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Project leader:	Amanda Jones, Edinburgh Napier University
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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.

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CONTENTS

GROWER SUMMARY

Headlines

- Shallow soil disturbance to reduce compaction before sowing a second crop of coriander, reduced yield decline in pot trials.
- Soil drying and sterilisation, both practices that altered soil microbe populations, were effective in reducing coriander yield decline in pots, providing further evidence for a microbiological cause.
- Soils from a healthy coriander crop had a very different fungal community to those of a coriander crop with yield decline at the same farm.

Background

Coriander (*Coriandrum sativum* L.) has been grown commercially in the UK since the 1970s. It is now the UK's most economically important herb, accounting for over 25% of the fresh herb market. The crop suffers from an acute form of yield decline which has impacted field production in recent years (Figure 1).

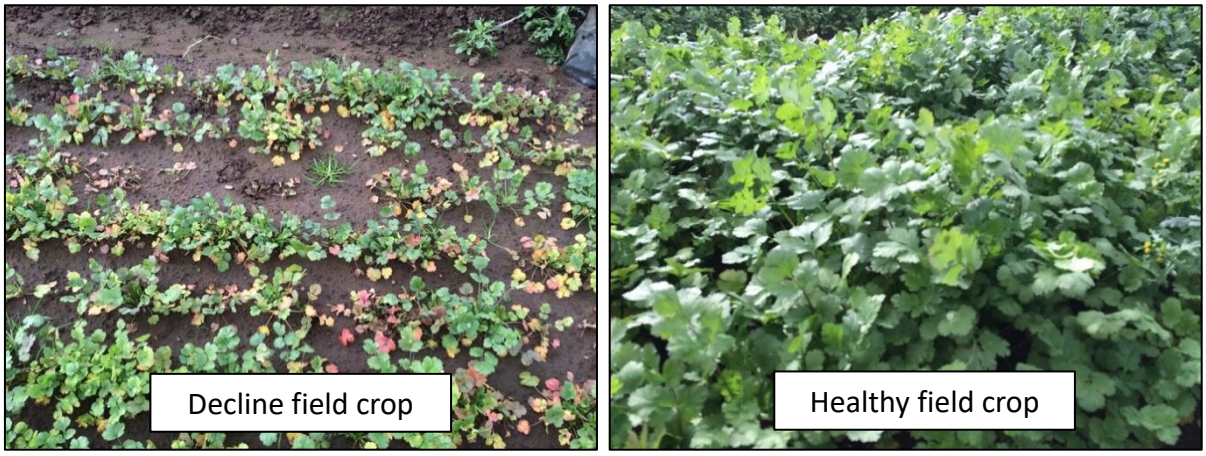

Figure 1. Comparing a yield decline coriander crop and a healthy crop

Although this problem is generally ill-defined, growers have reported yield losses of over 50%; an effect which is said to sometimes persist for up to 8 years. Growers who have access to large areas of land, tend to avoid the problem by using 4-5 year rotations. However, this is not possible for many growers, due to land availability constraints. This problem negatively impacts the UK herb market and new information and management options are urgently needed by growers. Part of the complexity of addressing coriander yield decline is the fact that coriander is grown under highly diverse agronomic practices. The present study was therefore done to i) investigate specific crop and soil management strategies which may reduce the occurrence of coriander yield decline and ii) to evaluate soil microbial communities (microbiome) associated with healthy coriander plants compared to plants showing yield decline. Following on from CP 117, this project involved a 'proof of concept' approach to evaluate whether methods capable of changing the soil microbial community are able to prevent or reduce coriander yield decline.

Summary

A series of glasshouse pot trials was carried out to assess crop and soil management practices to reduce coriander yield decline. Figure 2 illustrates the basic method for growing continuous coriander crops in the glasshouse. Coriander was grown for a first cycle in control soils (C1)—those without a prior coriander cropping history. Pots were then subjected to various treatments, before being sown again for a second crop cycle (C2) (using soils with one prior coriander crop) or occasionally a third crop cycle (C3) (using soils with two prior coriander crops).

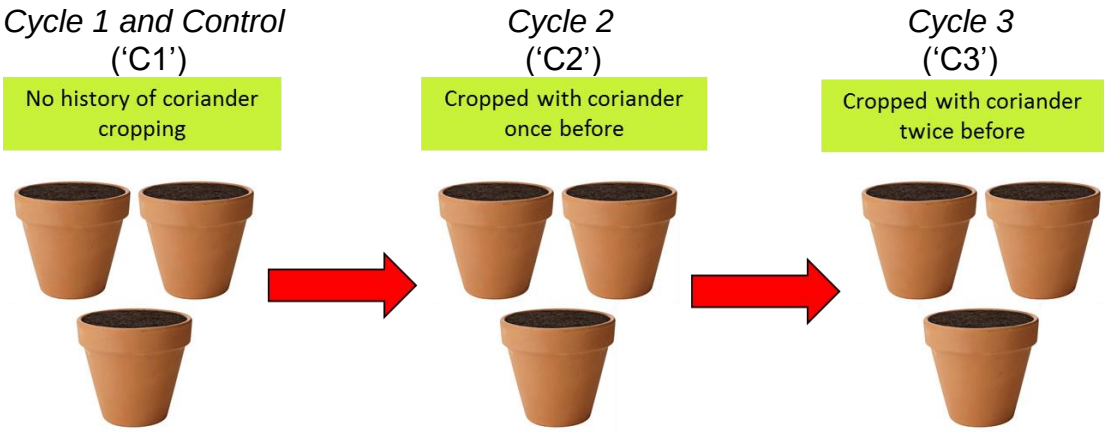

Figure 2. Basic method for growing coriander for two or three crop cycles

Above ground weights were calculated by measuring the combined shoot and leaf weight of individual plants. Total yield per pot was collected by harvesting the above ground plant material at approximately 3 cm above the soil surface.

Findings from selected pot trials are summarised below. Details of all pot trials are included in the Science Section. Overall findings were characterised by high variability within experiments, but suggested that certain practices helped to reduce levels of yield decline experienced in the glasshouse. Techniques which directly altered the soil microbiome (in particular, soil drying and sterilisation) had a greater impact on reducing the levels of decline, suggesting a partially microbiological cause. This effect was supported by the results of soil microbial community studies. While extensive soil drying and sterilisation may not be feasible for field-grown coriander in the UK, the results suggest that further investigation of practices that can 're-set' soil microbiological populations is warranted.

Assessing the impact of harrowing on coriander yield

A pot trial was carried out to investigate the impact of shallow soil disturbance (harrowing) on yields of coriander grown for a second crop in the same soils. This experiment involved three treatments:

(1) Coriander grown in fresh soil with no history of coriander cropping ('C1 Control')
(2) Coriander grown in soils that had contained one previous crop, and were harrowed before sowing ('C2 Harrowed')
(3) Coriander grown in soils that had contained one previous crop, but were not harrowed before sowing ('C2 Non-harrowed')

Coriander plants grown in the fresh control soils had significantly higher above ground fresh weights (shoots and leaves of individual plants) compared to C2 Harrowed plants ($p=0.006$); and C2 Non-harrowed plants ($p=0.003$). Total yield per pot for treatments was significantly greater in C1 Control pots (37.8 g) compared to C2 Non-harrowed pots (24.9 g) ($p=0.04$). However, the difference in yield between C1 Control pots and C2 Harrowed (30.4 g) pots was not statistically significant ($p>0.05$). This was reflected in the fact that relative to the C1 Control pots, the C2 Non-harrowed pots declined by 34%, compared to a decline of just 20% in the C2 Harrowed pots (Figure 3). Results indicated that harrowing limited the level of decline experienced when coriander was grown for a second cycle in the same soil.

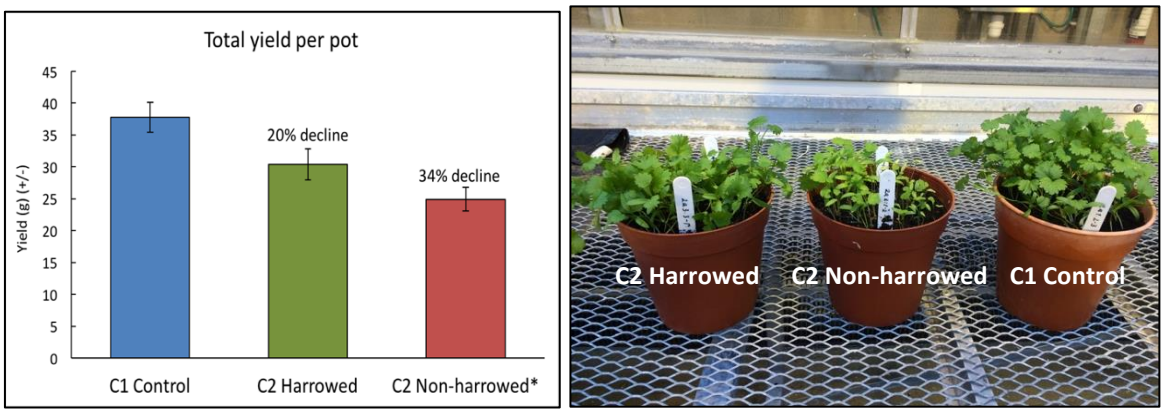

Figure 3. Comparison of mean total yields per pot for the harrowing experiment

Assessing the impact of drying out soils before re-sowing coriander

The soil microbiome of crops is highly influenced by agricultural management practices. One such practice is solarisation, which has been used in southern Spain to avoid the occurrence of coriander yield decline in continuous coriander cropping. Soil drying can perform some of the same functions as solarisation, reducing survival of soil-inhabiting microorganisms and affecting physical and chemical characteristics of a soil.

A pot trial was set up to examine the impact of drying out crop soils before sowing a subsequent crop of coriander in the same soils. This experiment involved three treatments:

(1) Coriander grown in fresh soil with no history of coriander cropping ('C1 Control')

(2) Coriander grown in soils that had contained one previous crop, where these soils were left to dry out for 4 weeks before re-sowing ('C2 Desiccated')

(3) Coriander grown in soils that had contained one previous crop, where these soils were watered daily for 4 weeks before re-sowing ('C2 Watered')

Total yields per pot for the C1 Control pots (41.2 g) and C2 Desiccated pots (39.6 g) were very similar and much larger than C2 Watered pots (16.3 g) (Figure 4). Due to inherent variability of means, this difference was not detected in ANOVA ($p>0.05$). Overall results of this experiment suggest that drying out crop soils before re-sowing coriander may help to alter soil physical or biological properties in a way which reduces the potential for yield decline to occur.

Figure 4: Coriander growth in the soil drying experiment

Assessing the impact of soil sterilisation on a yield decline affected field soil

A grower's field soil containing a healthy crop of coriander was used for a pot experiment. Prior to beginning the experiment, coriander was grown in this field soil in the glasshouse, confirming that yield decline would occur. Subsequent to this, an experiment was set up to determine the effect of sterilisation on yield decline and involved two treatments:

(1) Coriander grown in field soil that had contained one previous crop ('Non-sterilised')

(2) Coriander grown in the same field soil which was sterilised through autoclaving before sowing ('Sterilised').

