Project title:	Investigating the cause and potential treatment of coriander yield decline
Project number:	CP117
Project leader:	Kate Fraser, Newcastle University
Report:	Annual report (Year 1), September 2015
Previous report:	N/A
Key staff:	Dr. Ian Singleton Prof. Anne Borland
Location of project:	School of Biology, Devonshire Building, Newcastle University, Newcastle-Upon-Tyne, NE1 7RU
Industry Representative:	Dr. T M Davies, Malvern View Herbs, Rob Gibbs, Scot Herbs Ltd, Longforgan, Dundee, DD2 5HU Hugh Bullock, Herb Fresh, Southwood Manor Farm, Burhill Rd, Hersham, Walton-on-Thames, KT12 4BJ Gareth Taylor, Red Deer Herbs Ltd, Red Deer Farm, Earls Croome, Worcester, WR8 9DF
Date project commenced:	1st October 2014
Date project completed (or expected completion date):	30th September 2017

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

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.

[Name]

[Position]

[Organisation]

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

[Name]

[Position]

[Organisation]

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

Report authorised by:

[Name]

[Position]

[Organisation]

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

[Name]

[Position]

[Organisation]

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

CONTENTS

GROWER SUMMARY

Headline

- Coriander yield decline was successfully induced under controlled greenhouse conditions. Traditional microbiological studies suggest that coriander has a clear effect on soil microbial numbers surrounding the plant root (the rhizosphere). Interestingly, this rhizosphere effect varied with plant age and was different between healthy plants and those showing yield decline.

Background

Approximately 1500 hectares of coriander are grown annually. However, due to a lack of space for crop rotations, coriander is grown repeatedly in the same soil in successive years. It is common for plants grown in monoculture / short rotations to suffer from yield decline and coriander appears to be no exception, with growers reporting up to 50% losses in leaf yield. Interestingly, coriander yield decline has yet to be reported in the scientific literature and the phenomenon consequently remains an observation that requires confirmation. Coriander is the biggest selling herb in the UK, with over 30 million packets and bunches being sold in 2014, and so any decline in yield has a significant impact on the UK herb industry. Therefore, it is crucial that the cause of yield decline is established for a potential solution to this issue to be investigated.

There are many different interacting factors that can cause yield decline but observational evidence suggests that this issue has a soil microbiological basis. Hence, the initial aims of this project were to:

a) establish growers' practices and experiences of yield decline using a questionnaire;

b) recreate coriander yield decline under controlled greenhouse conditions to confirm growers observations and;

c) determine the effect of coriander on soil microbial numbers in both healthy and plants showing yield decline.

Summary

The overall aim of this project is to attempt to elucidate the cause of yield decline and to subsequently investigate potential remedies. Initial work focused on the use of traditional microbiological techniques to gain an insight into the effect of cropping coriander on the microbial numbers of both bulk soil, and soil associated with coriander roots (rhizosphere soil). Future work will utilise more complex molecular techniques to investigate these microbial populations in more detail. Furthermore microbe-plant interactions will be studied in more detail to elucidate the effect that microbes could be having on the plant. It is

anticipated that if a cause is found then attention can focus on soil management to prevent coriander yield decline.

Questionnaire Findings

The questionnaire asked growers to report the severity of yield decline they experienced. 2 out of 5 growers contacted reported that they suffered no yield decline. Both of these growers suggested that consistent ploughing in between crops may alleviate yield decline. Of the 3 growers who do suffer yield decline, all reported that the issue was worse earlier in the growing season and following cold, wet weather. Furthermore, 2 growers who suffer from yield decline indicated that they get higher yields of coriander when they use soil that has not experienced the crop before. One grower suggested that leaving soil clear of coriander for 4 years before replanting alleviates the issue. However another grower disagreed and reported that yield decline persists even with a 4 year break. When asked about growing regimes, all of the growers reported that they grow 2 crops of coriander in the same soil per season, though gaps left between crops in a season varies between 1 to 6 weeks. The 2 growers who did not experience yield decline had the shortest gap between crops as they ploughed between re-sowing. Most growers use a range of soil types (including sandy loam, sandy clay and silty clays) and varieties, though the most popular varieties were Santos (used by 4 growers) and Cruiser (used by 3 growers). Further communication, focusing on information required to grow coriander in greenhouse conditions, with growers took place after the questionnaire. These discussions provided information on temperature and light settings; fertiliser regimes; and varieties which were utilised in this reports experiments.

Objective 1: Can coriander yield decline be established under controlled greenhouse conditions?

Coriander var. Santos seeds were sown in pots (~12cm diameter) containing ~700g air dried sandy soil collected from a field at Newcastle University Cockle Park facilities, Northumberland with no previous history of coriander cropping. To replicate UK summer growing conditions, plants were exposed to 14 hours of light per day with a maximum air temperature of 20°C during the day and 16°C during the night. 10 weeks after sowing this initial crop was harvested and the same pots were replanted with coriander var. Santos seeds. Coriander var. Santos seeds were also sown in ~700g of fresh (previously unplanted), air dried sandy soil obtained from the same field. Therefore there were 2 treatments, grown under exactly the same growth conditions, with 3 replicates/ treatment: (1) coriander grown in fresh control soils; (2) coriander grown in soil previously planted with coriander once. 9

weeks after sowing both treatments were sampled for plant yield data: total plant fresh and dry weights; leaf area; and root and shoot lengths were measured.

While yield decline is observed in grower's fields, it was not known if this could be replicated under controlled conditions. However, after just 2 crops plants grown in control soil had a greater above ground biomass compared to those in previously planted soils (Fig. 1).

Figure 1. Comparison of observable difference in yield between coriander plants from control soils (treatment 1) and coriander plants from soils previously planted with coriander (treatment 2). Previously planted soil plants are shown on the right side of the picture with control plants on the left.

In particular, shoot and leaf fresh weights (above ground biomass) were over 50% greater in control plants compared to previously planted plants (Fig. 2). Therefore, this study successfully induced yield decline under controlled conditions, with plants grown in soil that had been previously planted with coriander exhibiting significant yield decline symptoms. However, further work is required to show this phenomenon occurs across a range of coriander cultivars and soil types.

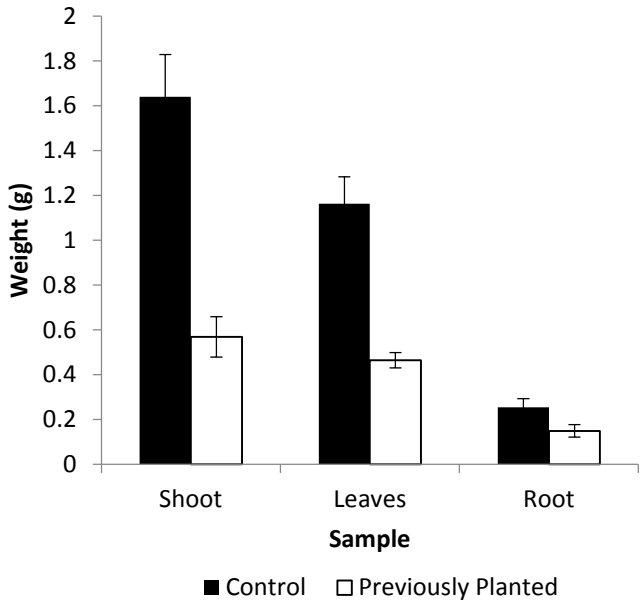

Figure 2. Comparison of mean fresh weights of different tissue types of coriander plants from control soils (treatment 1) and coriander plants from soils previously planted with coriander (treatment 2) (data for other yield parameters is shown in full science report).

