Project title	Embedding Masters-level students within the horticultural industry
Project number:	CP97
Project leader:	Dr. Ian Dodd, Lancaster University
Report:	Final Report, October 2014
Previous report	Not Applicable
Key staff:	Ms. Hazel Fielding Ms. Hannah Wright Mr. Matthew Naish
Location(s) of project:	Lancaster Environment Centre Myerscough College Produce World, Lincolnshire Khon Kaen University (Thailand)
Project coordinator:	Dr. Ian Dodd, Lancaster University
Date project commenced:	01/10/2012
Date project completed (or expected completion date):	31/10/2014
Key words:	ACC deaminase, ethylene, soil moisture, rootstocks, training,

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.

Hazel Fielding
Masters Student
Lancaster University

Signature 

Date 31/10/2013

Hannah Wright
Masters Student
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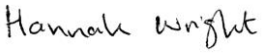
Signature 

Date 31/10/2013

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Report authorised by:

Dr Ian Dodd

Director of Study, Masters in Sustainable Agriculture & Food Security

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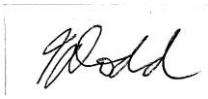
Signature .....  ..... Date..05/01/2016

CONTENTS

Grower Summary

- The plant growth promoting rhizobacteria (PGPR) *Variovorax paradoxus* 5C2 and 3C1 (that decreased plant ethylene production) successfully colonised the root systems of two species to which previously they had not been applied: calabrese (*Brassica oleracea* L.) and peanut *(Arachis hypogaea* L .)
- Adding PGPR to broccoli seedlings in the nursery increased early vegetative development, but no yield enhancement was detected when these seedlings were transplanted and grown to harvest in the field
- Although PGPR stimulated stomatal conductance of well-watered peanut plants, they decreased vegetative development during drought stress, but accelerated vegetative growth recovery following re-irrigation.
- Selecting new tomato rootstocks (from publically available resources) enhanced commercial yields under abiotic stress conditions compared to commercially available rootstocks

Background and expected deliverables

The UK higher education sector needs to train more high quality graduates in horticultural skills to fulfil the needs of the UK horticultural sector. Many UK companies currently search offshore for suitably qualified staff (eg. technical managers) to compensate for the lack of suitable UK graduates. In recognition of these concerns, and more global concerns about the world's ability to feed a burgeoning global population, Lancaster Environment Centre (LEC) has recently instituted a Masters degree (MSc) in Sustainable Agriculture & Food Security (MSAFS).

To encourage greater student engagement with the horticultural sector, this project aims to ensure that:

- academically able students apply for the MSAFS course administered by Lancaster University, thereby exposing them to the needs of the horticultural sector.
- these students engage with the horticultural industry by conducting all or part of their (4 month) dissertation projects within growers' holdings and/or horticultural research institutes

Summary of the project and main (training) conclusions

Three Masters bursaries (each worth £3000 off student tuition fees) were offered in academic year (AY) 2012/13; of which two were ultimately accepted. In addition to the 3

compulsory taught modules within this degree (Agriculture, Food Security & Climate Change; Crop Protection; Sustainable Soils Management), both students undertook 3 optional taught modules, as indicated in parentheses:

Ms. Hazel Fielding (Food Security Issues in China: International Summer School; Environmental Management; Data Analysis and Interpretation)

Ms. Hannah Wright (Food Security Issues in China: International Summer School; Environmental Management; Data Analysis and Interpretation)

Mr. Matthew Naish (Environmental Law; Biological effects of Air pollution and Climate change; Environmental Management)

Two of the students did dissertation projects that applied plant growth promoting rhizobacteria to crops to enhance plant growth and stress tolerance, while another looked at the impacts of tomato rootstocks, as indicated in parentheses:

Ms. Hazel Fielding *(Physiological effects of ACCd producing rhizobacteria on calabrese (Brassica oleracea))*

Ms. Hannah Wright (*Rhizobacterial colonisation of, and physiological effects on, peanut (Arachis hypogaea L.) under water-limited conditions*)

Mr. Matthew Naish (*Empirical modeling of hormone flows in reciprocally grafted tomato*)

