Project title:	OPTIMISING THE USE OF BIOCONTROL AGENTS TO IMPROVE THE CONTROL OF *B. CINEREA* IN KEY VEGETABLE AND FRUIT CROPS
Project number:	CP140
Project leader:	Prof. Xiangming Xu, NIAB EMR
Report:	Annual 04/2019
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Date project commenced:	05/10/2015
Date project completed (or expected completion date):	05/07/2019

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[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.]

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.

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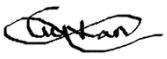
Signature  Date 24 April, 2019

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Contents

GROWER SUMMARY

Headline

The third year of the PhD project focused on characterising the effect of climatic factors on temporal population dynamics of the two commercial biocontrol agents (BCAs) (*B. subtilis QST 713* and *G. catenulatum J1446*) on lettuce and strawberry plants. We identified conditions suitable for phyllosphere establishment of the two BCAs, which can be used to optimise BCA application.

Background

BCAs are living organisms and as with every organism reproduction is critical to its survival. Understanding how environmental conditions affect survival, reproduction, and dispersal is crucial to maximise biocontrol efficacy. The overall aim of the study is to obtain ecological knowledge on BCAs available in the UK and utilize the knowledge to produce strategies for effective application of such BCAs to improve control consistency and efficacy against *B. cinerea* on lettuce and strawberry crops.

Summary

Establishing how abiotic factors influence the two BCAs temporal population dynamics is important to optimise their use on the two crops allowing successful colonisation. All the tested abiotic factors of temperature, relative humidity (RH) and dew point effected viable BCA population overtime, but the relationship between the BCAs and these factors in the phyllosphere was complex.

The viable population of the BCAs was monitored with the developed PMAxx^{TM}-qPCR method. Increasing temperature led to population survival and reproduction up to the optimum growth temperature under healthy plant transpiration rates (i.e. release of water through plants aerial parts at an appropriate rate in which the plants are not over or under transporting water) for the two BCAs on both crops. Increasing RH allowed population survival and reproduction especially in sub-optimal and optimal growth temperatures. The bacterial BCA thrived better in higher dew points, while the fungal BCA preferred lower dew points. The two BCAs can survive at least up to ten days in both crop systems, though the absolute viable population size decreased significantly in some conditions. There results can assist in the development of strategies for better timing of applications of BCAs to increase their survival and hence biocontrol efficacy. Currently we are developing mathematic models to capture such ecological knowledge of the two BCAs.

Financial Benefits

The knowledge can be used to increase the effectiveness of Serenade and Prestop against botrytis.

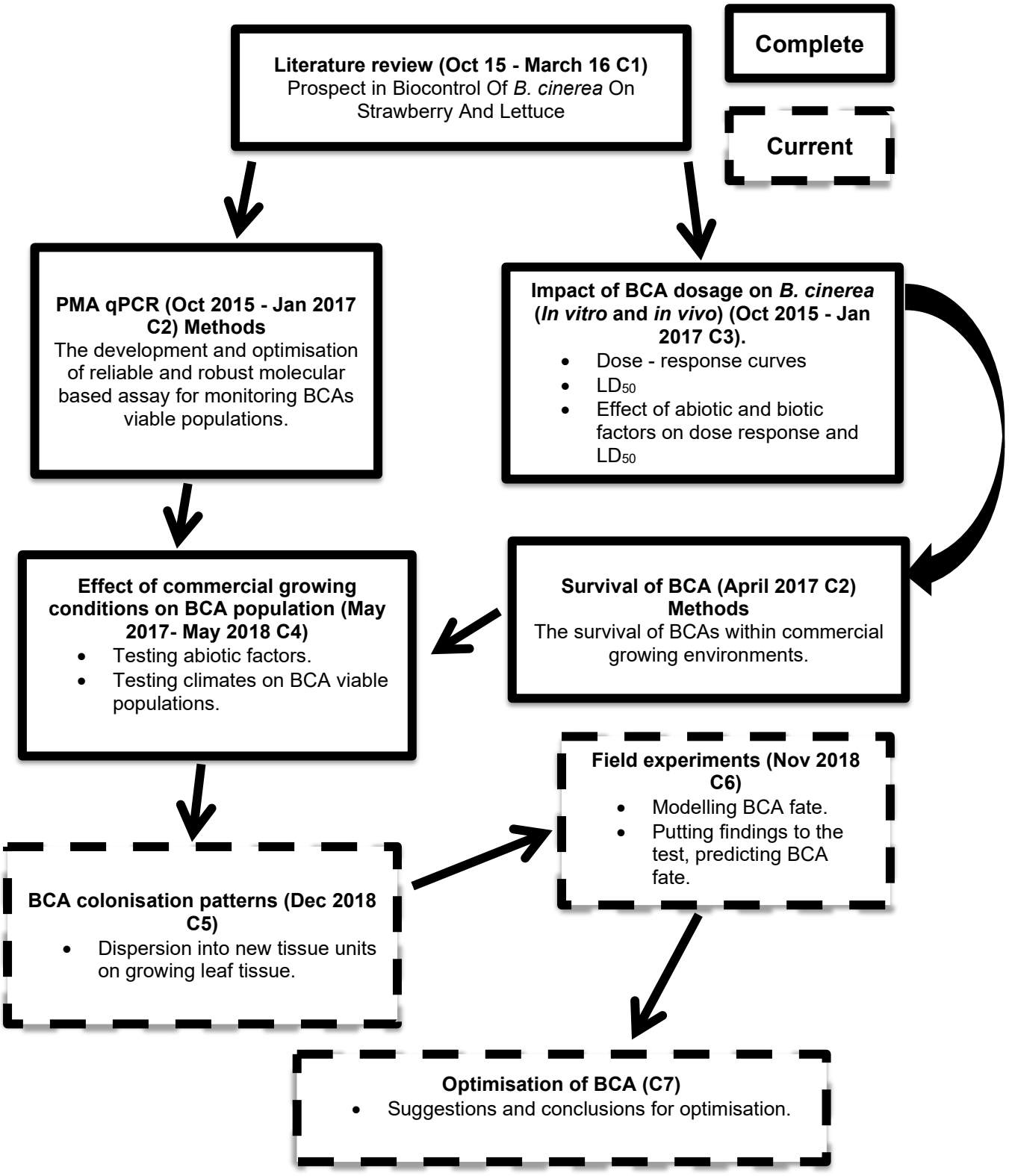
Action Points

Consider climatic conditions when applying Serenade and PreStop to improve their establishment.

SCIENCE SECTION

Introduction

This is the 4th (final) year of the PhD project; the overall project plan and the work achieved are displayed in the flow diagram below.



Please note that the completion dates in the flow diagram are for practical experimental work and do not include data collection, analysis and write up period. The research has progressed significantly since the last reported. For the two BCAs (*B. subtilis* QST 713 and *G. catenulatum* J1446), we studied their population dynamics under a range of climatic conditions on lettuce and strawberry plants. This ecological knowledge on how abiotic factors (temperature and RH) affect temporal population dynamics of the two BCAs on lettuce and strawberry plants will enable us to improve current application strategies to improve their establishment and survival. The BCA propagules were monitored in nineteen combinations of temperature and RH, representing UK commercial growing conditions for lettuce and strawberry.