Objective 2: Does the coriander rhizosphere have increased microbial numbers compared to bulk soil?

Coriander var. Santos seeds were sown in pots (~12cm diameter) containing ~700g of air dried sandy soil obtained from a field at Cockle Park, Northumberland with no history of coriander cropping. 15 plants were grown per pot with 5 pots per treatment. 2 experimental treatments were used: (1) control, fresh soil containing no coriander; (2) coriander, fresh soil with coriander planted. To replicate UK summer growing conditions, plants were exposed to 14 hours of light per day with a maximum air temperature of 20°C during the day and 16°C during the night. Chlorophyll content of plant leaves was used as an indicator of plant health, and was measured on a weekly basis beginning 5 weeks after sowing. Sampling took place on 2 separate dates: 9 weeks after sowing, and 10 weeks after sowing. At the 1st sampling date (9 weeks after sowing) 2 pots/treatment were sampled. Bulk and rhizosphere soils were sampled from treatment 2 (containing coriander) pots, whilst control bulk soils were sampled from treatment 1 (no coriander) pots. Rhizosphere soil samples consisted of soils adhering to coriander roots, whilst bulk soil samples were classified as soils that were not in close proximity to roots. The remaining 3 pots from each treatment were sampled, in the same manner, 10 weeks after sowing. At both sampling dates, the above ground biomass of any remaining plants from treatment 2 was removed whilst roots were left in the soil. Sampled

soils were diluted and isolated on selective media before any viable microbial colonies were counted.

In week 9 coriander rhizosphere soil samples total bacterial and fungal counts were ~$3.2x10^6$ and ~$1.4x10^4$ CFU/g soil, respectively; whilst in corresponding bulk soils microbial counts were at least 50% lower. This suggests that coriander roots have a marked effect (rhizosphere effect) on soil microbial populations. The rhizosphere effect is a known phenomenon where, typically, the number of microorganisms in the rhizosphere is greater than in the bulk soil. However, in week 10 coriander rhizosphere soil samples bacterial and fungal counts were reduced to ~$4.9x10^5$ and ~$6.6x10^3$ CFU/g soil, respectively; and were no longer different to corresponding bulk soil microbial counts. Therefore, the rhizosphere effect appears to decrease with plant age and decreasing chlorophyll content (Fig. 3). Chlorophyll content was measured on a weekly basis throughout the experiment. Chlorophyll content peaked 7 weeks after sowing but had fallen by 10 weeks after sowing. This drop in chlorophyll content coincides with a decrease in viable counts of rhizosphere soils between weeks 9 and 10 (Fig. 3).

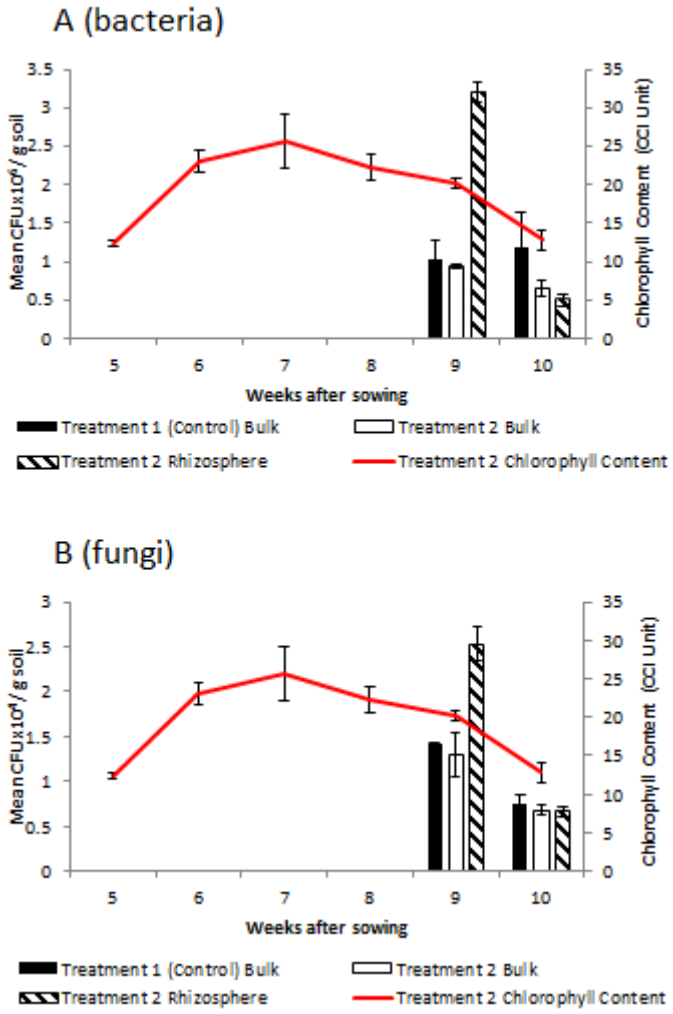

Figure 3. Comparison of mean total viable (CFU/g soil) bacteria (A) and fungi (B) from both treatment soils sampled on 2 separate dates. Sampling of soils took place at 9 and 10 weeks after sowing. Soil samples consisted of control bulk soils from fallow treatment 1 pots, and bulk and rhizosphere soils from coriander containing treatment 2 pots. The red line depicts mean chlorophyll content of the treatment 2 plants and was measured weekly beginning 5 weeks after sowing.

Objective 3: Are microbial numbers (viable counts) associated with plants exhibiting yield decline symptoms different to those found in 'healthy non – decline' plants?

The pots set up in objective 1 were also used to complete this objective. Briefly, there were 2 treatments, grown under exactly the same growth conditions, with 3 replicates/ treatment: (1) control, Coriander var. Santos grown in fresh control soils; (2) previously planted, Coriander var. Santos grown in soil previously planted with coriander once. The chlorophyll content of plant leaves was measured on a weekly basis 5 weeks after sowing. Sampling took place 9 weeks after sowing, 3 pots/ treatment were sampled with bulk and rhizosphere soils being collected from both treatments. At both sampling dates, the above ground

biomass of any remaining plants from both treatments was removed whilst roots were left in the soil. Sampled soils were diluted and isolated on selective media before any viable microbial colonies were counted. Interestingly, in plants showing yield decline symptoms an increased rhizosphere soil total bacterial and fungal population was sustained over the 9 weeks of plant growth. For example, in soils obtained from plants showing yield decline, the rhizosphere bacterial and fungal counts were ~1.6×10^6 and ~1.3×10^4 CFU/g soil, respectively; whilst in soils obtained from similarly aged control plants rhizosphere microbial numbers were at least 90% lower (detailed data is shown in the full report).

