New Project Summary Report for FV 421: rhizobacterial inoculants for enhancing lettuce production

Project Number	31304210
Title	Field evaluation of rhizobacterial inoculants for enhancing lettuce production
Short Title	FV 421
Lead Contractor	Lancaster University
Other Contractors	N/A
Start & End Dates	31 July 2013 - 30 March 2014
Industry Representative	David Norman, Precision Agronomy Ltd
Project Budget	£26,071
AHDB Contribution	£26,071

The Problem

Project summary

Previous work (within HDC Studentship CP54) has shown that rhizobacteria that modify crop hormone status (Variovorax paradoxus 5C-2, Bacillus subtilis IR-15) can stimulate lettuce head weight of crops grown under both well-watered and drying soil conditions in both pot experiments and small-scale (6 m2 cropping area per treatment) polytunnel experiments. More recent work (2012 season) conducted on grower's holdings showed that V. paradoxus 3C-1 increased iceberg lettuce head weight by 20%. The current proposal aims to determine suitable propagation, storage and inoculation techniques for large-scale inoculum production using both commercially available (Bacillus subtilis QST713, marketed as Serenade by AgraQuest) and experimental inoculants (available at Lancaster University). Inoculants will be produced (or purchased) and after different durations of storage, will be supplied (by either a soil drench or foliar spraying) at different concentrations to lettuce seedlings in pot trials. Rhizobacterial colonisation of the root system and plant growth will be measured. Having determined the optimum concentrations and method of application, 2 different inocula will be added to transplants in the nursery and then seedlings planted out to the field in covered ("managed drought") and uncovered experiments. Plants will be grown at two different levels of soil water availability (by automatic irrigation according to different soil moisture thresholds) until harvest. Head weight, disease incidence and rhizobacterial colonisation of the root system will be determined at harvest.

Benefits to industry

Applying rhizobacteria antagonistic to fungal pathogens may allow the creation of a rhizosphere environment that prevents fungal development and creates a desirable rhizobacterial community to both promote shoot growth and limit rootborne disease.

EU legislation limiting the availability of chemical biocides has stimulated research into alternative biological control mechanisms (leading to the marketing of bio-fungicides such as Serenade). There is a continuing need to develop new products and applications to expand the range of disease control options available.

Background

Plant growth promoting rhizobacteria (PGPR) can enhance crop productivity and resource use efficiency via a range of mechanisms, including altering phytohormone status thereby increasing root growth and thus water and nutrient uptake (Dodd & Ruiz-Lozano 2012; Current Opinion in Biotechnology 23, 236-242). Lancaster University has worked with several PGPR that can either produce the growth-enhancing phytohormones cytokinins (Bacillus subtilis IR-15), or decrease root production of the growth inhibitory phytohormone ethylene (Variovorax paradoxus 5C-2 and 3C-1), which have promoted growth of lettuce plants in both pot trials and field experiments (Arkhipova et al. 2007 Plant & Soil 292, 305-315; Teijeiro et al. 2011 Acta Horticulturae 898, 245-252). Although such work has trialled a range of inoculation procedures including incorporating the inoculum within the substrate, and adding a small volume of highly concentrated inoculum to the seedling base, other commercially relevant issues such as inoculum longevity and ability to supply the inocula as a foliar spray have not been assessed.

Nevertheless, a number of PGPR have been developed as commercially available products (Berg 2009 Applied Microbiology and Biotechnology 84, 11-18), mainly for control of fungal diseases. Bacillus subtilis QST713 (Serenade) has been licenced for use (and marketed by Fargro UK Ltd) within the UK as a bio-fungicide to control foliar diseases (eg. Botrytis spp. on several crops including lettuce (http://www.fargro.co.uk/prodmanl/serenade%20tecnote-0209.pdf), and additional work has shown that application of Serenade can stimulate lettuce yield (Mr. David Norman, personal communication). Preliminary work with the experimental inoculants used by Lancaster University (within HDC Studentship CP54) has shown that V. paradoxus 5C-2 decreased fungal colonisation of the lettuce rhizosphere, and prior to the recognition that Bacillus subtilis IR-15 produced cytokinins, it was shown to be antagonistic to Fusarium species (Prof Guzel Kudoyarova, personal communication). However, the effect of these experimental inoculants on plant disease incidence and severity has yet to be assessed under commercial conditions.