Effect of abiotic factors on temporal population dynamics of BCAs on the phyllosphere

Background for research

Our previous research on the dose-response relationships determined the median effective population density needed to control a high inoculum of *B. cinerea*. Identifying the required population density required for biocontrol is an important step in optimization of BCA formulations to control their target pathogens, especially in the phyllosphere (Gotor-Vila et al., 2017). Both biotic and abiotic factors impact on BCA population densities, especially in the phyllosphere of foliar surfaces. However, studies suggest that abiotic factors are the primary cause in the losses of the introduced inoculum populations and the biocontrol level achieved (Magan, 2001, Sui et al., 2015, Liu et al., 2013). Thus, it is important to examine the fate of *B. subtilis* QST 713 and *G. catenulatum* J1446 inocula under UK commercial production systems. For a BCA to be reliable, consistent and effective, establishment in the phyllosphere is essential. Previously, the inconsistent efficacy of BCAs was often attributed to the lack of BCA establishment under the environmental conditions. Resilience under changing abiotic factors is important characteristic of ecologically competent BCAs. There is lack of knowledge on the effect of temperature and relative humidity on *B. subtilis* QST 713 and *G. catenulatum* J1446 population dynamics in the plants' phyllosphere. Most studies have suggested that abiotic factors are the cause of loss in biocontrol efficacy.

To our knowledge the temporal population dynamics of these two BCAs under UK commercial growing climatic regimes in the phyllosphere of lettuce and strawberry crops has never been studied. The objective of the research is to study the impact of temperature, RH and dew point on temporal population dynamics of *B. subtilis* QST 713 and *G. catenulatum* J1446 under a range of climatic conditions on the phyllosphere of lettuce and strawberry crops.

2. Materials and Methods

2.1 BCA preparation

Refer to previous report. The following modifications were made: (1) for production of *G. catenulatum* J1446 inoculum, 5 g of PreStop powder was placed in 1 L of tap water and shaken vigorously; (2) The *B. subtilis* QST 713 colonies cultured on nutrient agar was cut into four equal parts and transferred to a 1 L vacuum filter flask containing pre-autoclaved tryptone soya broth (Sigma) and was grown on a rotary shaker (110 rpm) at 20 – 25 °C for 10 days. The concentration of both BCAs before each spraying event was determined by plate counts on three replicates of nutrient agar for *B. subtilis* QST 713 and malt extract agar for *G. catenulatum* J1446.

2.2 Plant propagation

Berry Plants provided strawberry plug plants (Malling Centenary), and Premier Plants provided lettuce cotyledons (Carter) in peat blocks. Plants were grown into pots (9 cm x 9 cm x 10 cm) using standard compost. Plants were grown in a semi-commercial pest and disease free glasshouse. Strawberry plants were at their early flowering stage, while lettuce plants were in early head development before experimentation. Under plant watering was adhered to on a daily basis.

2.3 Environmental treatments and experimental design

A randomized block design was followed to investigate the effect of climatic conditions on the temporal population dynamics of *B. subtilis* QST 713 and *G. catenulatum* J1446. Selected climatic treatments (see Table 1) represented UK climatic conditions for strawberry and lettuce production. The rationale in selecting these temperature and humidity combinations was to (1) cover a wide range of UK growing climates, (2) have several sets of common vapour pressure deficit (VPD in mbar) where feasible to study the effects of temperature under the same evaporative demand, (3) have an increasing relative humidity range for each temperature, and (4) have a large dew point range to represent the effect of combined increase in temperature and moisture within the climatic zone. This design allowed testing

the effect of changing temperature and relative humidity independently, and changing temperature and moisture together. Each climatic treatment was repeated at least twice, and contained a total of ten replicates (five replicates per repeat experiment) and each replicate containing approx. 6 leaves obtained from plants placed on a grid inside a controlled environment cabinet. The time period of each treatment was 10 days with samples taken every 48 h (D0, D2, D4, D6, D8 and D10). Refer to Appendix AHDB annual report 2 for details of the definition of the frequency and period of quantifying BCAs with the PMAxx^{TM}-qPCR technique. The experiments also contained negative controls which were untreated lettuce and strawberry plants in each climatic treatment.

Table 1 Experimental conditions and levels of temperature and relative humidity considered for representing commercial growing conditions in the U.K

Tempera-ture°C	1	Humidity	Dew point °C	2	Humidity	Dew point °C	3	Humidity	Dew point °C	4	Humidity	Dew point
10°C	**A**	65% (4.3mb)	3.7°C	**B**	75% (3.1mb)	5.8°C	**C**	85% (1.8mb)	7.6°C	**D**	95% (0.6mb)	9.2°C
16°C	**B**	60% (7.2mb)	8.2°C	**C**	68% (5.8mb)	10.1°C	**D**	76% (4.3mb)	11.8°C	**E**	90% (1.8mb)	14.4°C
22°C	**C**	55% (11.9mb)	12.5°C	**D**	64% (9.5mb)	14.9°C	**E**	73% (7.2mb)	16.9°C	**F**	84% (4.3mb)	19.2°C
28°C	**D**	50% (18.9mb)	16.6°C	**E**	59% (15.4mb)	19.2°C	**F**	68% (11.9mb)	21.5°C	**G**	81% (7.2mb)	24.4°C
34°C	**E**	45% (29.3mb)	20.4°C	**F**	55% (24.1mb)	23.7°C	**G**	64% (18.9mb)	26.2°C	**H**	78% (11.9mb)	29.6°C

2.4 General methodology description

All experiments followed seven common steps: (1) plant propagation and selection; plants were sown, grown and selected for being pest-disease free and healthy with a minimum of six leaves. (2) BCA cultivation and concentration calibration; plate counts were used to determine the concentration of the cultivated *B. subtilis* QST 713, and *G. catenulatum* J1446 (PreStop), and were adjusted as necessary to obtain a median effective dose (AHDB annual report 2). (3) Plant treatment; plants were sprayed with the BCA as a fine droplet setting just before run off. (4) Plant drying: after treatment plants were allowed to dry for 1 h in the

glasshouse and then placed into a climatic chamber, alongside sampling and image acquisition of D0 subjects. (5) Exposure to climatic regimes: all treatments had the same light dark cycles of 14 h light and 10 h dark (3 h at 30 % light, 3 hrs at 50 % light, 4 hrs at 70 % light, 2 h at 100% light, followed by 2 h at 10 % light, and 10 h of dark). (6) Sampling on days 0, 2, 4, 6, 8 and 10. The older leaves were collected from three pre-determined plants (two leaves per plant), imaged alongside a standard (Panasonic DMC-SZ3) and immediately placed into a falcon tube containing maximum recovery diluent (Sigma). (7) Surface washing, filtration and cell pellet collection; the leaves were soaked in the maximum recovery diluent until full, sealed and shaken on a rotary shaker at 100 rpm for 30 mins at 10 °C. The contents were filtered with a wet muslin cloth (four layers) and cells pelleted by centrifugation at 2000 × g for 15 minutes at 4 °C. The supernatant was decanted and the cell pellet supplemented with maximum recovery diluent solution and transferred into a 1.5 ml Eppendorf, and stored at 4 °C.

Constant temperature and relative humidity conditions (step 5) were achieved with two climatic chambers (Panasonic model MLR-352, and Sanyo format 650). Prior to experimentation climate chambers were calibrated using external data loggers (EasyLog EL-USB-2 standalone USB temperature and RH %, dew point data logger). The same data loggers were used for monitoring the temperature, relative humidity and dew point in each chamber throughout the experiment. The total surface area of the leaves in each replicate was calculated with image J.

2.5 PMAxx™ treatment

Refer to AHDB annual report 2 Material and Methods Section PMAxx™ treatment.