Financial Benefits

It is currently too early in the project to identify and deliver any financial benefits. However, the results so far are very interesting, the evidence for a rhizosphere effect means that more fundamental work is required to gain an understanding of what is occurring at the soil microbiological level. Further understanding could lead to the development of applied management methods to alleviate yield decline. Any such remedies would then deliver financial benefit to the UK herb industry.

Action Point

No clear change of practice can yet be recommended.

SCIENCE SECTION

Introduction

Coriander (*Coriandrum sativum* L.) is an annual herb belonging to the family Apiaceae (Diedrichsen 1996). *C. sativum* L. is cultivated in many countries for both its seeds and leaves which have both medicinal and culinary uses (Diederichsen 1996; Telc and Hışıl 2008; Nadeem et al. 2013). Demand due to a shift in diet trends towards healthier foods and with 40% of prescription drugs containing herbs has led to growth in the fresh aromatic herb market (Bashtanova and Flowers 2011; Nadeem et al. 2013). Coriander has been grown commercially in the UK since the 1970s with current production estimated at 1500 hectares annually (Tom Davies per comm.). Coriander is of huge economic importance with sales (over 30 million packs and bunches) exceeding those of other herbs in 2014 (Mail Online 2014).

Unfortunately, due to limited space for crop rotations, UK producers of coriander often repeatedly plant the crop in the same soil in successive years. It is common for crops grown in short rotations or monoculture to suffer from yield decline (Bennett et al. 2011) and coriander appears to be no exception with growers reporting yield declines of over 50% (Tom Davies per comm.). A recent review by Bennett et al. (2011) state that yield decline is probably caused by a number of interacting factors which can include deleterious rhizosphere microorganisms; unusual behaviour of mycorrhizal fungi; autotoxicity; and soil properties. Since decline can persist for up to 8 years (Tom Davies per comm.) this suggests toxic root exudates (autotoxicity) are likely not the cause as over time these will degrade and bind with the soil matrix. Furthermore, other crops can grow well in the same soil indicating that soil quality is not a cause. The most likely factor is thought to have a soil microbiological basis.

The initial aims of this project were to a) establish growers' practices and experiences of yield decline using a questionnaire b) recreate coriander yield decline under controlled greenhouse conditions to confirm growers observations and c) determine the effect of coriander on soil microbial numbers in both healthy and yield decline plants.

Materials and methods

Questionnaire Design and Distribution

At the start of the project it was important to collate information from various growers. A questionnaire was developed and distributed to growers of coriander early in the project: Herb Fresh, Hortopalma S.L., Red Deer Herbs Ltd., Scot Herbs Ltd., Zantra Ltd.. The questionnaire had a dual purpose of gaining information about how coriander was typically grown commercially, and to compare the severity of yield decline and how management practices varied between growers. A copy of the questionnaire design is shown in the Appendix 1.

Results of the questionnaire and further communication with the growers provided the information needed to grow coriander. It was hoped that growing coriander repeatedly in the same soil would result in yield decline similar to that observed by the growers. Greenhouse trials began at Newcastle University Cockle Park facilities in Northumberland, UK in December 2014 and are ongoing.

Establishing coriander yield decline under controlled greenhouse conditions

Experimental set-up

Approximately 30 Coriander var. Santos seeds were sown in pots (~12cm diameter) containing ~700g air dried sandy soil collected from a field at Cockle Park, Northumberland with no previous history of coriander cropping. After emergence of coriander all pots were thinned down to 15 plants/ pot. To replicate UK summer growing conditions, plants were exposed to 14 hours of light per day with a maximum air temperature of 20°C during the day and 16°C during the night. Plants were watered on a daily basis and there was a single application of 0.85g Scott's Peters Professional 20-20-20 fertiliser per pot at 4 weeks after sowing. This initial crop was harvested 10 weeks after sowing before the same pots were replanted with coriander var. Santos seeds. Coriander var. Santos seeds were also sown in ~700g of fresh (previously unplanted), air dried sandy soil obtained from the same field. Therefore there were 2 treatments, grown under exactly the same growth conditions, with 3 replicates/ treatment: (1) control, coriander grown in fresh control soils; (2) previously planted, coriander grown in soils that had been previously planted with coriander.

Sampling

9 weeks after sowing 3 pots/ treatment were sampled and 5 plants per pot were collected for yield data. Fresh and dry weights (plants dried for 24 hours at ~60°C); root and shoot lengths; and leaf area were subsequently measured. To measure leaf area, leaves were laid out on a white piece of paper before being scanned using a HP® scanjet 5530 and saved as a JPeg image file with a resolution of 300dpi. Leaf area was measured using Image J where, under 'analyze particles,' size was set to 0.5-infinity and circularity to 0.00-1.00.

Determining if the coriander rhizosphere has increased microbial numbers compared to bulk soil

Experimental set-up

Approximately 30 Coriander var. Santos seeds were sown in pots (~12cm diameter) containing ~700g air dried sandy soil collected from a field at Cockle Park, Northumberland with no previous history of coriander cropping. After emergence of coriander pots were thinned so that 15 plants were grown per pot with 5 pots per treatment. 2 experimental treatments were used: (1) control, fresh soil containing no coriander; (2) coriander, fresh soil with coriander planted. Light and temperature settings; watering regime; and fertiliser application remained as in experiment above (see 'establishing coriander yield decline under controlled greenhouse conditions').

Sampling

Chlorophyll content of plant leaves was used as an indicator of plant health, and was measured using an Opti-Sciences CCM-200 chlorophyll content meter on a weekly basis starting at 5 weeks after sowing. Sampling took place on 2 separate dates: 9 weeks after sowing, and 10 weeks after sowing. At the 1st sampling date (9 weeks after sowing) 2 pots/treatment were sampled. Bulk and rhizosphere soils were sampled from treatment 2 (containing coriander) pots, whilst control bulk soils were sampled from treatment 1 (no coriander) pots. Rhizosphere soil samples consisted of soils adhering to coriander roots, whilst bulk soil samples were classified as soils that were not in close proximity to roots. For both treatments, approximately 2g of bulk soil was taken per pot; to sample the rhizosphere 5 plants were collected from each treatment 2 pot. The remaining 3 pots from each treatment were sampled, in the same manner, 10 weeks after sowing. At both sampling dates, the above ground biomass of any remaining plants from treatment 2 was removed whilst roots were left in the soil.

Microbial counts

Of the 2g of bulk soil sampled from each of the treatment 1 pots, 0.5g was added to 2ml of 0.9% NaCl solution. This solution was shaken on an IKA® KS 260 Basic platform at 200rpm for 1 hour and then left to settle. 1ml of this soil suspension was used to prepare serial tenfold dilutions in 0.9% NaCl. 100µl of the 10^{-3} and 10^{-4} dilutions was spread onto Nutrient Agar and 100µl of the 10^{-1} and 10^{-2} dilutions was spread onto Rose Bengal Agar for the enumeration of total bacteria and fungi, respectively. NA plates were incubated at 37°C and RBA plates at 25°C. From each of the treatment 2 pots, 0.5g of bulk soil and 0.5g of plant roots plus tightly adhering soil were subjected to the same procedure as the treatment 1 soils.

