Project title:	Tomato: Addressing important knowledge gaps in the *Tuta absoluta* IPM programme
Project number:	PE 032
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Report:	Final report, 11 January 2018
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Date project commenced:	1 April 2017
Date project completed:	31 December 2017

DISCLAIMER

The results and conclusions in this report are based on a series of investigations 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.

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CONTENTS

GROWER SUMMARY

Headline

- The *Tuta absoluta* mating disruption system, Isonet-T, is an effective addition to the IPM programme in tomato.
- Side effects of NeemAzal, Prolectus, Reflect, Topas and Conserve have been categorised for *Macrolophus pygmaeus* adults and nymphs.

Background

Tuta absoluta arrived in the UK in 2009 and rapidly became the most important pest of commercially-grown tomatoes. By 2013, AHDB Horticulture (previously HDC) projects and associated studies, had developed a completely new IPM strategy for use against the pest and this was detailed in AHDB Factsheet 02/14. The programme was based on the predator, *Macrolophus pygmaeus*, integrated with some physical control measures and the chemical insecticides, spinosad (Conserve), chlorantraniliprole (Coragen) and indoxacarb (Steward). *Macrolophus* was released at the start of the growing season so that it would start to provide control of the pest by late spring. When the pest arrived, it was allowed to colonise the crop but population growth was slowed by applying Conserve through the irrigation system before the first generation of caterpillars completed their development. If necessary, a high volume spray of Coragen was applied as a second line of defence during the summer to keep the pest and predator populations in balance. If crop monitoring indicated that a clean-up spray was required at the end of the season, then Steward was used to reduce the number of *T. absoluta* surviving in the glasshouse to infest the next crop. The three insecticides were from different IRAC Mode of Action Classification Groups and, together with the biological control agent, should have formed a robust resistance management strategy.

The IPM programme was very successful and British tomato growers became complacent about the pest. However, during the 2015 and 2016 growing seasons several growers began to experience difficulties with Conserve and *Macrolophus*. Resistance to Conserve was confirmed at three nurseries and populations of *Macrolophus*, which had originally given good control of *T. absoluta* from May-June onwards, inexplicably crashed during the summer. This led to questions about variation between different strains of *Macrolophus* and the possibility that some had been harmed by Conserve and / or some recently approved fungicides.

These control failures made it clear that the British tomato industry had to take urgent measures to remain one step ahead of this potentially devastating pest.

Summary

Part 1: Mating disruption trial

The concept of mating disruption as a component of the *T. absoluta* IPM programme was first introduced to the UK tomato industry by Dr Jacobson at the 2011 'British Tomato Conference'. It involves artificially saturating the atmosphere in the glasshouse with a synthetic version of the sex pheromone that is naturally produced by female moths to attract males prior to copulation. As a consequence, the males become confused and are unable to find the females - so they do not mate. This product was considered to have the potential to slow down *T. absoluta* population growth in the early season while *M. pygmaeus* were becoming established - thereby providing an alternative to Conserve applied via the irrigation.

The TGA Technical Committee worked closely with CRD, CBC (Europe) and Fargro Ltd to facilitate the rapid authorisation of the pheromone-based mating disruption system, Isonet-T. The product was approved for use in the UK at the start of the 2017 growing season and a trial, hosted by Jan Bezemer & Sons, began immediately. The pest had been present in these crops at the end of the 2016 growing season and male moths continued to be caught in pheromone-based monitoring traps in the empty glasshouses during December. Isonet-T dispensers containing the sex pheromone were placed in crops in early January - either one week before or two weeks after the arrival of the tomato plants. Where placement was delayed by two weeks, a few active *T. absoluta* mines were seen during the first 4 weeks of the crop but none thereafter. Otherwise, no active mines were found during the following 22 weeks in any of the treatments. By that time, *Macrolophus* were well established and capable of controlling any subsequent *T. absoluta* infestation.

The power of the mating disruption system is also evident by exploring results obtained by commercial growers in parallel to this trial. Several TGA members, who already had significant *T. absoluta* infestations in their crops in February 2017, were watching the mating disruption trial with interest. They all opted to place Isonet-T dispensers in their crops and the pest population growth stopped immediately in all cases. The crops gradually 'cleaned up' as the old damage was removed by routine de-leafing. Only one of the growers saw any resurgence of *T. absoluta* by August 2017, and that was only in one area of one of his many glasshouses.

Despite these impressive results, it is important that we take account of a peer reviewed study

from the University of Liege (Belgium) which indicated that some female *T. absoluta* exhibited parthenogenesis; *i.e.* production of eggs without mating. It is quite possible that use of the mating disruption technique could select for a small proportion of female moths that exhibit parthenogenesis - just as the use of certain insecticides can select for resistance to that particular chemistry. This would clearly compromise the efficacy of the system and must be further investigated.

Part 2: Compatibility of three stains of *Macrolophus pygmaeus* with High Volume (HV) sprays of three fungicides and one insecticide

The purpose of this study was to evaluate the mortality effects of HV sprays of three fungicides and one insecticide on three different ‘strains’ of *Macrolophus.* The fungicides, Prolectus, Reflect and Topas, and the insecticide, NeemAzal, were applied at their recommended field application rates (Table 1). The three strains of *Macrolophus* were commercially available products from Bioline (strain 1), Biobest (strain 2) and Koppert (strain 3). For each strain, synchronised adult and nymph life cycle stages were tested separately. In addition, two different application methods were used for each chemical pesticide on each *Macrolophus* life stage; *i.e* surface residue and topical applications. The surface residues were applied to the base of the test arenas before releasing the insects while the topical applications were sprayed directly onto the predator.

Mortality over a 7 day period in each treatment was analysed to provide the LT_{50}; *i.e.* the time, in days, at which 50% mortality occurred. Additional analysis examined ‘hazard ratios’ which represent the individuals’ relative risk of death compared to controls treated with water only. While the hazard ratios clearly identify real differences between treatments, it can be quite difficult to visualise the impact those differences might have at a population level. To facilitate that, a method of presentation was adopted similar to that developed by the International Organisation for Biological Control (IOBC) and now utilised by all suppliers of biocontrol material in the UK. This method places the side-effects of pesticides on beneficial insects in one of the following four categories:

1. <25% reduction – *eg* designated as harmless or only slightly harmful
2. 25-50% reduction – *eg* designated as moderately harmful
3. 50-75% reduction – *eg* designated as harmful
4. >75% reduction – *eg* designated as very harmful

Table 1. Products tested for effect on *Macrolophus pygmaeus* adults and nymphs

Product	Active ingredient	Recommended dilution of product for field application
NeemAzal	1% azadirachtin A	3 litres / 1000 litres
Prolectus	500g/kg fenpyrazamine	1.2kg / 1000 litres
Reflect	125g/litre isopyrazam	1 litre / 1000 litres
Topas	100g/litre penconazole	0.5 litre / 1200 litres

There was considerable natural mortality of insects under the artificial test conditions and inherent variation within treatments, which is quite common in these types of experiments. None of the treatments caused catastrophic side-effects on any of the strains of *Macrolophus* adults or nymphs when the insects were subjected to either topical application or surface residues of the HV sprays. However, all the products caused a significantly higher mortality hazard than the water-treated control at some stage and it must be concluded that all the products, applied at their label rates, can have some adverse effects on *Macrolophus* survival. However, most of the significant effects represented less than 25% mortality of the insects being tested, thus fitting into the side-effect category 1. Only four of the treatments were classified as category 2 and none of the treatments fitted into any higher categories. Those in category 2 included three surface residue treatments (*i.e.* Reflect on strain 2 adults, Topas on strain 3 adults and Topas on strain 3 nymphs) and one topical application treatment (*i.e.* Reflect on strain 3 nymphs).

Part 3: Compatibility of three strains of *Macrolophus* with a systemic application of Conserve

The purpose of this study was to evaluate the mortality effects of a systemic application of Conserve (120g/litre spinosad) on two life cycle stages of each of three different ‘strains’ of *M. pygmaeus* obtained as commercial products from Bioline (strain 1), Biobest (strain 2) and Koppert (strain 3). The tests were done using 30-40cm tall tomato plants (cv Dometica), planted in rockwool, and supported by a wooden cane. A muslin bag was loosely fitted over each plant to retain the insects. Conserve was applied in the irrigation water at the rate of 1.2 litres product in 20,000 litres of irrigation water per hectare (EAMU 0325 of 2013). Mortality of insects was recorded after 10 days and statistical analysis performed on the data.

As with the HV spray experiments reported above, there was considerable natural mortality and inherent variation within treatments, but no catastrophic side-effects on any of the strains of *Macrolophus*. In the worst case scenarios, Conserve applied via the irrigation resulted in

70%, 68.7% and 56.1% mortality of adult *Macrolophus* strains 1, 2 and 3 respectively and 36.9%, 24.2% and 16.2% mortality of nymph strains 1, 2 and 3 respectively. When compared to the IOBC method of side-effect classification, the overall worst case scenarios would therefore be category 3 for adults and category 2 for nymphs.

