Project title:	Tomato: Phase 3 of contingency plans for the control of *Tuta absoluta*
Project number:	PC 302b
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Report:	Final report, November 2012
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Location of project:	Horticilha Agro-Industria S.A., Portugal. VHB, Runcton. Libraries at Fera and Lancaster, Leeds and York Universities. Statistics Department Warwick University.
Industry Representative:	Mr Philip Pearson Chairman, TGA Technical Committee
Date project commenced:	1 January 2011
Date project completed:	31 December 2012

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The results and conclusions in this report are based on an investigation conducted over a two-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.

Dr Rob Jacobson
Director
RJC Ltd

Signature .... Date ....

Report authorised by:

Mr Paul Howlett
Project Manager
Wight Salads Group

Signature .... Date ....

CONTENTS

GROWER SUMMARY

Headlines

- Nemasys® (*Steinernema feltiae*) provided 40-50% control of *Tuta absoluta* larvae.
- A spinosad containing product controlled *T. absoluta* larvae when applied through the irrigation system of a rockwool-grown crop.
- A UV light trap was shown to be attractive to both male and female *T. absoluta* moths.

Background

Tuta absoluta (Meyrick) was first intercepted in the UK on Spanish imports in March 2009 and there soon followed an outbreak in a commercial crop. The pest has since become established on several sites across the country. The larvae cause extensive damage by mining in leaves and fruit. It is currently considered to be the most important pest of UK tomato crops (Jacobson, 2012). One grower with a nursery in Portugal has been working towards a robust IPM programme for *T. absoluta* since the pest's arrival at their production site in Portugal in 2008. Based on experience gained in Mediterranean countries during 2009 and 2010, a theoretical season-long IPM strategy was designed based on the predatory bug, *Macrolophus* spp. Potentially useful components of that programme have been tested in this project and elsewhere using a 'modular' approach. This has involved testing each module independently to determine whether it is effective against *T. absoluta*, compatible with other components of the whole IPM programme and economically viable. This report describes the modular studies commissioned by HDC in 2010 and completed during 2011 and 2012.

Summary

The overall aim of the project was to develop cost-effective and sustainable IPM strategies for *T. absoluta* that were acceptable within organic tomato production in the UK. Specific technical objectives were to:

1. evaluate entomopathogenic nematodes against *T. absoluta*
2. refine methods of applying products through the irrigation system
3. evaluate combined pheromone and light traps

Evaluation of entomopathogenic nematodes against T. absoluta

A 'proof of concept' trial showed that two species of entomopathogenic nematodes, *Steinernema feltiae* (as Nemasys®) and *S. carpocapsae* (as NemasysC®) were capable of killing *T. absoluta* larvae within 2-6 days of application and producing offspring within dead larvae. However, the rates used (10m infective juveniles / litre), were relatively high and the cost was about seven times greater that of a standard chemical insecticide. There followed further trials to determine which was the more effective of the two nematode species and to establish the most cost-effective application rate. Based on those results, treatment with *S. feltiae* diluted to 1m infective juveniles / litre was carried forward to the next stage of the project.

There were two consecutive trials in a mature 1.17 ha organic tomato crop (cv. Sunstream). *Steinernema feltiae* was applied at dusk at 1m / litre using a Berg self-propelled, robotic sprayer. The unit carried two vertical booms, one spraying to the left of the sprayer, the other to the right. As most of the active *T. absoluta* mines were in the lower two thirds of the canopy, the booms were set up to target this stratum of the crop. The sprayer was calibrated to spray to the point of run off on both sides of the leaves delivering approximately 2,500 litres / ha. There were six monitored plots within the treated area. Within each plot, 40 infested leaflets were marked and monitored over 10 days post-treatment. There was an additional untreated plot in the first trial but not in the second due to the risk of substantial crop damage.

The results of both crop-scale trials are shown in Figure A. There was very little natural mortality. In trial 1, dead *T. absoluta* larvae were seen in the *S. feltiae* treatments three days post-treatment. By day 10, mortality in the plots ranged from 15% to 71% (overall mean 49.8%). At least one nematode was found in each dead *T. absoluta* larvae, thus confirming the cause of death. One plot showed considerably reduced mortality because some nozzles were temporarily blocked. If data from that plot were excluded, then the overall mortality rose to 56%. The results of the second crop-scale trial were similar to trial 1 but overall mortality was lower (range 32% to 53%; mean 40.3%). The environmental conditions for the second crop-scale trial were less favourable than the first trial for nematode activity on the leaf surface; the mean night temperature of 20.6^{o}C and relative humidity of 74% being 3.1^{o}C higher and 9% lower respectively. As a consequence, the spray dried on the leaves more rapidly, restricting the time available for nematodes to find an entrance hole.

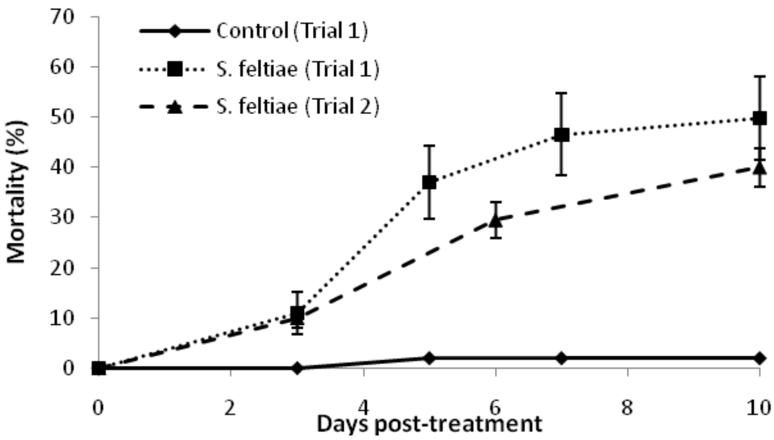

Figure A. The mean percentage mortality of *Tuta absoluta* larvae in infested leaves after crop wide applications of *Steinernema feltiae* using a robotic sprayer.