2.6 Grinding of *G. catenulatum* J1446 cells

Briefly, after PMA treatment, cells were pelleted by centrifugation at 5,000 × g for 10 minutes at 4°C. The supernatant was decanted and conidia were suspended in maximum recovery diluent solution with a final volume of 1 ml. After slow pipetting for homogenisation, stainless steel beads (6 mm) were transferred into each sample, and *G. catenulatum* conidia were ground with the use of the genome grinder 2000 set at 1750 rpm for 20 minutes. After samples were ground the steel beads were removed with a magnet and sterilized with 5 % bleach and 70 % ethanol.

2.7 DNA extraction

Refer to AHDB annual report 2 Material and Methods Section DNA extraction and qPCR.

2.8 Minimizing and suppressing of qPCR inhibitors

For improving the dissolution of the extracted DNA into 8 mM sodium hydroxide, five steel beads (6 mm) were transferred into each sample which contained a total volume of 1 ml and the DNA pellets were ground using the genome grinder 2000, set at 500 rpm for 1 minute followed by a cool down period of 2 minutes, the cycle was repeated fifteen times. After the grinding phase the DNA was pelleted at 1500 × g for 5 minutes at 4 °C and the supernatant (clear DNA suspended in 8 mM sodium hydroxide) was diluted into 8 mM sodium hydroxide. The dilution depended on the clarity of the DNA sample. Filtration of the diluted DNA was completed with a Millex-VV Syringe Filter Unit 0.1 µm (PVDF, 33 mm and gamma sterilized), and the DNA purity and concentration was measured on a Nanodrop spectrometer (NanoDrop ND-1000; NanoDrop Technologies, Wilmington, DE).

2.9 qPCR

Refer to AHDB annual report 2 for DNA extractions and qPCR methods. The following amendments were made: (1) The BIO-RAD CFX96TM real time PCR detection system (BIO-RAD) was used to quantify DNA, and (2) CFX Manager™ Software version 3.1 (BIO-RAD) used to analyse and calculate the Cq values automatically by adhering to the manufactures guidelines. (3) Reactions were prepared in a white/green semi-skirted 96 well qPCR plate (BIO-RAD). 4) The final volume in each reaction for both BCAs was 44 µL which contained 10 µL SensiFAST™ SYBR® No-ROX Kit (Bioline Meridian Bioscience, U.K). (5) Each reaction well contained 400 ng/µL of bovine serum albumin. (6) The cycling conditions used for both BCAs were 95 °C for 3 mins followed by 40 cycles of 45 s at 95 °C, 60 s at 61 °C and 60 s at 72 °C.

2.10 Statistical analyses

Before statistical analysis, the data sets of CFUs/mm^2 were transformed with the $\log_{10}$ function. One-way ANOVA was used to compare between all climatic treatments for each BCA on each crop, separated by time. When analysis proved statistically significant that climatic treatment and not time alone was affecting $\log_{10}$ CFUs/mm^2 an ANOVA was performed to test if differences were due to temperature, RH or dew point. For testing if differences were due to temperature, ANOVA's was performed on data sets with the same vapour pressure deficit at different temperatures. For testing if differences were due to RH ANOVA's was performed on data sets with the same temperature but different RH regimes. For testing if differences were due to dew point ANOVA's were performed for each BCA on each crop containing all dew points. The relationship of the BCA with each climatic regime on

each crop was defined using a general linear model. All data analysis was performed with the software package MiniTab (V. 17) at P=0.95 confidence level.

3. Results

One-way ANOVA showed that there were significant differences in temporal population dynamics of *B. subtilis* QST 713 on lettuce and strawberry plants, and of *G. catenulatum* J1446 on lettuce and strawberry among climatic treatments ($P < 0.05$). Two-way ANOVA indicated a highly significant effect of temperature, relative humidity and dew point on temporal population dynamics of *B. subtilis* QST 713 and *G. catenulatum* J1446 on both crops ($P < 0.05$).

Consideration of all the statistical analysis as a whole suggests that the temporal population dynamics of both BCAs in both crops was strongly influenced by temperature, relative humidity and dew point. However, the relationship between temporal population dynamics and these three factors in the phyllosphere was complex. The BCAs appeared to prefer specific climatic regimes (temperature and relative humidity combinations) ($P < 0.05$). For both BCAs in both crops, as temperature was increased, the viable population density increased ($P < 0.05$) but this was restricted to sub-optimal and optimal plant transpiration rates; the optimum plant transpiration rates for lettuce and strawberry plants are not known. But in general research suggests approx. 8 - 10 mb as ideal VPD for plants (Shamshiri, 2014). A clear pattern was found for the effect of RH for both BCAs with viable population density highest at temperature closest to their optimum for growth ($P < 0.05$). Viable population density increased on leaves of both crops for *B. subtilis* at dew points of 19 and 25 ($P < 0.05$), and for *G. catenulatum* at dew points of 7, 11, 14 ($P < 0.05$). The data in all figures is displayed as change from viable population Log_{10} (N – N0); meaning viable population at the time of X (i.e. day 2 / 4 / 6 / 8 / 10) minus the viable population at day zero. The data is displayed in this manner because it represents surpassing originally applied viable population as a positive value, and mortality as a negative value.

3.1 Effect of temperature on temporal population dynamics of BCAs

Figures 1 and 2 shows a small portion of the results illustrating the effect of temperature on the temporal population dynamics of the bacterial and fungal BCAs, respectively. This shows that for the bacterial BCA temperature significantly affected the temporal population dynamics on lettuce (4 out of 10 tests) and on strawberry (7 out of 10 tests). For the fungal BCA, on lettuce (6 out of 10 tests) and on strawberry (5 out of 10 tests) ($P < 0.05$). The temperature effect either leads to an increase in viable populations, relative survival or a decrease of the population overtime. This was directly influenced by vapour pressure deficit. An increase

and/or survival of the introduced applied populations of the BCAs in relation to temperature was found close to or at healthy plant transpiration rates (i.e. VPD of 6 and 8 mbar) (Shamshiri, 2014), which is close to, or at, the BCAs optimal temperatures, i.e., for *B. subtilis* QST 713 at 22 °C – 28 °C, and for *G. catenulatum* J1446 at 16 °C in both crops. The effect of increasing or decreasing temperature can be beneficial on the BCA population's overtime. However, the same temperature can also have a different effect if tested with a different VPD ($P < 0.05$). Overall, in both crops an increase in temperature favoured the increase and or survival of the bacterial BCA while a decrease in temperature favoured the increase and or survival of the fungal BCA. This is of course partially because of the different ecology of the two BCAs.

