Grower Summary

CP 162

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

2019 Annual Report
Disclaimer
While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

©Agriculture and Horticulture Development Board 2017. No part of this publication may be reproduced in any material form (including by photocopy or storage in any medium by electronic mean) or any copy or adaptation stored, published or distributed (by physical, electronic or other means) without prior permission in writing of the Agriculture and Horticulture Development Board, other than by reproduction in an unmodified form for the sole purpose of use as an information resource when the Agriculture and Horticulture Development Board or AHDB Horticulture is clearly acknowledged as the source, or in accordance with the provisions of the Copyright, Designs and Patents Act 1988. All rights reserved.

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
If you would like a copy of the full report, please email the AHDB Horticulture office (hort.info.@ahdb.org.uk), quoting your AHDB Horticulture number, alternatively contact AHDB Horticulture at the address below.

AHDB Horticulture,
AHDB
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel – 0247 669 2051

AHDB Horticulture is a Division of the Agriculture and Horticulture Development Board.
<table>
<thead>
<tr>
<th><strong>Project title:</strong></th>
<th>Characterising the molecular basis for insecticide resistance in the tomato leafminer <em>Tuta absoluta</em>.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Project number:</strong></td>
<td>CP 162</td>
</tr>
<tr>
<td><strong>Project leader:</strong></td>
<td>Charles Grant. University of Exeter</td>
</tr>
<tr>
<td><strong>Report:</strong></td>
<td>2019 Annual Report</td>
</tr>
<tr>
<td><strong>Previous report:</strong></td>
<td>2018 annual report</td>
</tr>
<tr>
<td><strong>Key staff:</strong></td>
<td>Professor Chris Bass University of Exeter</td>
</tr>
<tr>
<td><strong>Location of project:</strong></td>
<td>Penryn Campus. University of Exeter</td>
</tr>
<tr>
<td><strong>Industry Representative:</strong></td>
<td>Dr. Rob Jacobson</td>
</tr>
<tr>
<td><strong>Date project commenced:</strong></td>
<td>October 2016</td>
</tr>
</tbody>
</table>
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

Conserv

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.