Spinosad applied via the irrigation system

There were two consecutive trials in a mature 1.28 ha conventional rockwool-grown tomato crop (cv Mecano). The crop was bisected by a central concrete road which created two similar 'plots'. Each plot comprised 80 rows of plants trained in a 'V' formation and there were approx 24,320 heads per plot at the time of the trial. For the first trial, the west side of the glasshouse was the 'treated' plot and the east side was the untreated control. The treated area was served from an irrigation manifold positioned at the end of the central row beside the concrete road. The manifold split the irrigation flow with separate pipe work serving equal crop areas to the north and south. New valves were plumbed into each manifold pipe to connect a Dosatron diluter. The treatment was applied in week 28 2012 with the Dosatron on the 0.5% setting. 775 ml of a product containing spinosad at 120 g/l was diluted in 65 litres of water to provide the 'concentrate' and this was delivered to the plants in a total of 13,000 litres of water over 3 complete irrigation rounds.

Nine formal sample stations were set up throughout each plot to measure efficacy against *T. absoluta*. Each sample station comprised a batch of 10 plant heads giving a total of 90 inspected heads per plot. The top seven expanded leaves (*i.e.* approx 0.6m of growth) of each head were inspected immediately prior to treatment and the numbers of active *T. absoluta* mines recorded. The assessments were repeated in weeks 29, 30, 32 and 33. The size of the *T. absoluta* populations were thus compared between plots and the changes monitored over time. On the same dates, numbers of *Macrolophus* were assessed in three complete rows of plants. The head of every fifth plant was beaten over a white tray and numbers of *Macrolophus* adults and nymphs recorded.

The results in the first trial were extremely clear. There was an average of 14.4 active mines per plant head in the formal sample stations immediately prior to the application of the spinosad product. This declined to 0.1 active mine per plant head one week after treatment. No active mines were found in the sample stations during the subsequent four weeks. In contrast, numbers of active mines per plant head in the untreated control increased from 22.3 pre-treatment to 155.2 one week post-treatment. The untreated control was then deemed to have served its purpose and action was taken to prevent more serious crop damage. Numbers of *Macrolophus* steadily increased from 0.05 per plant head in week 28 to 2.9 per plant head in week 33 in the treated plot.

For the second trial, the east side of the glasshouse became the treated plot. The treatment was applied in week 29 2012 following the same procedure as in the first trial. Numbers of active *T. absoluta* mines and numbers of *Macrolophus* were recorded following the same procedures as described for the first trial. There was no untreated control in this trial and so the changes in size of the *T. absoluta* and *Macrolophus* populations were monitored over time. The results were as clear as the first trial. Numbers of active mines per plant head declined from 155.2 immediately before treatment to 1.7 per plant head one week post-treatment. No active mines were found in the sample stations during the subsequent four weeks. Numbers of *Macrolophus* increased from 0.1 per plant head in week 29 to 1.5 per head in week 33.

Evaluation of UV light traps

A series of four trials in 2.34ha glasshouses assessed the efficacy of different components of a prototype UV light trap and thereby determined the best overall combination. The first trial compared the attractiveness of UV strip lamps with and without diffusers fitted. The second and third trials compared sticky traps with water traps as a means of collecting the moths which were attracted to the lamps. The fourth trial compared the efficacy of the sticky base when orientated vertically and horizontally below the UV lamp. The traps were positioned below the leaf canopy among the bare plant stems. The UV lamps were illuminated for five hours each night with the lit period ending one hour after dawn. The numbers of moths captured was recorded and a sub-sample from each treatment was examined to determine their gender.

The traps performed better without the diffuser fitted to the UV lamp. Sticky traps proved to be more effective than water traps as a means of collecting the moths. There were consistently more adult moths on sticky traps when positioned horizontally than when positioned vertically with the overall mean being 2.4 times greater for the horizontal position.

It was presumed that the horizontal sticky platform provided a more favourable 'roosting' site for the moths. There were similar proportions of males to females in the traps.

Financial Benefits

- *Tuta absoluta* is currently the most important pest of tomato crops in the UK. For example, at one nursery during June and July 2012, 30% of fruit were damaged by the pest and graded out. This represented losses of approximately £50k per hectare to that grower for that period alone.
- The studies described in this report are contributing to the development of a robust control strategy for *T. absoluta* which can be integrated into the existing IPM programme for tomato crops. This will minimise the risk of losses for UK growers.

Action Points

- The entomopathogenic nematodes, *Steinernema feltiae* (as Nemasys®) can contribute to an overall IPM programme by slowing down *T. absoluta* population growth while the primary biological control agents, *Macrolophus*, become established in the crop. Their use in the first half of the growing season could be particularly important in organic crops where the limited number of allowed spinosad treatments can be held back until later in the season when fruit damage becomes a more serious issue.
- A spinosad product provided control of *T. absoluta* larvae when applied through the irrigation system of a rockwool-grown crop. It should be noted that this treatment is based on an EAMU which is only for use with a Plant Health Order specifically for the control of *T. absoluta*. The treatment was compatible with *Macroplophus* predators.
- A UV light trap may form part of the IPM programme and the optimum number of traps per hectare will be established in a later phase of the work.

SCIENCE SECTION

Introduction

Background

Tuta absoluta (Meyrick) was first intercepted in the UK on Spanish imports in March 2009 (Korycinska & Moran, 2009) and there soon followed an outbreak in a commercial crop (Fera, 2009). The pest has since become established on several sites across the country. The larvae cause extensive damage by mining in leaves and fruit. It is currently considered to be the most important pest of UK tomato crops. The team at WSG has been working towards a robust control programme for *T. absoluta* in organic crops since the pest's arrival at their production site in Portugal in 2008. Any control measures developed for organic crops have had a knock-on benefit for integrated pest management (IPM) in conventional tomato crops.