This may not be as damaging as it at first seems if considered at the predator population level during the main tomato growing season. At that time, Conserve is usually used as a second line of defence to support the primary biological control agents being used against *Liriomyza byoniae* and *Tuta absoluta* leafminers. Assuming the chemical treatment has greater impact on the pest populations than the biocontrols, then the overall effect should be successful in restoring the balance between pests and predators. It is also important to remember that in an established and growing population of *M. pygmaeus*, over 80% of individuals are likely to be in one of the immature stages upon which the treatment has least effect. Of course the impact on population growth will be greater if Conserve is applied through the irrigation system at the beginning of the season when *M. pygmaeus* are first released because a much greater proportion of individuals will be adults at that time.

Financial Benefits

Tuta absoluta is currently the most important pest of tomato crops in the UK. For example, at one nursery in 2012, 30% of fruit were damaged by the pest and graded out during June and July causing losses of approximately £50k per hectare to that grower for that period alone. The *Macrolophus*-based IPM programme prevented such damage for over three years but some components of that programme have now broken down. The industry urgently needed to find an alternative IPM compatible control measures which this project has done. The project has also answered questions about the compatibility of *Macrolophus* with important new pesticides. From the 2012 example cited above, payback from this project could be achieved by preventing damage in less than one hectare of tomatoes in one growing season.

Action Points

<u>Mating disruption</u>

- The mating disruption product, Isonet-T, when used as supplied by the manufacturers and applied at the maximum recommended rate of 1,000 dispensers per hectare, provides a very effective alternative to Conserve for slowing down *T. absoluta* population growth in the early season while *Macrolophus* predators are becoming established.
- There is a possibility that our use of the mating disruption technique could select for a small proportion of female moths that exhibit parthenogenesis thus compromising this

control measure. The TGA are now working with a team at Exeter University who are studying parthenogenesis in *T. absoluta*. Meanwhile, it is important that UK growers only use Isonet-T as part of an IPM programme with equal consideration given to other biological, physical and insecticidal products. Growers must also keep in mind that *T. absoluta* brought into the UK on imported fruit could have already been subjected to this selection process by mis-use of the mating disruption product in the country of origin.

<u>Compatibility of *Macrolophus* with various pesticides</u>

- There was no evidence of consistent significant differences between the three strains of *M. pygmaeus* currently available as commercial products in the UK.
- When applied at their label rates, all the pesticides tested had adverse effects on *Macrolophus* survival in some situations. The worst case scenarios are shown in Table 2.
- These tests have been carried out with care under the described conditions. However, side-effects can vary depending on crops, quality of insects, environmental conditions and specific methods of pesticide application. As a consequence, these results can only be used a guide to the possible side-effects of the tested products. If growers require more precise information about risks to beneficial insects in their own crops, then they are advised to organise modified tests which take into account their specific situation.

Table 2. Possible impact of five pesticides on *Macrolophus pygmaeus* expressed in the IOBC side-effect format

Product	Active ingredient	Method of application	Side effect category range	
			Adult	Nymph
NeemAzal	azadirachtin	HV spray	1	1
Prolectus	fenpyrazamine	HV spray	1	1
Reflect	isopyrazam	HV spray	2	2
Topas	penconazole	HV spray	2	2
Conserve	spinosad	via irrigation	3	2

SCIENCE SECTION

Introduction

Tuta absoluta arrived in the UK in 2009 and rapidly became the most important pest of commercially-grown tomatoes. By 2013, HDC projects PC 302 and PE 020, and associated studies, had developed a completely new IPM strategy for use against the pest and this was detailed in HDC Factsheet 02/14 (Jacobson, 2014). The programme was based on the predator, *Macrolophus pygmaeus*, integrated with some physical control measures and the chemical insecticides, spinosad (Conserve), chlorantraniliprole (Coragen) and indoxacarb (Steward). *Macrolophus pygmaeus* was released at the start of the growing season so that it would start to provide some control of the pest by late spring or early summer. When the pest arrived, it was allowed to colonise the crop but population growth was slowed by applying spinosad through the irrigation system before the first generation of caterpillars completed their development. If necessary, a high volume spray of chlorantraniliprole was applied as a second line of defence during the summer to keep the pest and predator populations in balance. If crop monitoring indicated that a clean-up spray was required at the end of the season, then the third insecticide, indoxacarb, was used to reduce the number of *T. absoluta* surviving in the glasshouse to infest the next crop. The three insecticides used in the IPM programme were from different Insecticide Resistance Action Committee (IRAC) Mode of Action Classification Groups and, together with the biological control agent, should have formed a robust resistance management strategy. Nonetheless, a strict warning about maintaining an effective insecticide resistance management strategy was incorporated in HDC Factsheet 02/14.

The IPM programme was very successful and British tomato growers admit that they became complacent about the pest. However, during the 2015 and 2016 growing seasons several growers began to experience difficulties with various components of the programme.

Resistance to Conserve has now been confirmed at three nurseries (via PE 028 and independent testing). Coragen treatment failures at two sites in 2016 are still under investigation but this may also be due to resistance. If so, this would be consistent with the development of resistant populations of *T. absoluta* in Italy and Greece (Rodiakis *et al.*, 2015). Steward has rarely been used against *T. absoluta* in the UK because the pest population has usually been reduced to an acceptable level by *M. pygmaeus* before the end of the growing season. However, one British grower has experienced treatment failures with this product (Holt & Jacobson, Pers. Com. 2011).

These control failures have made it clear that the British tomato industry must take measures to remain one-step ahead of this potentially devastating pest. The TGA TC worked closely with CRD, CBC (Europe) and Fargro Ltd to facilitate the rapid authorisation of Isonet-T (a pheromone-based mating disruption system). This product has the potential to slow down *T. absoluta* population growth in the early part of the season while *M. pygmaeus* are becoming established - thereby providing an alternative to Conserve via the irrigation in the IPM programme. Unpublished reports from mainland Europe, where Isonet-T was used in 2016, have been promising but a conflicting study has indicated that *T. absoluta* exhibit parthenogenesis (*i.e.* production of eggs without mating) which would clearly compromise the efficacy of this system (Megido *et al*, 2012). Crop-scale trials were urgently required to determine the true potential of this product.

Although the primary biocontrol, *M. pygmaeus*, had for several years given very good control of *T. absoluta* from May-June onwards, results in 2016 were inconsistent. Some populations crashed during the summer for inexplicable reasons. This has led to questions about variation between different strains of *M. pygmaeus* and the possibility that some strains have been harmed by Conserve and / or some of the fungicides that have recently become available to UK growers. This information was urgently required.

The TGA TC requested that the following studies be done as soon as possible (Minutes of TGA TC meeting, 7 December 2016):

- *Investigate variation between* Macrolophus *strains with respect to compatibility with Conserve (when applied via the irrigation) and the recently approved fungicides.*
- *Determine the efficacy of the Mating Disruption system on a commercial crop scale.*
- *Determine the compatibility of the insecticide, azadirachtin, with* Macrolophus *when applied as a high volume spray.*

Materials and methods

Part 1: Mating disruption trial

Due to the nature of the pheromone mating disruption system, this study had to be done as part of the IPM programme in whole commercial scale glasshouses. As a first step, criteria were established to help identify an ideal location for the trial. First and foremost, the chosen site must have had a serious infestation of *T. absoluta* in the previous season so that there was a high risk of re-infestation from the start of the crop. This was a relatively expensive treatment which would be paid for by the participating grower and it was important that the trial did not become prohibitively expensive. This meant seeking at least three moderate-sized similar glasshouses at the same location. Ideally, the crops would be of the same cultivar but, failing that, at least of the same genre of tomato. Most of the criteria were met by the crops at Jan Bezemer & Sons Ltd but there were inevitably some compromises in the range of glasshouses and experimental design.

The trial was done in three glasshouses (named 9, 10 & 12), which were of similar structure and row length, at Cleveland Nurseries, Stokesley, North Yorkshire. Glasshouses 9 and 10 were each approximately 8,200m^2 and glasshouse 12 was approximately 4,600m^2. All contained classic round tomatoes (cultivars Dometica, Arvento, Encore and Millandro). Glasshouses 10 and 12 were ‘planted’ in week 1, 2017 and glasshouse 9 in week 4, 2017. This proved to be an ideal arrangement for the planned timing of the treatments.

The mating disruption product, Isonet-T, was used as supplied by the manufacturers and applied at the maximum recommended rate of 1,000 dispensers per hectare. They were hung on the crop wires and carefully positioned to give an even distribution throughout each glasshouse. There were three Treatments. In Treatment 1 (glasshouse 9), the lures were applied one week before the plants arrived which coincided with the heating being raised to production temperature. In Treatment 2 (glasshouse 10), the product was applied two weeks after the plants arrived. Treatment 3 (glasshouse 12) was an untreated control with no action planned until the pest had become established.