Ms. Wright was also in receipt of Lancaster University Alumni & Development Office "Food Security" funding, allowing her to undertake her research project overseas. Since both projects involved field trials conducted between April and August, training in routine microbiological procedures, experimental design and plant culture was provided prior starting their dissertation projects. Brief progress updates were provided to HDC in April 2013 (Appendices 1, 2). The students successfully submitted their dissertations in September 2013 (Appendices 3, 4) and September 2014 (Appendix 5) respectively. Both the 2013 graduates attended the 4th International Conference on Positive Plant Microbe Interactions, for which conference proceedings papers (available on request) were prepared (Appendix 6). Data from Mr Naish's dissertation was presented at the annual Society of Experimental Biology meeting (Prague 2015).

The onward destinations of the students are as follows:

Ms. Hazel Fielding commenced a permanent position as Soils and Farming Systems Technician at the National Institute of Agricultural Botany (NIAB) in June 2014.

Ms. Hannah Wright commenced a PhD studentship at Lancaster University in October 2013 (funded by BBSRC's Doctoral Training Programme in Food Security) entitled *Benefits of controlled soil drying on crop yields: disentangling nutritional and phytohormonal effects.*

Mr. Matthew Naish commenced a PhD studentship at University of Warwick in October 2014 (funded by BBSRC's Doctoral Training Programme in Food Security) entitled *Epigenetic reprogramming during somatic cell regeneration*

All students have engaged more generally with the issue of food security by acting as ambassadors for the MSAFS course at University recruitment days; in the case of the 2013 students, presenting posters at the Waitrose Innovation Forum (February 2013); and with Mr. Matthew Naish presenting a poster at the Waitrose Science Day (February 2014).

Appendix 1: Training Update: Ms Hazel Fielding

HDC Masters Bursary Recipient:
Ms. Hazel Fielding
Project: *Accelerating the cropping cycle of Brassica*
Training and Skills Development Relevant to the HDC
February 2013

Research Initiative
A key objective of the Brassica Growers Association Research and Development Strategy (2011-2013), is to, 'Increase returns on investment through the efficient use of resources' with a 'continuity of supply' of produce. The proposed research project aims to increase profitability for the farmer by maximising on-farm efficiency and sustainability.

Background and Rationale
A novel method for increasing crop production whilst maximizing water (and possibly nutrient) use efficiency has been discovered by applying naturally occurring rhizospheric bacteria to crops. One of the key mechanisms involved in the plant-growth promoting properties of some rhizobacteria (PGPR), is their production of the enzyme ACC deaminase (ACCd); which degrades the precursor of the plant growth inhibitory phytohormone ethylene (Glick, et al., 2007). Lancaster University's research with the ACCd-containing PGPR *V. paradoxus* 5C-2 has demonstrated increased plant growth and yield of peas and potatoes under well watered and water stressed conditions (Belimov, et al., 2009a, b). More recent work with the model plant *Arabidopsis thaliana* (from the Brassicaceae family) shows that *V. paradoxus* 5C-2 stimulated flowering (Chen et al. in press), suggesting opportunities for rhizobacterial inoculation to stimulate growth, development and flowering of Brassica crops.
Preliminary trials were performed by the Bursary Recipient to develop essential laboratory techniques in preparation for a research project to be undertaken over summer. The research aimed to determine whether the bacteria would increase root growth and whether it could be successfully recovered from the rhizosphere post inoculation. Further experiments investigated whether direct seeding or transplanting affected the bacterial colonisation of the roots.

Results
Throughout October and November, various trials were set up to study the effects of different rhizobacteria on the root growth of two lettuce cultivars (All Year Round and Tom Thumb) and one pea cultivar *(Pisum sativum* cv. Progress #9). On this occasion, contrary to previous similar studies, there was no significant difference in root growth between the control and *V. paradoxus* 5C-2 inoculated seeds of either lettuce cultivar. However replications of this trial were limited.