Comparing microbial numbers of healthy soils to yield decline inducing soils

Experimental set-up

The pots set up in 'establishing coriander yield decline under controlled greenhouse conditions' (above) were also used to complete this objective. Briefly, there were 2 treatments, grown under exactly the same growth conditions, with 3 replicates/ treatment: (1) control, Coriander var. Santos grown in fresh control soils; (2) previously planted, Coriander var. Santos grown in previously planted soils. Light and temperature settings; watering regime; and fertiliser application were as described previously (see 'establishing coriander yield decline under controlled greenhouse conditions').

Sampling

Chlorophyll content of plant leaves was measured using an Opti-Sciences CCM-200 chlorophyll content meter on a weekly basis 5 weeks after sowing. Pots were sampled 9 weeks after sowing; for both treatments, approximately 3g of bulk soil was taken per pot. To sample the rhizosphere, 5 plants per pot were collected from each treatment. The above ground biomass of any remaining plants was removed whilst roots were left in the soil.

Microbial counts

Of the 3g of bulk soil sampled from pots from both treatments, 1g was added to 2ml of 0.9% NaCl solution. This solution was shaken on an IKA® KS 260 Basic platform at 200rpm for 1 hour and then left to settle. Serial dilutions and culturing on agar was then performed as described previously. 0.5g of plant roots plus tightly adhering soil, collected from both treatments, was subjected to the same procedure as the bulk soils.

Statistical Analysis

Data were analysed using Minitab 17 and graphs were produced using Microsoft Office Excel 2010. Normal distribution was tested using Anderson-Darling Normality test and significant differences between mean values were verified using a Tukey test ($p<0.05$) following one-way ANOVA.

Results

Questionnaire

The questionnaire asked growers to report the severity of yield decline they experienced. Only 2 out of 5 growers contacted reported that they suffered no yield decline. Both of these growers suggested that consistent ploughing in between crops may alleviate yield decline. Of the 3 growers who do suffer yield decline, all reported that the issue was worse earlier in the growing season and following cold, wet weather. Furthermore, 2 growers who suffer from yield decline indicated that they get higher yields of coriander when they use soil that has not experienced the crop before. One grower suggested that leaving soil clear of coriander for 4 years before replanting alleviates the issue. However another grower disagreed and reported that yield decline persists even with a 4 year break. When asked about growing regimes, all of the growers reported that they grow 2 crops of coriander in the same soil per season, though gaps left between crops in a season varies between 1 to 6 weeks. The 2 growers who did not experience yield decline had the shortest gap between crops as they ploughed between re-sowing. Most growers use a range of soil types (including sandy loam, sandy clay and silty clays) and varieties, though the most popular varieties were Santos (used by 4 growers) and Cruiser (used by 3 growers). Further communication, focusing on information required to grow coriander in greenhouse conditions, with growers took place after the questionnaire. These discussions provided information on temperature and light settings; fertiliser regimes; and varieties which were utilised in this reports experiments.

Can yield decline be established under controlled conditions?

Plant Yield Data

Plants grown in control, fresh soils and in soils where coriander had been grown once before were harvested 9 weeks after sowing for the collection of yield data. Plants grown in control, fresh soils had a significantly higher above ground biomass compared to those grown in soils where coriander had been grown once before; plants grown in soils previously planted with coriander exhibited symptoms of yield decline. Both fresh ($p=0.007$) and dry ($p=0.040$) shoot; and fresh ($p=0.005$) and dry ($p=0.009$) leaf weights were significantly greater in the control plants than in plants grown in soil where coriander had been grown before (Fig. 1). Leaf areas in the control plants (~8.7mm^2) were significantly greater than in plants grown in soil

which was previously used for coriander (~5.3mm^2; p=0.002; Fig. 2). Furthermore, shoot lengths were significantly greater in control plants (~16.9cm) than in plants grown in soil which was previously used for coriander (~11.5cm; p=0.005), whereas root lengths were not significantly different between the 2 treatments (Fig. 3).

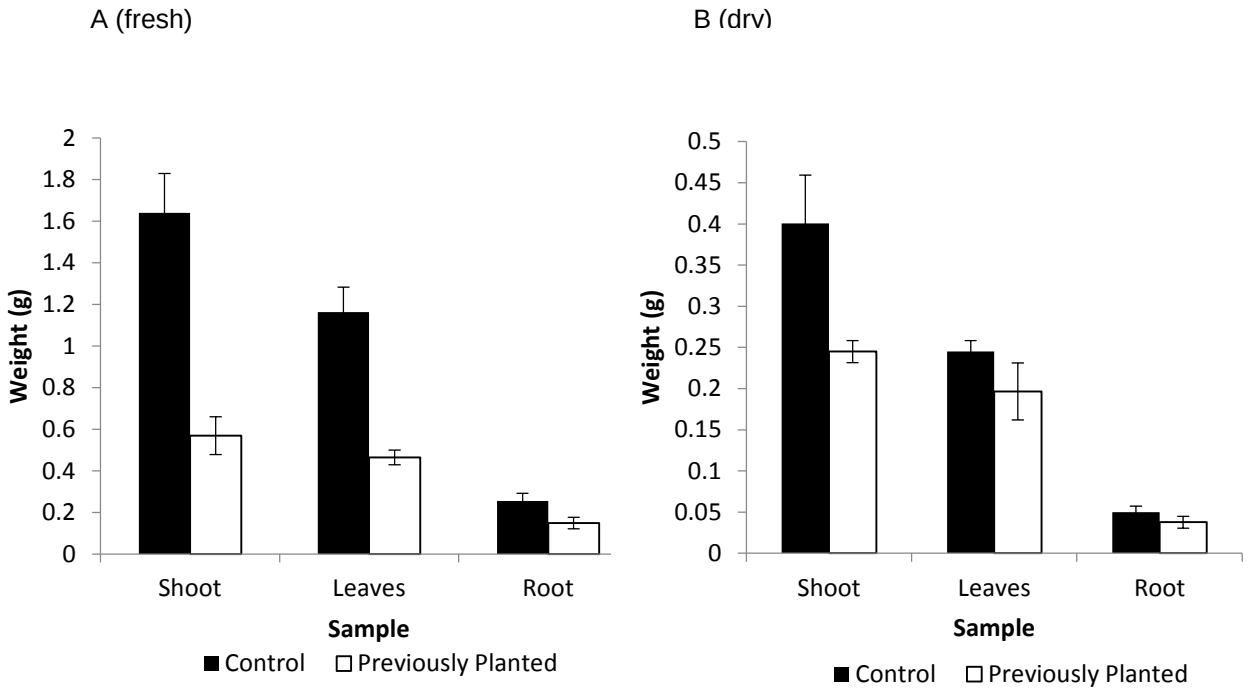

Figure 1. Comparison of mean fresh (A) and dry (B) weights of coriander plants from control soils (treatment 1) and coriander plants from soils previously planted with coriander (treatment 2). Values plotted are ±1 Standard Error (SE).

