Grower Summary

CP 105

Integrated protection of horticultural crops through enhancing endogenous defence mechanisms

Annual 2015

Disclaimer

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.

Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

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Project Number:	**CP 105**
Project Title:	**Integrated protection of horticultural crops through enhancing endogenous defence mechanisms**
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GROWER SUMMARY

Headline

Experiments have revealed that chitosan, the natural polysaccharide, and methyl-jasmonate (MeJA), the volatile organic compound and plant hormone derivative, can successfully induce resistance, having a positive effect at molecular level, in tomato, against the fungal pathogen *Botrytis cinerea*.

Background

Plants, especially crops, are threatened by numerous microbes; many of them can be actual pathogens causing devastating losses worldwide. Many of our crop protectants become ineffective as pathogens develop resistance due to extensive use or new pathogens emerge. Products are lost from the market also for regulatory reasons, and the market sometimes requires varieties to be grown that are susceptible. However, even susceptible plants have highly effective resistance mechanisms that, if triggered and expressed in a focussed, specifically-targeted way, could not only lead to better crop protection, but also substantially reduce the need for conventional pesticide use. This can be done with resistance 'elicitors' (RE). Resistance elicitors can mimic pathogen-induced defence mechanisms in the plant and thus trigger its defence mechanisms, enabling the plant to respond to actual pathogen threats faster, without damaging other species or the environment and with reduced operator hazard. Furthermore, pathogens don't develop resistance as elicitors target plant defences rather than killing pathogen directly, a common issue with other toxic crop protectants such as fungicides. Fungal resistance to benzimidazoles in the 1970's, due to extensive use of some newer fungicides such as dicarboximides, has subsequently led to the appearance of resistant *B. cinerea* strains (Pappas, 1997).

Pesticides are also limited by European regulations due to human health and environmental issues. The recent European Directives "Plant Protection Products Regulation" 1107/2009 and the "Sustainable Use Directive" 2009/128/EC are the latest in a series of legislative changes that aim to reduce pesticide use in Europe. Whilst elicitors can be regarded as pesticides as they result in pathogen control, their mode of action is mainly based upon a more effective plant defence induction and expression of defence mechanisms rather than direct toxicity against pathogens. However, elicitors can have costs in plant development due to the plant's complex metabolic pathways as defence induction can potentially have effects on yield quantity and quality. 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 pathways 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. We will use these approaches to determine both the phenotypic and molecular profiles of resistance elicitors. 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 project will focus on a single plant-pathogen system: *Botrytis cinerea* on tomato plants, testing a range of treatment types and regimes. Once effective treatment components and combinations have 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.

In particular, the project aims to (i) Identify novel resistance elicitors that result in effective induced resistance in *Solanum lycopersicum* (tomato) against *B. cinerea* ; (ii) to test the cost of induced resistance in plant development; (iii) to investigate the molecular basis to the plant defence response elicited by the treatment regime and to characterize the molecular response of the induced plant to the pathogen infection; and (iv) to evaluate the role and expression of the defence hormonal pathways in the plant in response to the pathogen attack and to investigate the role of the elicitors in the phytohormone cross-talk.

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. Besides, this research will establish the principles and potential for using resistance elicitors in robust integrated crop protection strategies.

Summary

Below, summaries and main findings are discussed within the framework of the project's four main objectives:

1. Identify novel resistance elicitors that result in effective induced resistance in *Solanum lycopersicum* (tomato) against *B. cinerea*

Chitosan and Methyl-Jasmonate-induced resistance in tomato cv. Money-maker against Botrytis cinerea

As seen in last year's report, the plant hormone methyl jasmonate (MeJA) and chitosan, the novel natural elicitor, are able to reduce disease in tomato (cv. Money-maker and Motelle) caused by the aggressive pathogen Botrytis (*Botrytis cinerea*) (Figure 8A).