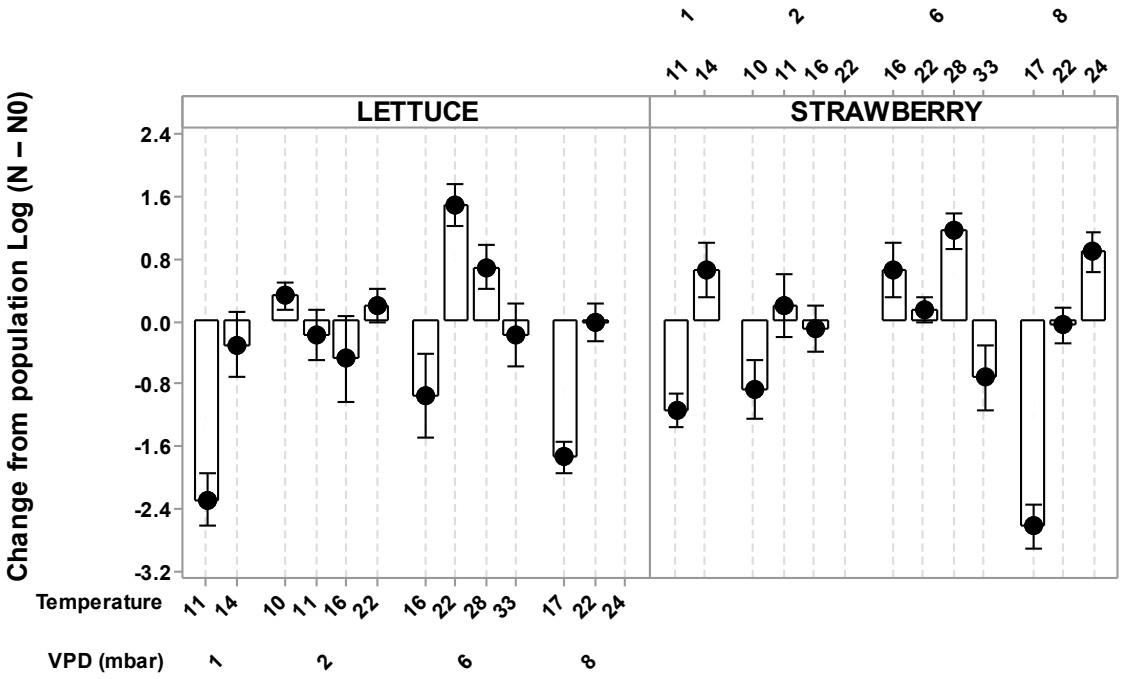


Figure 1 Change from the original populations of *B. subtilis* QST 713 on lettuce and strawberry plants measured every two days at different temperatures at the same VPD.

In Figure 1 - 6 the Y axis represents the BCA log-transformed mean population difference (N – N0) calculated by population at day X (2, 4, 6, 8, or 10 days) – population of day zero. Each mean in the figure contains up to ten replicates. The mean of the data sets displayed with a black circle are composed by change from the original population of all time points from the two experiments, and the standard errors of each mean are represented by interval bars. Negative controls lacked a positive

qPCR reaction (> 35 cq) and therefore the BCA population was assumed zero when Cq > 35. In Figure 3 and 4 the value and category scales were transposed (Y and X axis inverted).

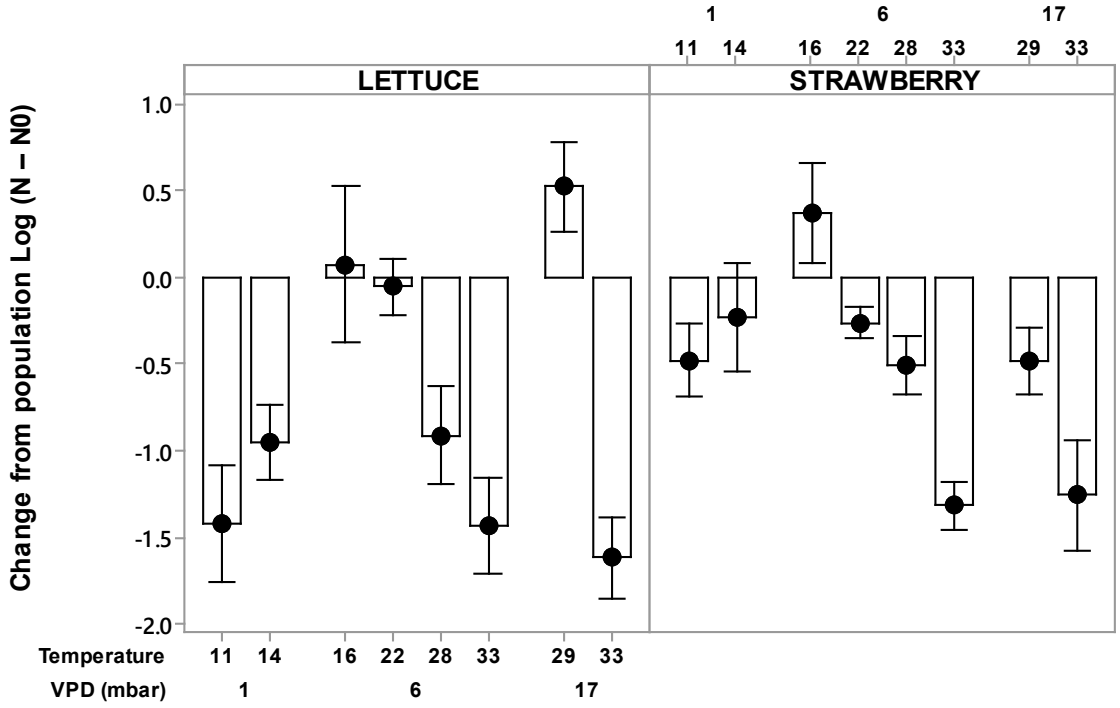


Figure 2 Mean changes from the original populations of *G. catenulatum* J1446 on lettuce and strawberry plants every two days at different temperatures at the same VPD.

Overall, the results suggest that temperature affects temporal population dynamics of both BCAs significantly under the same VPD, and the effect on populations can be positive, conserved or negative and this directly depends on the evaporative demand of the environment and the host. The only clear pattern in which a temperature increase was beneficial was when VPD is close to healthy plant transpiration rates. This suggests a strong interaction between the temporal population dynamics of the BCA × temperature × host × transpiration rates.

3.2 Effect of RH on temporal population dynamics of the two BCAs

RH significantly affected the temporal population dynamics of the two BCAs. In the five temperature groups, statistically significant differences due to RH were found for the bacterial BCA on lettuce (2 out of 5 tests), and on strawberry (3 out of 5 tests). For the fungal BCA, on lettuce (1 out of 5 tests), and on strawberry (2 out of 5 tests). Differences generally occurred at sub-optimal and optimal temperatures for growth of the BCAs ($P < 0.05$). The optimal growth temperature for *B. subtilis* can range from approx. 22 °C – 35 °C (Cook, 1996,

Schaechter et al., 2006), while for *G. catenulatum* optimal growth temperature ranges from 15 °C – 25 °C (Helyer et al., 2014).

Figures 3 and 4 show examples of the effect of RH on the bacterial and fungal BCA. For both BCAs on strawberry, in general, low RH across all the temperature treatments led to a greater decrease in the population densities overtime (averaged from difference between day 0 and day 2, 4, 6, 8 and 10). Preferences in terms of RH for both BCAs appeared to be both temperature and host specific, and this resulted in either an increase, or better survival of the introduced population inocula. For the bacterial BCA there was a significant benefit when RH was increased. This was observed in strawberry crops at 10 °C (A1-4), 16 °C (B1-4) and 22 °C (C1-4) as shown in Figure 3, and for the fungal BCA in both crops at 22 °C (C1-4) in Figure 4.