Three pheromone traps were placed in each glasshouse from week 48 2016. These traps were checked twice weekly and were used to record the presence of adult male moths before and during cropping. The traps were intended to demonstrate the presence of adult moths pre-treatment and also provide an indication of the ability of the male moths to find the monitoring traps during the mating disruption treatment.

Crop assessment procedures were planned with the project statistician, Dr John Fenlon (Warwick University), and were similar to those used in previous *T. absoluta* projects where treatments had to be applied to whole glasshouses; *i.e.* the plots were large and the effect on *T. absoluta* population growth was to be measured over time.

During the first few weeks of cropping, all plant rows were walked weekly and the total number of active mines recorded. It was intended that the assessment procedure would change six weeks into the crop when it was anticipated that the *T. absoluta* population would have progressed to the second generation of active mines. It was proposed that numbers of active mines would then be recorded in 5 sub-plots per glasshouse with 10 sample points per sub-plot. The major tool of analysis would be that of analysis of variance in which the sampling variation within each Treatment area would be combined and used as a test for differences between means of the samples from each plot.

Figure 1. Example of plants during the first few weeks of the trial when every row was walked weekly and the total number of active mines recorded.

Routine leaf trimming was done in three distinct stages during the first 14 weeks of each crop. The first stage removed the lowest five leaves at crop weeks 6-7. The second stage removed leaves from position 6 up to the side shoot which had been taken at about leaf 10 to form the second plant head. This was done during crop weeks 10-11. The third stage removed 2-3 leaves above the fork in crop week 14. This work was done primarily to improve air flow and reduce the risk of Botrytis establishment. However, each stage was also used as an opportunity to check for active and / or empty mines on that part of the plant as every removed leaf could be examined.

Figure 2. Example of plants after the third stage of trimming when leaves had been removed to just above the fork of the second plant head.

Part 2: Compatibility of three strains of *Macrolophus pygmaeus* with HV sprays of three fungicides and one insecticide

The purpose of this study was to evaluate the mortality effects of HV sprays of three fungicides and one insecticide on three different 'strains' of *Macrolophus pygmaeus.* The fungicides were Prolectus, Reflect and Topas, and the insecticide was NeemAzal (Table 2). The three strains of *M. pygmaeus* were commercially available products from Bioline (strain 1), Biobest (strain 2) and Koppert (strain 3). For each strain, synchronised adult and nymph life cycle stages were tested separately. In addition, two different application methods were used for each chemical pesticide on each *M. pygmaeus* life stage; *i.e* surface residue and topical applications. There were 5 replicates of each individual treatment with the exception of the surface residue tests on strain 1 – for which there were 10 replicates.

Each test unit consisted of a circular plastic Petri dish of approximately 79 cm^2 surface area. Shelter (to mitigate cannibalism), food (*Ephestia spp.* eggs) and moisture (a soaked cotton pad) were placed in the Petri dish prior to introduction of *M. pygmaeus* in all treatments.

The insects were ordered and received from the three suppliers as normal commercial products. Their health and general physical condition were checked before being added to the test units. Depending on the number of available insects, 5-10 were used in each individual test dish.

Surface residue treatments were applied to the test chambers before the insects were introduced. The pesticides were applied directly onto the test dish surface by a CO_2 powered spray apparatus and the mass of spray recorded using a microbalance. A spray volume of 200 l/ha is known to equate to 2 ± 0.2 mg/cm^2 (Candolfi *et al*., 2000), which is 158 mg ± 15 mg per 158 cm^2 Petri dish. Each test unit had 158 mg ± 15 mg of test item applied, at the field application concentration for that particular treatment (Table 2). The shelter, food, moisture pads and insects were then placed in the test unit.

For each topical application treatment, a 158 cm^2 Petri dish was filled with an excess of *M. pygmaeus* (>60) and then chilled to minimise insect movement. As with the surface residue treatments, 158 mg ± 15 mg of the test items were applied over the chilled *M. pygmaeus* at the field application concentration for that particular treatment (Table 2). The insects were then transferred to the test dishes along with the shelter, food and moisture pads.

Following application of treatments and introduction of insects, the test dishes were incubated in an environmentally controlled chamber maintained at a constant 20 ± 2^{o}C with a 16 hour light (400-800 lux) and 8 hour dark cycle. Mortality was recorded approximately 1 hour after treatment, and then 1, 3 and 7 days post-treatment. Where possible, mortality was also recorded 5 days after treatment.

Mortality over time was analysed by comparing the risk of death (hazard) in each group, as per OECD recommendations (OECD, 2006). A Cox proportional hazard model was used to compare the treated groups with the water control group. The resulting hazard ratios represent the individual's relative risk of death, compared to the water control group. Dependence of grouped observations within test chambers were taken into account by adding the test chamber as a clustering term in the Cox model. The LT_{50} values (time at which 50% mortality occurred) for each group were calculated with a Kaplan-Meier analysis. All statistical analyses were performed in R: A language and environment for statistical computing (version 3.4.1).

Table 2. Products tested for effect on *Macrolophus pygmaeus* adults and nymphs

Product	Active ingredient	Recommended dilution of product for field application
NeemAzal	1% azadirachtin A	3 litres / 1000 litres
Prolectus	500g/kg fenpyrazamine	1.2kg / 1000 litres
Reflect	125g/litre isopyrazam	1 litre / 1000 litres
Topas	100g/litre penconazole	0.5 litre / 1200 litres

Part 3: Compatibility of three stains of *Macrolophus pygmaeus* with a systemic application of Conserve

The purpose of this study was to evaluate the mortality effects of a systemic application of Conserve (120g/litre spinosad) on two life cycle stages of each of three different 'strains' of *M. pygmaeus* obtained as commercial products from Bioline (strain 1), Biobest (strain 2) and Koppert (strain 3). The tests were done using whole tomato plants with Conserve applied in the irrigation water. There were two test runs with between 3 and 6 replicates per treatment depending on the numbers of available healthy insects.

Each test unit consisted of a 30-40cm tall tomato plant (cv Dometica), planted in rockwool, and supported by a wooden cane. A muslin bag was loosely fitted over the plant. Twenty adult and 20 nymph *M. pygmaeus* individuals were released within the bag prior to it being secured around the base of the plants. Each plant was subsequently placed in a separate plastic tub to which irrigation water could be applied. Each test unit formed a single replicate in the study.

The application rate of Conserve was based on the recommended rate of 1.2 litres product in 20,000 litres of irrigation water per hectare (EAMU 0325 of 2013). There are approximately 20,000 tomato plants per hectare in a commercial crop, hence the equivalent application rate for this trial was 0.06ml Conserve in one litre of irrigation water per plant. The irrigation water also contained a commercial liquid plant food diluted according to the product label.

Application of treated irrigation water began two days prior to the release of the insects in aliquots of about 250ml per day. This continued as required by the plants until one litre had been applied to each test unit. Thereafter, watering continued as necessary using untreated irrigation water. An untreated control was used for all *M. pygmaeus* strains. These plants were provided with the same amount of water and feed as the test items but without Conserve.

The test units were held in a plant growth tent at 20 ± 5ºC with a Gavita pro propagation light and extractor fan. The timed light: dark cycle was 16:8. After 10 days, the test units were opened and the number of dead insects recorded.

Mortality of insects in the Conserve treatments was compared to untreated controls using a Welch Two Sample t-test. Each strain was assessed separately, as were adult and nymph life stages. All statistical analyses were performed in R: A language and environment for statistical computing (version 3.4.1).

Results and Discussion

Part 1: Mating disruption trial

Adult male moths were caught in the pheromone monitoring traps in the empty glasshouses throughout December and early January demonstrating that there was some survival from the previous crop. No moths were caught in the monitoring traps after the Isonet-T dispensers were placed in the glasshouses demonstrating that the mating disruption treatment was overpowering the weaker scent from the traps and preventing the males from finding those lures. From this we can infer that the male moths would also be unable to detect the even weaker scents produced by the adult females.

Active mines were found on plants around the periphery of the crops in glasshouse 10 (delayed Isonet-T treatment) and glasshouse 12 (untreated control) during the first four crop weeks. Thereafter no more active mines were found in these two treatments either during the formal assessments or during the routine leaf removal. In glasshouse 9, where the Isonet-T dispensers were applied the week before the plants arrived, no active mines were found at any time up to the end of the trial at the end of June 2017. Due to the lack of any mines, the assessment procedures were not changed at week 6 but instead we continued to walk the entire crops at two-week intervals until the end of the trial.