Plants grown in the field soil that was sterilised produced 70% bigger plants and 50% greater yields per pot, indicating a microbiological causal agent in this instance of coriander yield decline. These differences were significant between the treatments for fresh above ground weight ($p<0.001$), and total yield per pot ($p=0.012$). Figure 5 shows the dramatically larger mean yield per pot when the soil was sterilised (13.2 g), compared to soil that remained non-sterilised (6.2 g).

Figure 5: Comparison of Sterilised and Non-sterilised yield decline affected field soils

Microbial community studies of field soils and desiccation experiment soils

Plant and soil samples were collected from a grower's 'healthy' coriander crop and a coriander yield decline (CYD) crop in an adjacent field which showed two levels of decline (severe CYD and moderate CYD). Plants and soils were also collected from the desiccation experiment described above. DNA extracts were prepared for twelve plant samples in order to examine the associated microbial communities. Rhizosphere (RS) and bulk soils (BS) were collected, with 'rhizosphere' constituting soils clinging tightly to roots and 'bulk' soils as those not adhering to roots. Both rhizosphere and bulk soils were taken from the following six soils (for a total of 12 samples): 1) 'Healthy' field soil; 2) 'CYD Severe' field soil; 3) 'CYD Moderate' field soil; 4) Desiccation experiment control soil 'C1 Control'; 5) desiccation experiment 'C2 desiccated' treatment soil; and 6) desiccation experiment 'C2 Watered' treatment soil. DNA sequencing was carried out and classifications of fungi and bacteria were obtained for the twelve samples. Clearly defined differences in the relative abundance of bacteria were not observed within the field soils samples, or the desiccation experiment samples. However, shifts in the relative abundances of fungi were seen in both cases. This was particularly evident in the field soils (Figure 6), where the Dothideomycetes class of ascomycete fungi (with a corresponding reduction in Sordariomycetes), had a much greater relative abundance in the CYD field soil samples, compared to the healthy field soils samples. Dothideomycetes contains many agricultural plant pathogens with high economic impact. The three fungi which contributed most significantly to the abundance of Dothideomycetes in the rhizosphere of the yield decline samples were: *Bipolaris sorokiniana* (pathogen of wheat and barley); *Leptosphaeria maculans* (pathogen of *Brassica* spp., particularly oilseed rape); and *Cenoccocum geophilum* (a common ectomycorrhizal fungus). Overall results provide further support for a microbiological element (likely fungal) in the problem, at least in the case of an affected field soil.

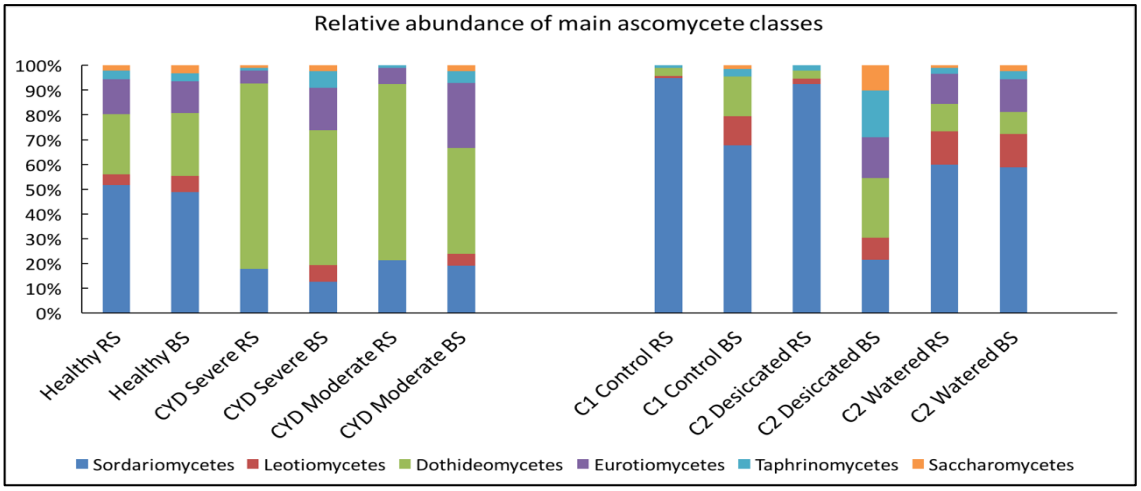

Figure 6: Relative abundance of the main ascomycete classes for two soil types

Figure shows the relative abundances of six ascomycete fungi classes for field soils (left) and desiccation experiment soils (right) with 'RS'=rhizosphere and 'BS'=bulk soil.

Pot trials suggested that practices such as soil drying and relieving surface compaction (e.g. harrowing) together with optimum planting densities (see Science Section) could help to reduce the impact of coriander yield decline in the field. However, these techniques need further assessment and field trials to confidently advise growers in changes to cropping practice. Soil sterilisation was effective at eliminating yield decline in an affected soil in pot trials, indicating a microbiological cause for yield decline, and that methods to alter microbial soil populations (e.g. biological soil disinfestation) warrant consideration. Results from the DNA studies also suggest that investigations into potential fungal causal agents could provide further insight into the microbiological causes of CYD.

Financial Benefits

No financial benefits have been quantified.

Action Points

There are no grower action points at this stage. Further research and/or field trials which take into account findings of this project is recommended.

SCIENCE SECTION

Introduction

Coriander (*Coriandrum sativum* L.) has been grown commercially in the UK since the 1970s. It is now the UK's most economically important herb, accounting for over 25% of the fresh herb market (Hargreaves, 2014). The crop suffers from an acute form of yield decline which has impacted field production in recent years. Growers report small, stunted plants, rather than obvious signs of pathogens (Fraser, 2017). Resulting yield losses can be as much as 50%; an effect which is said to sometimes persist for up eight years (Tom Davies, pers. comm. cited in Fraser, 2017). Growers with access to large areas of land tend to avoid this problem with four to five year crop rotations (Robert Gibbs, pers. comm., 2018). However, most growers are constrained by limited land availability and use shorter rotations. This problem negatively impacts the UK herb market and new information and management options are urgently needed for growers.

Part of the complexity of addressing coriander yield decline (CYD) in the UK is the fact that coriander is a relatively undeveloped crop that is grown under diverse agronomic practices. Therefore, the problem of CYD is generally ill-defined. The overall aim of this study was to assess a series of crop and soil management strategies for their potential in limiting CYD. A further aim was to provide follow-on support for AHDB study CP 117; adding 'proof of concept' towards a hypothesised microbial cause in CYD. The individual objectives of the present study are listed below:

- To assess tillage at different depths on the occurrence and severity of CYD.
- To evaluate seed planting density as a factor potentially influencing the occurrence and severity of CYD.
- To determine whether coriander can be grown for a second cycle without yield decline, if 'optimum' conditions for growth are met.
- To examine desiccation/drying of crop soils after harvest as a means to potentially alter microbial communities and soil structure; thus reducing CYD.

- To assess the effectiveness of soil sterilisation of field soils showing CYD, to prevent or reduce CYD.

- To evaluate soil microbial communities associated with healthy coriander plants compared to plants showing yield decline.

Materials and methods

Glasshouse conditions and general methods for growing coriander

General methods for growing coriander followed those used by Fraser in CP 117 (2017). Growth experiments for the present study were carried out at the glasshouse facilities of SASA (Science and Advice for Scottish Agriculture, 55.9237° N, 3.3429° W) in Edinburgh. These were maintained at 20ºC in the daytime (reaching a maximum of 25ºC), and 18ºC in the evenings. Glasshouse lighting (high pressure sodium *Papillon 270* 600 watt lamps, Papillon Luminaires) was set to achieve a photoperiod of 16 hours, mimicking UK summer conditions. Plants were watered daily and given a single application of Chempak 3 balanced (20-20-20 N, P, K) fertiliser, at a rate of 7.5 mg/cm^2 at 4 weeks' growth (this was later modified to include the addition of a base dressing of half this rate before sowing). Standardised potting compost (John Innes 2) and coriander var. Santos (sub-variety 'Rani') seeds were chosen for these trials (unless otherwise stated), as yield decline was previously established irrespective of soil type and coriander variety (Fraser, 2017). Planting density initially followed Fraser, 2017 (0.27seeds/cm^2), and later became an experimental factor in its own right.

Experimental set-up and design

Experiments were organised in randomised complete block designs using GenStat (VSN, 2011). Each crop cycle required approximately 8 weeks (or as few as 6 weeks, depending on season) to reach the optimum height and leaf number, as suggested by coriander growers and previous work (Robert Gibbs, pers. comm., 2017; Fraser, 2017). Figure 1 illustrates the basic method for growing continuous coriander crops in the glasshouse. Coriander was grown for a first cycle in 'virgin' or control soils (C1)—those without a prior coriander cropping history. Pots were then subjected to various treatments, before being sown again for a second crop cycle (C2) (using soils

with one prior coriander crop) or occasionally a third crop cycle (C3) (using soils with two prior coriander crops).

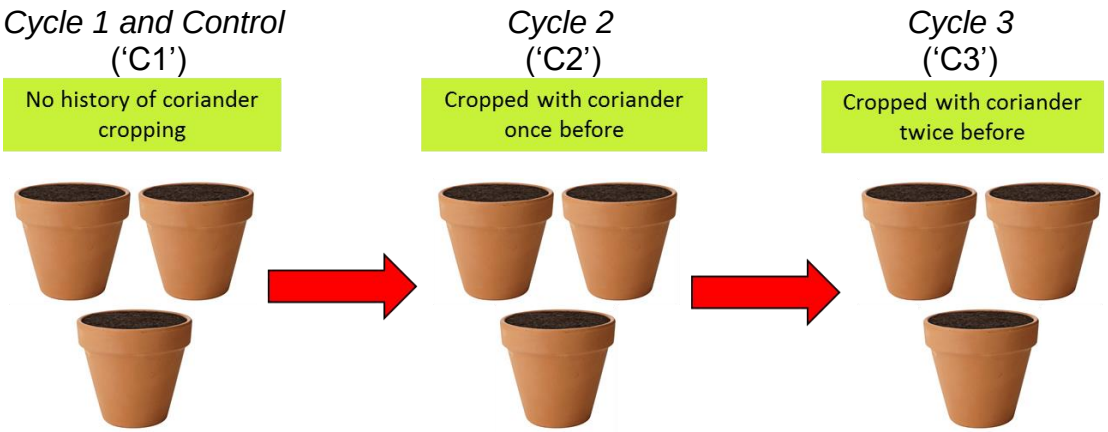

Figure 1: Basic method for growing coriander for two or three crop cycles

Data collection

Second and third cycle (C2 and C3) growth data were collected for 'individual plant biomass' and 'total yield per pot'. The former was determined by randomly sampling whole plants from each pot and measuring fresh and dry weights of roots, shoots, and leaves. A calculation was made for 'above ground weight', consisting of the combined weight of shoots and leaves; and 'below ground weight', consisting of the weight of the root only. Replications of plants sampled depended on the number of pots and size of plants (limited by the size of the drying oven) produced in the experiments. Plants were oven dried at 60°C for approximately 24 hours (according to Fraser, 2017). Total yield per pot was determined as a means of providing a more relevant form of data for growers. This was done by harvesting plants approximately three cm above the soil surface, to mimic the technique used by growers in the field (Robert Gibbs, pers. comm., 2017). All crop data were recorded and organised in Microsoft Excel workbooks, which were also used to produce bar charts.