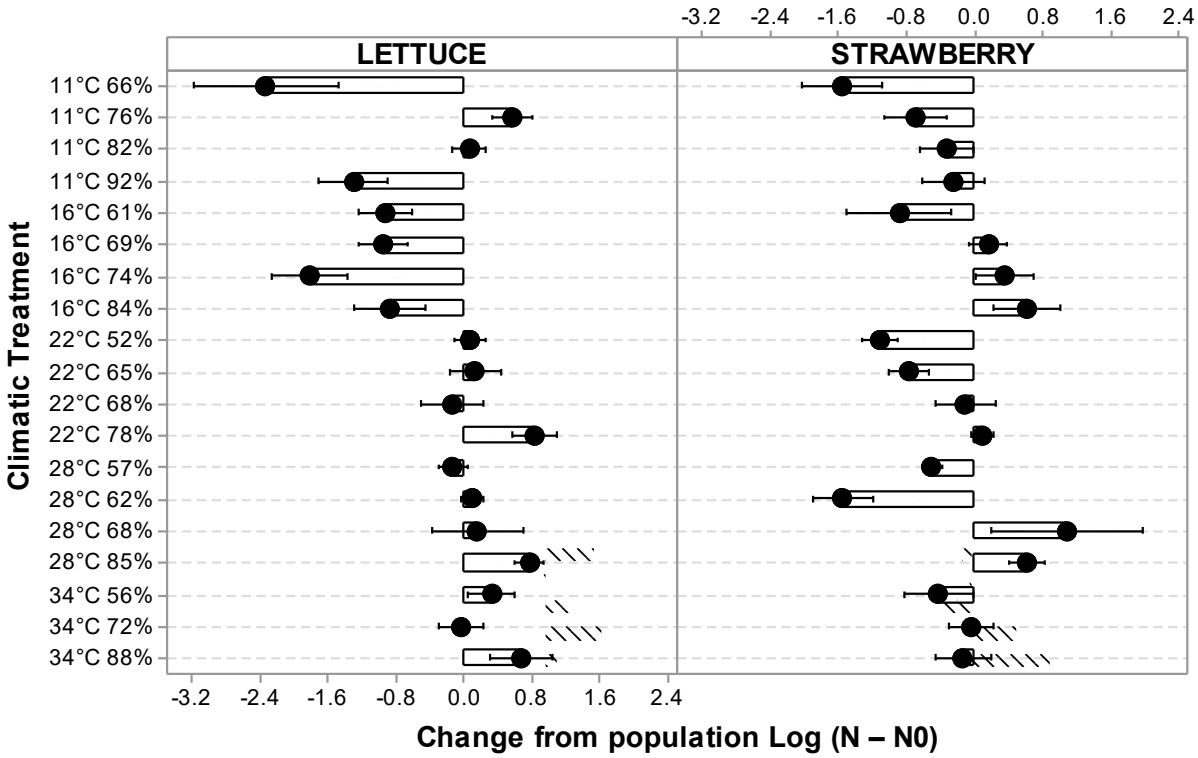


Figure 3 Change from the original populations of *B. subtilis* QST 713 on lettuce and strawberry plants every two days at a range of temperature and relative humidity regimes.

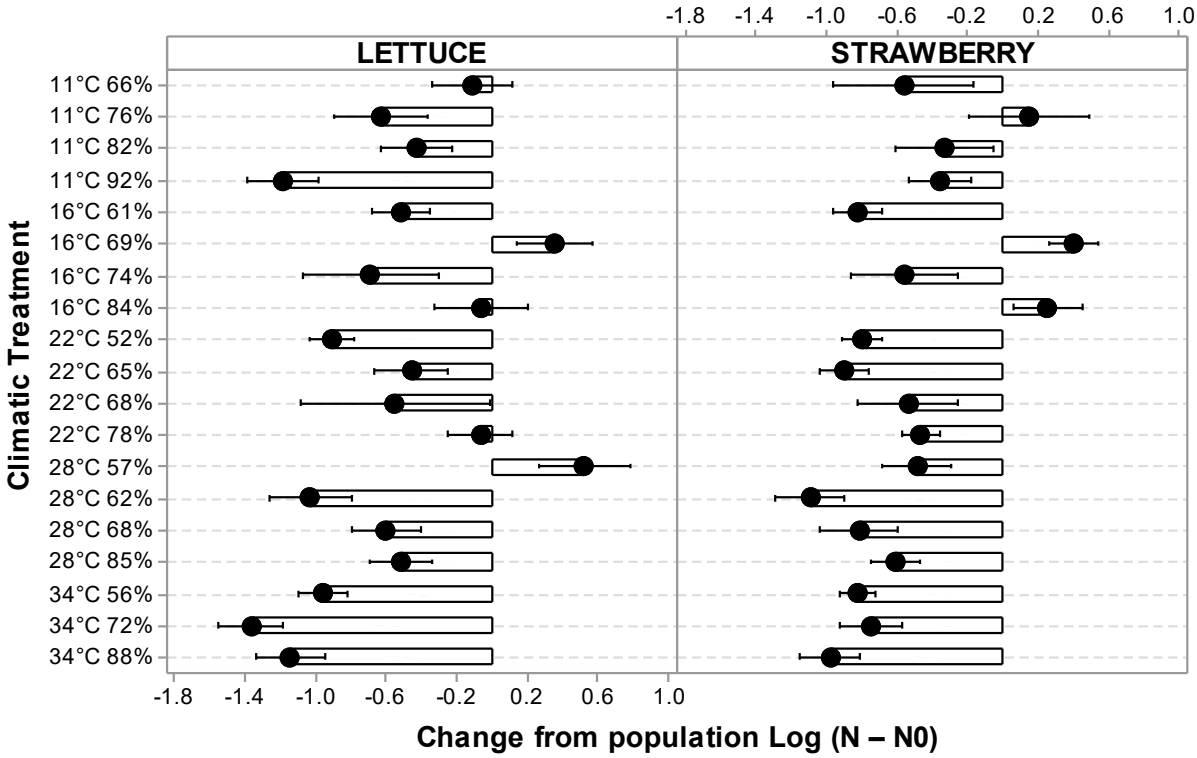


Figure 4 Change from the original populations of *G. catenulatum* J1446 on lettuce and strawberry plants every two days in a range of temperature and relative humidity regimes.

Increasing RH led to better survival of the introduced BCAs, but this was constrained to sub optimal and optimal growth temperatures of the BCAs. As a whole for both BCAs in both crops the favoured RH increased with temperature, but exceptions occurred at lethal temperatures for growth of the BCAs (i.e. 11 °C for *B. subtilis* QST 713 and 34 °C for *G. catenulatum* J1446 on both crops). The temporal population dynamics of these two BCAs differed depending on temperature, RH, and host; but there was no clear trend in the differences.

3.3 Effect of dew point on temporal population dynamic of the two BCAs

A total of twenty dew point treatments were available to use from all the available data. The change in the dew point significantly affected the temporal population dynamics of the BCAs. Out of the twenty dew points significances ($P < 0.05$) were found. A single statistical test containing the twenty dew points was performed for each BCA and crop combination. Thus, *B. subtilis* QST 713 on lettuce (9 out of 20 dew points) and, on strawberry (11 out of 20 dew points). For *G. catenulatum* J1446 on lettuce (12 out of 20 dew points) and on strawberry (16 out of 20 dew points). Figures 5 and 6 demonstrate the effects for some of the data when

dew point is increased for the bacterial and fungal BCAs, respectively. Low dew points on both crops resulted in a decrease in the bacterial viable population density overtime; in contrast for the fungal BCA this increased. High dew points in both crops resulted in an increase in the *B. subtilis* QST 713, and a decrease in the *G. catenulatum* J1446 viable population. For both BCAs on both crops in BCA optimal growth temperatures (i.e. for *B. subtilis* QST 713 at 22 °C – 28 °C, and for *G. catenulatum* J1446 at 16 °C) the closer the ambient air temperature to the dew point, the greater the viable population growth was observed. An increase in dew point positively was correlated with an increase in viable population survival and/or growth of *B. subtilis* QST 713 up to a dew point of 25 °C. A decrease in the dew point was positively correlated with an increase in phyllosphere viable population survival and/or growth of *G. catenulatum* J1446 down to a dew point of 7 °C.

