miccolis Angelini et al., 2019). The authors aimed to identify VOC biomarkers to monitor plant defence responses induced by elicitor application which may allow better determination of the conditions for optimal efficiency of such treatments. Release of VOCs was time-dependent and the profile of significant VOC emission in response to Bastid® was also variable among replicate experiments, with the sesquiterpene  $\alpha$ -farnesene being the only VOC observed in all three experiments. Nine VOCs were specifically induced in response to Bastid®, 6 in response to Redeli® and 5 in response to Romeo®. However, the authors could not conclude that they are markers of the grapevine response to the corresponding elicitor because they were not significantly induced in the three replicates of the experiment and VOC emission was temporally variable across experiments. Further work by Lemaitre-Guillier et al., 2022 aimed to determine whether leaf VOCs can be used as biomarkers to defence elicitors in the field. Three elicitors (Bastid®, copper sulphate and methyl jasmonate) were assessed at three phenological stages by monitoring stilbene phytoalexins and VOCs. Again, the response profiles depended on the type of elicitor, the phenological stage and the vineyard with weak levels of VOC emissions from both Bastid® and copper sulphate and strong emissions from methyl jasmonate. All three elicitors induced the emission of mainly monoterpenes such as ocimene and pinenes, but again the authors failed to highlight a specific elicitor induced VOC "biomarker", most probably because of the weakness of the two elicitors used in this study and the multiple parameters inherent to the fields. Chalal et al., 2015 found that sulfated

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laminarin increased the emission of terpenes and MeSA but reduced MeJA and (Z)-3-hexenyl acetate in grapevine emissions, which resulted in resistance to *Plasmopara viticola*. Some of the emitted sesquiterpenes, particularly (E,E)- $\alpha$ -farnesene, appeared to represent a specific response to PS3 treatment and the authors speculated these sesquiterpenes could be good candidates as biomarkers of elicitor-IR. This data suggests elicitor based bioprotectants such as COS-OGA, cerevisiane and laminarin based products which are commercially available and used in herb production in the UK may induce changes in VOC emission as part of their defence response. However, the effect is likely to be dependent on a myriad of variables including herb species, variety, crop phenological stage and environmental parameters. The strength and blend of VOC emissions are also highly likely to vary according to the elicitor being used. This area of research warrants further investigation in herbs.

# Sufficiency of bioprotectant to control slow developing pests or pathogens with longer latent periods

In this review we have looked at how the efficacy of specific bioprotectants can be affected by a range of features associated with the biology of their target pest or pathogen, such as growth and reproduction rate, susceptibilities of different life stages to the bioprotectants, and population size. There are also inherent features of bioprotectants that will determine their efficacy including speed of kill, effective concentration and persistence on the leaf surface. Whether the activity of bioprotectants with primarily contact activity can counteract slow developing pests or pathogens with longer latent periods can also be partially answered by looking at these factors.

For successful germination and colonisation of leaves, both *B. subtilis* and *G. catenulatum* require RH humidity of 80–98% and an optimal temperature of ca. 25°C. Studies conducted in AHDB project CP158 Application and Management of Biopesticides for Efficacy and Reliability (AMBER) also showed that there is a good survival rate of both bioprotectants on foliage for at least two weeks in the absence of a target pathogen. For the bioprotectant AQ10, which specifically targets powdery mildew and contains the mycoparasitic fungus *Ampelomyces quisqualis*, successful germination and parasitism of powdery mildew requires RH of 90-95% at the site of parasitism with efficacy decreasing rapidly below this, an optimal temperature around 25°C, and the presence of its target pathogen upon application. *A. quisqualis* has a long latent phase, and in the presence of powdery mildew takes 5-10 days to invade powdery mildew colonies on foliar tissues and complete its life cycle within the fungal host. Though the UK Registration Report for AQ10 states that without its powdery mildew host, viability of *A. quisqualis* is rapidly lost e.g., within a few days, the number of days as well as the rate of decline is not fully defined for particular crop situations.

While it is clear that humidity plays a key role in bioprotectant efficacy and growth rate of certain targets, temperature often plays a more important role in determing latent periods and growth rates of both organisms. Microbial bioprotectants of both pests and pathogens should ideally work under its target environmental temperature range, but also have a thermal performance curve that matches, or overlaps, that of its target organism. If the thermal performance curves of the bioprotectant and its target are different, then there is likely to be a set of temperatures at which the target can grow and reproduce but the bioprotectant cannot control it. If the thermal performance curves match, however, then both target and bioprotectant will respond similarly to temperature changes. In theory, this means that successful levels of crop protection can still occur at suboptimal temperatures. In summary, microbial bioprotectants which have shorter periods of growth and reproduction that are less temperature or humidity dependant, and have good survival rates in the absence of their target-which may be either slower reproducing, or existing on the phyllosphere in its latent phase, are likely to be more virulent against their target. This is something that has not been explored in great detail and requires attention in the future.

### Discussion

#### Survival and proliferation of microbial bioprotectants on herb surfaces

Three different forms of microbial protectant were chosen for consideration in the review entomopathogenic fungi for pest management (as Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii) and bacterial (as Bacillus subtilis) and fungal bioprotectants (as Gliocladium spp.) for disease management. No studies or information were identified in available public and published literature addressing the survival and proliferation of these microorganisms on rosemary, basil, or coriander. However, research is available from other horticulture crop species including strawberry, lettuce and cyclamen. Multiple studies show that while both *B. subtilis* and *G. catenulatum* can survive for long periods on the leaf surface, dispersal is very limited particularly under protected conditions where dispersal between leaves is achieved primarily from physical contact between leaves. The efficacy of microbial bioprotectants in practice is thus less likely to be affected by the survival of biocontrol microbes but more likely by other factors that influence the density of biocontrol cells in the phyllosphere, especially dilution due to rapid host leaf expansion, spray coverage and rain wash-off. It is likely that properties of herb species and their volatiles are limiting the ability of microorganisms to multiply, colonise and where relevant, to produce microbial compounds. The subject area warrants further investigation.