Assessing the impact of harrowing on CYD

Coriander seeds were sown at the standard density (36 seeds) in 13 cm pots in order to examine the effect of harrowing on coriander crop soils (in contrast to more compacted, non-harrowed soils). Pots were re-sown using the same parameters for the second crop with each of two treatments: 1) harrowing: disturbing only the upper

soil layers using a fork to a depth of five to seven cm, before re-sowing or 2) simply re-sowing by inserting seeds with minimal soil disturbance. Control pots were also sown. Individual plant biomass data were collected for 30 replicate plants, and for total yields per pot (four replicates).

Comparing the impacts of deep ploughing vs. harrowing on CYD

Coriander seeds were sown at the standard density (166 seeds) in 28 cm pots in order to assess the effect of a simulated deep plough on a second cycle coriander crop (in contrast to a more superficial harrowing). Pots were re-sown for a second cycle with the same general parameters for each of two treatments: 1) simulated ploughing to a depth of approximately 20 cm, or 2) harrowing to a depth of five to seven cm for a comparison. To simulate ploughing, 20 cm of crop soils were removed from pots, and inverted, so that the upper soil layers were roughly situated at the bottom of the pots. Harrowing was carried out as described in the previous experiment. Control pots were also sown. The second crop cycle was harvested after approximately 8 weeks. A third cycle was then sown in the same manner, and harvested at 8 weeks. Individual plant biomass data were collected for the second cycle crop (30 replicate plants), and total yields per pot (four replicates) were determined for both the second and third cycles.

Examining the effects of different seed planting densities on CYD

Coriander seeds were sown in 13 cm pots using three different planting densities:

- High planting density=36 seeds (0.27 seeds/cm^2) (used in CP 117)
- Low planting density=3 seeds (0.025 seeds/cm^2) (used by a grower—Robert Gibbs, pers. comm. 2017)
- Medium density=20 seeds (0.15 seeds/ cm^2) (an intermediate between the high and low planting densities)

A second crop cycle was then sown with the same parameters, with replicate pots sown for each of the three planting densities. Control pots for each of these densities were also sown. Individual plant biomass (ten replicate plants per treatment) and total yields per pot (two replicates) were collected.

Assessing 'optimum' growth conditions for their impact on CYD

Based on results obtained in the above experiments, an 'optimised' coriander growth trial was carried out to determine if CYD still occurred when coriander was grown under what was deemed to be optimum conditions. Coriander seeds were sown in 23 cm pots at a density of 0.088 seeds/cm^2 (37 seeds). The crop was grown for approximately 6 weeks, whereby plants were harvested and crop soils were allowed to dry out in their pots for 2 weeks. Pots were then harrowed, watered, base-dressed with fertiliser (1.5 g Chempak 3), and then re-sown with coriander (at the same density), alongside control pots. A second cycle was then grown, whereby total yields per pot were calculated (ten replicates).

Investigating the impact of desiccation of crop soils on the occurrence of CYD

Coriander seeds were sown in 13 cm pots at the standard density (36 seeds) and harvested after approximately 8 weeks. Three replicate pots with their crop soils (which had contained one previous coriander crop) intact were either: 1) left to dry out for a period of 4 weeks ('desiccated'), or 2) watered daily for 4 weeks ('watered'). A second crop was then sown in the same soils at the initial planting density, alongside C1 controls. Pots were then harvested, whereby individual plant biomass (30 replicate plants) and total yields per pot (three replicates) were collected.

Assessing the effect of soil sterilisation on a commercial grower's field soil exhibiting CYD

Two field soils were obtained from a grower who experiences CYD. One soil sample was taken from a healthy crop, and a second sample was collected from an adjacent field containing a poor crop with stunted growth symptomatic of CYD (Figure 2). The two crops had been sown one week apart. Both of these field soils were then used to grow crops in the glasshouse, which produced similar, severely stunted plants in each case. Plants and soil from the same fields were later used for the associated microbial studies. A glasshouse experiment was set up to test sterilisation as a possible means to alleviate CYD in a crop grown in the 'healthy' field soil (which had contained a previous crop in the field, and was confirmed to be CYD affected). To facilitate this, half of the soil was sterilised by running it through an autoclave for two cycles (high-pressure saturated steam reaching 121°C). Ten 12 cm pots (base dressed with 0.5 g Chempak 3) were sown at the standard density (30 seeds) consisting of: five pots with sterilised soil, and five pots with non-sterilised soil. After harvest, individual plant

biomass (20 replicate plants), and total yields per pot (five replicates) were collected for the two treatments.

Statistical analyses

Statistical analyses were performed using R (R Core Team, 2013). Normality of distribution of measured characteristics was tested using a Shapiro-Wilk test ($p \geq 0.05$). Data sets with non-normal distributions were transformed using the natural logarithm or square root. Analysis of variance (ANOVA) or a Welch two sample t-test (in the case of comparisons of two treatment levels) were performed to detect significant differences in means between treatments (*e.g.*, desiccation, ploughing, harrowing, different densities) and controls for each crop ($p<0.05$). Where normal distributions were not obtained, non-parametric tests were used (Kruskal-Wallis rank sum test or Mann-Whitney U test). Post-hoc analyses were carried out to determine individual differences between treatments (Tukey test 95% confidence interval (CI) groupings, or a Nemenyi Test for non-parametric analyses). Significant differences in treatment vs. control plant biomass and yield per pot were used to inform yield decline data.

Soil microbial community studies

Soil DNA extractions

Plant and soil samples were collected from the grower's field soils described in the sterilisation experiment. These represented a 'healthy' coriander crop and a yield decline coriander crop (Figure 2) with two levels of decline ('moderate CYD' and 'severe CYD') (Figure 3). Plants and soils were also collected from the desiccation experiment. DNA extracts were prepared for twelve plant samples in order to examine the associated microbial communities. Rhizosphere and bulk soils were collected, with 'rhizosphere' constituting soils clinging tightly to roots (Hilton, *et al*., 2013), and 'bulk' soils as those not adhering to roots. Rhizosphere and bulk soils were taken from the following six soils (for a total of 12 samples): 1) Healthy field soil; 2) Severe CYD field soil; 3) Moderate CYD field soil; 4) Desiccation experimental control (C1); 5) Desiccation experiment 'desiccated' treatment (C2); and 6) Desiccation experiment 'watered' treatment (C2). DNA extractions were performed using a PowerSoil® DNA isolation kit (MO BIO laboratories, Inc.).

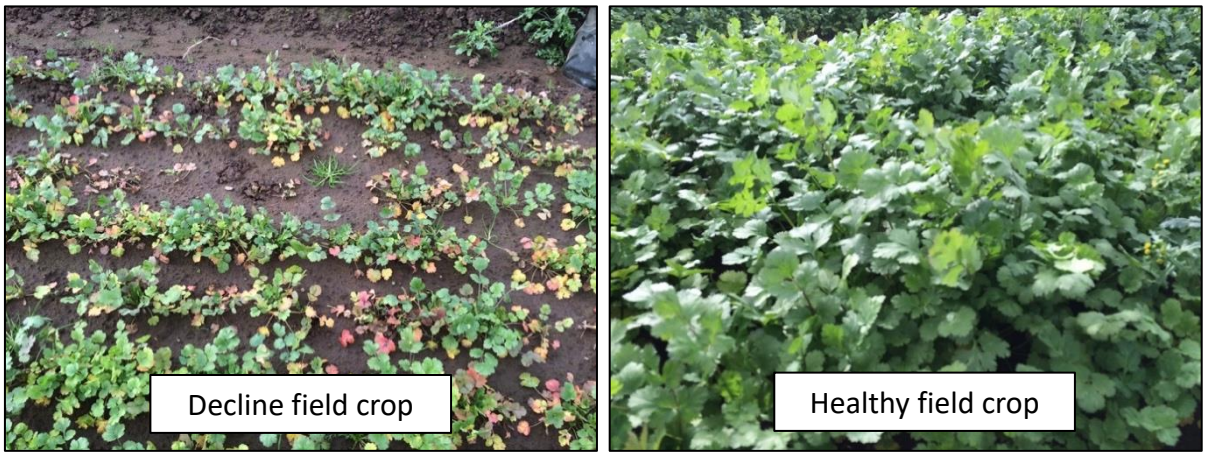

Figure 2: Comparing a yield decline coriander crop and a healthy crop
Figure shows a yield decline coriander crop (left) and a healthy crop (right) grown in an adjacent field; crops were sown one week apart.

Figure 3: Examples of plant samples taken from grower's field crops
Figure shows a plant from the healthy field crop (left) and two plants taken from the poor field crop (middle and right), which show two levels of CYD.

Polymerase Chain Reaction (PCR), amplification, and nanopore sequencing

PCR and amplification were carried out for fungi (ITS) and bacteria (16S). PCR primers ITS1 and ITS4 were used for ITS, and 27F and 1027R used for 16S; whereby standard PCR programs were run. A Rapid Barcoding Sequencing kit (SQK-RBK004)

(Oxford Nanopore Technologies (ONT)) was used according to the manufacturer's protocol, before performing 1D nanopore sequencing using MinION MK1B DNA Sequencer (ONT). The EPI2ME platform WIMP (What's In My Pot) (ONT) facilitated quantitative and qualitative sequence data, as well as broad taxonomic classification. The Kaiju program (Menzel*, et al*., 2013) was used for further taxonomic classification based on sequence comparisons to a reference database. Relative abundance percentages were calculated for bacterial phyla, fungal phyla, and classes within Ascomycota.

Results

Assessing the impact of harrowing on coriander yield and CYD

Harrowing coriander crop soils before sowing a subsequent crop in the same soils had an observable impact on the size of individual plants, and the overall yields per pot. Coriander plants grown in control (C1) soils had significantly greater fresh above ground weights (shoots and leaves) than C2 harrowed plants (confidence interval (CI) 95% p=0.006), and C2 non-harrowed plants (CI 95% p=0.003) (Figure 4). Likewise, the dry above ground weights of plants showed the same effect, with control (C1) plants larger than both the C2 harrowed plants (CI 95% p=0.035), and C2 non-harrowed plants (CI 95% p=0.038) (Figure 4). Control (C1) soils also produced the largest total mean yield per pot, which was significantly greater than that of the C2 non-harrowed pots (95%CI p=0.040), but not statistically different from the C2 harrowed pots (p>0.05). Figure 5 highlights this effect: C2 non-harrowed pots declined by 34% relative to the control (C1), while C2 harrowed pots declined by just 20%. Overall, results show that harrowed pots experienced less CYD in a second coriander crop.