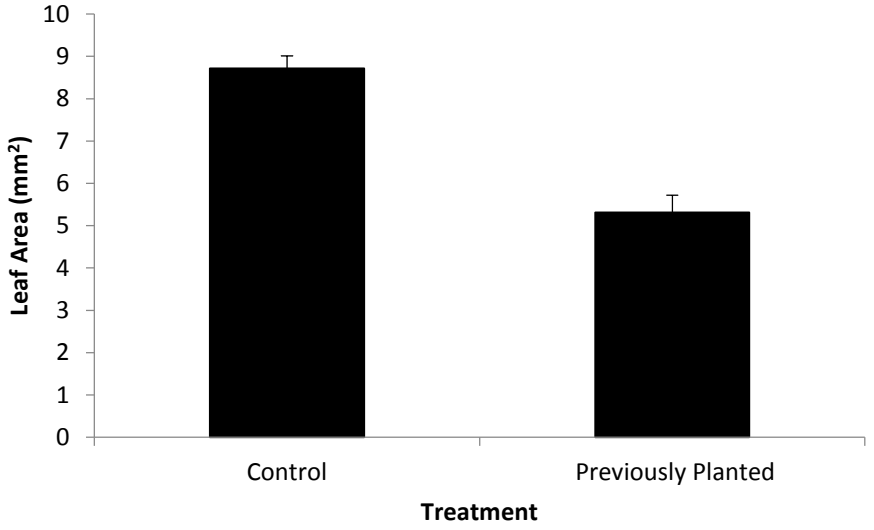

Figure 2. Comparison of mean leaf areas of coriander plants from control soils (treatment 1) and coriander plants from soils previously planted with coriander (treatment 2). Values plotted are ±1 Standard Error (SE).

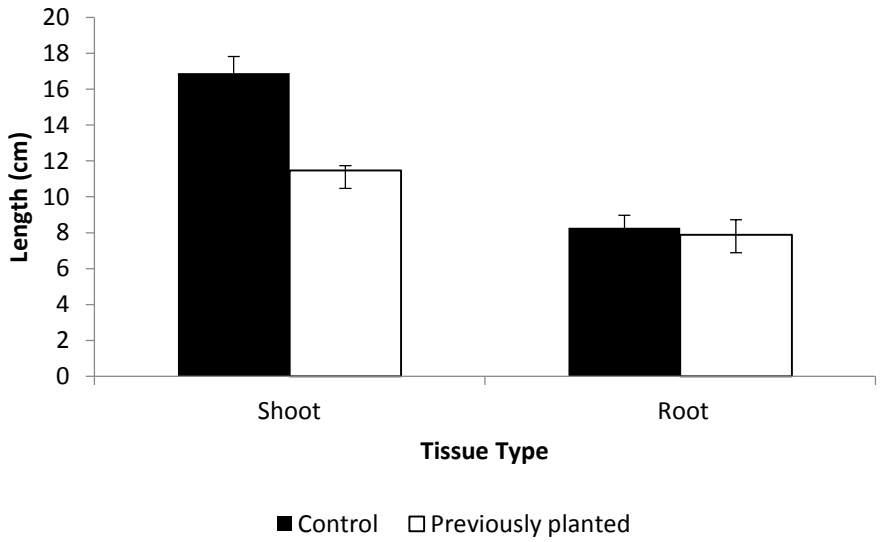

Figure 3. Comparison of mean tissue lengths of coriander plants from control soils (treatment 1) and coriander plants from soils previously planted with coriander (treatment 2). Values plotted are ±1 Standard Error (SE).

Establishing whether the coriander rhizosphere has increased microbial numbers compared to bulk soil

Physiological Parameters

In this experiment there were 2 treatments: (1) control, fresh soil containing no coriander; (2) coriander, fresh soil with coriander planted. Chlorophyll content of plants in treatment 2 (pots containing coriander) was measured weekly beginning 5 weeks after sowing. During the experiment mean chlorophyll content (MCC) peaked at week 7 (~25.6CCI units); by week 9 MCC was reduced, though not significantly, by ~21% (Fig. 4).

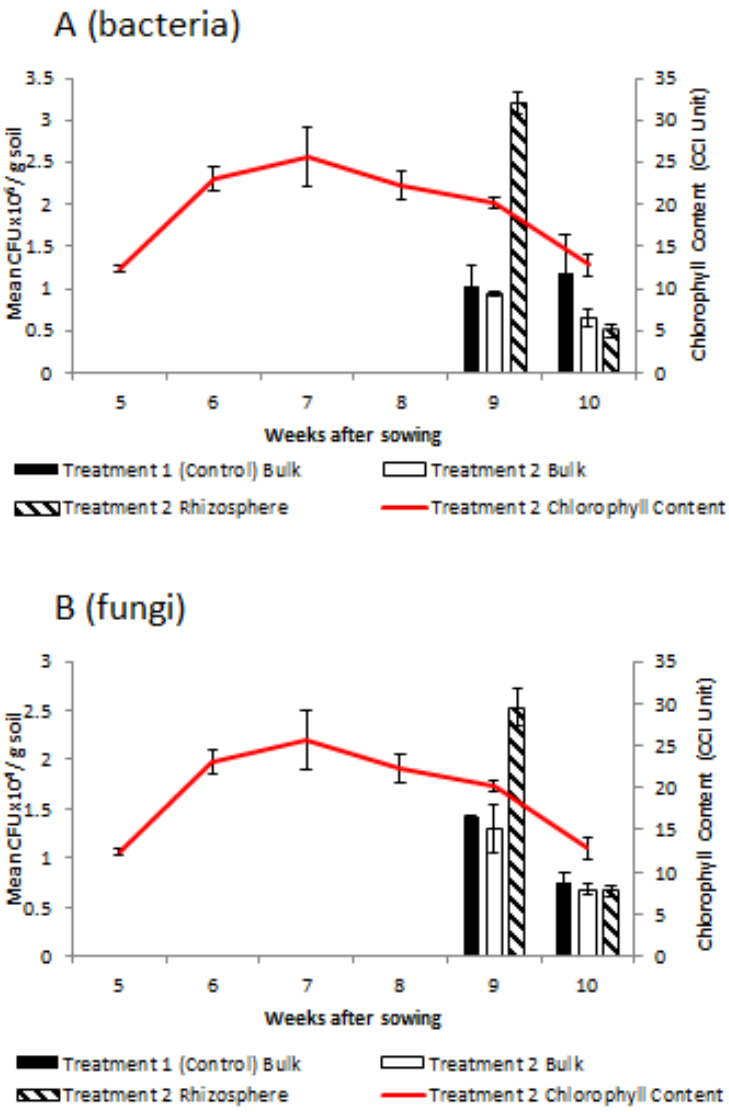

Figure 4. Comparison of mean total viable (CFU/g soil) bacteria (A) and fungi (B) from both treatment soils sampled on 2 separate dates. Sampling of soils took place at 9 and 10 weeks after sowing. Soil samples consisted of control bulk soils from fallow treatment 1 pots, and bulk and rhizosphere soils from coriander containing treatment 2 pots. The red line depicts mean chlorophyll content of the treatment 2 plants and was measured weekly beginning 5 weeks after sowing. Values plotted are ±1 Standard Error (SE).