Summary of previous work completed in project PC302

The HDC project began in August 2009 and focused on the development of a short-term solution that could be implemented immediately against *T. absoluta*. WSG were in a unique position in that they had crops in southern Europe that were already infested with this pest and in which efficacy trials could be done on a large-scale. Within 10 months, the project had successfully developed a short term control measure based on high volume sprays of spinosad (as Spintor 480SC®) (Jacobson & Morley, 2010).

Although this provided both organic and conventional tomato growers with a short term solution, there was concern that the treatment may be incompatible with the potentially important biological control agent, *Macrolophus* spp., and that the product may not reach *T. absoluta* larvae feeding within fruits and stems. One possible solution was to apply a formulation of spinosad through the irrigation system. The results of four such treatments using Spintor 480SC® in 2009 and 2010 looked promising and worthy of further investigation (Jacobson & Morley, 2010).

In addition, preliminary studies demonstrated that two species of entomopathogenic nematodes could enter mines and kill *T. absoluta* larvae (Jacobson & Morley, 2010). This also appeared to be worthy of further investigation.

Theoretical season-long control strategy

Based on experience gained in Mediterranean countries during 2009 and 2010, Dr Jacobson designed a theoretical season-long IPM strategy based on the predatory bug, *Macrolophus* spp. (Jacobson, 2011a & 2011b). For the purpose of this strategy, the growing season is divided into four distinct periods:

- The first period, from planting in December until mid-spring, is the key period for establishing *Macrolophus*. Other physical and cultural methods may be employed throughout this period to slow down *T. absoluta* population growth; *eg* aggressive deleafing, sticky floor treatments and mass trapping with pheromone and / or light traps.
- The second period is from mid- to late-spring. Despite the measures taken during the first period to delay *T. absoluta* population growth, it seems inevitable that at least one second line of defence (SLoD) treatment will be required before the predatory bugs start to have a significant impact. In 2010, the only IPM compatible and effective chemical SLoD treatment available to UK tomato growers was a high volume spray application of spinosad (as Conserve®). Biological options included entomopathogentic nematodes and *Bacillus thuringiensis* but both required further evaluation.
- The third period is from early summer through to early autumn. The predatory bugs should now be more numerous and suppress the *T. absoluta* population growth by feeding on eggs and larvae. However, careful monitoring is required to determine whether it becomes necessary to apply additional SLoD treatments.
- The fourth period is from early to late autumn when the main objective is to reduce the number of pests that survive to infest the following crop. This treatment must be effective against *T. absoluta* but need not be IPM compatible as the biological agents have by now served their purpose in that season.

Potentially useful components of this programme have been tested in the HDC project and elsewhere using a 'modular' approach. This has involved testing each module independently to determine whether it is effective against *T. absoluta*, compatible with other components of the whole IPM programme and economically viable (Jacobson & Morley, 2010; Jacobson, 2012). This report describes the modular studies commissioned by HDC in 2010 and completed during 2011 and 2012.

Objectives of project PC 302b

The overall aim of the project was to develop cost-effective and sustainable IPM strategies for *T. absoluta* that were acceptable within organic tomato production in the UK. Specific technical objectives are to:

1. evaluate entomopathogenic nematodes against *T. absoluta*
2. refine methods of applying products containing spinosad through the irrigation system
3. evaluate combined pheromone and light traps

Entomopathogenic nematodes as a SLoD against **Tuta absoluta**

Previous studies had developed techniques for evaluating the effect of entomopathogenic nematodes on *T. absoluta* larvae in tomato leaves. The results showed that both *Steinernema feltiae* and *S. carpocapsae* were capable of killing *Tuta absoluta* larvae within 2-6 days of application and producing offspring within dead larvae. However, the rates used in those preliminary experiments were relatively high and the cost was about seven times greater that of a high volume spinosad treatment. It was recommended that lower application rates of the nematodes be evaluated in small scale glasshouse trials and, if successful, tested on a commercial crop scale.

Spinosad applied via the irrigation system

The previous studies indicated that Spintor 480SC® applied through the irrigation system could control an existing *T. absoluta* population and prevent recolonisation of tomato plants for at least five weeks. However, those experiments used rates of Spintor 480SC® which exceeded the maximum quantity of active ingredient allowed per hectare in the UK.

During 2010 and 2011, the WSG team and the TGA Technical Committee took the initiative to work with Plant Health, the Chemicals Regulation Directorate, HDC and insecticide suppliers with a view to increasing the range and efficacy of the products available to UK growers for the control of *T. absoluta*. On 7 June 2012, an Extension of Authorisation and Consent for a Minor Use of a Plant Protection Product (EAMU) was granted which increased the quantity of a spinosad containing product which could be applied by high volume spray to a tomato crop and this was extended on 5 July 2012 to treatment via the irrigation system but only for use with a Plant Health Order for the control of *T. absoluta*.

Following consultation with the local Plant Health and Seeds Inspector, the newly approved rates of the spinosad product were tested via the irrigation system in a commercial tomato crop which was already heavily infested with *T. absoluta*. The trials and results are described in this report.

Combined pheromone and light traps

Russell IPM had recently developed Ferolite® (a combined pheromone and light trap), which was claimed to attract both male and female *T. absoluta* and thereby enhance pest monitoring techniques and contribute to the control of the pest population. Prototypes were offered to the WSG research team for evaluation in this project. Trials were designed with a statistician and initiated in tomato crops to compare the efficacy of the new product with standard pheromone water traps. There were numerous operating difficulties when the traps were used within commercial tomato crops. Of 15 prototype Ferolite® traps only seven operated correctly and, as a consequence, the trials had to be abandoned. We believe that the design of the Ferolite® traps has been improved since our aborted trials but the modified product was not available in time to test within this project. The WSG team switched their attention to 'home-made' UV light traps and it these trials which are described in the main body of this report.

