Grower Summary

CP105

Integrated protection of horticultural crops through enhancing endogenous defence mechanisms

Final Year Report 2017

Project title:	Integrated protection of horticultural crops through enhancing endogenous defence mechanisms
Project number:	CP105
Project leader:	Professor Adrian C Newton, The James Hutton Institute
Report:	Final Year Report, March 2017
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Location of project:	The James Hutton Institute (JHI), Invergowrie, Dundee, DD2 5DA
Industry Representative:	Neal Ward, Cantelo Nurseries Ltd (Left post 2016)
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The results and conclusions in this report are based on an investigation conducted over a three-year period but comprise separate individual experiments. 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.

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GROWER SUMMARY

Headline

Resistance elicitors are compounds that can protect commercial crop varieties (tomato cv. Money-maker and aubergine cv. Black Beauty) against fungal pathogens such as *Botrytis cinerea* (gray mold disease) by foliar spraying the crop.

Resistance elicitors could offer the potential to reduce fungicide use by including resistance elicitors, such as the natural polysaccharide chitosan and/or the phytohormone methyl-jasmonate, into crop protection strategies. Chitosan in particular can prime tomatoes for a more efficient and fine-tuned defence response against fungal pathogen *Botrytis cinerea*.

Background

Conventional crop protectants (pesticides) can lose their efficacy due to selection pressure for pathogen resistance caused by their widespread use. To date, there is a lack of genetic resistance in commercial crop varieties against fungal aggressive pathogens such as *Botrytis cinerea*, *Alternaria Solani* and *Sclerotinia sclerotium*. The aggressive fungal pathogen *Botrytis cinerea* infects almost all vegetable and fruit crops (>1400 plant species), including most of the crops of the *Solanaceae* family, trees and ornamentals. Botrytis kills the plant by inducing necrosis with degradation enzymes and manipulating its host defences. 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 benzimidazoles-resistant *Botrytis cinerea* strains. Pesticide availability and use is 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. Besides the above challenges to crop protection, market/consumer requirements sometimes require crop varieties to be grown that are susceptible. However, even susceptible plants have inducible defence mechanisms that, if triggered in a focussed, specifically-targeted way, can prevent disease and reduce the need for conventional fungicide use.

Resistance elicitors are compounds that can help plants to defend themselves. Elicitors can stimulate pathogen-induced defence mechanisms in the plant resulting in a broad-spectrum and more efficient resistance (Induced Resistance-priming) against pathogens such as *Botrytis cinerea.* Their effective application requires understanding of their associated defence responses (gene expression) and mode of action in the plant and the agronomy of the crop. Priming is based on a fine-tuned and enhanced resistance to biotic/abiotic stress that results in a faster and stronger expression of resistance after pathogen attack.

The aim of this PhD project was to determine the mode of action of specific resistance elicitors, to characterise their molecular function and to investigate their role in priming crops against *Botrytis cinerea.* Ultimately this project set out to identify novel alternatives in crop protection to reduce fungicide usage in the horticultural sector. This project used the *Botrytis cinerea* – tomato pathosystem as a model.