Microbial Counts

The total number of viable fungi and bacteria were measured in both the bulk and rhizosphere soil samples from treatment 2 (containing coriander) pots, and in the control bulk soils from treatment 1 (containing no coriander). Both treatments were sampled at 9 and 10 weeks after sowing. Treatment 2 rhizosphere soils, sampled 9 weeks after sowing, had total viable bacterial and fungal counts of ~3.2×10^6 and ~1.4×10^4 CFU/g soil, respectively. The treatment 2 rhizosphere soils had significantly higher bacterial ($p=0.014$) and fungal ($p=0.029$) counts

than both respective treatment 2 bulk soils as well as control (treatment 1) bulk soils. However, by 10 weeks after sowing, bacterial (p=0.002) and fungal (p=0.001) counts from treatment 2 rhizosphere soils were significantly reduced by ~84% and ~74%, respectively (Fig. 4). Total fungal and bacterial counts of these treatment 2 rhizosphere soils were ~4.9×10^5 and ~6.6×10^3 CFU/g soil, respectively, and were not significantly different microbial counts derived from either treatment 1 (total bacterial and fungal counts were ~1.2×10^6 and ~7.5×10^3, respectively) or treatment 2 (total bacterial and fungal counts were ~6.5×10^5 and ~6.8×10^3, respectively) bulk soils.

Do soils that induce yield decline have different microbial numbers compared to healthy soils?

Physiological Parameters

The 2 treatments in this experiment were as follows: (1) control, Coriander var. Santos grown in fresh control soils; (2) previously planted, Coriander var. Santos grown in soil that had been previously planted with coriander. Chlorophyll content of plants from each of the 2 treatments was measured weekly beginning 5 weeks after sowing. MCC in plants peaked 7 weeks after sowing in both treatment 1 and 2 (~15.4CCI units and ~16.1CCI units, respectively). However, by 9 weeks after sowing, MCC fell significantly by ~45% and ~37% in plants from treatment 1 ($p<0.001$) and treatment 2 (p=0.009), respectively (Fig. 5). Furthermore, the MCC of treatments 1 and 2 were ~11.6CCI units and ~12.6CCI units, respectively. Therefore there was no significant difference between mean chlorophyll content of the 2 treatments during this experiment (Fig. 5).

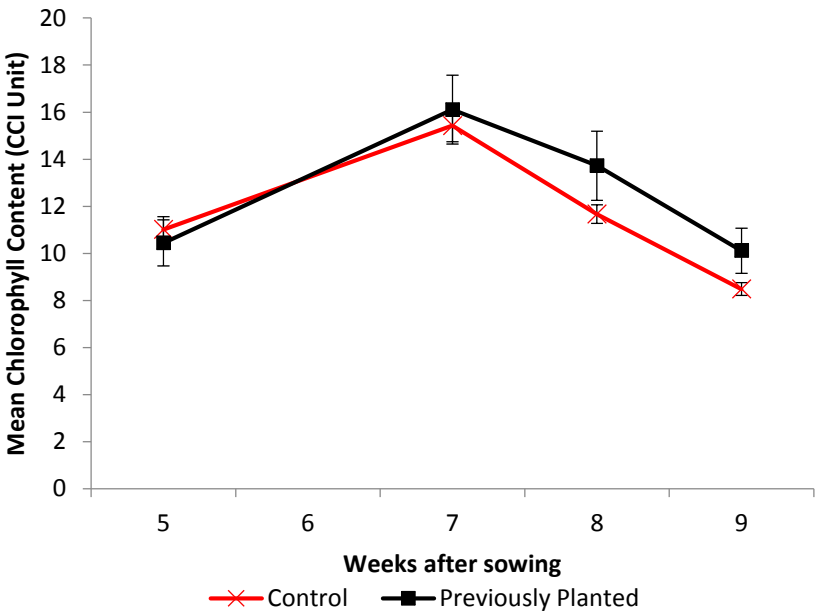

Figure 5. Comparison of mean chlorophyll content of coriander plants from control soils (treatment 1) and coriander plants from soils previously planted with coriander (treatment 2). Values plotted are ±1 SE.

Microbial Counts

The total number of viable fungi and bacteria were measured in both the bulk and rhizosphere soil samples from both treatments. Rhizosphere soils sampled from control (treatment 1) pots at 9 weeks after sowing had significantly (p=0.028) lower fungal counts (~4.1x10^3 CFU/g soil) than bulk soils (~1.2x10^4 CFU/g soil) from the same pot. The same control rhizosphere soils also had higher, though not significantly, bacterial counts compared to bulk soils (~4.9x10^5 CFU/g soil and ~3.0x10^5 CFU/g soil, respectively). Though not significant, viable counts of total bacteria and fungi from previously planted rhizosphere soils (~1.6x10^6 CFU/g soil and ~1.3x10^4 CFU/g soil, respectively) were higher compared to corresponding bulk soil samples (~7.4x10^5 CFU/g soil and ~1.0x10^4 CFU/g soil, respectively) from the same pots (Fig. 6).

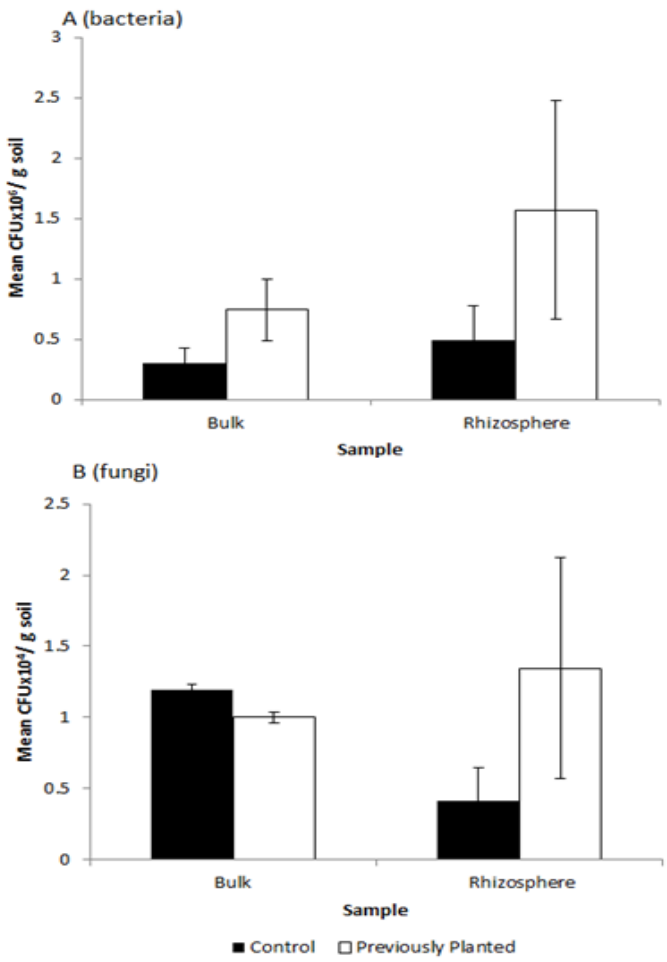

Figure 6. Comparison of mean CFU/g soil of culturable bacteria (A) and fungi (B) from control soils (treatment 1) and soils previously planted with coriander (treatment 2). Values plotted are ±1 Standard Error (SE).