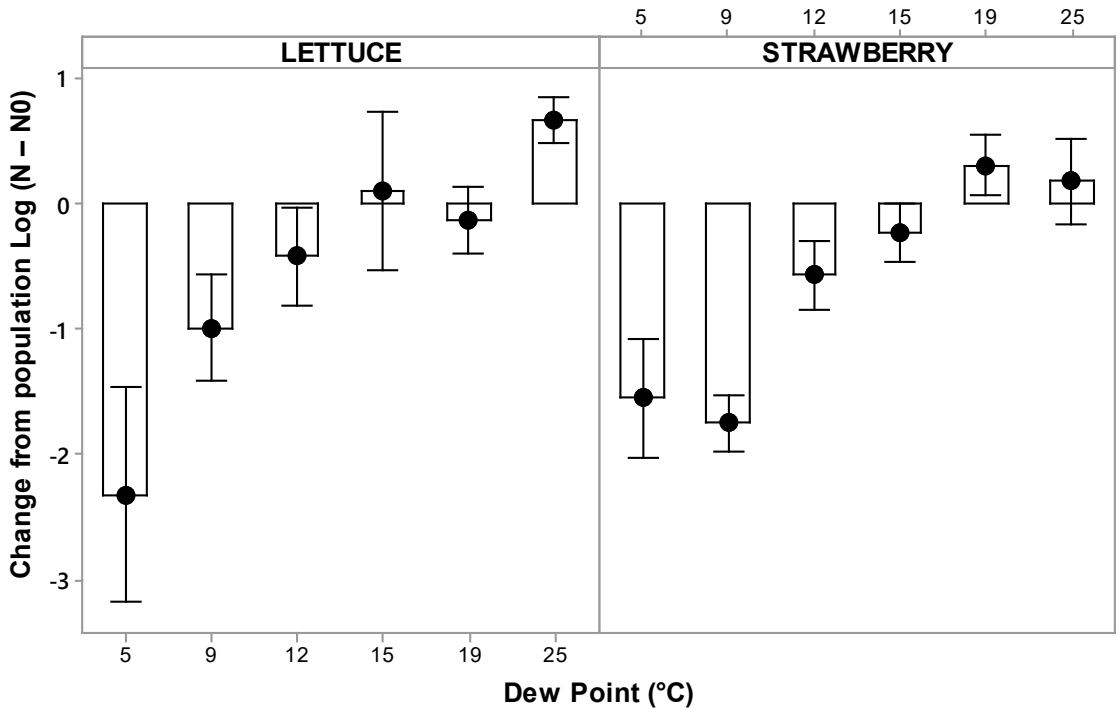


Figure 5 Change from the original populations of *B. subtilis* QST 713 on lettuce and strawberry plants every two days at a range of dew points.

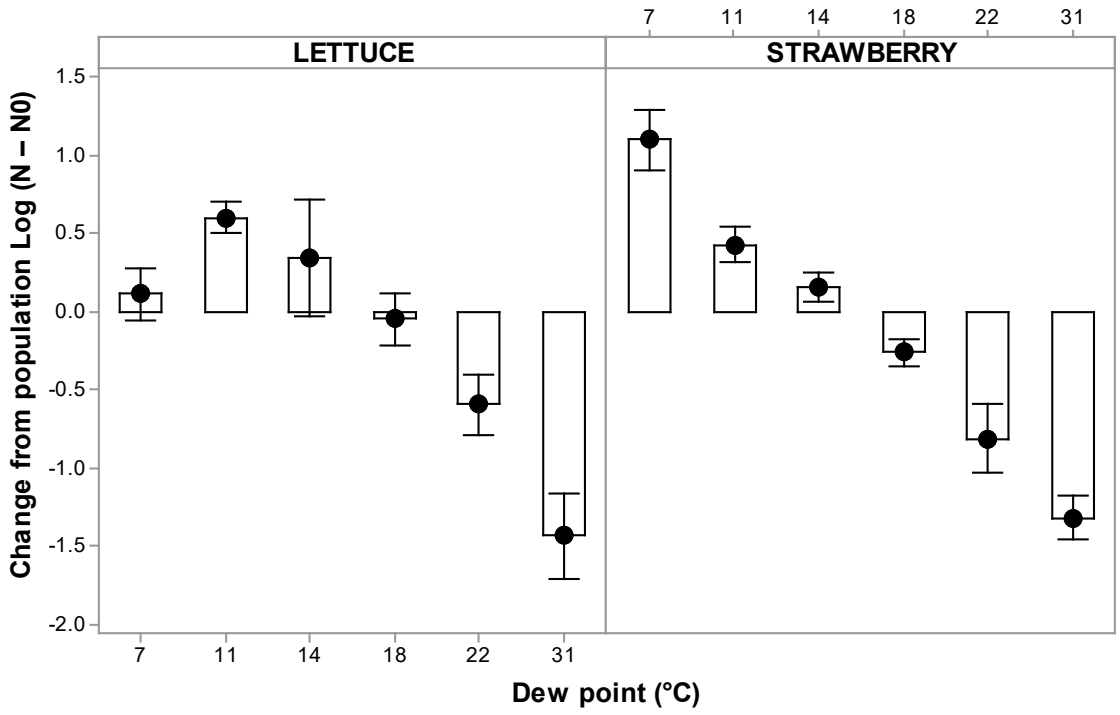


Figure 6 Change from the original populations *G. catenulatum* J1446 on lettuce and strawberry plants every two days at a range of dew points.

3.4 UK commercial growing climates

Table 2 presents the average population changes for *B. subtilis* QST 713 and *G. catenulatum* J1446 on both crops. Also to understand the relationship and compatibility between climates and the BCAs on their target hosts a ANOVA general linear model was fitted (Table 3) the data models coding was (-1, 0, +1).

Table 2 Summary of log change from the original viable population of the two BCAs (Log N – Log N0) under UK commercial growing climates for ten days.