Since lettuce is a relatively shallow-rooted crop, it is usually grown under non-limiting water availability, which may pre-dispose the crop to attack by opportunistic “damping-off” pathogens (Pythium, Phytopthora). Preliminary work (conducted within HDC Studentship CP54) has shown that automated irrigation (feedback regulated by commercially available soil moisture sensors) can control soil water availability to impose a mild stress (that decreased head yield by 23% three weeks after transplanting), but that plants inoculated with V. paradoxus 5C-2 showed no yield reduction under these conditions. Increasing concerns about water availability for horticulture (less of an issue within UK production, but critical for “out-of-season” production which typically moves to Mediterranean regions) suggests that technologies that can sustain productivity, despite limited soil water availability, will become essential for the industry. One of the difficulties with integrating environmentally-friendly bio-technologies (such as mycorrhizae and rhizobacteria) into commercial production systems is that they may be susceptible to plant protection products commonly used for disease control. While this issue has obviously been addressed for commercially-available inocula, there is relatively little information available for the experimental inocula. During current trials on lettuce production in the Murcia region that plan on using the copper-based product Sergomil for disease control, the copper sensitivity of V. paradoxus 3C-1 was determined in vitro and the likely Sergomil application rates shown to be below a threshold inhibiting rhizobacterial growth. Since testing each rhizobacterial inoculant for sensitivity to each biocide used in commercial production is logistically challenging, an alternative is to compare the levels of rhizobacterial colonisation of the root system in response to a typical commercial spraying schedule, and control plants where spraying is not implemented. While root colonisation of some rhizobacteria can be assessed using dilution plates using defined antibiotics (Belimov et al. 2009; New Phytologist 181, 413-423), there is a need to develop more specific molecular methods based on extracting DNA from the rhizosphere, to determine the success or failure of different inoculation techniques.

Aims and Objectives

Project aim

- To determine whether commercially available, and recently developed, rhizobacterial inocula can enhance lettuce productivity under “managed drought” and typical field production conditions

Project objectives

This research aims to:

- Determine suitable inoculation techniques for several different rhizobacterial inocula intended for field lettuce production

- Evaluate the impact of different inoculation techniques on both rhizobacterial colonisation of the rootzone, incidence of plant disease and crop productivity for two different rhizobacterial inoculants

- Determine whether rhizobacterial inocula can improve crop water use efficiency by allowing crops to be produced with less water in either the nursery production or field stages of growth

- Inform the horticultural industry of this research by producing an article for HDC News, a Final Report, and (where appropriate) relevant scientific publications

Approach

Methods

Molecular methods to assay root colonisation:

Since root colonisation by V. paradoxus 3C-1 (a strain we anticipate developing as a commercial inoculant) cannot be assessed on the basis of dilution plate tests, more specific molecular techniques will determine whether different inoculation techniques have allowed effective colonisation within different experiments. 16S RNA will be isolated from the strain and used to produce 16S RNA primers specific for 3C-1 (based on homology with the sequence of V. paradoxus S110 - available on NCBI - which will be used as a template - Han et al. 2001 Bacteriology 193, 1183-1190). DNA will be extracted using the heat shock method (Adbulamir et al. 2010 African Journal of Biotechnology 9, 1481-1492), prepared for PCR (Qiagen taq PCR core kit), purified and sequenced, allowing primers to be chosen as probes to assay V. paradoxus 3C-1 rhizosphere colonisation. Primers will be used to conduct PCR on rhizosphere samples extracted from plants to which V. paradoxus 3C-1 was applied.

Aim: Develop a robust method to determine rhizobacterial colonisation in the field

Pot trials:

High concentration liquid inocula (> 1010 cells mL-1) will be either purchased (Serenade from AgraQuest), or produced at Lancaster University (Variovorax paradoxus 5C-2 and 3C-1, Bacillus subtilis IR-15), and stored until required for experiments. Lettuce transplants will be sourced from commercial suppliers at the two-leaf stage, and supplied with different inocula concentrations as either soil drenches or foliar sprays. Plants will be grown under typical nursery conditions until the stage that they would normally be transplanted to the field. At this point, shoot fresh weight and rhizobacterial colonisation of the root system will be assessed. Inoculum longevity will be assessed by repeated experiments with the same inoculum source, conducted at various times since inoculum production.

Aim: Select appropriate inoculum and inoculation technique for field experiments

Field experiments:

Two (± rhizobacterial inoculation) x 2 (levels of soil water availability) x 2 (levels of biocide application) experiments will be conducted at Myerscough College under cover in a polytunnel (to prevent rainfall compromising the experimental design) and on grower's holdings with inoculated and uninoculated lettuce transplants. Two irrigation regimes will be applied: according to usual grower practice and feedback-control based on soil moisture sensors, since there is evidence that rhizobacterial inoculants can stimulate root growth allowing greater water capture from the soil. Since one of the potential benefits of rhizobacterial inoculation is control of fungal diseases, half the plants will be grown in the absence of chemical fungicide applications while the remainder will use a typical spraying schedule (as required). At harvest, shoot fresh weight and rhizobacterial colonisation of the root system will be assessed. Lettuce heads will be removed to cold storage to assess whether the treatments impact on shelf-life (assessed visually).

Aim: Determine impacts of inocula on crop productivity