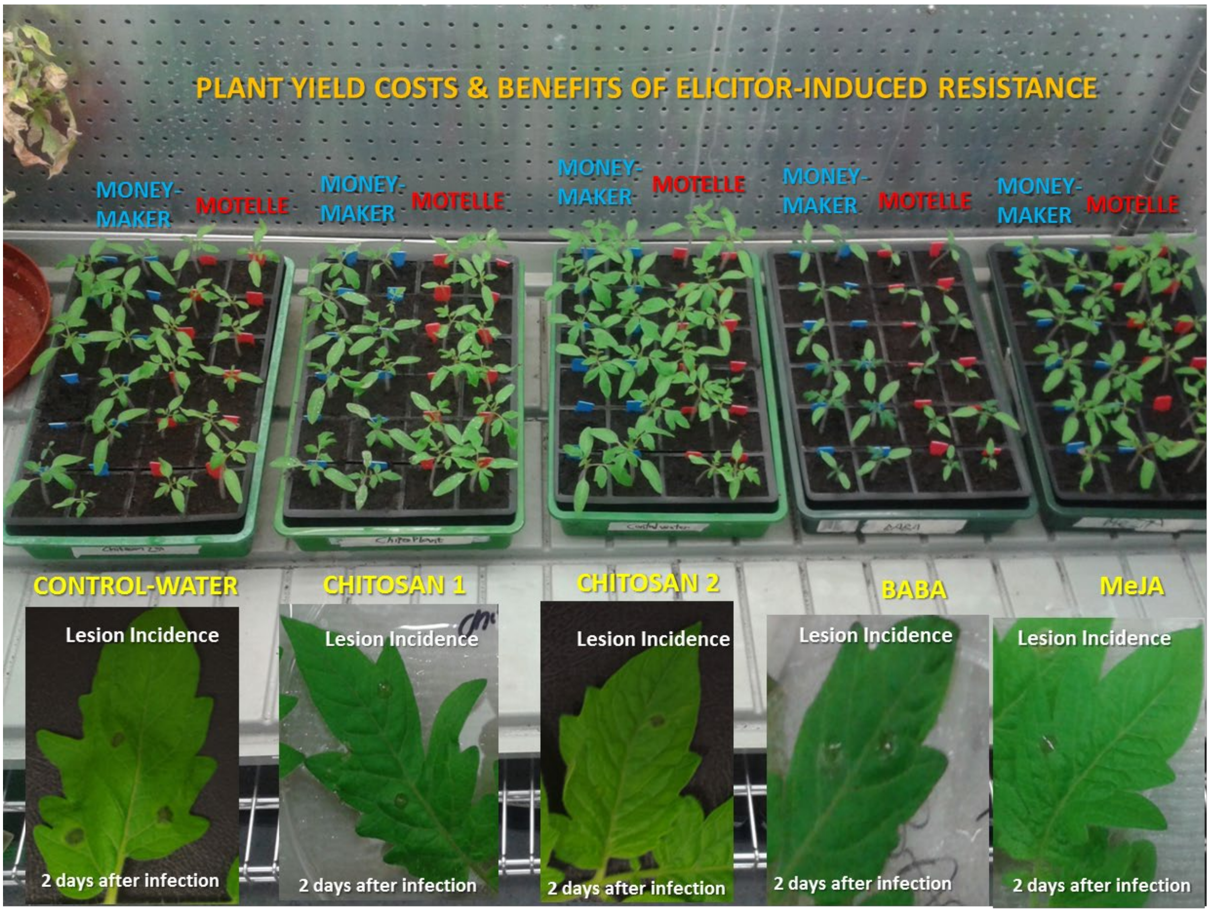
Summary

A selection of resistance elicitors were evaluated for their ability to induce resistance in the *Botrytis cinerea* – tomato pathosystem as follows; methyl-jasmonate (MeJA, a plant phytohormone), Benzothiadiazole (BTH, A chemical analogue of Salicylic Acid marketed by Syngenta under the tradename 'BION'), beta-aminobutyric acid (BABA, an amino acid compound) and chitosan (a low molecular weight and water soluble formulation marketed under the trade name 'ChitoPlant'). All four resistance elicitors were shown to induce resistance by significantly reducing gray mould disease (*Botrytis cinerea*) on two 4-weeks old tomato varieties (tomato cv. Moneymaker and cv. Motelle).

MeJA and ChitoPlant also induced resistance in 4-week-old *Arabidopsis thaliana* (thale cress, a model plant) cv. Columbia0 and Aubergine cv. Black Beauty plants and significantly decreased *Botrytis cinerea* infection in comparison to water treated control plants. Treatments were applied by foliar spraying the solutions once on to the shoot 4 to 17 days before Botrytis infection demonstrating long-lasting induced resistance.

Resistance elicitors can induce "early acting" defence mechanisms in the plant by reinforcing plant cell-wall. Experiments showed that the phytohormone elicitors MeJA and chitosan were able to reinforce the cell-walls of tomato plant cells by inducing callose deposition on tomato epidermal cells. Callose is a plant polysaccharide that can delay pathogen penetration in the epidermis. Furthermore, MeJA and chitosan were able to induce plant defences such as peroxidases and H_2O_2 production, involved in the fight against fungal and bacterial toxic compounds upon attack.

Elicitors can reduce plant growth when applied at medium-high concentrations. To help understand more about the balance between the value of priming crop defences and elicitor interaction with plant development, the costs of the elicitor-induced resistance were measured in relation to crop yield. Treatment of tomato seedlings (1-2 wks old) with the resistance elicitors revealed that BABA had a strong growth repression effect (GS Figure 1). BABA reduced relative growth rate by an average of 41% across the different cultivars tested. The other elicitors tested did not lead to reduced growth at the concentrations tested.