		***B. subtilis* QST 713 on lettuce**					***B. subtilis* QST 713 on strawberry**				
		Time (days)					**Time (days)**				
≈	**≈**	**2**	**4**	**6**	**8**	**10**	**2**	**4**	**6**	**8**	**10**
Temp°C	**RH%**										
11	**66**	-2.67	-1.53	-1.83	-4.59	*	-0.77	-0.41	-1.75	-1.78	-3.09
11	**76**	0.39	0.15	0.51	1.29	0.52	-0.20	-0.37	-0.45	-1.21	-1.20
11	**82**	0.06	0.34	0.49	-0.32	-0.20	0.16	0.10	0.47	-1.23	-1.19
11	**92**	-0.86	-2.29	-1.02	-1.02	-1.29	0.49	0.05	-0.18	-0.88	-0.75
16	**61**	-1.21	-0.33	-0.82	-1.06	-1.19	-0.59	-1.29	-0.29	-1.40	-0.86
16	**69**	0.03	-1.31	-1.14	-1.18	-1.16	0.70	0.29	-0.05	0.11	-0.50
16	**74**	-0.08	-1.77	-1.62	-2.26	-3.24	2.24	-0.07	-0.28	0.05	-0.25
16	**84**	0.74	-0.71	-1.32	-1.46	-1.57	1.67	0.89	0.85	-0.30	-0.03
22	**52**	-0.06	0.71	-0.10	-0.07	-0.08	-0.41	-1.06	-1.33	-1.83	-0.90
22	**65**	0.49	0.63	0.69	-0.64	-0.44	-0.83	-1.45	-0.49	-0.40	-0.62
22	**68**	0.44	1.31	-0.24	-0.45	-1.67	0.00	0.51	0.49	-1.91	-0.96
22	**78**	1.14	1.15	0.58	0.78	0.58	0.25	-0.19	0.01	0.19	0.22
28	**57**	0.24	-0.43	-0.70	0.40	-0.39	-0.93	-0.46	-0.24	-0.44	-0.47
28	**62**	-0.06	0.27	0.04	0.11	0.14	-1.14	-0.73	-2.57	-2.05	-2.05
28	**68**	1.56	*	-0.02	-1.11	0.27	1.32	2.39	3.49	-0.57	-1.25
28	**85**	1.11	0.74	1.38	0.03	0.65	0.16	0.46	1.30	0.73	0.38
34	**56**	0.64	1.07	0.11	-0.58	0.12	-0.64	-0.67	0.38	-0.32	-0.84
34	**72**	-0.73	0.42	0.91	-0.13	-1.17	-0.14	0.74	0.35	-0.80	-0.33
34	**88**	1.21	1.28	0.46	0.62	-0.08	-0.76	0.90	0.44	-0.46	-0.80
		***G. catenulatum* J1446 on lettuce**					***G. catenulatum* J1446 on strawberry**				
		Time (days)					**Time (days)**				
≈	**≈**	**2**	**4**	**6**	**8**	**10**	**2**	**4**	**6**	**8**	**10**
Temp°C	**RH%**										
11	**66**	0.39	0.01	-0.16	-0.77	0.00	-0.67	0.35	-0.73	-1.46	-0.29
11	**76**	-0.83	-0.96	-0.50	-0.24	-0.61	0.55	0.51	-0.12	0.03	-0.23
11	**82**	-0.53	-0.86	-0.40	0.03	-0.36	0.21	-0.17	-0.40	-0.20	-1.09
11	**92**	-0.61	-1.13	-0.84	-1.45	-1.92	0.21	0.13	-0.28	-1.06	-0.78
16	**61**	-0.41	-0.56	-0.19	-0.81	-0.60	-0.53	-1.04	-0.84	-0.99	-0.71
16	**69**	0.89	0.41	0.49	-0.34	0.38	0.94	-0.12	0.34	0.43	0.39
16	**74**	-0.81	-0.72	-0.94	-0.60	-0.06	-0.06	-0.37	-0.42	-1.21	-0.92
16	**84**	0.60	0.69	-0.87	-0.70	-0.02	0.78	0.14	-0.06	0.48	-0.12
22	**52**	-0.74	-0.91	-1.06	-0.75	-1.09	-0.33	-0.56	-0.99	-0.72	-1.37
22	**65**	0.30	-0.32	-0.92	-0.48	-0.86	-0.73	-0.76	-1.38	-0.83	-0.79
22	**68**	-0.76	-1.09	0.80	0.72	-1.10	0.03	-0.72	-0.68	-0.66	-0.19
22	**78**	0.47	0.37	-0.16	-0.13	-0.86	-0.30	-0.25	-0.45	-0.60	-0.71
28	**57**	0.64	0.52	0.40	0.22	0.86	-0.38	0.12	-0.42	-1.02	-0.74
28	**62**	-0.53	-0.90	-1.22	-1.14	-1.36	-0.49	-0.74	-1.28	-1.76	-1.19
28	**68**	-0.14	-0.34	-0.73	-0.65	-1.13	0.10	-0.45	-1.19	-1.13	-1.41
28	**85**	-0.83	-0.40	-0.70	-0.43	-0.22	-0.58	-0.34	-1.00	-0.67	-0.45
34	**56**	-0.85	-1.14	-0.92	-1.19	-0.68	-0.49	-1.37	-0.60	-0.99	-0.67
34	**72**	-1.87	-1.61	-0.83	-1.28	-1.24	-0.77	-1.50	-0.43	-0.28	-1.03
34	**88**	-1.86	-1.40	-1.12	-0.82	-0.52	-1.42	-1.26	-0.68	-0.96	-0.57

The symbol (*) indicates population was not quantifiable.

Table 3 Expected change in viable phyllosphere population densities of the two BCAs under UK commercial growing climates every two days.

≈ Temp°C	≈ RH%	*B.subtilis* lettuce	*B.subtilis* strawberry	*G.catenulatum* lettuce	*G.catenulatum* strawberry
11	66	-0.533	1.613	-0.163	-0.289
11	76	0.571	0.558	0.403	0.340
11	82	0.099	0.059	-0.221	-0.150
11	92	0.632	0.741	0.136	-0.015
16	61	0.020	0.051	0.072	-0.067
16	69	0.627	1.529	0.834	0.710
16	74	0.108	0.924	-0.095	-0.060
16	84	0.545	1.277	0.371	0.518
22	52	-0.005	-0.283	0.128	-0.188
22	65	0.363	-0.197	0.572	0.287
22	68	1.218	1.169	-0.408	-0.165
22	78	-0.694	-1.024	0.352	0.335
28	57	-2.070	-1.725	0.028	0.214
28	62	0.293	-0.598	-0.218	-0.218
28	68	-0.639	-0.380	-0.502	-0.213
28	85	0.268	-0.665	-0.341	-0.057
34	56	-0.301	-1.332	-0.347	-0.233
34	72	-0.521	-1.160	-0.246	-0.419
34	88	*	*	*	*

The climatic treatment of 34 °C at 88 % RH could not be predicted as the variance inflation factor was not producible (meaning the variance between the replicates were too large for prediction).

4. Discussion

This is the first study to assess the impact of abiotic factors (temperature and RH, and hence dew point) on the temporal population dynamics of these two BCAs in the phyllosphere of lettuce and strawberry crops. These abiotic factors all significantly affected BCA temporal population dynamics. When BCAs were subjected to their sub-optimal and optimal growth temperature, increasing RH led to an increase in the viable population for both BCAs. For *B. subtilis* QST 713 increasing dew point improved its survival and/or reproduction; the opposite was observed for *G. catenulatum* J1446.