Interactions between herb-produced volatiles and microbials used for pest and disease control

Seven studies were identified which directly addressed the interaction between essential oils or associated VOCS from rosemary, basil, and coriander and the microbial bioprotectants chosen for inclusion in the review. Additional studies were identified which addressed the interaction between relevant VOCs and either B. bassiana or B. subtilis in vitro, or the interaction between essential oils from species with a strong VOC emission profile e.g., lavender, eucalyptus, or coniferous trees. Content et al., 2022 showed that certain growing practices such as open field production, temperature ranges between 11-20°C, soils high in nutrients, irrigation and fertiliser use can all cause an increase in volatiles abundance for some of the herb species included in this review. This suggests that different growing practices are indirectly manipulating the composition and abundance of volatile compounds that could have a detrimental effect on bioprotectant efficacy. Use of essential oils or VOCs in vitro affected germination and germ tube development, hyphal growth and sporulation of the EPFs B. bassiana, M. anisopliae and L. lecanii. But available evidence was not consistent across organisms or treatment e.g. Nardoni et al., 2018 demonstrated that M. anisopliae showed limited sensitivity against essential oils of eucalyptus, lavender and pelargonium while B. bassiana appeared to be sensitive to 1% lavender oil. Liu (2012) showed that cymene had a stimulatory effect on germ tube development of *B. bassiana* at the lowest concentration tested but inhibited germ tube development at the highest concentration (50mM). For B. subtilis, the evidence was clearer that VOCs such as  $\beta$ -pinene and q-cymene have antimicrobial properties against intact Bacillus subtilis spores by directly interacting with the hydrophobic regions of membrane proteins in the cell. No evidence was available on the impact of essential oils or VOCs on G. catenulatum, however data is available on fungal pathogens including B. cinerea, F. oxysporum and Verticillium dahliae. It is highly likely that volatiles being produced by herb crops are affecting plant defence responses for individual herb species and the efficacy of microbial bioprotectants.

#### Repellent or attractant effects of herb volatiles on pest species and/or their natural enemies

Three studies were identified that specifically addressed the interaction between essential oils and VOCs from culinary herbs and their associated pests or natural enemies. Additional studies were identified which addressed the interaction between crop species containing relevant VOCs- specifically sweet pepper and melon cotton aphids, whiteflies, and their natural enemies, either *in vitro* or *in planta*. These studies showed that endophytic colonisation by EPFs including *B. bassiana* and *M. brunneum* have the potential to induce characteristic blends of volatiles which can influence olfactory behaviour and performance of aphids and whitefly, but the effects are small and dependant on the EPF strain used. It was also clear that emission of individual volatiles was not likely sufficient to elicit repellent or attractant effects, and that any effects were a consequence of individual VOCs acting in a

blend with other VOCs. Several studies demonstrated that whitefly preference on rosemary is repellency based, with preference recorded as a continuum of repellencies. Based on the evidence it is highly likely that volatiles being produced by adjacent herb crops are interfering with the attraction and distraction of pests affecting herb species and their natural enemies.

The role of bioprotectants, specifically elicitors in activating specific plant defence pathways and/or induced systemic resistance (ISR) mechanisms in herbs

Limited research to date does indicate that elicitors can trigger VOC emission in plants but comparative studies between different elicitors are rare and no publicly available research was identified which addressed the role of bioprotectants in ISR mechanisms in herbs. The available research suggests that it is highly unlikely that plant defence responses are limited or absent in herbs such that there is no induced systemic resistance. Available research from other pathosystems suggests that elicitor based bioprotectants such as COS-OGA, cerevisiane and laminarin based products which are commercially available and used in herb production in the UK may induce changes in VOC emission as part of their defence response. However, the effect is likely to be dependent on a myriad of variables including herb species, variety, crop phenological stage and environmental parameters. The strength and blend of VOC emissions are also highly likely to vary according to the elicitor being used. This area of research warrants further investigation in herbs.

## Conclusions

- Growers' hypotheses that lack of success with bioprotectants on herb crops may be due to intrinsic characteristics of the herb species being cultivated has been partly confirmed through limited evidence identified through completion of this review. But the effects are likely to be small and dependant on a myriad of compounding, interacting factors including the bioprotectant, the growing environment and crop species.
- The efficacy and survival of microbial bioprotectants in practice is less likely affected due to biochemical characteristics of herbs, and more likely by other factors that influence the density and further dilution of these microorganisms on foliar surfaces. This dilution in concentration of the microbial protectant will be compounded by crop growth and abiotic factors including slow or fast host leaf expansion, spray coverage and rain wash-off.
- Outcomes from this review on the biochemical and physical characteristics of herbs which affect bioprotectant efficacy should form the basis for more detailed research through other methods of funding, e.g., through a BBSRC-CASE Studentship or through IUK.

## Knowledge and Technology Transfer

The review has been discussed with growers and scientists. This report will be disseminated to all relevant levy payers, appropriate research organisations and contractors.

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