Materials and methods

Objective 1. Evaluation of entomopathogenic nematodes against T. absoluta

Proof of concept trial

Sprays were applied to infested tomato (cv. Dimple) leaflets both on the plant (Part 1) and removed from the plant in trays (Part 2). There were four treatments; i) untreated control, ii) positive control comprising Spintor 480SC® (spinosad) at 25ml product per 100 litre water, iii) *Steinernema feltiae* (as Nemasys®) diluted to 10m infective juveniles (IJ) / litre, and iv) *S. carpocapsae* (as NemasysC®) diluted to 10m IJ / litre. Treatments were applied at dusk to extend drying time and thus allow the nematodes more time to find entry holes to mines. Sprays were applied to the point of run off with a manual Matabi pneumatic sprayer fitted with a standard lance / nozzle.

Part 1 - For each treatment, 40 leaflets, each containing a medium sized *T. absoluta* larva, were selected and clearly marked. Leaflets were sprayed, collected when dry the following morning, kept in culture boxes with tissue paper refuges at 21+/-2^{o}C and examined daily until larvae were dead or pupated. Cadavers were dissected to determine the presence of nematodes. A sub-sample of cadavers was retained to monitor the nematodes' development.

Part 2 – 160 leaflets, each containing medium sized *T. absoluta* larva, were divided into sets of 40 and each set placed in a plastic tray. One treatment was applied to each tray using the Matabi pneumatic sprayer. Thereafter, the leaflets were treated as in Part 1.

To determine cost effective application rates (March 2011)

The overall approach was the same as the 'proof of concept' trial but based on tomato cv. Sunstream. On this occasion, there were seven treatments in Part 1 and five treatments in Part 2; *i.e.* untreated control, *S. feltiae* diluted to 10m (Part 1 only), 3m and 1m IJ / litre, and *S. carpocapsae* diluted to 10m (Part 1 only), 3m and 1m IJ / litre.

Commercial crop scale evaluations (March and April 2011)

There were two consecutive trials in a mature 1.17 ha organic tomato crop (cv. Sunstream). *Steinernema feltiae* was applied at dusk at 1m IJ / litre using a Berg self-propelled, robotic sprayer. The unit carried two vertical booms, one spraying to the left of the sprayer, the other to the right. As most of the active *T. absoluta* mines were in the lower two thirds of the canopy, the booms were set up to target this area of the crop. The sprayer was calibrated to spray to the point of run off on both sides of the leaves delivering approximately 2,500 litres / ha. There were six monitored plots within the treated area. Within each plot, 40 infested leaflets were selected and subsequently treated the same as in the previous trials. There was an additional untreated plot in the first trial but not in the second due to the risk of substantial crop damage.

For the most part, statistical analysis is informal, and results are presented in graphical form. End-point analysis (days 7, 8 and 10) is used for all the trials, though the cumulative mortality is indicated in the plots.

Objective 2. Spinosad applied via the irrigation system

There were two consecutive trials in a mature 1.28 ha conventional rockwool-grown tomato crop (cv Mecano). The crop was bisected by a central concrete road which created two similar 'plots'. Each plot comprised 80 rows of plants trained in a 'V' formation. There were 170 rockwool cubes per row, initially with two 'plants' taken from each cube (*i.e.* 340 heads). Extra heads had been taken during the season giving approx 24,320 heads per plot at the time of the trial.

For the first trial, the west side of the glasshouse was the 'treated' plot and the east side was the untreated control. The treated area was served from an irrigation manifold positioned at the end of the central row beside the concrete road. The manifold split the irrigation flow with separate pipe work serving equal crop areas to the north and south. New valves were plumbed into each manifold pipe to connect the Dosatron (Figure 1).

Figure 1. Dosatron in position for treatment (image left) and showing attachments to manifold (image right)

The treatment was applied on 11 July (week 28 2012) with the Dosatron on the 0.5% setting. 775 ml of the product (containing spinosad at 120g/l) was diluted in 65 litres of water to provide the 'concentrate' and this was delivered to the plants in a total of 13,000 litres of water over 3 complete irrigation rounds.

Nine formal sample stations were set up throughout each plot to provide an indication of efficacy against *T. absoluta* throughout the whole area. Each sample station comprised a batch of 10 plant heads giving a total of 90 inspected heads per plot. The top seven expanded leaves (*i.e.* approx 0.6m of growth) of each head were inspected immediately prior to treatment and the numbers of active *T. absoluta* mines recorded per head. In addition, other rows were 'walked' to monitor the general level of infestation with particular attention given to the gable ends and corners of the plots (*i.e.* the furthest points from the manifolds). The assessments were repeated in weeks 29, 30, 32 and 33. The size of the *T. absoluta* populations could therefore be compared between plots and the changes monitored over time.

On the same dates, numbers of *Macrolophus* were assessed in three complete rows of plants using a technique developed in HDC Project PC 240 (Jacobson & Morley, 2006). The head of every fifth plant was beaten over a white tray and numbers of *Macrolophus* adults and nymphs recorded. This provided a total sample size of 456 plants per plot per assessment date.

For the second trial, the east side of the glasshouse became the treated plot. The treatment was applied on 20 July (week 29 2012) following the same procedure as in the first trial. Numbers of active *T. absoluta* mines and numbers of *Macrolophus* were recorded following the same procedures as described for the first trial. There was no untreated control in this trial and so the changes in size of the *T. absoluta* and *Macrolophus* populations were monitored over time.

Objective 3. Evaluation of UV light traps

A series of four trials assessed the efficacy of different components of a prototype UV light trap and thereby determined the best overall combination of the components. The first trial compared the attractiveness of UV strip lamps with and without diffusers fitted. The second and third trials compared sticky traps with water traps as a means of collecting the moths which were attracted to the lamps. The fourth trial compared the efficacy of the sticky base when orientated vertically and horizontally below the UV lamp.

Each trial was done in a 2.34ha glasshouse. There were 12 traps in each trial including six replicates of each treatment. The traps were left in situ for two weeks with positions of the treatments being switched at the end of the first week. The traps were positioned below the leaf canopy among the bare plant stems (Figure 10). The UV lamps were illuminated for five hours each night with the lit period ending one hour after dawn. At least two illuminated traps were visible to the human eye from any position in the glasshouse at night.