GS Figure 1. Two-week-old tomato cv. Motelle (red) and Moneymaker (blue) seedlings one week after elicitor treatment (Chitosan2, ChitoPlant/Chitosan1, BABA, MeJA and water as a non-treated control). BABA-treated plants were smaller than the rest of the treatments and Motelle-BABA treated plants were even smaller than Moneymaker BABA-treated seedlings. Seventeen days after elicitor-treatment, tomato plants were infected with Botrytis spores and 2 days after infection necrotic lesions were measured. In all elicitor-treated plants lesion incidence was lower than water-treated plants and gray mould disease expansion was significantly slower compared to water-treated control plants.

To determine the duration of elicitor-induced resistance in the crop tomato seedlings were foliar sprayed with the different resistance elicitors or distilled water (as a control). Seventeen days after elicitor treatments plants were infected with spore solution of *Botrytis cinerea* and necrotic lesion expansion was measured in order to look for an induced resistance phenotype. We saw that all elicitors were able to significantly reduce Botrytis lesion expansion (GS Figure 1 inset) up to 4 days after infection in both tomato varieties tested. Therefore, we can conclude that elicitors can prime tomato plants for a stronger defence response for over two weeks from application.

Of the four types of Resistance Elicitor tested, Chitosan was chosen for more in depth study as it was the most effective at reducing disease lesion expansion following inoculation of Botrytis across a range of host species tested (tomato, thale cress and aubergine).

In vitro tests, in which chitin was exposed to Botrytis in petri dishes, demonstrated that Chitosan has a direct fungicide effect; either stopping or reducing fungal spore germination and fungal hypha growth in a concentration dependent manner (>0.1% w/v).

Chitosan foliar treatment was shown to have a phytotoxic effect on young (<4 week) tomato and aubergine plants, in a concentration-dependent manner (>0.1% w/v).

The mode of action of the resistance eliciting properties of Chitosan was revealed using a large scale gene expression analysis approach called Microarray analysis. This approach enabled the comparison of gene expression between chitosan-treated and Botrytis-infected tomato plants verses water-treated and Botrytis-infected tomato plants. The main findings were:

- Chitosan-treated plants displayed stronger and faster defence mechanisms by differentially expressing more than 2,100 defence-related genes after Botrytis infection compared to water treated plants which only differentially expressed 363 genes.
- Chitosan-treated tomatoes were able to repress Botrytis virulence genes expression (used by the pathogen to facilitate infection) while water-treated plants couldn't avoid Botrytis gene expression.
- Analysis on the 2,100 genes induced by chitosan revealed key pathways involved in tomato defences against Botrytis, such as cell-wall modification genes (lignin and cellulose synthesis), regulatory and signalling genes, jasmonate and ethylene-dependent genes, redox state (glutaredoxins, involved in the cell oxidative stress) and secondary metabolites (phenylpropanoids).

These results unveiled potential molecular pathways involved in chitosan-induced priming for resistance in tomato against the aggressive fungal pathogen *Botrytis cinerea*, which may also be applicable to other crops (e.g. aubergine). This information may assist breeders to develop commercial crop varieties with increased expression of genes in the key pathways identified in this study.

Finally, the effects of a resistance elicitor combination treatment were evaluated. Low-dose combination of chitosan (0.01% w/v) + methyl-jasmonate (0.1mM) applied to the foliage of tomato plants showed increased protection against Botrytis compared to the each treatment alone. This result suggests a potential synergistic effect of chitosan in combination with other elicitors (i.e. methyl-jasmonate).

Financial Benefits

Botrytis cinerea is a fungal pathogen that infects almost all vegetable and fruit crops and annually causes \$10 billion to \$100 billion in losses worldwide (Weiberg et al., 2013). Understanding how elicitors, such as chitosan, can prime horticulture crops against Botrytis infection may facilitate novel antifungal strategies and therefore reduce crop losses due to pre- and post-harvest fungal infection.

Outcomes of this project are in the form of preliminary knowledge which may lead to novel crop protection products. This PhD project has relevance to a number of different sectors because the nature of the research is to investigate common mechanisms of plant defence. This research begins to explore the principles and potential for using resistance elicitors in integrated crop protection strategies of the future.

Action Points

- There are few resistance elicitors currently licenced for use on horticultural crops (Harpin, SiTKO-SA, Amistar). AHDB project FV 417 demonstrated that elicitors can interact with fungicides and they may have beneficial effects on controlling bacterial diseases on red onion, cabbage and broccoli, depending on the elicitor and the crop
- Chitosan was approved as a *basic substance* in 2014 by the EU, thus it may be used on fruit and vegetables in the field or in the greenhouse
- Resistance elicitors, such as chitosan, can lead to phytotoxic effects in a concentration dependent manner
- Key defence pathways identified in this work may lead to future breeding targets for plant breeders