From November to December investigation into the effects of *V. paradoxus* 5C-2 on plant physiology, in autoclaved vs. non autoclaved soil, and through direct seeding or transplanting was executed. The key findings are presented below:

- Colonisation of the roots by *V. paradoxus* 5C-2 was successful in the inoculated plants of all treatments.
- No significant difference in number of CFU (colony forming units per ml) retrieved from transplanting or direct seeding, thus in further experimentation the easier and faster method of direct seeding will be used.
- No significant difference in the number of CFU retrieved from autoclaved or non-autoclaved soil. This is important to note if application were to be extended to the field for commercial use.
- No significant difference in leaf area or shoot fresh weight between the control and inoculated plants for direct seeded or transplanted treatments.
- Variance in leaf area and shoot fresh weight was reduced upon *V. paradoxus* 5C-2 inoculation in all treatments.

Future research

The goal of this line of research is for it to be put to commercial use to increase crop production. As research has been carried out studying the effects of *V. paradoxus* 5C-2 on maize, wheat and pea, investigation into the effects of this PGPR on Brassica species seems necessary. Thus a project to investigate the effects of V. paradoxus on Brassicas (specifically broccoli), to potentially accelerate the cropping cycle as means of providing security to a crop will be undertaken. Bacterial inoculation is hypothesised to decrease the risk of a failed crop by reducing the dependence on rainfall and irrigation, and mitigate the consequential production of ethylene in response to drought stress as this hormone can delay crop growth. This will pertain to a consistently high quality crop all year round.

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Appendix 2: Training Update: Ms Hannah Wright

. **HDC Masters Bursary Recipient: Ms. Hannah Wright**
Project: *Microbial inoculants for legumes grown under water-limited conditions*
Training and Skills Development Relevant to the HDC
February 2013

Research initiative(s) addressed from HDC Strategies
Increasing returns on investment through efficient use of resources is the major objective of the Field Vegetable Research and Development Strategy for Legumes addressed through this research (HDC, 2012). Identifying novel practices to overcome limitations to resource use efficiency and yields faced by several crop sectors, and also to specific problems on individual crops, are principle objectives for horticultural research in general (HDC, 2013).

Rationale and Background
Plant growth promoting rhizobacteria (PGPR) can promote growth and increase yields of a wide range of crops, by manipulating physiological responses to environmental conditions via a diversity of mechanisms. Increased shoot growth of pea inoculated with rhizobacteria that utilise ACC, the immediate biochemical precursor of the growth inhibitor ethylene, has been reported (Arshad et al., 2008; Belimov et al., 2009). Applying PGPR as inoculants has been suggested as a low-cost, low-technology strategy to optimise the use of available water and nutrient resources. However, further trials are required to develop effective commercially available inoculants for specific cropping systems.

Investigations were carried out to develop skills in preparing and applying rhizobacterial inoculants and assaying effects on growth. A pot trial using pea (*Pisum sativum* cv. Progress #9) aimed to test the hypotheses that: 1)*Variovorax paradoxus* 5C-2 colonies could be recovered from the rhizosphere 23 days after inoculation; 2) shoot growth would be promoted in inoculated compared to control plants; 3) colonisation and shoot biomass would differ according to whether seeds were directly sown or transplanted following germination; 4) colony recovery and higher shoot biomass production would depend on the provision of a sterilised (autoclaved) soil environment.

Results and Implications
1) ***V. paradoxus* colonies were successfully recovered** from the rhizosphere of the PGPR treatments.
2) There was **no difference between direct seeded and transplanted treatments** in rhizobacterial colonization of the root system (colony forming units per gram fresh root) *(F*(1, 16)= 3.135; *p*= 0.096), signifying that direct

seeding would be an appropriate method in further trials. This is consistent with the objective to increase the efficiency of labour and resource management within production systems (HDC, 2012).