At first sight, it may seem that the failure to find adult male *T. absoluta* in monitoring traps or active mines in the plants in the untreated control indicates that the pest was absent from these crops. However, we do know that it was present up to the time that the Isonet-T dispensers were placed in the adjacent glasshouse. We also know from our personal experience at many sites over the previous 8 years that once *T. absoluta* is present in a crop it does not simply go away. In fact, the population grows rapidly and can cause serious damage to the plants by the third generation. We now believe that the Isonet-T treatment was so potent that leakage of pheromone through the partition glass wall and connecting door from the adjacent treated glasshouse was sufficient to cause mating disruption in the untreated crop. In fact, we imagine that the permeable partition wall was acting like a huge and extremely powerful pheromone trap attracting the male moths away from the untreated crop and any female moths therein. Once the males had been 'pulled' over to that wall, their instincts would not allow them to return to the crop.

All the crops at Jan Bezemer & Sons remained free of *T. absoluta* infestation until the trial finished at the end of June 2017. By that time, *M. pygmaeus* predators were well established and capable of controlling any subsequent *T. absoluta* infestation. Isonet-T was therefore shown to provide an effective alternative to Conserve, applied via the irrigation, in the IPM programme; *i.e.* it will slow down *T. absoluta* population growth in the early part of the season while *M. pygmaeus* are becoming established.

The power of the mating disruption treatment is also evident by exploring results obtained by other commercial growers in parallel to this trial. Several TGA members (including Wight Salads, R&L Holt, Flavourfresh, Eric Wall Ltd) already had significant *T. absoluta* infestations in their crops in February 2017 and had been following this trial with interest. They all opted to deploy Isonet-T dispensers at around that time using the same rate as was being tested in this trial. Their results were, without exception, beyond our expectations. The pest population growth stopped immediately and the crops gradually became 'clean' as the old damage was removed by routine leaf removal. Furthermore, only one of those sites had suffered any re-infestation by *T. absoluta* up to the end of June 2017, and then in only two small localised areas.

Despite these quite spectacular results, it is important that we do not dismiss the reports of parthenogenesis published by the University of Liege (Megido *et al,*, 2012). It is quite possible that our use of the mating disruption technique could select for a small proportion of female moths that exhibit parthenogenesis - just as the use of certain insecticides can select for resistance to that particular chemical. The TGA are now working with a team at Exeter University who are beginning to study parthenogenesis in *T. absoluta* with particular reference to the possibility of Isonet-T selecting for this trait from within populations of the pest (Bass & Grant, Pers. Com., June 2017). Meanwhile, it is important that UK growers only use Isonet-T as part of an IPM programme with equal consideration given to other biological, physical and insecticidal products. We must also keep in mind that *T. absoluta* brought into the UK on imported fruit could have already been subjected to this selection process by mis-use of the mating disruption product in the country of origin.

Part 2: Compatibility of three stains of *Macrolophus pygmaeus* with HV sprays of three fungicides and one insecticide

Overall, mortality in the supplied populations of *M. pygmaeus* ranged from 48% to 97% for adults and from 24% to 100% for nymphs in the water-treated controls across the various test runs / replicates. Within individual *M. pygmaeus* strains, mortality in water-treated controls averaged 97%, 66% and 78% for adults and 61%, 75% and 44.5% for nymphs from strains 1, 2 and 3 respectively. There was relatively large inherent variation within treatments. This was taken into account when processing the data but impacted on statistical significance of the results.

Several methods of processing and displaying data have been used. Figures 3-5 show the general trends in survival of *M. pygmaeus* adults and nymphs over 7 days following application of all treatments and allow cursory comparisons with the water-treated controls. However, these are only general trends with no statistical analysis and do not show whether any of the apparent differences are real. For each treatment, there followed calculation of the LT_{50}, *i.e.* the time, in days, at which 50% mortality occurred. These data were statistically analysed and the results are summarised in Tables 2, 3 and 4. Additional analysis examined hazard ratios, which represent the individuals' relative risk of death compared to water-treated controls. While the hazard ratios clearly identify real differences between treatments, it can be quite difficult to visualise the impact those differences might have at a population level. To facilitate that, a method of presentation was adopted similar to that developed by the International Organisation for Biological Control (IOBC) and now utilised by all suppliers of biocontrol material in the UK. The IOBC method places the side-effects of pesticides on beneficial insects in one of the following four categories:

1. <25% reduction – *eg* designated as harmless or only slightly harmful
2. 25-50% reduction – *eg* designated as moderately harmful
3. 50-75% reduction – *eg* designated as harmful
4. >75% reduction – *eg* designated as very harmful

The present method of categorisation calculated the difference in mean mortality between insects in each treatment and insects in their corresponding water-treated control seven days post-treatment. The calculated balances are presented in Figure 6. These charts incorporate thresholds for side-effect categories equivalent to those utilised by the biocontrol suppliers. It must be stressed that these charts lack precision and are only intended to provide an approximate guide to the impact of the pesticides on the insect populations.

The mean survival of *M. pygmaeus* strain 1 adults and nymphs over 7 days following application of the four pesticide treatments, with comparisons to water-treated controls, are shown in Figure 3. The trends are similar throughout with no evidence of catastrophic side-effects. The LT_{50} values and hazard ratios are listed in Table 2. Significant differences are highlighted with explanatory notes provided at the foot of that Table. However, the identified differences are relatively small, with some even implying a slight beneficial effect, and they are unlikely to have a major impact at the population level.

Figure 3. Mean survival of *M. pygmaeus* strain 1 adults and nymphs over 7 days following application of four pesticide treatments with comparisons to water-treated controls.

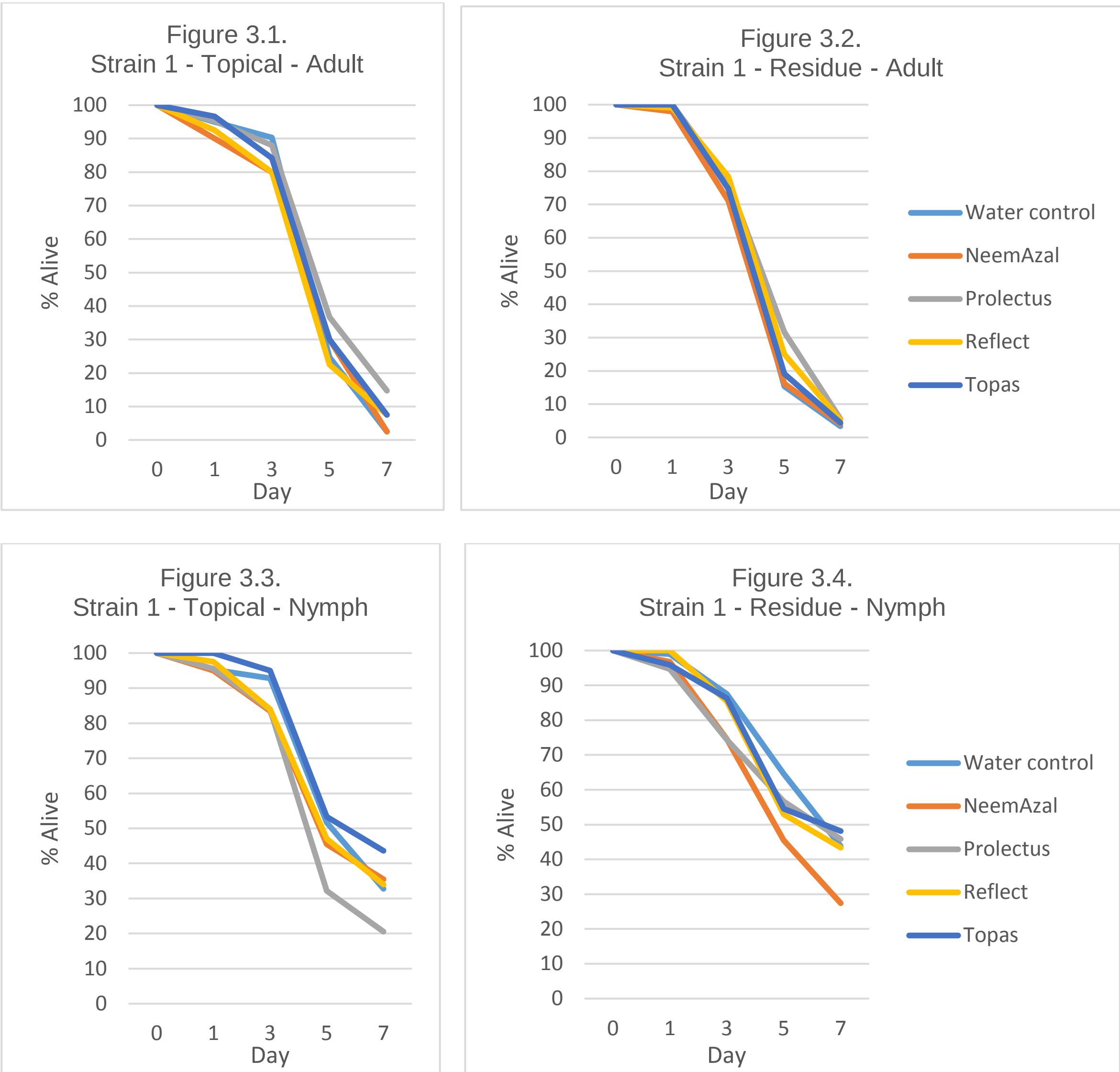


Table 2. LT_{50} values and hazard ratios for all treatments involving *Macrolophus pygmaeus* strain 1.