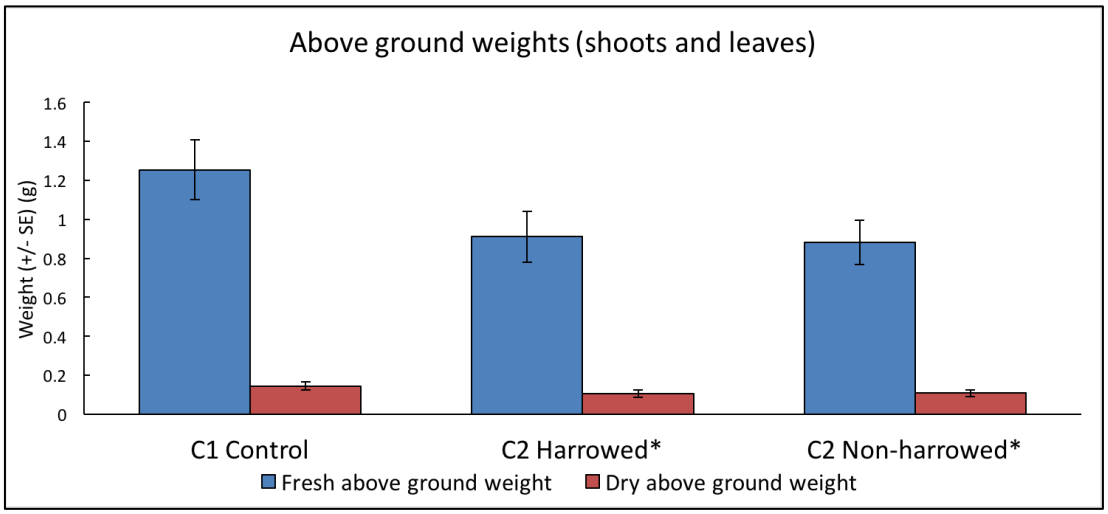

Figure 4: The effect of harrowing on the above ground weights of coriander
Figure shows mean above ground weights (fresh and dry) for C1 Control, and C2 Harrowed and C2 Non-harrowed treatments with significant differences (*) between both treatments vs. Control (C1) (fresh and dry weights). (Means calculated from 30 replicate plants/treatment).

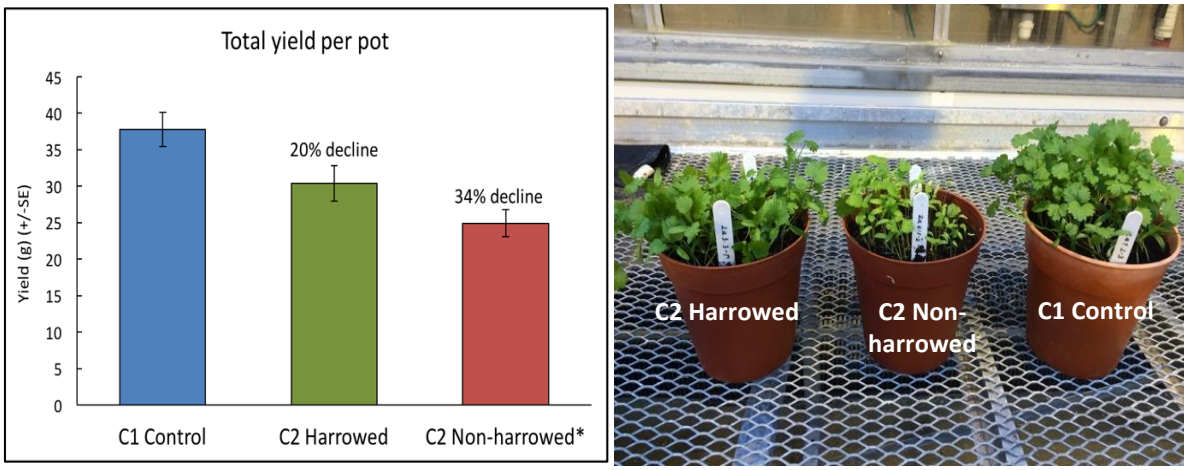

Figure 5: The effect of harrowing on coriander total yield per pot
Figure (left) shows mean total yields per pot for C1 Control, and C2 Harrowed and C2 Non-harrowed treatments with significant difference (*) only between Control (C1) and C2 Non-harrowed pots; relative declines from the Control are also shown; also shown (right) an example of coriander for each treatment at four weeks' growth. (Means calculated from four replicate pots per treatment).

Comparing the impacts of ploughing vs. harrowing on coriander yield and CYD

Coriander grown in fresh control (C1) soils produced greater overall yields per pot than coriander grown in soils that had contained one previous crop (C2) or two previous crops (C3), irrespective of the tillage treatment applied. Statistically speaking, the control (C1) pots produced significantly greater yields than the C2 ploughed pots (CI 95% p=0.031), and the C2 harrowed pots (CI 95% p=0.042). To illustrate this, Figure 6 shows that C2 ploughed pots experienced a decline of 27% relative to the control (C1), compared to a decline of 15% in C2 harrowed pots. This

situation was reversed for Cycle 3, where C3 harrowed pots declined by 32% relative to the control, compared to a 26% decline in yields of C3 ploughed pots (Figure 6). In measuring individual plant biomass for Cycle 2 pots (only total yields per pot were calculated for Cycle 3), no statistical differences ($p>0.05$) were found for any of the individual plant metrics; therefore, no data is shown.

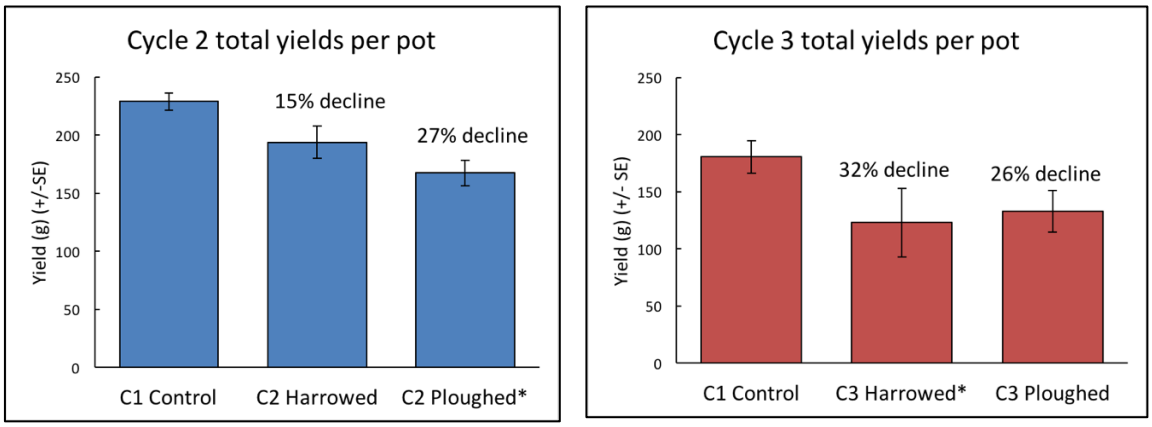

Figure 6: The effect of harrowing and ploughing on coriander total yields per pot Figure shows mean yields per pot (C2 on left, and C3 on right), with two treatments and a control (C1); also showing relative percent decline from the C1 Controls ('*' indicates statistically significant difference from control). C2 ploughed pots yielded significantly less than the control, while in C3, the same was true for harrowed pots. (Means calculated from four replicate pots per treatment).

Examining the effects of different seed planting densities on CYD

Coriander grown in fresh control (C1) soils and coriander grown for a second cycle in the same soils (C2) both indicated that planting density significantly influenced coriander growth. This was seen in the individual plant biomass and total yields per pot. Coriander plants grown at the low density of just three seeds per pot (both in C1 soils and C2 soils) produced the largest above and below ground weights (Figure 7). This difference in biomass was found to be statistically significant for: fresh above ground weights ($p= 3.12^{e-14}$), dry above ground weights ($p=1.34^{e-12}$), fresh below ground weights ($p=3.97^{e-10}$), and dry below ground weights (1.53^{e-07}). Although a defined difference in total yields per pot can be seen between the three different densities in Figure 8, no significant variance was detected in an ANOVA ($p>0.05$). Relative to C1 control pots, the C2 high density pots experienced the highest rate of decline at 41%, with the C2 low-density pots at 34%, and the C2 medium density pots showing the least decline at 13% (Figure 8).

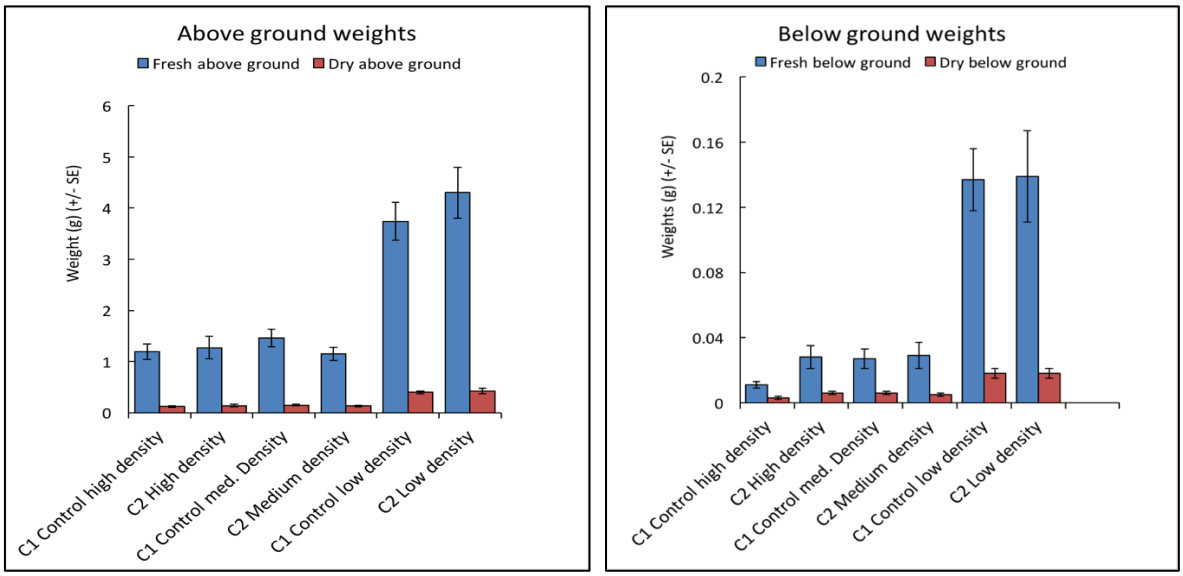

Figure 7: The effect of planting density on individual plant
Figure shows mean above ground weights (left) and mean below ground weights (right) for controls (C1) and C2 for the three densities; low density pots produced significantly larger plants (above and below ground). (Means calculated from ten replicate plants per treatment).

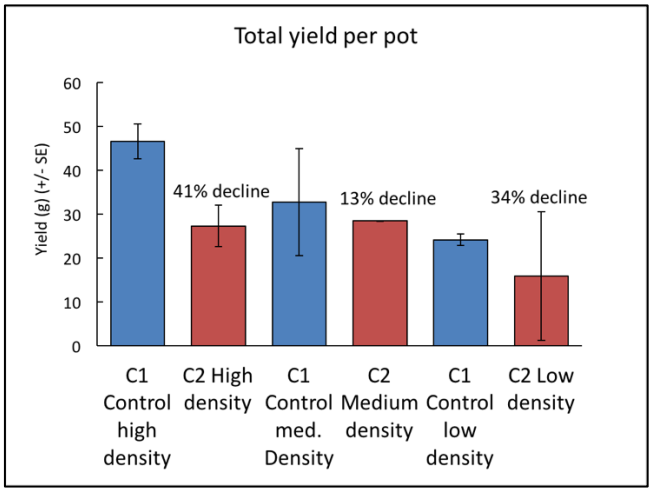

Figure 8: The impact of planting density on coriander total yield per pot
Figure shows total mean yield per pot with percent C2 declines from relative C1 controls for each density. (Means calculated from two replicate pots per treatment).