3) The interactive effect between the two factors was not significant ($F(1, 16)= 1.795$; $p= 0.199$), justifying further analysis of rhizobacterial colonization of the root system for only the direct seeded treatments. There was **no difference between autoclaved and non-autoclaved soil** ($t(8)= 1.354$; *S. E.*= 0.217; $p= 0.213$), signifying that survival did not depend on soil sterilisation. Thus further inoculant trials in field environments (where soil sterilisation is impractical) may be successful.
4) For direct seeded and for transplanted treatments, shoot fresh weight or leaf area between control and inoculated plants did not differ. However, the **coefficient of variance was lower in the inoculated treatments** compared to the controls for shoot fresh weight (control: 26.5; inoculated: 19.6) and for leaf area (control: 26.4; inoculated: 14.5), indicating that rhizobacterial inoculation increased crop uniformity. Thus inoculation with PGPR may increase the uniformity and resilience of production systems and therefore contribute to achieving the objectives within the overarching HDC strategy (2013[b]).

Future directions

Skills developed and knowledge gained will contribute to the development of additional pot and field trials. Preliminary findings, supported by previous studies reported in the literature, have indicated further opportunities to investigate whether applying inoculants containing ACC-utilising PGPR promote growth for *different legume crops and genotypes in water-limited conditions.* To more fully understand the local and systemic effects of *V. paradoxus* on plant growth, root and productivity traits also need to be measured and the effects assayed over the duration of the crop cycle. To ensure increased returns from field-scale production systems, evaluation of yield, quality and resource use efficiency is essential.

References:

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Appendix 3:

Physiological Effects of ACC deaminase Producing Rhizobacteria on *Brassica oleracea*

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Submitted September 2013

This dissertation is submitted in partial fulfillment of the degree of MSc Sustainable Agriculture & Food Security

ENTIRE DOCUMENT AVILABLE AS A SEPARATE PDF (OR WORD) FILE DUE TO FORMATING ISSUES ASSOCIATED WITH INCORPORATING INTO HDC REPORT TEMPLATE

Appendix 4:

Rhizobacterial colonization of, and physiological effects of, peanut (*Arachis hypogaea* L.) under water-limited conditions

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Submitted September 2013

This dissertation is submitted in partial fulfillment of the degree of MSc Sustainable Agriculture & Food Security

ENTIRE DOCUMENT AVILABLE AS A SEPARATE PDF (OR WORD) FILE DUE TO FORMATING ISSUES ASSOCIATED WITH INCORPORATING INTO HDC REPORT TEMPLATE

Appendix 5:

Empirical Modeling of Hormone Flows in Reciprocally Grafted Tomato Plants

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This dissertation is submitted in partial fulfillment of the degree of MSc Sustainable Agriculture & Food Security

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Appendix 6: Publications and Poster Presentations Arising

Publications:

HR Wright, W Noypang, N Vorasoot, S Jogloy, IC Dodd (2013) Rhizobacterial colonisation of, and physiological effects on, peanut (*Arachis hypogaea* L.) under water-limited conditions. *Aspects of Applied Biology* 120, 95-100.

HA Fielding, IC Dodd (2013) Physiological effects of ACC deaminase producing rhizobacteria on broccoli *(Brassica oleracea). Aspects of Applied Biology* 120, 109-114.

IC Dodd, AA Belimov, ED Elphinstone, L Chen, RG Teijeiro, C Kemp, **HA Fielding, HR Wright** (2013) Exploiting rhizobacteria that mediate plant hormone status. *Aspects of Applied Biology* 120, 29-34.

Poster Presentations:

4th International Conference on Positive Plant Microbe Interactions (Lincolnshire; 2-3 December 2013)

Physiological effects of ACC deaminase producing rhizobacteria on broccoli *(Brassica oleracea)*