[LT_{50} = Lethal time 50 (time, in days, at which 50% mortality occurred), * significant increase compared to the water-treated control, Cox proportional hazard model ($p<0.05$). N/A = LT_{50} values are not given where 50% mortality was not reached by the end of the study.]

Application Method	Life cycle stage	Treatment group	LT_{50}	LT_{50} Lower 95 % C.I.	LT_{50} Upper 95 % C.I.	Hazard ratio (compared to the water control)	p value (compared to water control)
Residue on surface	Adult	Water control	5	5	5	N/A	N/A
		NeemAzal	5	5	5	0.80	0.20
		Prolectus	5	5	5	**0.72* (3)**	**0.02**
		Reflect	5	5	5	0.77	0.21
		Topas	5	5	5	0.88	0.35
	Nymph	Water control	N/A	N/A	N/A	N/A	N/A
		NeemAzal	7	5	7	**1.46*(1)**	0.03
		Prolectus	7	5	N/A	1.13	0.46
		Reflect	7	5	N/A	1.00	0.98
		Topas	7	5	N/A	0.98	0.86
Topical application	Adult	Water control	5	5	5	N/A	N/A
		NeemAzal	5	5	5	**0.77* (3)**	0.05
		Prolectus	5	5	7	**0.56* (3)**	**<0.01**
		Reflect	5	5	5	1.00	1.00
		Topas	5	5	N/A	0.87	0.29
	Nymph	Water control	7	5	N/A	N/A	N/A
		NeemAzal	5	5	5	1.05	0.75
		Prolectus	5	5	5	**1.39*(2)**	**0.02**
		Reflect	5	5	N/A	1.23	0.16
		Topas	7	5	N/A	**0.69* (3)**	0.02

Notes:

1. NeemAzal significantly increased mortality relative to the water control ($p<0.001$). The risk of death (hazard) was 1.46 times that of the water control group in the NeemAzal.
2. Prolectus significantly increased mortality relative to the water control ($p<0.001$). The risk of death (hazard) was 1.39 times that of the water control group in the Prolectus.
3. All these treatments have a significantly lower hazard ratio than the control implying a beneficial effect.

The mean survival of *M. pygmaeus* strain 2 adults and nymphs over 7 days following application of the four pesticide treatments, with comparisons to water-treated controls, are shown in Figure 4. As with *M. pygmaeus* strain 1, the trends were broadly similar throughout with no evidence of catastrophic side-effects. The LT50 values and hazard ratios are listed in Table 3 with significant differences highlighted and explanatory notes provided at the foot of that Table. Mortality of adults was significantly greater than the water-treated control when they were subjected to residues of all of the four pesticides - hazard ratios being 1.89, 2.55, 2.87 and 2.42 for NeemAzal, Prolectus, Reflect and Topas respectively. When the pesticides were applied topically to adults, mortality was only significantly greater than the water-treated control for Reflect and Topas – hazard ratios being 1.70 and 1.67 respectively. Mortality of nymphs was significantly greater than the water-treated control when they were subjected to residues of Reflect and Topas – hazard ratios being 1.87 and 3.49 respectively. When the pesticides were applied topically to nymphs, mortality was only significantly greater than the water-treated control for NeemAzal and Prolectus – hazard ratios being 1.79 and 1.45 respectively.

Figure 4. Mean survival of *M. pygmaeus* strain 2 adults and nymphs over 7 days following application of four pesticide treatments with comparisons to water-treated controls.

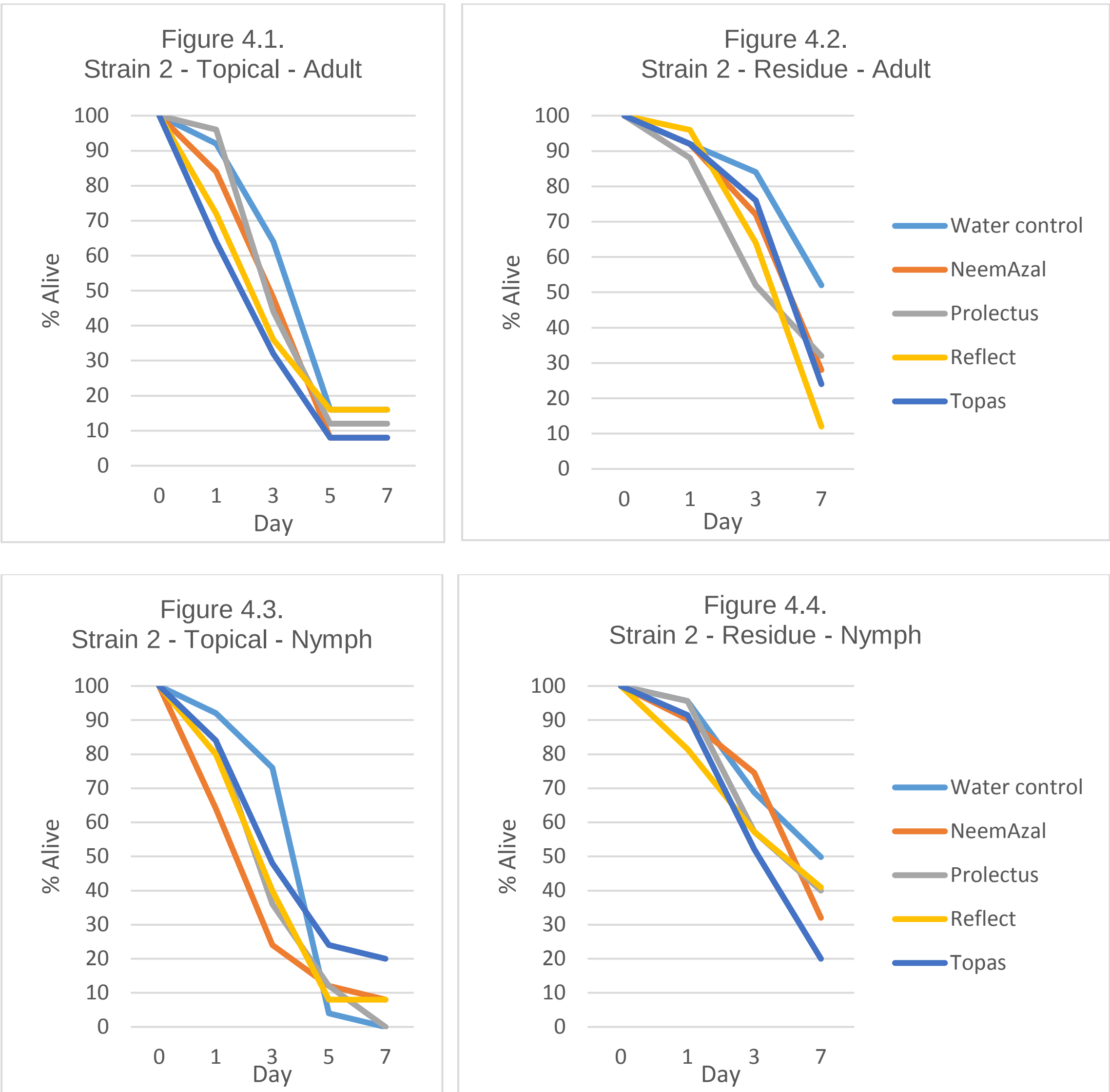


Table 3. LT_{50} values and hazard ratios for all treatments involving *Macrolophus pygmaeus* strain 2.

[LT_{50} = Lethal time 50 (time, in days, at which 50% mortality occurred), * significant increase compared to the water-treated control, Cox proportional hazard model ($p<0.05$). N/A = LT_{50} values are not given where 50% mortality was not reached by the end of the study.]