Discussion

Questionnaire Responses

Results of the questionnaire highlighted differences between growers' management practices and the severity of decline they observed. For instance, 2 of the growers observed no yield decline; both only left a gap of 1-2 weeks between crops in a season and were the only growers who consistently ploughed in between crops. This suggests that future studies should be done to examine the effects of ploughing compared to rotovation on coriander yield decline. Santos was the most popular variety amongst the growers and so this was used for initial experiments. However, the growers use a variety of soil types and cultivars, therefore more studies need to be done to investigate whether yield decline can be induced in a range of soil types and coriander varieties.

Confirming the yield decline phenomenon

While yield decline is observed in growers' fields, there are no reports of this phenomenon in the scientific literature (as far as we are aware), and so this observation needed to be confirmed. Therefore, the first objective of this project was to establish yield decline under controlled greenhouse conditions. To confirm yield decline was occurring plant yield was assessed via measurements of the above and below ground biomass of plants from both treatment soils. It was found that plants grown in control soils (healthy plants) had significantly greater above ground biomass compared to those in soils previously planted with coriander (yield decline plants). This is similar to the reports, from 2 of the growers, that higher yields are achieved in fields that have not experienced the crop before. This study successfully induced yield decline under controlled greenhouse conditions, and enabled the generation of yield decline soil for further experiments. However, further work is required to show this phenomenon occurs across a range of coriander cultivars and soil types.

The effect of coriander on soil microbial numbers over time

With the yield decline phenomenon confirmed, the next step was to investigate the effect of coriander on soil microbial populations. A detailed literature search revealed that little or no work had been carried out to investigate soil microbial communities associated with coriander. An experiment was therefore conducted to establish whether the coriander rhizosphere increased microbial numbers; and how the rhizosphere populations changed with time. Total viable counts, a traditional microbiological technique, were used initially to achieve these aims. This study found that rhizosphere soil samples sampled 9 weeks after sowing had significantly higher viable counts than in bulk soils. This would suggest that coriander root does have a marked effect (rhizosphere effect) on soil microbial populations. The rhizosphere effect is a known phenomenon; the difference in the number of microorganisms between the bulk and rhizosphere soils can act as a measure of the rhizosphere effect. Typically, and as found in this study, the number of microorganisms in the rhizosphere is found to be greater (Semenov et al. 1999) than in the bulk soil. The effect is believed to be due to rhizodeposition, including exudates, secretions and lysates from dead cells influencing the soil microbial communities of the rhizosphere (Semenov et al. 1999; Singh et al. 2004; Dotaniya and Meena 2015).

By 10 weeks after sowing there was no significant difference in microbial counts between rhizosphere and bulk soils suggesting that this rhizosphere effect decreases with plant age. Despite an extensive literature search, there is very little mention of a decreasing rhizosphere effect. However, the rhizosphere is a dynamic environment and so it is expected that the

microbial communities within it will change with time (Micallef et al. 2009). Temporal shifts in bacterial communities have been previously reported (Marschner et al. 2002 Micallef et al. 2009). In particular Marschner et al. (2002) demonstrated a correlation between microbial community changes and plant age; they suggest changes in community are due to changes in organic acid exudation. Therefore, in future it may be useful to investigate plant-microbe interactions, including the effect of coriander root exudates on microbes and coriander itself, in more detail.

Chlorophyll content of coriander leaves over time

Chlorophyll content was used as an indicator of plant health and was measured on a weekly basis. Chlorophyll content peaked at 7 weeks after sowing but by 10 weeks after sowing had significantly decreased. This drop in chlorophyll content coincides with a decrease in total microbial viable counts seen in both control and previously planted rhizosphere soils between 9 and 10 weeks after sowing. Further studies could be carried out to elucidate whether a decrease in rhizosphere microbial counts over time correlates with a decrease in chlorophyll content. In such studies chlorophyll content could act as an indicator of plant health, soil samples could be collected when plants reach their maximum and then minimum chlorophyll contents. Viable counts from soils collected when plants are at their maximum and minimum chlorophyll contents could be compared to see if there is any such correlation.

Comparison of healthy and yield decline soils

Finally, this project looked at microbial numbers associated with plants exhibiting yield decline symptoms. Rhizosphere soils sampled from soils that had been previously planted with coriander (yield decline plant) had greater total fungal and bacterial counts compared to control (healthy plant) soils. Furthermore, previously planted rhizosphere soils had greater fungal and bacterial counts compared to bulk samples taken from the same pot. This suggests that in plants showing yield decline symptoms, an increased rhizosphere soil bacterial and fungal population was sustained over 9 weeks of plant growth.

Conclusions

- Yield decline has been successfully replicated under controlled conditions, making it possible to now study microbial populations associated with yield decline.

- It would appear that coriander has a significant effect on soil microbial populations. While there is a rhizosphere effect, this appears to decrease with plant age and coincides with a decrease in chlorophyll content. Further understanding of these effects could lead to potential methods to control yield decline.

Knowledge and Technology Transfer

Site visit to Red Deer Farm to discuss and observe the yield decline issue
Networking at HDC Student Conference 2015
Newcastle University Postgraduate Poster Conference 2014

Newcastle University Postgraduate Conference 2015

References

Bashtanova, U.B. and Flowers, T. J. (2011) Diversity and physiological plasticity of vegetable genotypes of coriander improves herb yield, habit and harvesting window in any season. Euphytica 180:369–384.

Bennett, A.J., Bending, G.D., Chandler, D., Hilton, S., Mills, P. (2011) Meeting the demand for crop production: the challenge of yield decline crops grown in short rotations. Biological Reviews 87:52-71.

Diederichsen, A. (1996)Coriander (Coriandrum sativum L.) Promoting the conservation and use of underutilized and neglected crops. 3. Institute of Plant Genetic and Crop Plant Research Gatersleben/International Plant Genetic Resources Institute, Rome.

Dotaniya, M.L. and Meena, V.D. (2015) Rhizosphere Effect on Nutrient Availability in Soil and Its Uptake by Plants: A Review. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 85:1-12.

Garbeva, P., van Elsas, J.D., van Veen, J.A. (2008) Rhizosphere microbial community and its response to plant species and soil history. Plant and Soil 302:19-32.

Mail Online (2014) Coriander is now Britain's favourite herb: Ingredient sees surge in sales thanks to popularity of Asian curries and stir fries. http://www.dailymail.co.uk/news/article-2777211/Coriander-Britain-s-favourite-herb-Ingredient-sees-surge-sales-thanks-popularity-Asian-curries-stir-fries.html. Accessed 9/3/15.

Marschner, P., Neumann, G., Kania, A., Weiskopf, L., Lieberei, R. (2002) Spatial and temporal dynamics of the microbial community structure in the rhizosphere of cluster roots of white lupin (Lupinus albus L.). Plant and Soil 246:167-174.

Micallef, S.A., Channer, S., Shiaris, M.P., Colón-Carmona, A. (2009) Plant age and genotype impact the progression of bacterial community succession in the Arabidopsis rhizosphere. Plant Signaling Behaviour 4:777-780.

