New Project Summary Report for CP 105: Integrated protection of horticultural crops through enhancing endogenous defence mechanisms

Project Number	31101050
Title	Integrated protection of horticultural crops through enhancing endogenous defence mechanisms
Short Title	CP 105
Lead Contractor	Mylnefield Research Services (MRS)
Other Contractors	N/A
Start & End Dates	30 September 2013 - 29 September 2016
Industry Representative	Neal Ward, Cantelo Nurseries Ltd
Project Budget	£67,650
AHDB Contribution	£67,650

The Problem

Background:

Many of our crop protectants either become ineffective as pathogens develop resistance, products are removed from the market for regulatory reasons, new pathogens emerge, or we need to grow varieties that are susceptible for market reasons. New active ingredients in crop protection products permitted for horticultural crops are all too rare and late, and for bacterial and viral pathogens there are few options available anyway. However, the plants themselves have highly effective resistance mechanisms that if primed and expressed in a focussed, specifically-targeted way, could not only lead to better crop protection, but also substantially reduce the need for toxic crop protectant interventions. This can be done with resistance 'elicitors' (Walters, Newton & Lyon, 2007).

Pathogens are not the only organisms on and in crops and several horticultural crops have been at the centre of food safety issues in recent years leading to a new understanding of the role of plants in general in the colonisation, propagation and distribution of microorganisms and bacteria in particular. The plant variety, its environment and all agronomic treatments have an impact on its microbial community, but those aspects of the environment that are specifically meant to target microbes will obviously have the

greatest impact, so crop protectants including the resistance elicitors referenced above will influence food safety in horticultural crops. This aspect to the best of our knowledge has hitherto not been considered and offers great potential.

Resistance elicitors 'prime' plant defence mechanisms and enable the plants to respond to actual pathogen threats faster. The underlying mechanism is mainly based upon a more effective induction and expression of defence mechanisms. However, being mediated through the plant's complex metabolic pathways where many feedback and trade-off mechanisms operate, the result of resistance priming and induction can potentially affect non-disease resistance mechanisms too. These may result in either positive or negative effects on yield quantity, quality and its components. To develop resistance induction crop protection approaches, a detailed knowledge of the timing and amplitude of defence induction as well as the consequences on target and non-target end-products is required. The molecular tools for such studies and our understanding of the mechanisms in model and crop systems have advanced considerably in recent years. Our previous data has shown that elicitors present in yeast cell-wall extracts were able to substantially reduce symptomatic disease caused by Botrytis cinerea on lettuce plants (Reglinski et al., 1993 (HDC PV/FV135); 1995).

In the PhD project outlined, we will use resistance elicitors thought to induce specific pathways and characterise their activity for phenotypic disease control levels as well as on a molecular level. The work will focus on an important plant pathogen with a wide host range, B. cinerea, which is a major cause of yield loss as well as post-harvest spoilage The project will also include a human pathogen because there is growing evidence showing that some human pathogens are able to adapt to plant hosts and can colonise them, posing a public health risk for the produce industry. The molecular study will encompass microarray-based gene expression studies following recognition of known pathogen molecules (PAMPs and effectors) by cognate plant receptors to identify marker genes. Quantitative RT-PCR assays will be developed for informative marker genes identified from the microarray study to measure the amplitude and response time of plants following treatments with the elicitors and pathogens. Furthermore, we will determine both the phenotypic and molecular profiles of defence activator combinations that should prove synergystic. We will further examine how nutrients and key adjuvants or co-formulants affect these individual and synergystic effects. This will give crucial information about how key signalling pathways interact in various crops and the mechanisms of trade-offs associated with disease reduction. The research has relevance to a number of different sectors because the nature of the research is to investigate common mechanisms of defence, rather than for example, focus on fungicides that are limited to a single group of crops. Therefore, the work can be seen as under-pinning crop protection mechanisms.

Aims and Objectives

Project aim: The project aims to (i) establish a robust and reproducible beneficial effect with an elicitor regime on a 'model' plant pathogen system; (ii) to investigate the molecular basis to the plant defence response elicited by the treatment regime and (iii) to test whether this same response is triggered in different plant species. The project will focus on a single plant-pathogen system: Botrytis cinerea on tomato plantlets, testing a range of treatment types and regimes. Once effective treatment components and combinations has been established and the response characterised, the treatments will then be tested on other plant species that are also infected by B. cinerea to determine whether there are commonalities in the mode of elicitor action. We will also determine what the effects are on non-target organisms, and here we will focus on bacteria with the potential to a pose food safety risk, (non-typhoidal) Salmonella enterica. Our pilot studies indicate that some elicitors can reduce the presence of human pathogens on plants. We have also found that S. enterica can colonise tomato plants to very high levels and that some serovars appear to have the ability to modulate the plant defence response (unpublished).

The project aims to provide detailed information on the mode of action of elicitors. This is an important aspect of alternative treatment strategies and will ultimately assist growers in providing targeted crop protection strategies. Since the response in a range of plants will be tested, the project will encompass three sectors within the HDC: Protected Edibles, Field Vegetables, and Soft Fruit. The elicitors used and their formulation will be informed by other projects with commercial and research partners as appropriate to access the latest technological and commercial developments in the field.

Key questions to be addressed:

1) Effectiveness of novel elicitors products (some may be provided by the commercial company):

a. do any novel products provide protection in the B. cinerea-tomato pathogen system?

b. if protection is provided by the novel products, (i) is protection conferred to the same or greater level than previously tested products; (ii) how long does protection last; (iii) does protection extend into subsequent generations of plants?

c. what effect does the addition of elicitors that are effective in crop protection have on crop development and yield?

d. what are the effects of elicitors on non-target organisms on the plants, particularly bacteria?

2) Molecular basis to the response:

a. which plant genes are triggered by the elicitors?

b. does the plant gene expression profile change in the presence of the pathogen?

3) Breadth of the response

a. does the beneficial effect of the elicitor regime extend to other susceptible hosts of B. cinerea, such as lettuce, strawberry, cauliflower.

b. for any other plant hosts that can be induced to elicit a response against B. cinerea, is the same

molecular pathway triggered?

c. is the molecular basis to the elicitor-induced plant defence response the same for tomato plants colonised by human pathogens (S. enterica serovar Senftenberg) as it is for the plant pathogen B. cinerea?

Approach

Project Methodology:

To test induced resistance in living plants, plants will be propagated under conditions that are most appropriate for the plant-pathogen systems. To address Key Question 1), variations in the dose, timing of elicitor application and formulation (e.g. presence / absence of adjuvant) will be trialled. For Key Question 2) the most effective elicitor regime will be used. Genomic sequence information is available for both the plant host (tomato) and pathogen (B. cinerea). Already established tomato DNA microarrays will be utilised to interrogate expression of plant genes during different stages of infection and treatment. For additional plant species for which there is only a limited amount of genetic information (addressed in Key Question 3), individual genes that are key markers of defence response will be examined using quantitative reverse-transcription PCR.