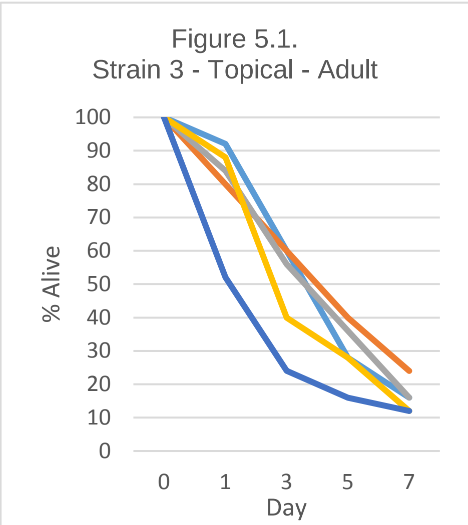
Application Method	Life cycle stage	Treatment group	LT_{50}	LT_{50} Lower 95 % C.I.	LT_{50} Upper 95 % C.I.	Hazard ratio (compared to the water control)	p value (compared to water control)
Residue on surface	Adult	Water control	N/A	7	N/A	N/A	N/A
		NeemAzal	7	7	N/A	**1.89* (1)**	0.006
		Prolectus	7	3	N/A	**2.55* (1)**	<0.001
		Reflect	7	3	7	**2.87* (1)**	<0.001
		Topas	7	7	7	**2.42* (1)**	<0.001
	Nymph	Water control	N/A	7	N/A	N/A	N/A
		NeemAzal	N/A	7	N/A	1.52	0.104
		Prolectus	7	3	N/A	1.58	0.074
		Reflect	7	3	N/A	**1.87* (2)**	0.013
		Topas	7	3	7	**3.49* (2)**	<0.001
Topical application	Adult	Water control	5	3	5	N/A	N/A
		NeemAzal	3	3	5	1.29	0.146
		Prolectus	3	3	5	1.30	0.146
		Reflect	3	3	5	**1.70* (3)**	0.003
		Topas	3	1	5	**1.67* (3)**	0.004
	Nymph	Water control	5	5	5	N/A	N/A
		NeemAzal	3	1	3	**1.79* (4)**	<0.001
		Prolectus	3	3	5	**1.45* (4)**	0.023
		Reflect	3	3	5	1.22	0.250
		Topas	3	3	5	1.21	0.270

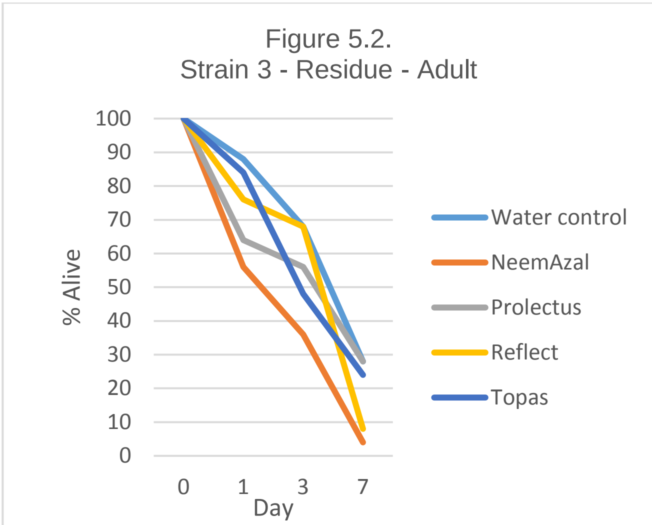
Notes:

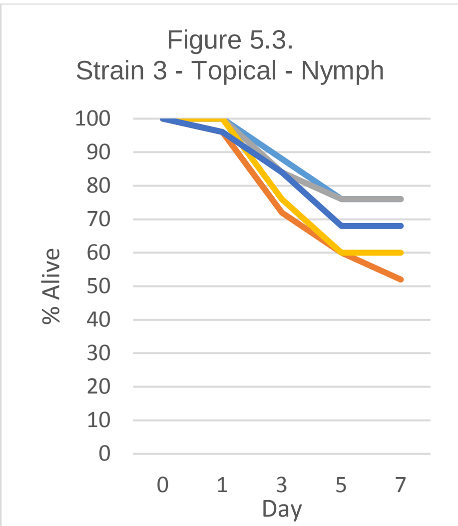
1. NeemAzal, Prolectus, Reflect and Topas significantly increased mortality ($p<0.01$ in all cases) with hazard ratios of 1.89, 2.55, 2.87 and 2.42 respectively.
2. Reflect and Topas significantly increased mortality ($p<0.01$ in each case) with hazard ratios of 1.87 and 3.49 respectively.
3. Reflect and Topas significantly increased mortality ($p<0.01$ in each case) with hazard ratios of 1.70 and 1.67 respectively.
4. NeemAzal and Prolectus significantly increased mortality ($p<0.01$ in all cases) with hazard ratios of 1.79 and 1.45 times respectively.

The mean survival of *M. pygmaeus* strain 3 adults and nymphs over 7 days following application of the four pesticide treatments, with comparisons to water-treated controls, are shown in Figure 5. As with *M. pygmaeus* strains 1 and 2, the trends were broadly similar throughout with no evidence of catastrophic side-effects. The LT50 values and hazard ratios are listed in Table 4 with significant differences highlighted and explanatory notes provided at the foot of that Table. Mortality of adults was significantly greater than the water-treated control when they were subjected to residues of NeemAzal and Reflect - hazard ratios being 2.44 and 1.48 respectively. When the pesticides were applied topically to adults, mortality was only significantly greater than the water-treated control for Topas with a hazard ratio of 2.45. Mortality of nymphs was significantly greater than the water-treated control when they were subjected to residues of NeemAzal, Reflect and Topas – hazard ratios being 1.77, 1.96 and 1.75 respectively. When the pesticides were applied topically to nymphs, mortality was significantly greater than the water-treated control for NeemAzal, Reflect and Topas – hazard ratios being 2.69, 2.08 and 1.97 respectively.

Figure 5. Mean survival of *M. pygmaeus* strain 3 adults and nymphs over 7 days following application of four pesticide treatments with comparisons to water-treated controls.







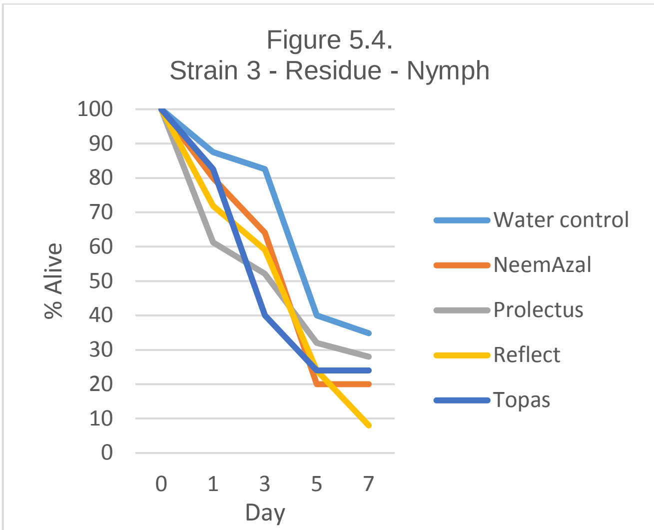


Table 4. LT_{50} values and hazard ratios for all treatments involving *Macrolophus pygmaeus* strain 3.

[LT_{50} = Lethal time 50 (time, in days, at which 50% mortality occurred), * significant increase compared to the water-treated control, Cox proportional hazard model ($p<0.05$). N/A = LT_{50} values are not given where 50% mortality was not reached by the end of the study.]

Application Method	Life cycle stage	Treatment group	LT_{50}	LT_{50} Lower 95 % C.I.	LT_{50} Upper 95 % C.I.	Hazard ratio (compared to the water control)	p value (compared to water control)
Residue on surface	Adult	Water control	5	5	N/A	N/A	N/A
		NeemAzal	3	1	N/A	**2.44* (1)**	<0.001
		Prolectus	5	1	N/A	0.94	0.770
		Reflect	5	3	7	**1.48* (1)**	0.030
		Topas	3	7	7	1.24	0.260
	Nymph	Water control	5	5	N/A	N/A	N/A
		NeemAzal	5	3	5	**1.77* (2)**	0.003
		Prolectus	5	1	N/A	1.33	0.165
		Reflect	5	5	5	**1.96* (2)**	<0.001
		Topas	3	3	7	**1.75* (2)**	<0.004
Topical application	Adult	Water control	5	3	7	N/A	N/A
		NeemAzal	5	3	7	0.86	0.404
		Prolectus	5	3	7	1.13	0.488
		Reflect	3	3	7	1.25	0.210
		Topas	3	1	3	**2.45* (3)**	<0.001
	Nymph	Water control	N/A	N/A	N/A	N/A	N/A
		NeemAzal	N/A	5	N/A	**2.69* (4)**	<0.001
		Prolectus	N/A	N/A	N/A	1.26	0.496
		Reflect	N/A	5	N/A	**2.08* (4)**	0.019
		Topas	N/A	N/A	N/A	**1.97* (4)**	0.030

Notes:

1. NeemAzal and Reflect significantly increased mortality ($p<0.01$ in each case) with hazard ratios of 2.44 and 1.48 respectively.
2. NeemAzal, Reflect and Topas significantly increased mortality ($p<0.01$ in all cases) with hazard ratios of 1.77, 1.96 and 1.75 respectively.
3. Topas significantly increased mortality ($p<0.01$) with a hazard ratio of 2.45.
4. NeemAzal, Reflect and Topas significantly increased mortality ($p<0.01$ in all cases) with hazard ratios of 2.69, 2.08 and 1.97 respectively.