The numbers of captured adults in each trap were recorded at the end of each week. A sub-sample of approximately 20 moths from each treatment was examined to determine their gender.

Results and Discussion

Objective 1. Evaluation of entomopathogenic nematodes against T. absoluta

The results of the 'proof of concept' trial are shown in Figure 2. There was very little mortality of *T. absoluta* in the untreated controls, while those treated with spinosad (as Spintor 480SC®) died within six days of the spray application. Some dead *T. absoluta* larvae were recorded in the *Steinernema* treatments two days post-application and all were dead by day 6. Although there was no formal replication the results from the two separate trials were very similar with the 'in tray' trial being temporally advanced by 1-2 days relative to the crop trial. Dead larvae dissected on day 4 contained nematodes at a range of development stages with the most advanced being adult females. By day 8, there was evidence of offspring within female nematodes and these had been released into the cadaver by day 10. These results were broadly consistent with the parallel findings of Batalla-Carrera *et al* (2010).

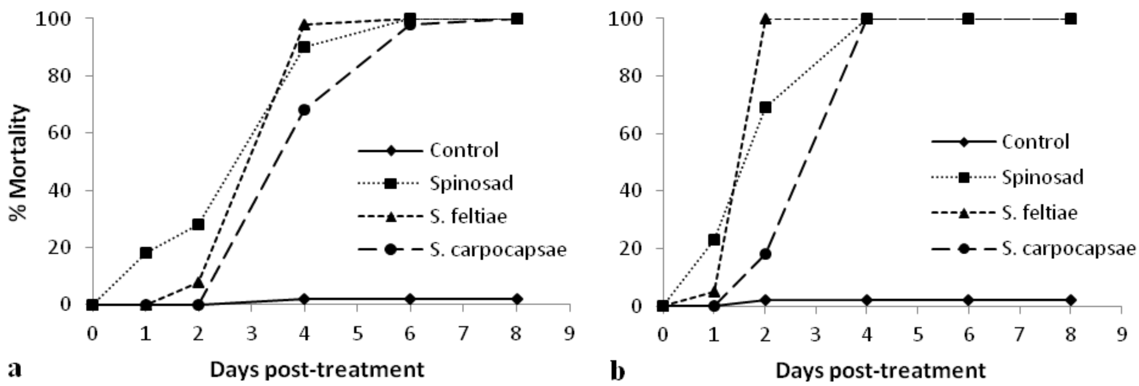

Figure 2. Mortality of *Tuta absoluta* larvae after treatment with Spintor 480SC® (spinosad), *Steinernema feltiae* or *S. carpocapsae* to infested leaves either in a) the crop or b) contained in trays.

The results of the trial to determine a more cost-effective application rate are shown in Figure 3. A probit analysis on the *S. feltiae* data was inappropriate as mortality exceeded 80% on all concentrations, whereas it produced a LD_{50} for *S. carpocapsae*, but 50% at around a dose of 2m IJ / litre is ineffectual. Based on these results, the treatment with *S. feltiae* diluted to 1m IJ / litre was carried forward to the next stage. The cost of 1m IJ / litre at 2,500 litres / ha was approximately 350 Euros / ha, which was comparable to the cost of Spintor 480SC®.

The results of both crop-scale trials are shown in Figure 4. A standard error is presented for each mean (six replicates). In trial 1, very little natural mortality was observed in the untreated controls. Dead *T. absoluta* larvae were seen in the *S. feltiae* treatments three days post-treatment. By day 10, mortality in the plots ranged from 15% to 71% (overall mean 49.8%). At least one nematode was found in each dead *T. absoluta* larvae, thus confirming the cause of death. One plot showed considerably reduced mortality because some nozzles were temporarily blocked. If data from that plot are excluded, the overall mortality rises to 56%.

The results of the second crop-scale trial (Figure 4) were similar to trial 1 but overall mortality was lower (range 32% to 53%; mean 40.3%). The environmental conditions for the second crop-scale trial were less favourable than the first trial for nematode activity on the leaf surface; the mean night temperature of 20.6^{o}C and relative humidity of 74% being 3.1^{o}C higher and 9% lower respectively. As a consequence, the spray dried on the leaves more rapidly, restricting the time available for nematodes to find an entrance hole.

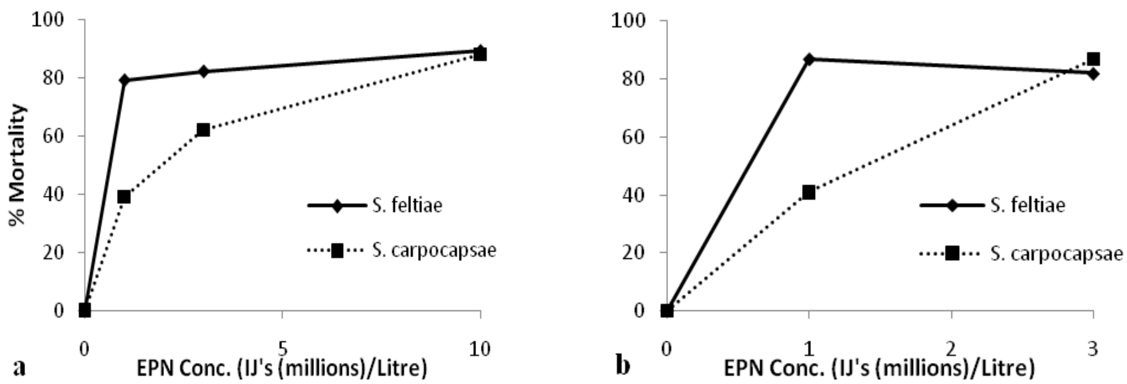

Figure 3. The effect of different concentrations of infective juveniles (IJ's) of the entomopathogenic nematodes (EPN), *Steinernema feltiae* or *S. carpocapsae*, applied to tomato leaves in a) the crop or b) contained in trays on the percentage mortality of *Tuta absoluta* larvae 7 days after treatment.