Nadeem, M., Anjum, F.M., Khan, M.I., Tehseen, S., El-Ghorab, A., Sultan, J.I. (2013) Nutritional and medicinal aspects of coriander (Coriandrum sativum L.). British Food Journal 115:743-755.

Semenov, A.M., Van Bruggen, A.H.C., Zelenev, V.V. (1999)Moving Waves of Bacterial Populations and Total Organic Carbon along Roots of Wheat. Microbial Ecology 37:116–128.

Singh, B.K., Millard, P., Whiteley, A.S., Murrell, J.C. (2004) Unravelling rhizosphere-microbial interactions: opportunities and limitations. Trends in Microbiology 12:386-393.

Stefanis, C., Alexopoulos, A., Voidarou, C., Vavias, S., Bezirtzoglou, E. (2013) Principal methods for isolation and identification of soil microbial communities. Folia Microbiologica 58:61-68.

Telc, Hışıl, Y. (2008) Biomass Yield and Herb Essential Oil Characters at different harvest Stages of Spring and Autumn Sown Coriandrum sativum. European Journal of Horticultural Science 73:267-272.

Appendix 1

Coriander Yield Decline Survey Date: 13/10/14

Section A: Contact Details

Name	
Company	
Company Address	
Post code	
Telephone	
Mobile	
E-mail	
Date survey completed	

Section B: Coriander Growth and Management

1. What is the approximate annual area of your field coriander crop (including re-sowing):

   ……
   ….

2. How many coriander crops are grown on the same ground per season?

   ……
   ….

3. Do you leave a gap between crops during a growing season? If so, for how long?

   ……
   ….

4. What are your earliest and latest coriander sowing dates (approximately)?
   Earliest sowing date: ……
   Latest sowing date: …….

5. Do you leave land fallow between seasons? If yes, please indicate duration in table.
   Please tick:

Yes		Duration of fallow:	
No			

6. If answered no to above, is a break crop used instead? If so, can you specify?

……
……..

7. What approximate plant spacings do you use when sowing coriander?

……
……..

8. What is your soil type?

……
……..

9. Which varieties of coriander do you grow?

……
……..

10. If you grow more than one variety of coriander, please provide information on any differences between the varieties e.g. time of year grown:
Please answer in box below

11. Is there any crop debris that remains after a harvest? If so, is it treated in anyway before the next crop?
Please answer in box below

12. Please provide information below of any fertiliser, irrigation and weed control measures:
    Please answer in box below

Section C: Coriander Yield Decline

13. Do you have a problem with coriander yield decline?
    Please tick:

Yes	
No	

If answered yes to above question, please now answer questions 14-21:

14. How severe is the decline on a scale of 1-5 (where 1 = fairly severe and 5 = extremely severe)
    Please tick:

1	
2	
3	
4	
5	

15. Have you made any observations of changes to plant roots; leaves yellowing; or any other potential signs of decline?
    Please answer in box below

16. Is there a particular time of year/ weather condition when coriander yield decline is worse?
    Please answer in box below

17. Is there a particular variety of coriander that is affected more severely? If so please provide details:

……
….

18. Do you grow coriander on new ground that has not experienced the crop previously?
Please tick:

Yes	
No	

19. If answered yes to above, how do yields from such fields compare with other coriander areas on your farm?
Please answer in box below

20. Have you observed any pattern of coriander yield in relation to previous cropping? If so, please provide details:
Please answer in box below

21. Have you observed any management practices that appear to alleviate coriander yield decline?

Please answer in box below

22. Please provide any additional comments, relating to any part of this questionnaire, which you consider important.
    Please answer in box below

***

Please check that you have completed all sections before 1/11/14 and are returning the questionnaire to:

Kate Fraser
School of Biology
Devonshire Building
Newcastle University
Newcastle-Upon-Tyne
NE1 7RU
United Kingdom
e-mail: k.l.fraser@ncl.ac.uk

Many thanks for your co-operation.

Figure 7. Blank questionnaire design distributed to a selection of coriander growers: Herb Fresh, Hortopalma S. L., Red Deer Herbs Ltd., Scot Herbs Ltd., and Zantra Ltd.

Appendix 2

Table 1. Raw data for fresh and dry plant weights during experiment to establish yield decline (all values measured in grams and are to 2d.p.)

Fresh Weights	Control Pots			Previously Planted Pots		
Tissue Type	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
Shoot	1.57	2.00	1.35	0.40	0.71	0.59
Leaves	1.05	1.40	1.04	0.41	0.53	0.46
Root	0.19	0.32	0.25	0.13	0.20	0.12

Dry Weights	Control Pots			Previously Planted Pots		
Tissue Type	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
Shoot	0.31	0.51	0.38	0.16	0.27	0.16
Leaves	0.25	0.26	0.22	0.11	0.17	0.10
Root	0.04	0.06	0.05	0.03	0.05	0.03

Table 2. Raw viable count data for determining if the coriander rhizosphere has increased microbial numbers compared to bulk soil (all values measured as CFU/g soil and are to 2d.p.)

Bacteria	Week 9 Sampling		Week 10 Sampling		
Soil Type	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 3
Control Bulk	1.2×10^6	8.02×10^5	8.16×10^5	6.42×10^5	2.10×10^6
Coriander Bulk	9.22×10^5	9.54×10^5	8.29×10^5	4.90×10^5	6.42×10^5
Coriander Rhizosphere	3.08×10^6	3.32×10^6	4.13×10^5	6.36×10^5	4.45×10^5

Fungi	Week 9 Sampling		Week 10 Sampling		
Soil Type	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 3
Control Bulk	1.43×10^4	1.42×10^4	9.45×10^3	6.95×10^3	6.33×10^3
Coriander Bulk	1.54×10^4	1.06×10^4	8.02×10^3	6.15×10^3	6.24×10^3
Coriander Rhizosphere	2.34×10^4	2.72×10^4	7.63×10^3	5.72×10^3	6.68×10^3

Table 3. Raw chlorophyll content data from experiment determining if the coriander rhizosphere has increased microbial numbers compared to bulk soil (all values measured as CCI Unit and are to 2.d.p.)

Week	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
5	11.62	11.56	12.56	12.56	13.70
6	21.98	26.36	21.48	19.36	25.94
7	27.50	31.74	15.88	33.78	19.16
8	23.26	28.42	20.74	18.36	20.44
9	21.56	18.78	18.72	21.26	20.54
10			13.26	14.70	10.48

Table 4. Raw viable count data for determining if yield decline inducing soils have increased microbial numbers compared to 'healthy' soils (all values measured as CFU/g soil and are to 2d.p.)

Bacteria	Control			Previously Planted		
Soil Type	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
Coriander Bulk	2.90×10^5	5.20×10^5	9.50×10^4	4.50×10^5	1.25×10^5	5.23×10^5
Coriander Rhizosphere	3.76×10^5	8.75×10^5	2.30×10^5	9.06×10^5	2.06×10^6	1.74×10^6

Fungi	Control			Previously Planted		
Soil Type	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
Coriander Bulk	/	8.70×10^3	1.23×10^4	1.02×10^4	1.05×10^4	9.23×10^4
Coriander Rhizosphere	3.53×10^4	3.32×10^4	5.47×10^4	5.38×10^4	2.61×10^4	8.92×10^4