Assessing a set of 'optimum' conditions for their impact on CYD

For the optimum growth conditions experiment, coriander was grown and harvested. Before sowing a second cycle (C2), crop soils were left to dry out for two weeks, harrowed, base-dressed with fertiliser, and then sown at a medium-low planting density alongside C1 control pots. Even after growing coriander under these seemingly optimum conditions, C2 yields still declined significantly (p=0.005). This

difference is illustrated by the 28% decline experienced in C2 pots, relative to C1 control pots (Figure 9).

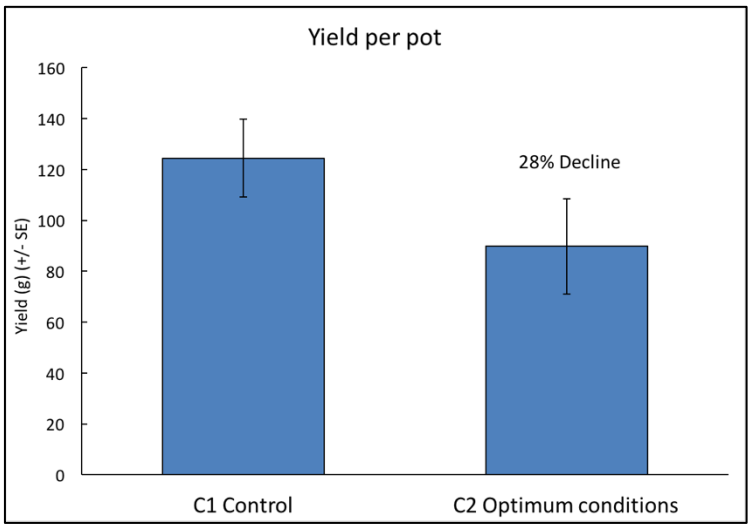

Figure 9: The impact of optimum growth conditions on total coriander yield per pot
Figure shows significantly greater mean total yields for C1 Controls vs. C2 optimum conditions coriander; C2 optimum conditions declined by 28% relative to C1 Control pots. (Means calculated from ten replicate pots per treatment).

The effect of soil desiccation on CYD in a second crop cycle

The desiccation experiment showed a pronounced difference in coriander growth depending on the two treatments applied before sowing the second crop ('C2 watered' vs. 'C2 desiccated'). This was evident in the size of individual plants, and also for total yields per pot. Soils which had been watered daily before sowing a second coriander crop produced the largest plants (above and below ground), but the lowest total yields per pot. The fresh above ground weights of C2 watered plants were significantly greater than those of C2 desiccated plants ($p=0.002$), and C1 control plants ($p=2.00^{e-04}$). However, dry above ground weights were not statistically different ($p> 0.05$). Significantly larger fresh root weights were obtained for C2 watered plants, compared to C1 control plants ($p=5.90^{e-06}$), and C2 desiccated plants ($p=2.46^{e-05}$). Dry below ground weights showed a similar effect, with C2 watered treatment plants again significantly larger than C1 controls (95% CI $p=0.005$) and C2 desiccated treatment plants (95% CI $p=0.011$). Figures 10 and 11 clearly show that differences in total yields per pot for the C1 control pots (41.2 g +/- 8.3) and C2 desiccated pots (39.6 g +/- 9.0) were negligible, while C2 watered pots produced much lower yields (16.3 g +/- 13.4). However, this difference was not detected in ANOVA due to inherent variability ($p>0.05$).

Figure 10*: *The impact of soil desiccation on growth of a second coriander crop
Figure shows soil desiccation experiment plant growth at time of harvest: C1 Control pots and C2 Desiccated pots had similar yields; C2 Watered pots produced visibly lower yields.

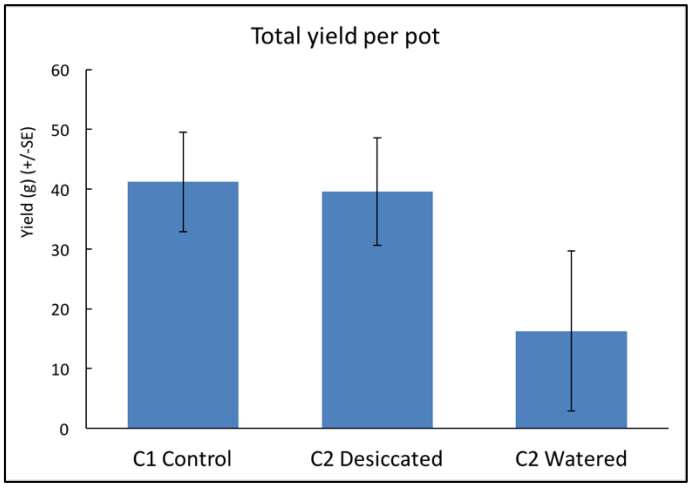

Figure 11: The impact of soil desiccation on coriander total yields per pot
Figure shows: C1 Control pots and C2 Desiccated pots had similar yields, C2 Watered pots gave lower yields, but with high variability. (Means calculated from three replicate pots per treatment).

The effect of soil sterilisation on a commercial grower's field soil exhibiting CYD

A grower's field soil (which exhibited CYD when used to grow a subsequent crop in the glasshouse) produced significantly larger plants and greater total yields per pot after sterilisation. A Welch two sample t-test (95% CI) confirmed significant differences for all measured characteristics of sterilised vs. non-sterilised soils. Figure 12 illustrates the dramatic difference in above and below ground biomass for treatments: sterilised soils produced approximately 70% larger plants (combined above and below ground weights). Total yields per pot were approximately 50% greater in the sterilised soils, compared to non-sterilised soils (Figure 13).

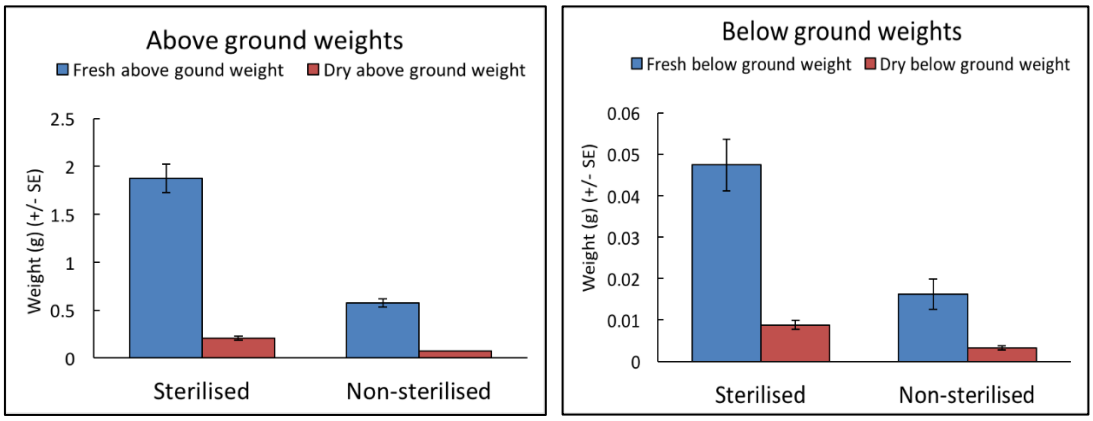

Figure 12: The effect of soil sterilisation on individual plant biomass of coriander
Figure shows the dramatic difference between treatments for above ground weights (left) and below ground weights (right), with sterilised soils producing much larger plants. (Means calculated from 20 replicate plants per treatment).

Figure 13: The effect of soil sterilisation on coriander total yield per pot
Figure shows total yield per pot for two soil treatments (left) with corresponding photo of growth at harvest time (right). (Means calculated from five replicate pots per treatment).

Microbial community study results for field soils and desiccation soils

MinION sequencing (ONT) produced 1,032,110 reads that passed the quality filter, with a total yield of 65.2 million bases. Average sequence length was 635 base pairs, with an average quality score of 9.95 (out of 12) (EPI2ME WIMP, ONT). The broad taxonomic breakdown of sequences was: <1% Archaea, <1% Viruses, 19% Eukaryota, and 81% Bacteria (EPI2ME WIMP, ONT). The number of reads classified for each sample are shown in Appendix 3.

Phylum level analysis

Identification at phylum level was carried out for bacteria and fungi to give a broad overview of potential microbial community changes within the field soil samples and

desiccation experiment soil samples. Bacterial taxa classified for the twelve samples did not show clearly defined changes within either of the soil sample groups. Proteobacteria and Firmicutes were interchangeably dominant in both the field soils and in the desiccation experiment soils, throughout the rhizosphere and bulk soils. Distribution of fungal phyla across samples showed more pronounced shifts between soils, as illustrated in Figure 14. This was particularly evident in the field soil samples, where relative abundances of ascomycetes in the rhizosphere of the CYD soils (both for severe CYD 81% and moderate CYD 82%) were nearly double that of the healthy field soil rhizosphere (44%). Within the desiccation experiment soils, the most notable difference between samples is the dominance of basidiomycetes classified in the bulk soil of the C2 desiccated sample, at 48% relative abundance (Figure 14). This is in contrast to basidiomycetes classified for bulk soils of C1 control (24%), and C2 watered soils (15%).

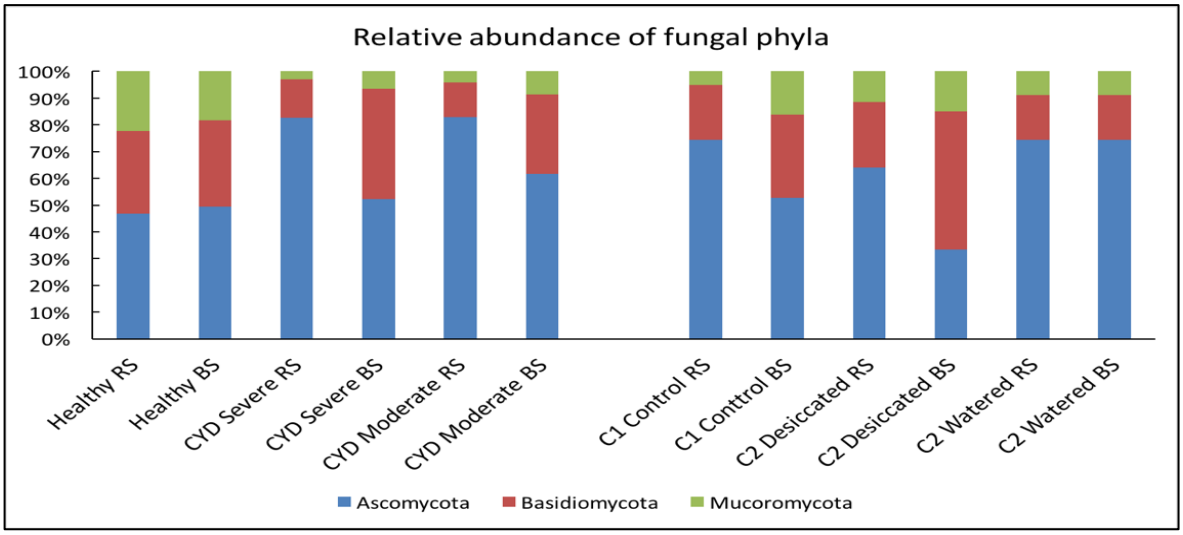

Figure 14: Relative abundances of fungal phyla for twelve soils samples
Figure shows the relative abundances of three fungal phyla classified for field soils (left) and desiccation experiment soils (right) with 'RS'=rhizosphere and 'BS'=bulk soil; (relative abundance % for total fungi classified for each sample).

