Grower Summary

CP 162

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

Annual report 2019

Project title:	**Characterising the molecular basis for insecticide resistance in the tomato leafminer *Tuta absoluta.***
Project number:	
Project leader:	Charles Grant. University of Exeter
Report:	2019 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
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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: 1/10/19

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GROWER SUMMARY

Headlines

- Coragen remains effective in the UK although target-site resistance genes are present in UK populations.
- Spanish strains of *T. absoluta* show strong levels of resistance to Coragen.
- Over expression of detoxification genes confers metabolic resistance in Spanish strains.
- No quarantine on *T. absoluta* to the UK.
- Implementation of new control measure (Isonet-T - mating disruption) has been successful in control of *T. absoluta.*
- UK population could have the capacity to reproduce asexually.

Background

The tomato leaf miner *Tuta absoluta* is a highly destructive pest of economically important crops in the family solanaceae including tomatoes. It arrived in the UK from Spain in 2006(1) and is now so well established in tomato grow houses it is no longer identified as a quarentinable insect. The larvae of this moth are capable of devastating whole crops if left unchecked. Integrated Pest management (IPM) strategies have been extremely successful, however, control levels have fluctuated in glasshouses over the years due to arms-race dynamics initiated by population suppression through the introduction of novel control measures (insecticides and mating disruptors), followed by population resurgence driven by adaptive evolution (resistance).

Previous project reports describing the characterisation of the molecular basis of insecticide resistance in *T. absoluta* (2017, 2018) have clearly shown how the efficacy of the economically important bio-pesticide (conserve) was neutralised through evolution of novel target-site resistance, a resistance resulting in a >480-fold decrease in pesticide effectiveness. These reports also warned of how resistant genotypes to a second contemporary pesticide coragen (class: diamide), were not only present in UK populations but could be further selected for, resulting in a >4000-fold resistance increase (2).

Both previously described resistance mechanisms involve the alteration of the insecticides target-sites. Target-site resistance comes about through mutations that alter the chemical properties of transcribed proteins, typically nerve receptor proteins (3). This reduces the affinity of the pesticide inducing functional inhibition of the receptor. These mutations are then selectively amplified over subsequent generations to fixation culminating in the emergence of population-level resistance. However, a population of *T. absoluta*, originating from Spain, showed strong resistance to the pesticide but genetic analysis reviled an absence of any target-site mutations. This finding suggests other genes may be involved in generating resistance to coragen. The varying resistance phenotypes could potentially arise from differential expression of metabolic detoxification enzymes (4). One aim of this report is to identify the evident second mechanism of resistance to coragen in *T. absoluta*. We test for metabolic resistance, the detoxification of the compound, through the analysis of differentially expressed genes in the two Spanish strains of *T. absoluta*. These strains express either a resistant or susceptible phenotype. Identification of resistance mechanisms will allow for detection of resistant populations in the field informing the response of IPM strategies

IPM strategies have been greatly aided by the introduction of Isonet-T, a mating disruptor. Isonet-T was introduced to the market in 2017 works by inundating a closed glasshouse environments with a synthetic version of the female sex pheromone. This prevents mate location and stops reproduction. Although highly

successful some glasshouses reported lower levels of control. Previous reports into European population of *T. absoluta* show an ability to reproduce through a process called parthenogenesis (asexual reproduction)(5). This process could have the effect of reducing the level of control implemented by the mating disruptor as well as allowing population re-emergence in the absence of the control. Populations of *T. absoluta* were collected from UK glasshouses that had been using Isonet-T in their IPM and observed some variability in their results.

Summary

T. absoluta strains showing both a susceptible and resistant phenotypes were collected from Spain and evaluated for resistance. The ryanodine receptor, a nerve receptor and the target-site for coragen was assessed for mutations but was found to be free from any resistance associated alterations. This suggests other mechanisms of resistance must be functioning. To test for this second mechanism the transcriptomes (all the RNA) were sequenced from resistant and susceptible strains. This provided a list of all the genes that were expressed by the pest. These libraries of expressed genes were then mapped to the *T. absoluta* genome allowing the assessment of their levels of expression. The more RNA molecules of a particular gene, the greater the expression of that gene by the pest. A short list of candidate genes was created by comparing the expression profiles of susceptible and resistant strains of *T. absoluta.* This comparison provided a list of 20 genes that were significantly differentially expressed. These genes were then checked against online databases to assess their function. From this annotated list one gene stood out as a candidate resistance gene, a UGT-glucosyl transferase enzyme. The function of this gene is to detoxify compounds through the binding of a sugar molecule making the toxic compound highly water soluble and therefore easy to excrete. Further analysis of the function of this gene is under way to fully associate its role in the allowing resistance to diamides in *T. absoluta.*

The second aspect of this report was to assess the propensity of *T.absoluta* to reproduce parthenogenetically as this may influence the effectiveness of Isonet-T. The assessment showed that *T. absoluta* did indeed have the capcity to produce offspring in the absence of sex. Virgin females were place in isolated containers with a food source and tomato leaves. 91% of females laid eggs with an average of 14 eggs per female. The average life span of the females was 21 days. Only 4 of these females laid viable eggs resulting in 9 active mines. 4 of these larvae survived to adulthood producing 1 male and three females. These females were isolated at pupa (before sexually mature) and placed in test chambers with food and tomato leaves. 23, 27 and 41 eggs were laid by the females but no mines were detected. These results show that *T.absoluta* may persist in the absence of sex (induced by mating disruption) although with greatly reduced fecundity. This doesn't rule out the potential for the evolution of greater success of parthenogenesis if it has a genetic basis and is under strong selection from mating disruption.

Financial Benefits

- Assessment of current resistance status in UK populations of *T.absoluta* is vital to informing integrated pest management strategies.
- Early detection of resistance populations within the glasshouses can prevent misuse of control measures, inhibit fixation of resistance genes and slow the evolution of resistance.
- It ensures the adaptation of IPM minimising yield loss.

Action Points

- Monitor overall levels of control of current IPM.
- Continue to monitor resistance status of UK populations to diamides using the knowledge gained of resistance mechanisms evolved in *T. absoluta.*
- Where molecular resistance occurs suggest hiatus of diamide use to prevent total loss of the compound.
- Continue to monitor efficacy of Isonet-T. Reassess asexual reproductive capacity over time in the presence of the mating disruptor with a specific emphasis on glasshouses with *T. absoluta* presence.