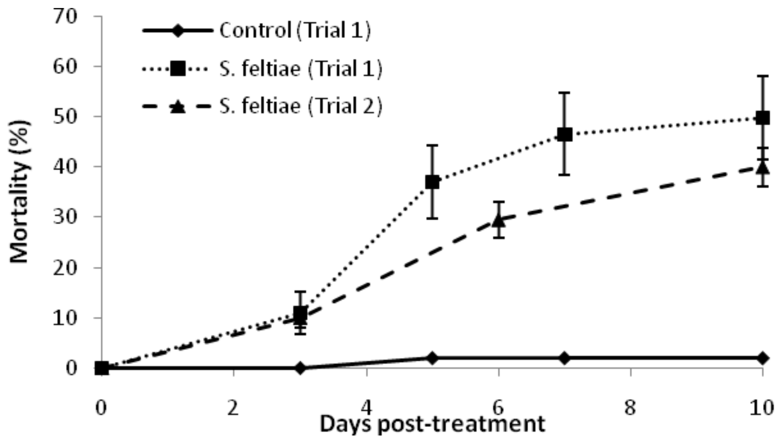

Figure 4. The mean percentage mortality (± 1 S.E.) of *Tuta absoluta* larvae in infested leaf samples taken after crop wide applications of *Steinernema feltiae* using a robotic sprayer.

In warm climates such as Portugal, it seems most probable that nematode treatments will be restricted to the period from mid-Autumn to early Spring. However, there should be more opportunities to use such treatments throughout the cooler UK growing season.

While the level of control with nematodes can probably be further improved by fine tuning the spray technique and by incorporating wetters / spreaders to improve leaf coverage, it seems unlikely that nematodes will become the primary control measure against *T. absoluta*. Nonetheless, we believe that such treatments can contribute to an overall IPM programme by slowing down *T. absoluta* population growth while the primary biological control agents, *Macrolophus*, become established in the crop. Their use in the first half of the growing season will also allow the limited number of allowed spinosad treatments to be held back until later in the season when fruit damage becomes a more serious issue. This will be particularly important in organic crops for which there are no other chemical options.

Objective 2. Spinosad applied via the irrigation system

The results in the first trial were extremely clear and did not require statistical analysis. There was an average of 14.4 active mines per plant head in the formal sample stations immediately prior to the application of the spinosad product. This declined to 0.1 active mine per plant head one week after treatment (Figure 5). No active mines were found in the sample stations during the subsequent four weeks. In contrast, numbers of active mines per plant head in the untreated control increased from 22.3 pre-treatment to 155.2 one week post-treatment (Figure 5). The untreated control was then deemed to have served its purpose and action was taken to prevent more serious crop damage. Numbers of

Macrolophus steadily increased from 0.05 per plant head in week 28 to 2.9 per plant head in week 33 in the treated plot.

Figure 5. Typical condition of sampled leaves in treated (image left) and untreated (image right) plots one week post-treatment in the first trial.

The results in the second trial were equally clear. Numbers of active mines per plant head declined from 155.2 immediately before treatment to 1.7 per plant head one week post-treatment. No active mines were found in the sample stations during the subsequent four weeks. Figure 6 clearly illustrates how the plants recovered from the *T. absoluta* infestation and remained free of the pest during the following four weeks. Numbers of *Macrolophus* increased from 0.1 per plant head in week 29 to 1.5 per head in week 33 in this plot.

Top of canopy – no active mines on new growth

Middle of canopy – no active mines on new growth

Lower canopy – no active mines but showing the damage from the original infestation prior to treatment in week 29.

Figure 6. Example of the crop canopy on the east side of glasshouse four weeks post-treatment (*i.e.* in week 33).

In summary, the product containing spinosad at 120g/l, applied through the irrigation system at the rate of 1.2 litres of product per hectare, effectively controlled established *T. absoluta* populations on two occasions and provided protection from the pest for at least 4-5 weeks. Although there were still no active mines in the formal sample stations at the end of this period, more general crop walking did reveal a few small localised colonies of active mines. While this represented only very small overall populations of the pest, it did indicate that the protection from the spinosad-based treatment was beginning to break down.

Macrolophus numbers increased 58 fold over five weeks in the first trial and 15 fold over 4 weeks in the second trial. This clearly demonstrated that the spinosad product applied via the irrigation was compatible with the predatory bug.

Samples of ripe fruit taken two days post-application of the spinosad product in the first trial were tested for residues of the chemical. They contained between 2% and 18% of the MRL (*i.e.* overall mean of 8.6% of MRL).

Objective 3. Evaluation of UV light traps

The results of the following four trials should be interpreted with caution because there was only limited replication in each trial and statistical analysis was deemed to be inappropriate. Furthermore, direct comparisons between the trials were not possible because each trial was done in a different crop with a different sized *T. absoluta* population. Nonetheless, there were quite clear trends which have proved to be of considerable help in designing UV traps for use in commercial-scale tomato crops.

The mean numbers of moths captured per trap per week in each of the two treatments in the first trial are shown in Figure 7. Approximately 3.4x as many moths were caught without the diffuser present over the UV strip lamp and there were similar proportions of males and females (approx 1:1) in each treatment. Diffusers were not attached to the UV lamps in subsequent trials.

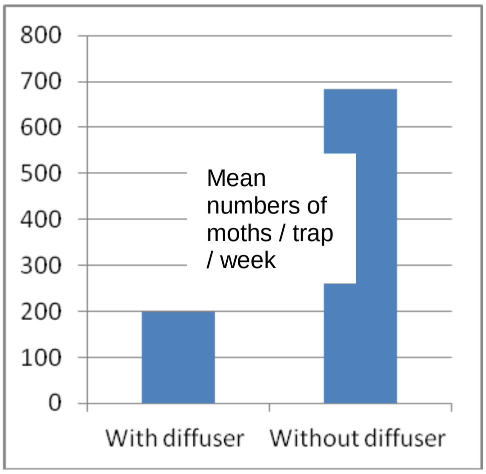

Figure 7. Mean numbers of moths captured per trap per week with and without a diffuser fitted to the UV strip lamp.