Higher taxonomic resolution of fungi

Considering the overall dominance of ascomycete fungi classified, further taxonomic resolution within Ascomycota was carried out to provide additional insight into potential microbial community change. Figure 15 shows the relative abundance of six ascomycete classes. The most defined difference in comparing the six field soil samples is the shift from a dominance of Sordariomycetes in the healthy field soils

(rhizosphere and bulk soil), to a dominance of Dothideomycetes in the CYD soils. Dothideomycetes particularly characterise the rhizosphere of the CYD samples (both levels). The desiccation experiment soils present a very different pattern of relative ascomycete abundance. The C1 control and the C2 desiccated samples show a similar distribution of rhizosphere fungi with a dominant relative abundance of Sordariomycetes, at 91% and 87%, respectively. However, the C2 watered soils show no clear distinction between rhizosphere and bulk soil fungi. C2 desiccated bulk soils have a very different distribution of ascomycetes compared to the other samples: 15% Taphrinomycetes (compared to <3% in the other two bulk soil samples), and 8% Saccharomycetes (compared to <2% in the other two bulk soil samples).

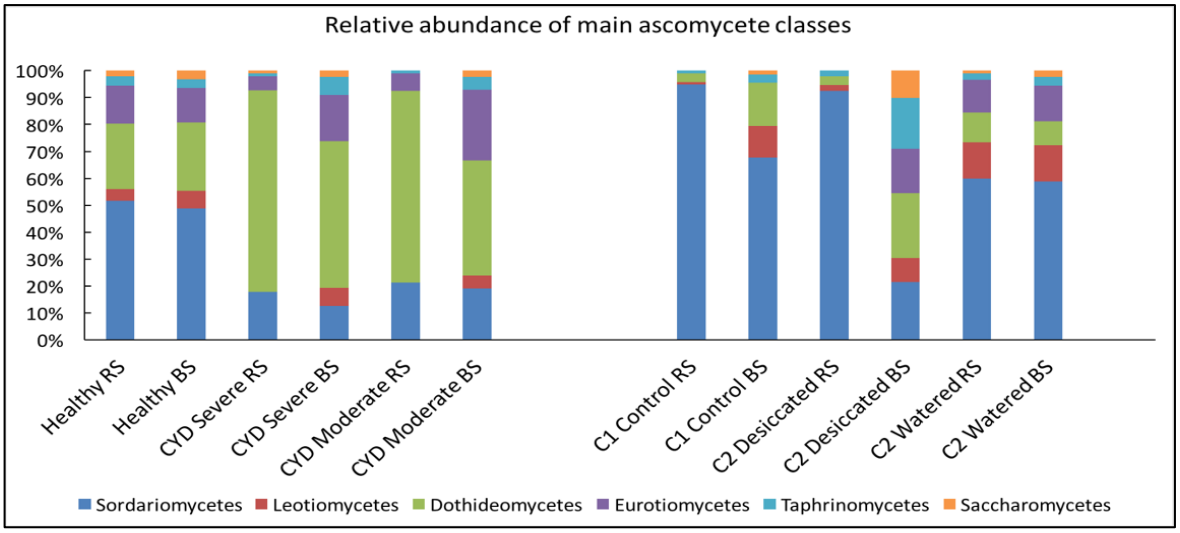

Figure 15: Relative abundances of ascomycete classes for twelve soil samples
Figure shows the relative abundances of six ascomycete classes for field soils (left) and desiccation experiment soils (right) with 'RS'=rhizosphere and 'BS'=bulk soil; (relative abundance % for total ascomycetes classified for each sample).

Discussion

Coriander yield decline is a real phenomenon that has been observed in a grower's field, and also in glasshouse pot trials. In this study, plants showing 'decline' did not exhibit obvious symptoms of disease. CYD was observed in the form of lower yields due to lack of emergence, smaller plants, or both. The overall results of this study succeeded in meeting the objectives outlined in the proposal for CP 117a: to investigate potential methods to prevent coriander yield decline which are aimed at altering the soil microbial community. Results showed that CYD is likely the product of a combination of interacting factors, and that some management practices used in

glasshouse pot trials impacted the occurrence or severity of CYD experienced. However, it was found that methods which directly altered the soil microbiota (drying out crop soils and sterilisation) were the most effective at treating CYD. This gives further 'proof of concept' to the microbial nature of the problem, hypothesised in CP 117.

The impact of crop and soil management techniques which indirectly alter soil microbial communities

The tillage experiments in this study produced mixed results. The ploughing vs. harrowing experiment did not provide conclusive evidence to support deep ploughing as a practice to mitigate CYD, at least in a glasshouse scenario. All second and third cycle pots showed significant decline in total yields, compared to control soils. The ploughing treatment showed the greatest decline (compared to harrowing) in the second cycle, and slightly less decline in the third cycle. This may indicate that the ploughing effect achieved in pot trials did not sufficiently reflect the process of inversion used in the field (large pots maintained dry, friable soils which were difficult to fully invert). As this practice is used by some growers who do not get CYD (Fraser, 2017), it would be a worthwhile experiment to assess more realistically in field trials. Harrowing was proposed as an experimental treatment for this study, as soil compaction before sowing the second cycle of coriander may have influenced the level of CYD achieved in CP 117(Fraser, 2017; Kim Parker, pers. comm., 2017). It is unclear whether UK coriander growers always harrow before sowing a coriander crop. Results of the harrowing experiment in this study showed that overall yields for harrowed pots were 14% greater than yields for non-harrowed pots. However, a considerable level of decline was still seen in the second crop cycle, with significantly smaller plants (above ground weights) being produced for both treatments. This may partly reflect the lower nutrient levels in second cycle pots, as smaller plants were also obtained when pots were not base-dressed before sowing in the 'Fertiliser regime experiment' in Appendix 1. Notwithstanding, results showed that harrowing had a significant positive impact on coriander yield in a second cycle. For this reason, harrowing was included as a factor in the provision of 'optimum' conditions for coriander growth in subsequent experiments. Whilst reducing compaction may be an

important consideration in management efforts to limit CYD in the field, this practice would need further study within the context of a field environment.

Planting density was investigated in this study because growers use a wide variety of planting densities in the field. Furthermore, a previous AHDB report found the growth of pot-grown coriander to be highly influenced by different planting densities in the glasshouse (Flowers & Bashtanova, 2008). In pot trials, low density pots produced much larger above and below ground plant parts—reflecting the greater availability of light, nutrients, and rooting volume (Poorter, *et al*., 2012). The resulting total yields per pot suggested that planting density impacted the level of CYD experienced. Although replication was low, the medium density showed limited decline (13%), compared to the low density (34%) and the high density (41%). Further support for the impact of density was shown in the '*Improved growing conditions experiment'*, presented in Appendix 2, where low planting density pots had significantly greater levels of decline than those sown at a high density. Particularly with the lower density plants in this study, the second crop cycle showed a pronounced loss of soil structure associated with greater exposure to water and potential waterlogging. Clearly, plant spacing achieved by drilling rows in the field is very different to the planting density facilitated in pots. Determining optimum density in the field also requires the consideration of a complex set of environmental factors. Nonetheless, results of these experiments suggest that facilitating an optimum planting density for the particular growing environment could be an important consideration in reducing/limiting CYD.

The optimum conditions experiment in this study used a combination of factors aimed at facilitating an ideal growing environment for a second cycle of coriander, based on previous experimental results. However, even after attempts at providing these conditions, the second crop cycle still declined significantly (28%) from control pots. It is likely that a longer period of desiccation would have improved growth in this case. Pots were relatively large, but soils were only allowed to dry out for a period of two weeks; as opposed to the period of four weeks used successfully in the desiccation experiment (conducted in 13 cm pots). Additionally, it is possible that the density chosen was not optimum in this case, in combination with the other experimental factors. The '*Improved growing conditions experiment'* (see Appendix 2) produced a much lower level of decline (13%), which was achieved using similar parameters, but

smaller pots and a higher planting density. In any case, results indicate that a further mechanism also was contributing to the occurrence of CYD in this case—a microbiological influence.

The impact of management strategies which directly alter soil microbial communities

The soil microbiome of crops is highly influenced by agricultural management practices (Chaparro, *et al*., 2012). One such practice is solarisation, which has been used in southern Spain to avoid the occurrence of CYD in continuous coriander cropping (Victoria Langdale, pers. comm., 2017). Of relevance to the desiccation experiment in this study, is the fact that soil drying can perform some of the same functions as solarisation. Kaiser, *et al*., (2015) found that one of the major effects of air-drying soil (at ambient temperature), was the death of a large proportion of the soil-inhabiting microorganisms. Besides affecting soil biotic properties, air-drying soils can profoundly change the physical and chemical characteristics of a soil (Kaiser, *et al*., 2015). The results of the desiccation experiment in this study showed that soils that were dried out for 4 weeks before sowing a second crop, did not experience yield decline. The difference in mean total yields for C2 dessicated pots and C1 control pots was negligible; and the mean above ground fresh weight of C2 desiccated plants was actually slightly higher than that of C1 controls. Importantly, the fact that the desiccated treatment pots did not experience yield decline, differentiates soil drying as a treatment which may directly alter the soil microbiome; as opposed to other treatments used in this study, which likely have less effects on soil microorganisms (e.g. ploughing, harrowing, and planting density). It is also probable that the effect of drying out the crop soils after growing coriander helped to maintain the soil physical structure in pots. Air-drying soils has been shown to facilitate the stability of soil aggregates, which helps to store soil organic matter and maintain overall soil structure and plant productivity (Kaiser, *et al*., 2015; Six, *et al*., 2004). But equally importantly, it appears that the microbial community composition of these soils was altered in the drying process. In this case, drying soils may have had a beneficial effect on soil microorganisms; specifically, resulting in very different fungal communities to those of the poor-performing C2 watered treatment pots. Whilst the exact parameters required to achieve sufficient drying in the field would require further experimentation

and field trials, it appears that this technique could potentially be beneficial in limiting CYD, in combination with other management practices.

Along with solarisation and air-drying of soils, other non-chemical means have been used to control soil pathogens and also address yield decline associated with microbial causes. As an example, steam sterilisation was used to improve growth of *Angelica sinensis,* another Apiaceae herb crop which suffers from yield decline in continuous cropping systems (Zhang, *et al*., 2016). Interestingly, this method is also used by a UK coriander grower in Guernsey to avoid CYD in a covered cropping system (not in pots) (Simon Harty, pers. comm., 2018). The level of soil sterilisation achieved in the present study was not determined prior to sowing coriander. However, the contrast in coriander yields between the sterilised and non-sterilised field soils confirmed an effect from the sterilisation. This difference was pronounced, both in the individual plant size, and the total yield per pot. The overall results suggest that the sterilisation process eliminated a deleterious microbial element from the soils. This gives further weight to a microbial cause in CYD, at least in the case of the affected field soil. Importantly, it also confirms that a deleterious effect may occur in healthy coriander crop soil, when a subsequent crop is introduced into the same soil. Another consideration in this experiment, is the fact that soil sterilisation has also been shown to facilitate increased availability and acquisition of nutrients; which may have further contributed to the effect of improved growth (Troelstra, *et al*., 2001; Costa, *et al*., 2006). Results showed that sterilisation successfully eliminated CYD in pot trials. Implementing this in the field may be a costly, and labour-intensive process (Simon Harty, pers. comm., 2018); but warrants further investigation.