The occurrence of significantly different mortality effects across all the treatments is summarised in Table 5. No consistent pattern has emerged. The effects appear to be randomly distributed across *M. pygmaeus* strains and life cycle stages without evidence to suggest that any of the insects are more vulnerable to topical application than to chemical residues on the surface of the test chamber. However, in most cases those effects represented less than 30% mortality. Only surface residues of Reflect caused greater mortality (c. 40%) but this effect was only seen with adults of *M. pygmaeus* strain 2.

Figure 6 presents the calculated balance in mean mortality between insects in each treatment and insects in their corresponding water-treated control 7 days post-treatment. The charts incorporate thresholds for side-effect categories equivalent to those utilised by the UK biocontrol suppliers. The majority of mortality balances were less than 25%, which fitted into Category 1 and would be designated ‘harmless or only slightly harmful’. Three surface residue treatments fitted into the higher Category 2, which is designated ‘moderately harmful’. They were Reflect on strain 2 adults, Topas on strain 3 adults and Topas on strain 3 nymphs. One topical application treatment was in Category 2; *i.e.* Reflect on strain 3 nymphs.

Table 5. Summary of significant mortality effects across all treatments compared to water-treated controls.

[T = Topical application, R = Surface residue, * = Significance p<0.01]

Macrolophus pygmaeus Strain 1															
NeemAzal				Prolectus				Reflect				Topas			
Adult		Nymph		Adult		N		Adult		N		Adult		N	
T	R	T	R*	T	R	T*	R	T	R	T	R	T	R	T	R
Macrolophus pygmaeus Strain 2															
NeemAzal				Prolectus				Reflect				Topas			
Adult		N		Adult		N		Adult		N		Adult		N	
T	R*	T*	R	T	R*	T*	R	T*	R*	T	R*	T*	R*	T	R*
Macrolophus pygmaeus Strain 3															
NeemAzal				Prolectus				Reflect				Topas			
Adult		N		Adult		N		Adult		N		Adult		N	
T	R*	T*	R*	T	R	T	R	T	R*	T*	R*	T*	R	T*	R*

Figure 6. Mean mortality of *Macrolophus pygmaeus* in all treatments in relation to side-effect categories similar to those utilised by the UK biocontrol suppliers.

[S1=Strain 1, S2=Strain 2, S3=Strain 3, S4 = Strain 4, A= Adult, N= Nymph, T= Topical application, R= Surface residue]

Figure 6.1. Seven days post-treatment with NeemAzal

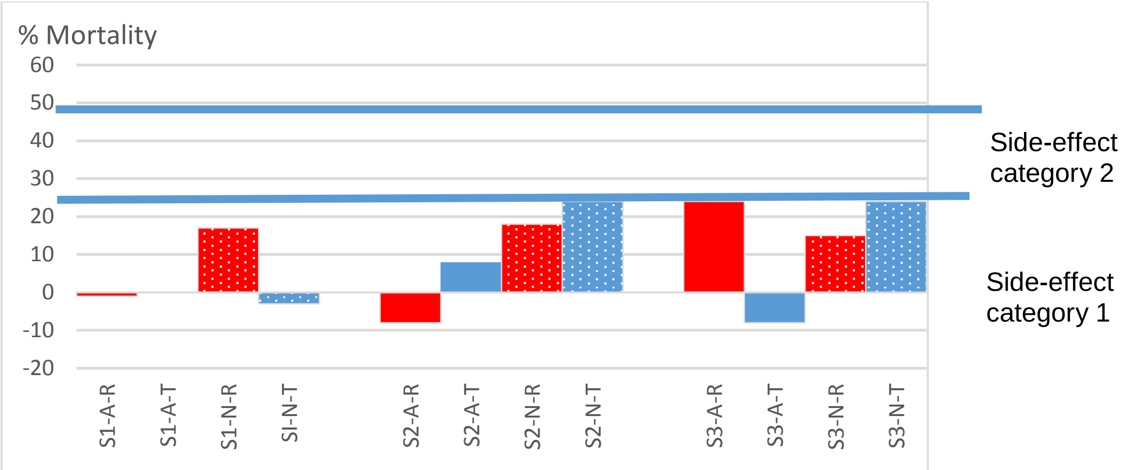


Figure 6.2. Seven days post-treatment with Prolectus

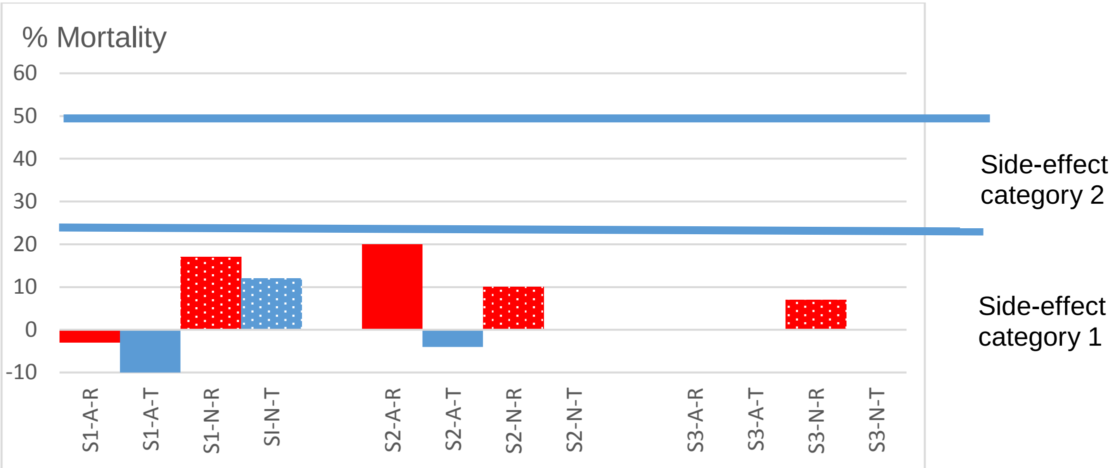


Figure 6.3. Seven days post-treatment with Reflect

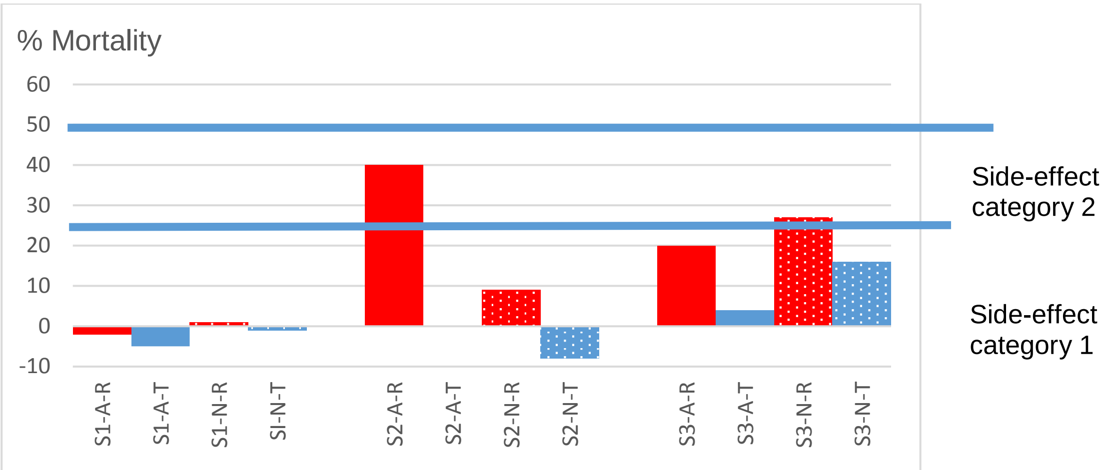
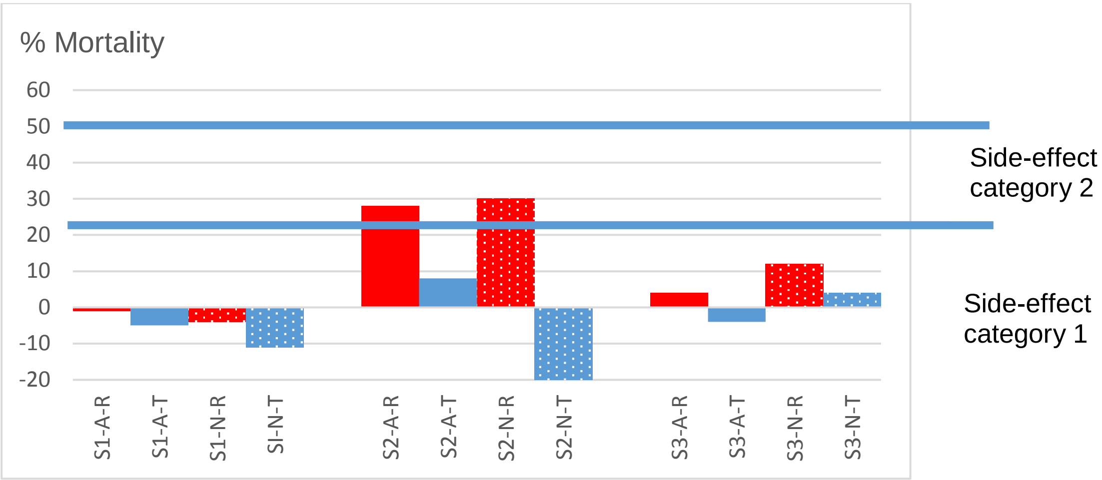


