Grower Summary

CP 162

Insecticide resistance in the Tomato leafminer

Annual Report 2017

Project title:	Insecticide resistance in the Tomato leafminer
Project number:	CP 162
Project leader:	Chris Bass, University of Exeter
Report:	Annual report, May 2018
Previous report:	
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Location of project:	Exeter University
Industry Representative:	Rob Jacobson, Rob Jacobson Consulting
Date project commenced:	1st October 2016
Date project completed (or expected completion date):	30th September 2019

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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.

A**U**THENTICATION

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.

Prof C. Bass

Project Leader

University of Exeter

Signature .... Date ....

Charles Grant

PhD Student

University of Exeter

Signature .... Date ....

Report authorised by:

Dr R. Jacobson

Industry Representative

Rob Jacobson Consulting

Signature .... Date ....

GROWER SUMMARY

Headline:

- Resistance to important IPM compatible chemicals has been confirmed in some UK populations.
- Molecular basis of this resistance has been determined.
- Molecular-based resistance diagnostics are under development.
- Ability to 'resist' novel mating disruptors is investigated in UK populations.

Background:

Tuta absoluta.

T. absoluta is a micro lepidopteran species belonging to the Gelechiidae family which consists of about 500 genera and 4700 species. It is endemic to South America, first described in Peru in 1917, and genetic studies reveal that its expansion throughout Europe, Africa and the Middle East came from a single introduction into Eastern Spain from central Chile in 2006. Its arrival in the UK is believed to be due to importation of infected produce from Spain in 2009. *T. absoluta* arrived in the UK armed with resistance to many traditional insecticides.

T. absoluta host plants include economically important crops in the family solanaceae and since the outbreak of *T. absoluta* in 2009 its economic impact has been felt by many UK tomato growers. Its life cycle lasts about one month and females have been recorded laying in excess of 200 eggs. Favourable temperatures for development are similar to those of tomato glasshouses in Europe and its host plant preference is greater for cultivated tomato varieties than that of wild types. Control of this pest after its insurgence required the development of an integrated pest management (IPM) strategy. This included biocontrol from the predatory hemipteran bug *Macrolophus pygmaeus* and applications of two pesticides with novel modes of action, Coragen and Conserve. A more recent addition in the fight to control *T. absoluta* is the introduction of Isonet-Ts mating disruptor.

Conserve.

The active compound in Conserve is spinosad, a bio-pesticide derived from the soil bacteria *Saccharopolyspora spinosa*. This insecticide targets the nicotinic acetylcholine receptor (nAChR), a nerve receptor involved in rapid neurotransmission. nAChR has long been recognised as a target for insecticidal action. Binding of spinosad to the nAChR causes loss of function of the receptor, allowing the movement of chemical charge, depolarising the muscle membrane. This electrochemical transduction activates many responses in the cell including contraction of muscles. Intoxication by spinosad ultimately results in paralysis, feeding cessation and death.

High levels of spinosad resistance have been reported in field populations of South American and European *T. absoluta.* An amino acid substitution in the nAChR was identified in a resistance-selected strain, resulting in alteration of the structure of nAChR. The same mutation was observed in other resistant insects.

A second mechanism of resistance in *T. absoluta* is exon skipping. Exon skipping is the exclusion of discrete regions of RNA that are usually present in normal RNA transcripts, RNA transcripts which then go on to direct the assembly of the protein receptors. This 'missing' region of RNA results in the loss of a whole section of the protein. 100% of resistance-selected *T. absoluta* expressed receptor protein transcripts lacking the normally present exons. This resulted in the loss of the spinosad binding region of the nAChR.

Coragen.

Coragens active compounds are in the chemical class diamides which have excellent effectiveness at controlling lepidopteran pests. Diamides have a novel mode of action, targeting another nervous system receptor, the ryanodine receptor (RyR). This receptor is involved in calcium signalling which regulates many processes in insects, including muscle contraction. In spite of Coragens great lepidopteran profile, resistance has recently evolved in the field. 2010-2011 South Chinese populations of Diamond back moth *Plutella xylostella* developed target-site resistance resulting in 2000-fold resistance increase. A mutation resulting in an amino acid substitution was presumed to confer resistance in Thailand, Philippine and Chinese populations. Resistance to diamides has also been reported in *T. absoluta.* Italian populations showed 2414-fold resistance and in resistant Greek populations an amino acid alteration at the same site as the resistant *P. xylostella* was identified as being causal.

Isonet-T

Mating disruptors were introduced in 2016 and after great success in controlling *T. absoluta,* have immediately become an integral part of IPM. They work by inundating the glass house atmosphere with a synthetic sex pheromone identical to that released by females. This confuses males as to the location of females, preventing reproduction, and has proved a highly effective control measure. While this introduction has relaxed the pressure on the chemical controls there are reports in the scientific literature of parthenogenesis (asexual reproduction) in *T. absoluta*. With *T. absoluta's* track record of overcoming control measures and reports of control failure at one U.K. site this year, it is important to asses the U.K. populations ability to reproduce parthenogenetically.