Microbial community differences were found between healthy and yield decline soils

In addressing the causes of yield decline, there is increasing support for the involvement of communities of microorganisms which are not specific pathogens. DRMOs (deleterious rhizosphere microorganisms) have been linked to numerous examples of yield decline in crops grown in monoculture and shortened rotations (Bennett, *et al*., 2012). Some of the deleterious activities of DRMOs include altering a plant's uptake of water, ions, and plant growth substances; through limiting root growth and function (Schippers, *et al*., 1996; Schippers, *et al*., 1987). Even with the

availability of adequate soil nutrients, the implication is that soil microbial communities may be partly responsible for yield reduction in crops grown in monoculture and shortened rotations. Soil sterilisation experiments have provided evidence to support the existence of DRMOs in yield decline soils, and transfer studies have shown that such organisms may also be harboured in crop debris (Bennett, *et al.*, 2012; Nehl, *et al*., 1997).

In examining the field soils sampled in this study, a pronounced difference in fungal taxa was found between the healthy and yield decline samples. There was a clear dominance of ascomycetes in the CYD field soil samples. Higher taxonomic resolution showed a defined increase in relative abundance of Dothideomycetes in the CYD field soil samples, particularly in the rhizosphere. Dothideomycetes contains many agricultural plant pathogens with high economic impact (Ohm, *et al*., 2012). The three specific fungi which contributed most to the abundance of Dothideomycetes in the rhizosphere of the yield decline soil samples were: *Bipolaris sorokiniana* (pathogen of wheat and barley); *Leptosphaeria maculans* (pathogen of *Brassica* spp., particularly oilseed rape); and *Cenoccocum geophilum* (a common ectomycorrhizal fungus). While the functional modes of these fungi could not be ascertained in this study, it is possible that they are functioning as DRMOs in this case. These results provide further support for a potential fungal cause in the problem, which was one of the hypotheses proposed in CP 117 (Fraser, 2017). The elucidation of specific fungal groups also provides direction for further studies.

The desiccation experiment soil samples in this study presented distinct microbial communities from the field soil samples. This agrees with findings in CP 117 (Fraser, 2017), that separate incidences of yield decline may not share a single microbial cause. This is not surprising, given the very different physical and biological properties of the compost, compared to field soil; and the very nature of growing plants in pots in a controlled environment. But like the field soils, the desiccation experiment soils showed a pronounced difference in fungal taxa between the healthy and yield decline samples. At phylum level, the most notable difference between samples was the higher relative abundance of basidiomycetes in the C2 desiccated bulk soil, compared to C1 control bulk soil, and C2 watered bulk soil. This indicates that the process of drying changed the fungal composition of the soil. In examining

the classes of Ascomycota in the desiccation soil samples, the rhizospheres of the C1 control and C2 desiccated samples were very similar; and characterised by the dominant abundance of Sordariomycetes. This may reflect the similarly high yields of these two crops, as the C2 desiccated plants showed no CYD.

Future work

Before advising growers on changes to coriander cropping, potential management strategies require further assessment through more extensive replication and field trials. Additional studies into the soil microbial communities associated with CYD would also be beneficial. This may further elucidate the involvement of specific microorganisms, and could drive more targeted management options. Biofumigation is a potential avenue of treatment, which was recommended as a management option in CP 117 (Fraser, 2017), and could be a worthwhile study avenue. Furthermore, the use of biocontrol treatments to eliminate fungal DRMOs may provide another valuable study area.

Coriander is not a developed crop, in the sense of major crop species. Further research is needed to facilitate yield optimisation, and also a better understanding of the factors which contribute to CYD. In directing future work, it must be noted that the most successful method for increasing or maintaining crop yields is to extend the length of the crop rotation used (Bennett, *et al.*, 2012; Bullock, 1992; Karlen *et al*., 1994;). Long rotations may be impractical for most coriander growers, but the parameters of an effective rotation to avoid CYD have not been determined. The crop species used by coriander growers during rotation breaks may not be effective at disrupting the yield decline incurred in subsequent coriander crops. It may also be that cropping frequency is another contributing factor to CYD—perhaps the tendency to 'double-crop' is not an advisable practice for all coriander cropping systems. A longer-term study into effective rotations and cropping frequency of coriander would therefore be highly beneficial in further addressing CYD.

Conclusions

This study showed that management practices which indirectly altered soil microbial communities impacted the severity of CYD incurred in the glasshouse, but were not effective in preventing it. Sterilisation and drying of crop soils effectively treated CYD

in two different cropping systems. Results of the soil microbial community studies give further weight to a fungal cause in the problem, particularly in the case of CYD in a grower's field soil. Further studies are required to confidently advise management options for growers, and also elucidate further microbial mechanisms in CYD.

Knowledge and Technology Transfer

- Visit to growing operation for collection of yield decline and healthy coriander plants and soils (Herbs Unlimited, Thirsk) (09/2017)
- BHTA Herb event, networking and workshops (Surrey) (09/2017)
- AHDB six-month progress update, meeting and presentation (SASA, Edinburgh) (02/2018)
- BHTA Spring conference, presentation: 'MRes Coriander Yield Decline: project progress and future directions' (Kenilworth, Warwickshire) (02/2018)
- Microbiological research group joint presentation: 'From Farm to Fork: the fresh produce supply chain' (Edinburgh Napier University) (03/2018)
- Coriander yield decline MRes presentation for the SASA March seminar series (SASA, Edinburgh) (03/2018)
- Post graduate conference, coriander yield decline poster and presentation (Edinburgh Napier University) (04/2018)

Glossary

References

Bennett, A.J., Bending, G.D., Chandler, D., Hilton, S. and Mills, P. (2012). Meeting the demand for crop production: the challenge of yield decline in crops grown in short rotations. *Biological Reviews*, *87*(1): 52-71.

Berendsen, R.L., Pieterse, C.M. and Bakker, P.A. (2012). The rhizosphere microbiome and plant health. *Trends in plant science*, *17*(8): 478-486.

Chaparro, J.M., Sheflin, A.M., Manter, D.K. and Vivanco, J.M. (2012). Manipulating the soil microbiome to increase soil health and plant fertility. *Biology and Fertility of Soils*, *48*(5): 489-499.

Costa, R., Götz, M., Mrotzek, N., Lottmann, J., Berg, G. and Smalla, K. (2006). Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. *FEMS Microbiology Ecology*, *56*(2): 236-249.

Davies, T. Personal Communication, cited in Spencer, K. (2017). MPhil University of Newcastle/AHDB project CP 117: Investigating the cause and potential treatment of coriander yield decline.

Flowers, T., and Bashtanova, U. (2008). Report: Effects of variety and growth conditions on shelf life and appearance in-pot and field-grown coriander. Agriculture and Horticulture Development Board. https://horticulture.ahdb.org.uk/sites/default/files/research_papers/PC%20259%20final%20report%202008.pdf (Accessed 16/07/2017).

Fraser, K. (2017). MPhil Thesis, University of Newcastle/AHDB project CP117: Investigating the cause and potential treatment of coriander yield decline.

Gibbs, R. (AHDB CP 117a industry representative, coriander grower Langmead Farms) (2017 and 2018). Personal communications and email correspondence: Edinburgh Napier University, SASA (Science and Advice for Scottish Agriculture), and BHTA Summer Herb Event, Surrey.

Hargreaves, C. (2014). *Coriander: how the 'marmite herb' went from zero to hero in Britain*. Independent. http://www.independent.co.uk/life-style/food-and-drink/features/coriander-how-the-marmite-herb-went-from-strength-to-strength-9753528.html.

Hilton, S., Bennett, A.J., Keane, G., Bending, G.D., Chandler, D., Stobart, R. and Mills, P. (2013). Impact of shortened crop rotation of oilseed rape on soil and rhizosphere microbial diversity in relation to yield decline. *PLoS One*, *8*(4): 598-599.

Kaiser, M., Kleber, M. and Berhe, A.A. (2015). How air-drying and rewetting modify soil organic matter characteristics: an assessment to improve data interpretation and inference. *Soil Biology and Biochemistry*, 80: 324-340.

Langdale, V. (2017). Personal Communication, Email correspondence, Hortopalma SL, Spain.

Mendes, R., Garbeva, P. and Raaijmakers, J.M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS microbiology reviews*, *37*(5): 634-663.

Nehl, D.B., Allen, S.J. and Brown, J.F. (1997). Deleterious rhizosphere bacteria: an integrating perspective. *Applied Soil Ecology*, *5*(1): 1-20.

Ohm, R.A., Feau, N., Henrissat, B., Schoch, C.L., Horwitz, B.A., Barry, K.W., Condon, B.J., Copeland, A.C., Dhillon, B., Glaser, F. and Hesse, C.N. (2012). Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycetes fungi. *PLoS Pathogens*, *8*(12):1003037.

Parker, K. (AHDB CP117 a representative). (2017). Personal communication by telephone and at Edinburgh Napier University, and SASA (Science and Advice for Scottish Agriculture) Edinburgh.

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J. and Postma, J.A. (2012). Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology*, *39*(11): 839-850.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Schippers, B., Bakker, A.W., Bakker, P.A.H.M., Weisbeek, P.J. and Lugtenberg, B. (1986). Plant growth-inhibiting and stimulating rhizosphere micro-organisms. In *FEMS symposium-Federation of European Microbiological Societies*.

Schippers, B., Bakker, A.W. and Bakker, P.A. (1987). Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. *Annual review of Phytopathology*, *25*(1): 339-358.

Six, J., Bossuyt, H., Degryze, S. and Denef, K. (2004). A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research*, *79*(1): 7-31.

Troelstra, S.R., Wagenaar, R., Smant, W. and Peters, B.A.M. (2001). Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. *New Phytologist*, *150*(3): 697-706.

VSN International (2011). GenStat for Windows, 14th Edition. VSN International, Hemel Hempstead, UK. https://www.vsni.co.uk/.

Zhang, X.H., Lang, D.Y. and Zhang, E.H. (2016). Effects of Soil Sterilization on Growth of *Angelica sinensis* Plant and Soil Microbial Populations in a Continuous Mono-cropping Soil. *International Journal of Agriculture & Biology*, *18*(2).

Appendices

Appendix 1

Fertiliser regime experiment: investigating the impact of fertiliser on coriander growth and the occurrence of CYD

Coriander seeds were sown in 13 cm pots at the standard density (36 seeds). A second cycle was sown using the same parameters, for two levels of treatment: 1) 'fertilised' with a base-dressing of 0.5 g Chempak 3; or 2) 'non-fertilised'. Three replicate pots were sown for each treatment, alongside three controls for each treatment, for a total of 12 pots. Plants were grown for approximately 8 weeks, and all were given the standard application of fertiliser at 4 weeks' growth. Data were collected for individual plant biomass (21 replicate plants per treatment) and total yields per pot (three replicate pots).

