Grower Summary

CP 162

Characterising the molecular basis for insecticide resistance in the tomato leafminer *Tuta absoluta*.

Annual report 2018

Project title:	**Characterising the molecular basis for insecticide resistance in the tomato leafminer *Tuta absoluta.***
Project number:	CP 162
Project leader:	Charles Grant. University of Exeter
Report:	2018 annual report
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Key staff:	Professor Chris Bass University of Exeter
Location of project:	Penryn Campus. University of Exeter
Industry Representative:	Dr. Rob Jacobson
Date project commenced:	October 2016

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[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.

Charles Grant

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Signature: Charles Grant Date: 7/12/2018

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GROWER SUMMARY

Headlines

Conserve (Spinosad)

- Strong Conserve insecticide resistance prevalent in UK populations of *Tuta absoluta.*
- Two unique mechanisms of resistance Identified.
- Development of diagnostic for one mechanism is completed and shows good detection of resistance genes.

Coragen (Chlorantraniliprole)

- Low level of resistance detected
- Selected lines showed potent resistance
- All known UK and European resistant strains of *Tuta absoluta* sequenced for robust diagnostic capable of rapidly detecting any resistance-associated mutation.

Background

The UK consumes around 500 000 tonnes of fresh tomatoes each year, of which 92 000 tonnes are UK grown and these have a retail value of approximately £190 million. The invasion of *Tuta absoluta* into tomato crops can result in massive yield losses. Developing, implementing and maintaining effective control measures has been an urgent requirement for the UK tomato growing industry. This control has come about through the development of an integrated pest management strategy (IPM), incorporating biocontrol agents with pesticide applications and pheromone mating disruptors. However, in recent years, this IPM has struggled to take total control of the pest in certain glasshouses. This

is due to, either the evolution of resistance genes within populations or the importing of populations already resistant to the chemistries used, Conserve (spinosad) and Coragen (chlorantraniliprole). Furthermore, the loss of effectiveness of mating disruptors has been reported. These outcomes are alarming and bring into question the long-term effectiveness of this contemporary IPM.

Summary

Conserve

Bioassay results have confirmed the widespread presence of resistance to Conserve in UK populations. Last year's report identified the mechanism of resistance in two populations, an RNA alteration resulting in a truncated acetylcholine receptor (a receptor in the nervous system which is the target of Conserve). A molecular approach to identifying this is possible but currently slow and expensive. We are currently assembling the genome of *T. absoluta* which will help us elucidate the genomic mutation that caused the truncation of the nerve receptor protein. Once this is known, simple, cheap and fast diagnostics will allow rapid assessment of the gain/loss of resistance genes in glasshouse populations allowing 'realtime' resistance assessments.

This year we identified a second mechanism of resistance to Conserve. This mechanism was also an alteration in the same nerve receptor, however this alteration is a deletion of three genomic nucleotides, resulting in exclusion of one single amino acid. The position of this amino acid in the finished folded protein is in a region of the receptor suspected to be the binding site of Conserve. To associate the amino acid deletion with survival to the application of Conserve, individual *T.absoluta* larvae were exposed to a dose of the pesticide expected to kill about 50 % of the population. The dead and alive individuals were then collected and analysed using a specially designed target mutation assay (TaqMan ®). Genotype was scored by the colour of fluorescence

from one of two specific probes which bind discriminately to either the genotype, with the deletion or without the deletion. There was a strong correlation between individuals surviving the test and having the deletion present, as well as between individuals dying in the test and them lacking the deletion. The overall frequency of resistance genes in the population was 0.57. Thirty one individuals were homozygous susceptible, 88 were heterozygous and 56 were homozygous resistant.

Coragen

The low-level presence of Coragen resistance was identified in UK populations using leaf dip bioassays. A low-frequency mutation in the ryanodine receptor (a nerve-muscle junction-receptor in the nervous system) at a location associated with Coragen binding was observed. Suggestion that this was the mechanism of resistance was confirmed when the strain was selected for resistance. The mutation rose in frequency to 100% in the population along with their resistance to the pesticide, which after the final selection experiment was over 4000 times more resistant than susceptible strains. Growers have reported control failure of *T. absoluta* by Coragen in this season's crops. Samples from these glasshouses will be tested to assess any rise in frequency of this mutation within the population as predicted by the selection experiment.

The development of a molecular diagnostic is under way. The genetic mutation we are targeting with diagnostic is already present at low frequency in the population and so, it is likely that this resistance mechanism was imported from European populations. To ensure the robustness of the diagnostic we are collaborating with research groups in Europe in an attempt to incorporate all known resistance associated mutations into one diagnostic. This would mean the diagnostic would be sensitive to any further imported resistant genotypes.

Financial Benefits

- Molecular diagnostics are cheaper and faster than traditional bioassays.
- Early detection of resistance populations within the glasshouses.
- Inform whether pesticide application will have the desired effect.
- Reduce mismanagement of pesticides.
- Slow evolution of resistance genes.

Action Points

- Stop the use of Conserve (spinosad) and continue to monitor resistance in the field to see if there is any return of efficacy.
- Monitor effectiveness of Coragen (chlorantraniliprole) closely and report any application failures so resistance to those populations can be assessed.