Project title:	Organic tomato: Phase 1 of contingency plans for the control of *Tuta absoluta* and *Nesidiocoris tenuis*
Project number:	PC 302
Project leader:	Dr P Morley, Wight Salads Group
Report:	Final report
Key staff:	Project Manager - Dr R Jacobson Agronomic input - Dr P Morley, Dr P Challinor, Mr P Howlett, Mr J Pettersson, Mr P Townsend, Mr R Alves Biometrics - Mr J Fenlon, Warwick University Interface with PHD/PHSI – Dr H Moran (early stages of project)
Location of project:	Horticilha, Cilha Queimada, Alcochete, Portugal Wight Salads Group, Arreton, Isle of Wight, PO30 3AR RJC Ltd, Bramham, W. Yorks, LS23 6TH
Project coordinator:	Mr P. Pearson, Chairman, TGA Technical Committee
Date project commenced:	1 August 2009
Date project completed (or expected completion date):	31 July 2010
Key words:	Tomato, organic production, *Tuta absoluta*, *Nesidiocoris tenuis*, IPM, spinosad, natural pyrethrins, entomopathogenic nematodes, pheromone traps, *Bacillus thuringiensis*, *Trichogramma achaeae*

The results and conclusions in this report are based on investigations conducted over a four-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 P Morley
Senior Company Agronomist
Wight Salads Group

Signature ....Philip S Morley........... Date...19th July 2010.........................

Dr R Jacobson
Director
Rob Jacobson Consultancy Ltd

Signature ......R J Jacobson...... Date ......19 July 2010......

Report authorised by:

Dr P Challinor
Technical Director
Wight Salads Group

Signature.... PF Challinor.................... .. Date ....19th July 2010...........

CONTENTS

HEADLINES

- High volume sprays of spinosad provide a short-term solution to *Tuta absoluta* infestations.
- Entomopathogenic nematodes offer a biological alternative to spinosad.

BACKGROUND AND EXPECTED DELIVERABLES

Tuta absoluta and *Nesidiocoris tenuis* are becoming increasingly important pests in southern Europe. They are extremely damaging and are currently controlled in those countries by intensive applications of chemical pesticides. However, such products are not compatible with biological control agents and would threaten the continued use of IPM if adopted in UK tomato crops. Organic crops are particularly vulnerable to these pests because growers would not be allowed to use synthetic pesticides and retain organic status, even under PHSI instruction. It was therefore vital that contingency plans were prepared for the control of both pests.

When the concept note for this project was presented to the TGA Technical Committee and HDC PC Panel in June 2009, *Tuta* had already been detected in several UK tomato pack houses. By the time the project began in August 2009, it had been found in a conventional tomato crop in Essex and PHSI had implemented an intensive control programme. The project was split into three phases, with Phase 1 focusing on the development of a short-term solution that could be implemented immediately against *Tuta*. 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. In addition, a desk study was prepared for Phase 2 by identifying potentially useful IPM compatible products to be tested against *Nesidiocoris*. This was completed in December 2009 and will be incorporated into the Phase 2 report.

Preliminary desk studies and small-scale trials at WSG's nursery in Spain in 2009 indicated that high volume sprays of spinosad could control *Tuta* larvae within the leaf and could provide the short-term solution. This insecticide is derived from naturally-occurring soil fungi and is therefore allowed in organic growing systems. Larger scale trials were subsequently done in 12 hectares of crops at the WSG nursery in Portugal following the general approach that had been successfully developed in HDC project PC 240. This approach immediately identified any important interactions with current agronomic practice and eliminated the need for an additional exploitation phase to transfer the technology to the commercial situation.

In addition to the work on spinosad, the team recorded and collated information relevant to the use of pheromone traps and biological control. This knowledge will pave the way towards longer-term more sustainable control measures that may be further investigated in Phase 3.