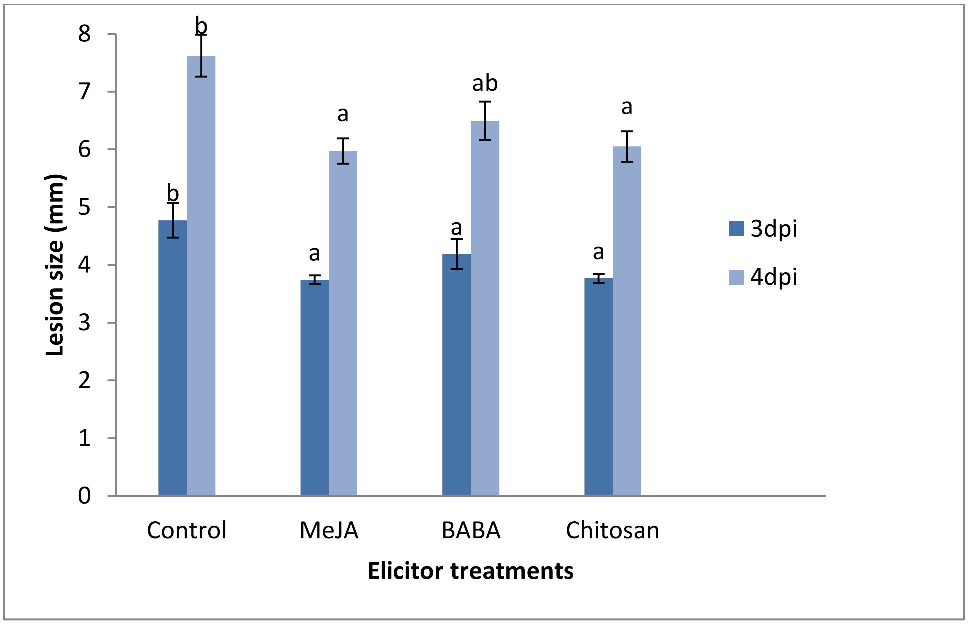

Figure 8A. Quantification of MeJA, BABA and Chitosan-induced resistance against *Botrytis cinerea* at 3 and 4 days post-infection in tomato cv. Money-Maker. Values presented are means ± SEM. Different letters indicate statistically significant differences.

Results also showed that beneficial bacteria *Bacillus subtilis* and *Bacillus amyloliquefaciens* GB03 strain can act as biocontrol agents and induce resistance against *B. cinerea* through significantly reducing necrotic lesion expansion at a late stage of the infection (72 hpi) (Figure 8).

2. Evaluate the cost of induced resistance in plant development

Elicitor-induced growth reduction in tomato cv. Money-Maker and Motelle

MeJA and the commercial chitosan formulation (ChitoPlant) were then chosen as the main significant elicitors to provide resistance in tomato against *B. cinerea* with no fitness costs in plant development. However, the synthetic elicitor BABA showed a strong growth repression in both tomato varieties (see 1st year annual report).

3. Investigate the molecular basis to the plant defence response elicited by the treatment regime and characterize the molecular response of the induced plant to the pathogen infection

***Early acting pathogen-inducible defence responses in tomato* - B.cinerea**

Plant's early defences, such as callose, the plant polysaccharide, and reactive oxygen species (i.e. H2O2), can play a crucial role in reducing pathogen penetration and giving the plant "more time” to display its late acting and fine-tuned defences, such as hormone pathways and chromatin/DNA modifications.

MeJA and Chitoplant (the chosen successful chitosan formulation) are able to significantly reduce botrytis necrotic lesion expansion in tomato and the model plant *Arabidopsis thaliana* (Figure 4). ChitoPlant defence induction is characterised by plant cell-wall fortification through callose deposition in tomato leaves before and after pathogen challenge (Figures 5 and 6).

Furthermore, both natural compounds can reduce and limit pathogen infection through localizing H_2O_2 production to the infection site (Figure 2), potentially reducing pathogen manipulation of its host defences (still to be further investigated).

4. Evaluate the role and expression of the defence hormonal pathways in the plant in response to the pathogen attack and to investigate the role of the elicitors in the phytohormone cross-talk

Jasmonic acid-dependent priming of gene expression in tomato -B.cinerea interaction

As expected, MeJA was able to trigger one of the main tomato defence pathways, the jasmonic acid-signalling pathway, through expression of key defence genes. Tomato leucine aminopeptidase (LapA) gene and tomato lipoxygenase D (LoxD) gene were triggered enabling the plants to respond to the pathogen infection faster (Figures 10 and 11). Both genes are involved in the jasmonic acid biosynthesis pathway, which is crucial for plant defence against necrotrophic pathogens as well as defence against herbivores.

In summary, it has been demonstrated that both chitosan and methyl-jasmonate play a crucial role in tomato defences against B. cinerea. Both elicitors act through “activating” the jasmonic acid-signalling pathway and through enhancing plant’s early acting defences, such as cell-wall reinforcement (callose deposition) and H_2O_2 production surrounding the infection site. The chitosan findings may be extrapolated to the model plant Arabidopsis thaliana, which suggest a promising commonalty in chitosan mode of action between plant families. However, their efficacy may vary depending on the pathogen strain, crop cultivar and infection time point.

Financial Benefits

Outcomes of this project will be in the form of knowledge that enables product replacement with more benign alternatives, and principles for their use. We see this as maintaining profitability by providing the tools to continue to achieve effective crop protection that might otherwise be compromised by loss of crop protection products or their reduced efficacy. Any specific knowledge that identifies either improved crop protection over conventional approaches or results in increased marketable or quality crop will be calculated in terms of financial benefit on a case-study basis as appropriate.

Action Points

There are few resistance elicitors currently licenced for use on horticultural crops and experimentation to determine which of these are effective for particular crops is being carried out in another project. This PhD project will help determine the principles whereby such products can be used, and particularly how they might be combined effectively. The latter will be as much about avoiding detrimental combination and practices as identifying those that might be additive or synergistic.