Summary

Objectives.

The main aims of this research project are to test for resistance in the field, characterise the molecular basis of resistance to Conserve and Coragen. Once the molecular basis for these pesticides has been identified, DNA based diagnostics will be developed with the aim of replacing the classical leaf dip assays.

As this is an applied study, the aims of this project must stay flexible and track changes as they occur in the grow houses around the U.K. - this includes changes in levels of resistance. Often the evolution of resistance occurs through a trade-off with pest fitness, so discontinuation of pesticide use may cause population level susceptibility to return, thus restoring the efficacy of the pesticide. This project also aims to monitor new mating disruption control products and to asses the ability for *T. absoluta* to reproduce asexually.

Results and discussion.

Overview.

Previous work on U.K. populations has described spinosad resistance and this report not only confirms this, but also describes alternative mechanisms for this resistance and a rapid method for its detection. This work also shows low-level field resistance to diamides and further laboratory selection of this population generated high levels of resistance. These findings have a serious implication, potentially rendering current IPM strategies used by many growers in the U.K. severely restricted. However evidence of *T. absoluta's* ability to reproduce in the absence of sexual reproduction was not detected.

Conserve.

Leaf dip assays showed high levels of resistance in U.K. strains. These populations were then tested for target site alterations. Amplified nAChR transcripts from resistant populations showed reduction in size compared to the susceptible strain. Disparity in size between the resistant and susceptible amplified transcripts can be observed by a process called gel electrophoresis. This gives a distinct picture of the variation in transcript size and therefore resistance status.

The redundancy of spinosad was confirmed from the bioassay results, and analysis of the nAChR gene transcript clearly showed exon 4 had been skipped when compared to the full complement of exons in a susceptible population. The exclusion of exon 4 from this reading frame resulted in a 109 nucleotide deletion and a shift in the reading frame, producing a premature stop codon in exon 5 at position. RNA translation would produce a massively truncated protein and would structurally render the protein completely non-viable.

More likely however is that nonsense mediated decay (NMD) would digest RNA before energy was wasted, translating it to a non-functional protein. NMD is a surveillance pathway that prevents expression of genes with PSC translating into incomplete proteins.

This result however does offer the ability to create an RNA based diagnostic capable of identifying the size difference between full nAChR transcripts and those with exon 4 skipped, based on PCR and gel electrophoresis. This would drastically reduce the time taken to assess frequencies and locations of resistant phenotypes from ~2 months to 24-48 hours.

Coragen

Bioassays for Coragen resistance showed some moderate tolerance to diamides in field populations. The RyR transcripts were amplified, targeting the regions surrounding the binding site of diamides. Sequencing of these amplicons showed no obvious mutations when compared to the susceptible population in most cases. However, low level variation at one target site was indicated in a population with some tolerance. Selection of this population with increasingly higher doses of diamides caused a rise in the frequency of this mutation within the population until it was present in all individuals tested.

This mutation is present in resistant European populations and so is likely to have been imported with U.K. populations at low frequency. This means it is only a matter of time before it is selected for in the field.

Isonet-T

90 virgin females across three populations were tested for their ability to reproduce asexually. Although over 90% of these females laid eggs, none of the eggs developed into larvae. Results from these experiments did not support previously reported findings and revealed no evidence to support the claim that *T. absoluta* can reproduce parthenogenetically.

Since this research has been conducted, much of the *T. absoluta* problems faced by growers has been alleviated by the introduction of a new control measure - the Isonet-T mating disruptor. However control failure has been reported in one grow house this season. Understanding this failure is vital to optimising the use of this control measure and maintaining its effectiveness.

Maintaining optimum IPM effectiveness requires a plethora of control measures each targeting a different aspect of the insect's biology. Over use of any particular control measure can select for fixation of resistance rendering the control measure useless. The loss of any control measure increases selection for resistance to remaining control measures. If Coragen and Conserve are used without prior knowledge of resistance in the population they could lose their efficiency and be lost altogether. Although the mating disruptor may seem to be a 'silver bullet', reliance solely on this will select for resistance. Coragen and Conserve need to be preserved to support the mating disruptor and maintain a diverse array of control measures.

Financial benefits

Insecticide resistance tests currently done by scientists are time consuming, expensive and slow to provide results. Molecular assays could reduce the time taken from 8-10 weeks to within 2-3 days and reduce the cost from £2000 to around £100 - £200.

The careful monitoring of control measure effectiveness will dictate their integration into glasshouse specific management strategies. This will result in the choice of the most effective control measure, as well as a relaxation of resistance selection on less effective measures preventing their redundancy. Reliance on only one control measure will result in resistance and resurgence of *T. absoluta* populations, causing losses of up to £50k per ha.

Action Points

Further research

- Test UK population's ability to overcome mating disruptors.
- Uncover potential mechanism if resistance is present
- Test diagnostic capability to asses UK resistance frequency.
- Advise growers on use of control method combinations based on resistance profile of populations.