On the phyllosphere the bacterial and fungal BCAs temporal population dynamics are complex and depend on the interaction between temperature, humidity and host. In the majority of UK commercial growing climates represented by temperature and RH

combinations differences in the temporal population dynamics for both BCAs in lettuce and strawberry phyllospheres were found. The viable population sizes varied with specific climatic conditions, indicating that these factors have influenced BCA mortality and/or reproduction. Of the abiotic factors examined, temperature has usually been suggested to be the most important factor influencing the development of an organism. *B. subtilis* cell growth becomes limited at < 11 °C (Price, 2000) and is optimal over the range 25 – 37 °C (Cook, 1996, Schaechter et al., 2006). The fungal BCA, *G. catenulatum* is active at 5 - 34 °C, but optimal temperatures for growth is between 15 – 25 °C (Helyer et al., 2014). The temporal population dynamics for both BCAs in the present study correlated with their RH requirements. Unfavourable temperatures may cause mortality, while optimal temperatures lead to population growth in the crop phyllosphere. In addition, the viable population density were highest at optimal temperatures for each BCA, but was affected by RH. Overall, temperature appeared to be a major abiotic factor for the establishment and development of the BCA population in strawberry and lettuce phyllospheres. Previous studies on *B. brongniartii,* an entomogenous fungal BCA (Kessler et al., 2003), *B. bassiana* an ascomycetal fungal BCA (Studdert and Kaya, 1990), *Staphylococcus aureus* a gram-positive, bacterium (Valero et al., 2009), *B. subtilis* IB-15, *B. polymyxa*, Bacillus sp. 739 (Melent'ev et al., 2000), and yeast BCAs (Artes et al., 1995) have all concluded that temperature significantly influenced the development and survival of their BCA, and was probably the key factor. However, these studies did not examine the interaction of temperature with RH. When BCAs are subjected to non-optimal temperatures, a higher rate of mortality occurs, reducing BCA viable population sizes and hence affecting biocontrol. Temperatures < 22 °C caused a reduction in the population density of *B. subtilis* QST 713, while temperatures > 22 °C led to a reduction in the population density of *G. catenulatum* J1446 overtime. Extreme low and high temperatures were lethal for the BCAs in the phyllosphere of lettuce and strawberry in the present study. Extended periods in unfavourable temperatures, especially on foliar surfaces, will thus impact BCA performance. This has also been found with some other BCAs (Kessler et al., 2003, Melent'ev et al., 2000).

The other important factor that influenced the development of BCA populations in the present study was RH. This is a critical bottleneck as often RH ranges of 95 – 100 % are required for population establishment, especially in the phyllsphere of crops (Magan, 2001, Hallsworth and Magan, 1999). The present study suggests the RH preferences exist for both BCAs, depending on air temperature as well as host plant. An increase in the BCA population size occurred with increasing RH. This pattern was most evident in the optimum growth temperature ranges for these two BCAs. A study with the BCA *Candida oleophila* found that excess water was required for rapid reproduction in apple wounds to achieve biocontrol

(Mercier and Wilson, 1995). The present study is one of the few studies that have demonstrated the impact of RH alone and when interacting with other factors on the temporal population dynamics in a bacterial and fungal BCA. We clearly showed that for effective phyllosphere establishment RH is a critical factor together with temperature. Perhaps formulation of the BCA then becomes critical in influencing the potential for effective establishment in the phyllosphere of different target crops. In the phyllosphere RH plays an important role and the formulation medium may prevent desiccation and improve BCA survival (Magan, 2001, Barbosa-Cánovas et al., 2008). Absence of a relationship between BCA population size and RH in non-optimal temperatures on both crops may also suggest that the host can adequately provide water requirements in non-optimal temperatures for BCA survival. The presence of a relationship between BCA population size and RH in optimal temperatures on both crops suggests that this is a key factor for BCA growth under suitable temperatures. As RH increases, the amount of moisture for a given temperature rises which also results in a reduction of the available oxygen in the air. Whilst *B. subtilis* can swiftly adapt to an anaerobic environment (Härtig and Jahn, 2012), no such evidence exists for *G. catenulatum*. This supports the observed bell shaped curve patterns in which low RH caused desiccation, and high RH caused oxygen deprivation, as well as shedding light onto why in some non-optimal temperatures the BCAs preference in low RH contrasted to high. Overall, the preference in RH is dependent on both temperature and phyllosphere of the host.

Grounds for investigating dew point came from the effect of beneficial temperature being constrained to healthy plant transpirations rates which was estimated from the influence of VPD (Vadez et al., 2014, Yang et al., 2012, Shamshiri, 2014), and the effect of beneficial RH being constrained to sub optimal and optimal BCA growth temperatures. Dew point represents both the heat energy and the amount of water vapour available in the air, and therefore can be used to observe the combined effect of temperature and RH of a climatic treatment. Both temperature and RH impact on BCA temporal population dynamics and are further influenced by host factors, which results in complex interactions among the factors affecting BCA population dynamics. Thus consideration needs to include interactions between these factors including temperature, RH, potential source of water in the environment and plant surface characteristics (Ruinen, 1961, Beysens, 1995, Andrews and Hirano, 2012). Because dew point was capable of representing these factors it was considered, and the tested range was between 5 °C – 31 °C. Overall, dew point affected the temporal population dynamics of both BCAs in the phyllosphere of lettuce and strawberry in the same manner. Thus, dew point increased the viable population sizes of *B. subtilis* QST 713 up to 25 °C overtime. In contrast, as dew point was decreased the viable population size of *G. catenulatum* J1446 increased up to a dew point of 7 °C on the phyllosphere. For *B.*

subtilis QST 713 dew points < 15 °C and for *G. catenulatum* J1446 dew points > 18 °C were harmful to the introduced BCAs. Dew point was the sole climatic factor for both BCAs that followed the same correlation pattern in the current experimental conditions. The effect of increasing dew point either positively (*B. subtilis* QST 713) or negatively (*G. catenulatum* J1446) impacted on the temporal population dynamics. A few exceptions to the correlation existed, which may be due to the existence of native microbiota. An increase in dew point raises available water potential, which controls the competitive activity between the BCA and the native microbiota. Thus, the better adapted to a specific climatic regime may outcompete the other (Edel-Hermann et al., 2009, Toyota et al., 1996).

On the phyllosphere the BCAs temporal population dynamics depends on abiotic factors as we demonstrated. Therefore the climatic regimes the BCAs are subjected to determine their survival and reproductive success. The present findings highlight the importance of such ecological understanding of BCAs in order to improve their biocontrol performance. The same temperatures at different RH and the same RH at different temperatures do not produce the same response. The present findings can improve the development of strategies for the timing of applications of BCAs with regard to climatic regimes and the relevant host for optimising the potential for their establishment in the phyllosphere to maximise disease control.

Conclusions

The present research initially developed the molecular tool PMAxx™-qPCR for quantifying viable population of *B. subtilis* QST 713 and *G. catenulatum* J1446 in formulation and on aerial plant surfaces.In year 3, we studied the effects of abiotic factors (temperature and RH) on the temporal population dynamics of *B. subtilis* QST 713 and *G. catenulatum* J1446 in lettuce and strawberry. BCA population size is highest when temperature is close to the optimum for the growth and development. High RH encourages survival and/or reproduction in sub-optimal and optimal growth temperatures of the BCA. Increasing dew point encourages survival and/or reproduction of *B. subtilis* QST 713, and discourages survival and/or reproduction of *G. catenulatum* J1446 populations.

Knowledge and Technology Transfer

- 2016 AHDB Crops PhD Studentship Conference poster presentation.

- Visit to Laurence J Betts Ltd and knowledge transfer with their agronomist as well as field inspections for *B. cinerea*.
- Visit to an Anglia salads Ltd and JEPCO for commercial glasshouse and hydroponic growing of lettuce and knowledge transfer with their agronomist as well as inspections for *B. cinerea*.
- 2016 EMR Association/AHDB Soft Fruit Day poster presentation.
- 2017 AHDB Crops PhD Studentship Conference poster presentation.
- 2017 AHDB Crops PhD Studentship Conference research presentation.
- 2017 NIAB and NIAB EMR PhD outreach event presentation.
- 2017 NIAB and NIAB EMR PhD outreach poster presentation.
- 2018 Collaboration efforts with the Amber project.
- 2018 Presentation of results at conferences attended by Amber team.
- 2018 Meeting at ADAS Cambridge with Aoife O' Driscoll (Amber team).

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Appendices

N/A

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