Figure 6.4. Seven days post-treatment with Topas



Part 3: Compatibility of three stains of *Macrolophus pygmaeus* with a systemic application of Conserve

Natural mortality in the supplied populations of *M. pygmaeus* ranged from 41% to 69% for adults and from 7% to 24% for nymphs over 10 days post-treatment in these test runs / replicates. This led to relatively large inherent variation within treatments, which had to be taken into account when processing the data. As a consequence, apparent mortality trends in the treatments were not always statistically significant.

The mean numbers of dead adults and nymphs per replicate, corrected for natural mortality, are shown in Table 6. These data are also presented as percentage mortality compared to the equivalent untreated controls. Adult mortality in test run 1 was 11.9%, 65.2% and 41.6% for strains 1, 2 and 3 respectively but none of these results were statistically significant at the 95% confidence level. Adult mortality in test run 2 was 70%, 68.7% and 56.1% for strains 1, 2 and 3 respectively with results for strains 1 and 2 being significant ($P < 0.01$). Mortality of nymphs in test run 1 was very low at 1.3%, 3.5% and 1.0% for strains 1, 2 and 3 respectively. None of these mortalities were significantly different to the untreated controls. However, mortality of nymphs was greater in test run 2 at 36.9%, 24.2% and 16.2% for strains 1, 2 and 3 respectively with results for strains 1 and 3 being significant ($P < 0.05$).

As previously described, all suppliers of biocontrol material in the UK use a simplified method of expressing side-effects of chemical pesticides on their biological products based on a system originally developed by the IOBC. Table 7 shows the results of the present experiments transferred to a comparable format. Combining the results from both test runs allocates side-effect categories of 1-3, 3 and 1-3 to adults of strains 1, 2 and 3 respectively. Nymphs of strains 1, 2 and 3 fit into categories 1-2, 1 and 1-2 respectively. Given the overlapping nature of the results, the response of the three strains of *M. pygmaeus* to the test treatment must be considered to be similar. Overall, adults fit into categories 1-3 and nymphs into categories 1-2.

Table 6. Mean numbers of dead *Macrolophus pygmaeus* adults and nymphs per replicate, and percentage mortality compared to untreated controls, following systemic application of Conserve.

Life cycle stage	Test run	Strain of *Macrolophus pygmaeus*	Mean number dead per replicate (*i.e.* greater than untreated control)	Percentage mortality compared to untreated control	Statistical significance
Adult	1	1	1.59	11.9%	P = 0.285
		2	4.13	65.2%	P = 0.060
		3	3.33	41.6%	P = 0.476
	2	1	7.00	70.0%	**P = 0.008***
		2	5.50	68.7%	**P = 0.006***
		3	2.34	56.1%	P = 0.108
Nymph	1	1	0.25	1.3%	P = 0.377
		2	0.60	3.5%	P = 0.659
		3	0.17	1.0%	P = 0.100
	2	1	6.75	36.9%	**P = 0.022***
		2	3.20	24.2%	P = 0.238
		3	2.87	16.2%	**P = 0.048***

Table 7. Impact of a systemic application of Conserve on two life cycle stages of three 'strains' of *Macrolophus pygmaeus* expressed in the style of the IOBC side-effects format.

Strain of *Macrolophus pygmaeus*	Life cycle stage	IOBC category range (1-4 scale)
1	Adult	1-3
	Nymph	1-2
2	Adult	3
	Nymph	1
3	Adult	2-3
	Nymph	1
Overall	Adult	1-3
	Nymph	1-2

The variation encountered here is not unique to this experimental situation. Even greater variation is encountered in commercial crops due to condition of biological material, size of plants, type of growing media, rate of uptake of irrigation water, temperature and other environmental conditions. It is therefore important to consider the worse scenario when predicting the impact of chemical pesticides on biological control agents. In this case, growers should anticipate up to 75% mortality of *M. pygmaeus* adults and up to 50% mortality of *M. pygmaeus* nymphs following treatment. This may not be as damaging as it at first seems if considered at the predator population level during the main tomato growing season. At that time, Conserve is usually used as a second line of defence to support the primary biological control agents being used against *Liriomyza byoniae* and *Tuta absoluta* leafminers. Assuming the chemical treatment has greater impact on the pest populations than the biocontrols, then the overall effect should be successful in restoring the balance between pests and predators. It is also important to remember that in an established and growing population of *M. pygmaeus*, over 80% of individuals are likely to be in one of the immature stages upon which the treatment has least effect. Of course, the impact on population growth will be greater if Conserve is applied through the irrigation system at the beginning of the season when *M. pygmaeus* are first released because a much greater proportion of individuals will be adults at that time.

Conclusions

<u>Mating disruption</u>

- The mating disruption product, Isonet-T, when used as supplied by the manufacturers and applied at the maximum recommended rate of 1,000 dispensers per hectare, provides a very effective alternative to Conserve for slowing down *T. absoluta* population growth in the early season while *Macrolophus* predators are becoming established.
- There is a possibility that our use of the mating disruption technique could select for a small proportion of female moths that exhibit parthenogenesis thus compromising this control measure. This is being further investigated.

<u>Compatibility of *Macrolophus* with various pesticides</u>

- There was considerable natural mortality of insects under the artificial test conditions and inherent variation within treatments, which is quite common in these types of experiments.
- None of the treatments caused catastrophic side-effects on any of the strains of *M. pygmaeus* adults or nymphs when the insects were subjected to either topical application or surface residues of HV sprays of NeemAzal, Prolectus, Reflect or Topas, or to systemic application of Conserve via the irrigation system.
- Following the HV sprays, there was no consistent pattern in significant mortality effects, which appeared to be randomly distributed across all *M. pygmaeus* strains, life cycle stages and application methods. Nonetheless, all four pesticides caused a significantly higher mortality hazard than the water-treated control at some stage and it must be concluded that all the products, applied at their label rates, can have some adverse effects on *M. pygmaeus* survival. However, most of the recorded significant effects represented less than 25% mortality of the insects being tested. When compared to the IOBC derived side-effect method of classification, these results would all fit into Category 1, which is designated as ‘harmless or only slightly harmful’. Only four of the treatments would be classified as Category 2, which is designated ‘moderately harmful’, and none of the treatments would fit into any of the higher Categories. Those in Category 2 included three surface residue treatments (*i.e.* Reflect on strain 2 adults, Topas on strain 3 adults and Topas on strain 3 nymphs) and one topical application treatment (*i.e.* Reflect on strain 3 nymphs).
- In the worst case scenarios, Conserve applied via the irrigation resulted in 70%, 68.7% and 56.1% mortality of adult *M. pygmaeus* strains 1, 2 and 3 respectively and 36.9%, 24.2% and 16.2% mortality of nymph strains 1, 2 and 3 respectively. When compared to the IOBC method of side-effect classification, the overall worst case scenarios would therefore be Category 3, designated ‘harmful’, for adults and Category 2 for nymphs.

- These tests have been carried out with care under the described conditions. However, side-effects can vary depending on crops, quality of insects, environmental conditions and specific methods of pesticide application. As a consequence, these results can only be used a guide to the possible side-effects of the tested products. If growers require more precise information about risks to beneficial insects in their crops, then they are advised to organise modified tests that take into account their specific situation.

Knowledge and Technology Transfer

- Jacobson (2017). Regular informal updates to members of the TGA 'Tuta Think Tank' group throughout the period from December 2016 to July 2017.
- Jacobson (2017). Report to TGA Technical Committee meeting 1 March 2017.
- Jacobson (2017). Presentation to members of the Tomato Working Party at Jan Bezemer & Sons Ltd, Stokesley, North Yorkshire on 16 May 2017.
- Jacobson (2017). Report to TGA Technical Committee meeting 7 June 2017.
- Jacobson, Bass & Grant (2017). Article prepared for AHDB Grower journal planned for September 2017 issue.

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