By H A FIELDING and I C DODD

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Summary

Plant growth promoting rhizobacteria can stimulate plant growth by multiple mechanisms, including the production or metabolism of plant hormones (such as abscisic acid, ABA and ACC, the ethylene precursor). Since ACC deaminase (ACCd)-containing rhizobacteria increased early vegetative growth of multiple crop species (including pea, potato and maize), and farmers are interested in shortening crop cycle durations, the effects of the ACCd-containing rhizobacteria *V. paradoxus* 5C-2 and 3C-1 on broccoli (*Brassica oleracea*) growth were investigated under well watered and soil drying conditions in both pot and field trials. Drought and rhizobacterial impacts varied between trials. Treatment with *V. paradoxus* 5C-2 seven days after planting increased seedling shoot fresh weight (SFW) and leaf area (LA) by 50% and 43% respectively when compared to the control. Conversely, when treated at seeding, SFW and LA were significantly decreased by 20% and 16% respectively. As expected, drought significantly reduced shoot dry weight of *Brassica oleracea* plants grown in pots in the greenhouse, but contrary to previous research, there was no effect of 5C-2 treatment during the drought period**.** Drought significantly increased leaf ethylene and ABA levels, but 5C-2 apparently diminished accumulation of both of these phytohormones. Field trials under commercial conditions indicated no effect of drought or rhizobacterial treatment with *V. paradoxus* 3C-1 on head weight. Thus rhizobacterial stimulation of early vegetative growth did not increase marketable yield.

Key words: Abscisic acid, ACC-utilising PGPR, drought, ethylene, *Variovorax paradoxus* 5C-2 and 3C-1

4th International Conference on Positive Plant Microbe Interactions (Lincolnshire; 2-3 December 2013)

Rhizobacterial colonisation of, and physiological effects on, peanut (*Arachis hypogaea* L.) under water-limited conditions.

Hannah R. Wright[1], Worawut Noypang[2], Nimitr Vorasoot[2], Sanun Jogloy[2], Ian C. Dodd[1]

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Summary

Previously, the plant growth promoting rhizobacterium (PGPR) *Variovorax paradoxus* 5C-2 (5C-2) increased growth and yields of a temperate legume (pea) under water-limited conditions (Belimov et al., 2009). To determine whether 5C-2 could promote legume (peanut) growth in a tropical soil, four genotypes were inoculated at Khon Kaen Field Crops Research Centre and grown along with uninoculated controls. Mid-season drought (MSD) was imposed by withholding water between 30 and 60 days after planting (DAP) followed by re-watering; irrigated controls were maintained at field capacity (FC). 5C-2: colonised roots of each genotype and proliferated for at least 37 days; promoted stomatal conductance of three genotypes before MSD; had genotype-specific effects on leaf relative water content (RWC) during MSD; and decreased leaf extension rate (LER) during MSD but promoted it following re-irrigation. These effects suggest that rhizobacterial inoculation promoted more opportunistic water use when water was available, but more conservative water use otherwise.

Keywords: Mid-season drought, peanut, ACC-utilising PGPR, leaf water relations, growth.

Society of Experimental Biology Annual Meeting
(Prague, Czech Republic, 30 June-3 July 2015)

Root to shoot signalling of single and multiple abiotic stresses

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Although the paradigm of root-to-shoot signalling is established in the literature, it is often assessed simply by measuring xylem sap composition, without discriminating the source (root or shoot) of any changes. To distinguish these possibilities, grafting surgically attaches genetically distinct shoot (scion) and root (rootstock) genotypes, thereby evaluating the role of the root system. When wild-type (WT) tomatoes were self- and reciprocally grafted with the ABA-deficient *flacca* genotype, root xylem ABA concentration was solely rootstock-mediated, independently of whether xylem sap was collected by spontaneous root exudation or via root pressurization to ensure sap flowed at transpirational flow rates. In contrast, collecting xylem sap from intact leaves by pressurizing the root system revealed that xylem ABA concentrations of reciprocally grafted WT and *flacca* plants was intermediate between leaf xylem ABA concentration of self-grafts, implying modification of the ABA signal in transit from roots to shoots. When WT scions were grafted onto WT or the ABA-overproducing *sp12* rootstock, abiotic stress magnified rootstock impacts on leaf xylem ABA concentration. However, combined stress effects were non-additive, with combined salt (75 mM NaCl) and drought (50% of control irrigation) having the same effect as drought alone. Although stomatal conductance and leaf water potential were rootstock-independent, the *sp12* rootstock inhibited leaf area. Similar experiments are under way in the EU ROOTOPOWER project (Grant # 289365), to identify the role of other root-sourced phytohormones in regulating physiological responses to single and combined abiotic stresses, in the hope of identifying rootstocks with improved tolerance to these stresses.