Results: Investigating the impact of fertiliser regime on coriander growth and the occurrence of CYD

Fertiliser regime (base-dressing pots with fertiliser vs. not base-dressing with fertiliser before sowing a second coriander crop) was a further influential factor on the size of individual plants, but not on the overall yields per pot. Significant differences were found for each of the plant metrics, with the exception of dry above ground weights ($p>0.05$). The fresh above ground weights of plants were significantly different between treatments ($p= 0.022$); fertilised plants (grown in both fresh control (C1) soils and in C2 soils) had greater fresh above ground weights (Figure A1a). Although post-hoc analyses failed to reveal specific pairwise differences (95% CI $p>0.05$), fertilised pots produced fresh above ground weights approximately 20-30% greater than those of non-fertilised pots. The fresh weights of plant roots differed significantly between treatments ($p= 0.003$), but with a contrasting result. Figure A1a shows that the largest fresh root weights were found in C2 non-fertilised plants (which failed to show pairwise significance in post-hoc analyses, $p>0.05$). Total yields per pot were significantly different ($p=0.002$). Coriander yields per pot for fresh control soils (C1) were greater

than yields per pot for coriander grown for a second cycle (C2) (Figure A1b), with pairwise significant differences (CI 95%) shown below:

C2 Non-fertilised vs. C1 Control non-fertilised	p= 0.005
C2 Fertilised vs. C1 Control fertilised	p= 0.041
C2 Non-fertilised vs. C1 Control fertilised	p=0.003

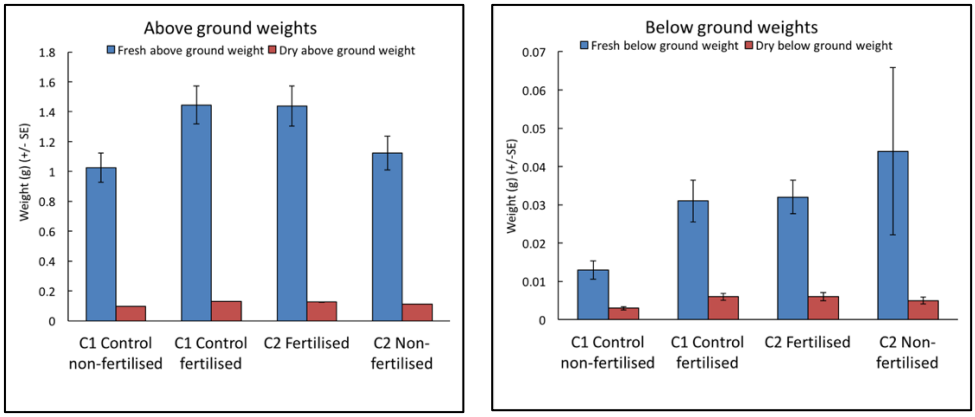

Figure A1a: The impact of fertiliser regime on individual plant biomass

Figure shows above ground weights (left), and below ground weights (right). (Means calculated from 21 replicate plants per treatment).

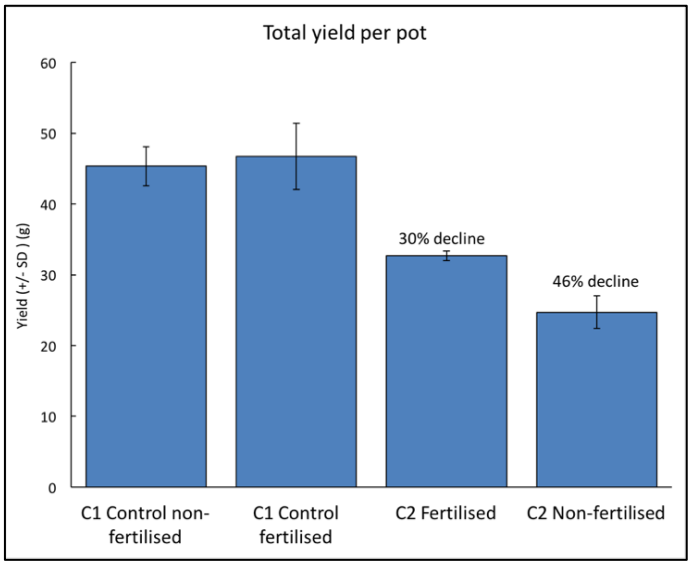

Figure A1b: The impact of fertiliser regime on coriander total yield per pot

Figure shows total yields per pot, with percent C2 declines from relative C1 control pots. (Means calculated from three replicate pots per treatment).

Appendix 2

Improved growing conditions experiment: Investigating whether CYD can be reversed through the improvement of growing conditions for a second crop cycle

Twenty-eight replicate 12 cm pots were sown with coriander seeds at the standard planting density (30 seeds) and grown for approximately six weeks. Two basic treatments were applied to pots before re-sowing a second crop cycle: 1) 'improved' conditions were facilitated by letting crop soils dry out in their pots for a period of two weeks, followed by harrowing the pot soils before re-sowing; 2) 'poor' conditions were facilitated by watering crops daily for two weeks, and then simply inserting seeds, rather than harrowing. Both treatments (14 replicate pots each) were base-dressed with fertiliser (0.5 g Chempak 3); half of each of the treatment pots (seven replicates) were sown at a high density (30 seeds), and half were sown at a low density (10 seeds). Seven control pots were sown for each of the two densities, totaling 42 pots for the experiment, with the following six combinations of experimental variables:

1. Improved conditions low density
2. Improved conditions high density
3. Poor conditions low density
4. Poor conditions high density
5. Control low density
6. Control high density

The second crop cycle was harvested at approximately 5 weeks' growth, whereby yields per pot was calculated (seven replicates per treatment).

Results: Investigating whether CYD can be reversed through the improvement of growing conditions for a second crop cycle

A significant decline effect for yield was seen in the CYD reversal experiment when comparing treatments, with and without considering planting density. A significant difference in yield was found between three general treatments ('improved conditions', 'poor conditions', and 'control') ($p=7.96^{e-06}$) (Figure A2a). Pots with improved conditions showed much less decline (37%) than pots subjected to poor condition (67%). Post-hoc analyses showed significant differences in yield between the control and the poor condition pots (CI 95% $p=6.30^{e-06}$); and the improved

conditions vs. poor conditions pots (CI 95% p=0.002). No statistical difference was detected between the control and the pots with improved conditions (p>0.050). Figure A2b shows the relative percentage of decline from respective controls, with the high density improved condition pots showing the least decline at 13%, and the low density poor condition pots yielding very poorly with 89% decline.

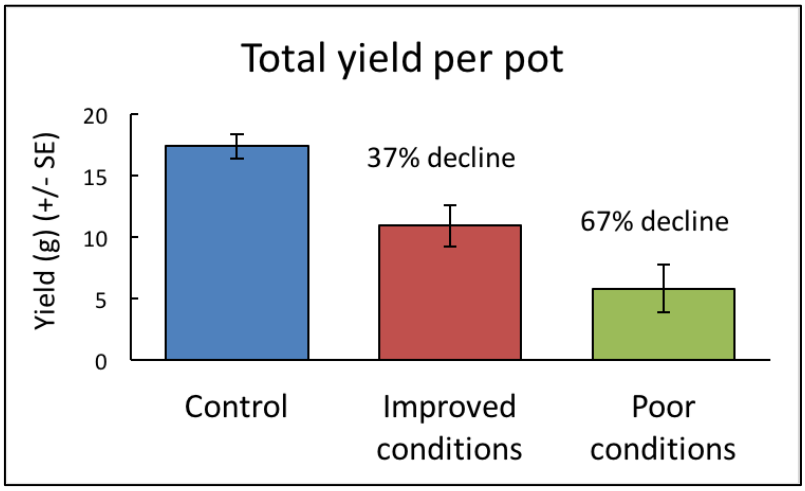

Figure A2a: The impact of improved vs. poor conditions on coriander yield
Figure shows mean total yields for three general treatment levels in the improved growing conditions experiment (without the factor of planting density), with relative percentage of decline from the control.

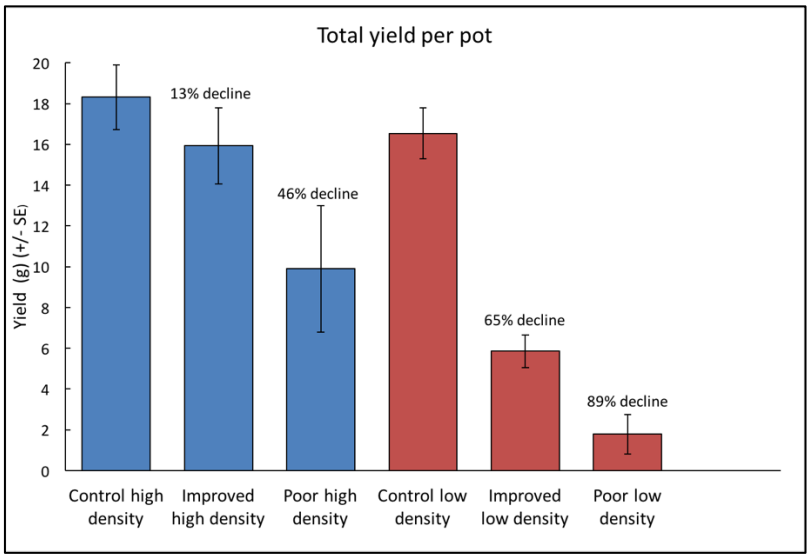

Figure A2b: ***The impact of improved vs. poor conditions plus density***
Figure shows mean total yields for the improved growing conditions experiment with six levels of treatment (conditions plus density) and relative percentages of decline from respective controls.

Appendix 3

The Kaiju program classification results for MinION nanopore sequencing

Table 2 shows the number of reads produced in MinION (ONT), with the corresponding number of taxonomic classifications from the Kaiju program. This shows an overall 'rhizosphere effect', (Berendesen, *et al*., 2012), particularly evident for the field soil samples, with more than three times the number of reads for the rhizosphere soils compared to corresponding bulk soils. This effect was also seen in the C1 control of the desiccation experiment (with twice the number of classifications for rhizosphere vs. bulk soil), but not for the other two soil treatment samples.

Sample	***Total reads***	***Reads Classified***
1) Healthy rhizosphere	58,740	45,029
2) Healthy bulk soil	17,726	13,249
3) Severe CYD rhizosphere	65,921	89,547
4) Severe CYD bulk soil	19,767	28,248
5) Moderate CYD rhizosphere	79,898	108,554
6) Moderate CYD bulk soil	23,748	33,219
7) C1 Control rhizosphere	63,535	97,939
8) C1 Control bulk soil	33,965	45,487
9) C2 Desiccated rhizosphere	68,748	102,431
10) C2 Desiccated bulk soil	73,172	103,996
11) C2 Watered rhizosphere	64,347	94,746
12) C2 Watered bulk soil	62,127	92,162

Table 2: Kaiju classification results

Table shows the total number of quality reads produced in MinION and number of reads classified in Kaiju. Samples 1-6 represent field soil samples from two coriander crops (healthy and CYD with two levels of decline); samples 7-12 were taken from the desiccation experiment and represent the two levels of C2 treatment and a C1 control.