The mean numbers of moths captured per trap per week in each of the treatments in the second and third trials are shown in Figure 8. Considerably more adult moths were captured on sticky traps than in water traps regardless of whether the sticky traps were orientated vertically or horizontally beneath the UV lamps. Water traps were not used in any subsequent studies. As in the first trial, there were similar proportions of males and females in each treatment (approx 1:1). Trials 2 and 3 were in different glasshouses with different sized *T. absoluta* populations, so it was inappropriate to make direct comparisons between the results for the vertically and horizontally positioned sticky traps.

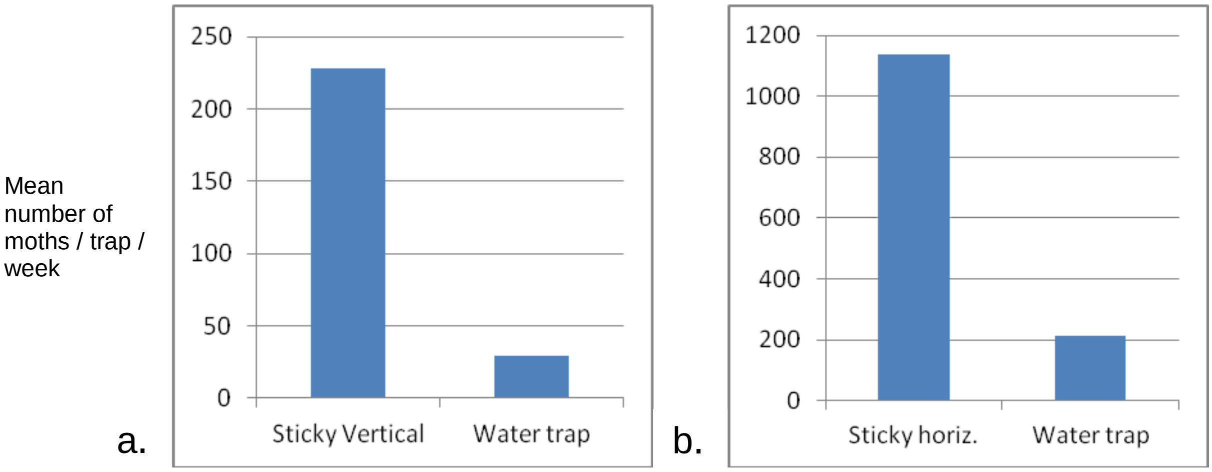

Figure 8. Mean numbers of moths captured per water trap per week compared to mean numbers captured on sticky traps per trap per week when the latter were positioned vertically (a) and horizontally (b) beneath the UV strip lamp.

The mean numbers of moths captured per trap per week in each of the treatments in the fourth trial are shown in Figure 9. There were consistently more adult moths on sticky traps when positioned horizontally than when positioned vertically with the overall mean being 2.4 times greater for the horizontal position. This was presumed to provide a more favourable 'roosting' site for the moths. Once again, there were similar proportions of males and females in each treatment (approx 1:1).

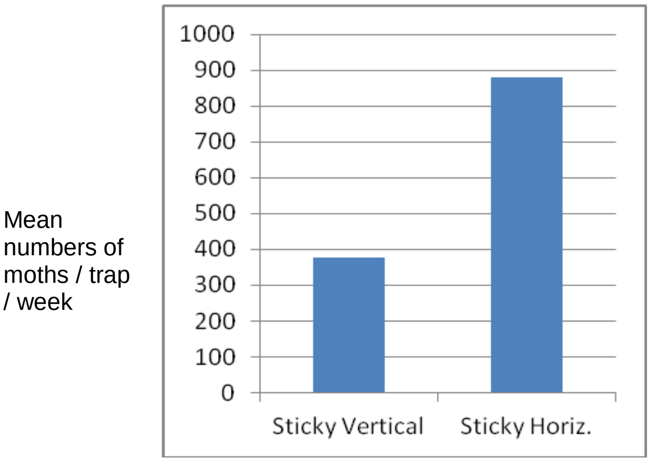

Figure 9. Mean numbers of moths captured per trap per week with the sticky base positioned either vertically and horizontally beneath to the UV strip lamp.

The current trap design, showing the sticky base orientated in the horizontal position, is illustrated in Figure 10. It is possible that a white sticky base may be more attractive to moths than yellow as it would reflect some UV light. This will be tested in a later phase of the work. It is not possible to tell from these results how much impact the traps have on the overall *T. absoluta* population. However, we believe that at least twice as many traps would be required to significantly reduce population growth. This will be taken into consideration in a later phase of the work.

Figure 10. The current UV trap design with the sticky base in the horizontal position.

Conclusions

Three modules of the theoretical season-long IPM strategy for the control of *T. absoluta* were evaluated in this HDC project:

- The entomopathogenic nematodes, *Steinernema feltiae* (as Nemasys®), provided 40-50% control of medium-sized *T. absoluta* larvae when applied to the point of foliar run-off at the rate of 1million infective juveniles per litre. The sprays were applied at dusk to the lower two thirds of the crop canopy using a Berg self-propelled robotic sprayer calibrated to deliver 2,500 litres per hectare.
- Spinosad, which is derived from naturally occurring soil fungi, provided control of *T. absoluta* larvae when applied through the irrigation system of a rockwool-grown crop at the rate of 1.2 litres of product per hectare. The foliage remained free of active mines for 4-5 weeks despite the presence of a declining population of adult moths in the glasshouse. The treatment was applied with a Dosatron diluter plumbed into an irrigation manifold. 775 ml of the product containing 120g/l spinosad was diluted in 65 litres of water to provide the 'concentrate' and this was delivered to the plants in a total of 13,000 litres of water over three complete irrigation rounds.
- A prototype UV light trap was shown to be attractive to both male and female *T. absoluta* moths. The trap consisted of a UV strip lamp (without diffuser) positioned above a horizontal sticky platform. The optimum number of traps per hectare will be established in a later phase of the work.

These findings, along with the results of modular studies completed elsewhere, have allowed the emerging IPM strategy to be refined as follows:

- The first stage, from planting in December until mid-spring, remains the key period for establishing *Macrolophus*. It is now proposed that the pest be allowed to colonise the crop at the start of the season but, following the relevant liaison with Plant Health to permit approval, the spinosad-based product is applied via the irrigation system before the first caterpillars complete their development to aid short term *T. absoluta* population growth. This treatment should be supplemented by physical control methods including deleafing, sticky floor treatments and mass trapping with pheromone and / or light traps.
- The second period is from mid- to late-spring. It is proposed that chlorantraniliprole be the first choice for a second line of defence (SLoD) treatment in conventional crops as it is compatible with *Macrolophus* and introduces different insecticidal chemistry. Application of chlorantraniliprole products is also subject to an EAMU which is only for use with a Plant Health Order. Other options for organic crops include spinosad sprays,

entomopathogenic nematodes and *Bacillus thuringiensis*, depending on the type of damage and type of crop.

- The third period is from early summer through to early autumn. The predatory bugs should now be more numerous and suppress the *T. absoluta* population growth by feeding on eggs and larvae. However, careful monitoring is required to determine whether it becomes necessary to apply additional SLoD treatments.
- The fourth period is from early to late autumn when the main objective is to reduce the number of pests that survive to infest the following crop. It is proposed that indoxacarb (Steward®) is used for this treatment in conventional crops because it brings additional chemistry to the programme and thus further contributes to resistance management.

Variations of this season-long IPM programme are now ready to be tested in commercial crops as complete packages.

Knowledge and Technology Transfer

- Presentation to TGA Technical Committee, 3 June 2009
- Presentation to Tomato Conference 2009 (24 September 2009, Coventry); 'Two more threats: *Tuta absoluta* and *Nesidiocoris tenuis*'.
- Article in HDC News (May 2009, 15); 'A step ahead of the moth from the south'.
- Presentation to Tomato Pest and Disease Seminar (14 January 2010, Stoneleigh); '*Tuta absoluta*: Biology and control'.
- HDC Factsheet (January 2010, 03/10); 'Tomato leafminers'.
- Article in HDC News (March 2010, 14); 'Keep your guard up against exotic threats'.
- Article in HDC News (May 2010, Vol 163, 18-19); 'Research catching up with *Tuta*'.
- PC 302 Project Update to TGA Technical Committee, 2 June 2010
- Invited presentation to Tomato Conference 2010 (September 2010, Coventry); 'Update on *Tuta absoluta* and *Nesidiocoris tenuis*'.
- Article in Horticulture Week (15 October 2010, page 29). 'Study says parasites key to Tuta control'.
- Article in HDC News (November 2010, Vol 168, 26-27); '*Tuta* meets its match'.
- Invited presentation to Tomato Conference 2011 (29 September 2011, Coventry); '*Tuta absoluta*: A realistic IPM approach'.
- Jacobson, R.J. & Martin, G. (2011). A potential role for entomopathogenic nematodes within IPM of Tuta absoluta (Meyrick) on organic tomato crops. *IOBC/WPRS Bulletin*, 68 (1), 71-74.
- Jacobson, R.J. (2011). *Tuta absoluta*: A season-long IPM strategy based on predatory bugs. *EPPO/IOBC/FAO/NEPPO International Symposium on management of Tuta absoluta (tomato borer).* Agadir, Morocco, 16-18 November 2011. 33-34
- Article in HDC News (September 2011, Vol 176, 26-27); 'Fortified defences against tomato moth'.
- Article in Horticulture Week (14 October 2011, page 30). *Tuta absoluta* options move closer.
- Martin, G. & Jacobson, R.J. (2011). IPM of Tuta absoluta; A potential role for entomopathogenic nematodes in tomato production. "*Advances in Biocontrol*". Grantham. 30 November 2011. P25
- Presentation to TGA Technical Committee, 15 February 2012
- Article in *TGA Newsletter* (July 2012. 5-7). *Tuta absoluta*: A season-long IPM strategy based on predatory bugs.
- Article in *TGA Newsletter* (August 2012. 5-7). More weapons in the armoury against

Tuta absoluta

- Presentation to TGA Technical Committee, 5 September 2012
- Invited presentation to Tomato Conference 2012 (27 September 20121, Coventry); *Tuta absoluta*: Another piece in the IPM jigsaw.
- Presentations to spray operators on NRoSo courses in Kent (9 October 2012), Evesham (23 October 2012) and Chichester (24 October 2012). ' Introduction to the biology and control of the new tomato pest – *Tuta absoluta*.

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Jacobson, R.J. and Morley, P (2006). Organic tomato: Development and implementation of a robust pesticide-free IPM programme. Horticultural Development Company Annual report for project PC 240, December 2006, 70pp

Jacobson, R.J. and Morley, P (2010). Organic tomato: Phase 1 of contingency plans for the control of *Tuta absoluta* and *Nesidiocoris tenuis.* Horticultural Development Company Final report for project PC 302, July 2010, 51pp.

Jacobson, R.J. (2011a). *Tuta absoluta*: A realistic IPM approach. In: *Abstracts, HDC/HRA 2011 Tomato Conference, Coventry, 29 September 2011* (ed J. Wooley), 1-15

Jacobson, R.J. (2011b). *Tuta absoluta*: A season-long IPM strategy based on predatory bugs. EPPO/IOBC/FAO/NEPPO International Symposium on management of Tuta absoluta (tomato borer). Agadir, Morocco (16-18 November 2011)

Jacobson, R.J. (2012). *Tuta absoluta*: More pieces in the IPM jigsaw. In: *Abstracts, HDC/HRA 2012 Tomato Conference, Coventry, 27 September 2012* (ed G. Choto), 101-114.

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