SUMMARY OF THE PROJECT AND MAIN CONCLUSIONS

Use of Tuta absoluta pheromones:

Female *Tuta* produce a sex attractant pheromone which has been synthesised and utilised as a very effective lure in sticky traps, often detecting males before there is any other evidence of the pest in the crop. Water traps fitted with lures are also effective but it is more difficult to sort the catch. The lures have a limited life and we may expect them to continue releasing pheromone for 4-6 weeks in the UK. The initial release is relatively large and this is reflected in the size of the catch. Thereafter, the release declines progressively and the size of the catch may follow the same pattern. Each time the lure is replaced there will be a surge in both the quantity of pheromone released from the trap and the size of the catch even if the size of the insect population remains constant. As a consequence, the data collected from the traps can be misleading when used to monitor population trends and effects of insecticidal treatments. Our solution was to have multiple traps, replace them in sequence and then average the counts.

Although the pheromones are highly selective, it can not be assumed that all the moths caught are *Tuta*. At least three other species of moths have been found in traps; *i.e. Blastobasis lignea* and two species of *Brytropha* spp. While none of these species are pests, it is difficult to separate them from *Tuta* and their presence could result in misleading monitoring results.

Considerable research has been carried out in southern Europe on methods of monitoring the pest with pheromone baited traps. However, the results have been varied and more work is required to establish treatment thresholds. In particular, researchers must be consistent when reporting pheromone dose rates so that comparisons can be drawn between separate studies.

The sensitivity of the male moth to the pheromone means that there is potential to control the pest using mating disruption techniques. However, a definitive paper on this subject has stated that “there is a need to obtain the synthetic sexual pheromone at much lower cost in order to obtain a commercial product that meets the agro-economic needs of the end-user”.

Monitoring Tuta absoluta populations in WSG crops:

Tuta were consistently caught on traps around the outside of the Portuguese site from planting to termination of the crops. The numbers caught appeared to be influenced by infestations in local potato crops and by weather conditions. The results demonstrated that the crops were under constant invasion pressure and all control measures were given a very severe test.

All eight organic blocks were fitted with screened roof ventilators and double entrance doors to reduce pest invasion while the four conventional crops had unscreened ventilators and doors. The benefits of screening the glasshouses were very clear. Significant numbers of moths were caught within the unscreened glasshouses from week 46 (2009) and treatments were applied from week 48. In contrast, very few moths were caught in the screened glasshouses until week 8 (2010) and no treatments were applied before week 10.

Notes on biology and behavior of Tuta absoluta:

At the start of this project, our knowledge of *Tuta* was based on data from warmer climates and there was little information of direct relevance to the UK. By collating data, we estimated that the life cycle would take between 3 and 6 weeks. However, there were many gaps in our knowledge and a spin-off project (HDC Project PE 002) was encouraged to generate relevant life cycle data.

It was noted that there were few mines in the top 0.8 m of the plant canopy, which represented about 2-3 weeks of new growth. It is possible that eggs were laid on those leaves but, due to the time to hatch, the mines were not seen until the leaves were lower down the plant. The nursery adopted an aggressive deleafing policy resulting in only 5-6 weeks of leaf growth remaining on the plant. This removed many mines in which *Tuta* larvae had not yet completed their development and there was little doubt that this slowed the pest's population growth. However, this practice could also have an adverse effect on the biological control of whiteflies.

Evaluation of high volume sprays of spinosad against Tuta absoluta:

It was difficult to interpret the results of early plant scale experiments because many larvae evacuated the leaves following treatment and it was impossible to determine whether they had survived or died. The worst possible scenario was that the treatment had driven larvae from the leaves and they had moved into fruit, thus increasing the risk of damaged produce reaching the retail customer. Techniques were developed to overcome this experimental difficulty.

Using two approaches, spinosad (as Spintor 480SC at 25 ml per 100 litres water) was applied to the point of run-off to leaves containing *Tuta* larvae. The effect was compared to untreated controls over eight days. By day four, most of the larvae had moved out of the untreated mines and by day eight most had pupated. In the spinosad treatments, some mortality was noted the day after the leaves were sprayed but the majority of larvae succumbed to the chemical between two and four days post application. Approximately half died within the leaves while the rest had moved out into containers which were used to retain them. All were dead by day six.

In addition to that formal study, we took every opportunity to gain more information about the use of spinosad on a commercial crop scale and the impact that the treatment would have on *Tuta* at the population level. In week 10 (2010), a crop was treated with Spintor 480SC at 25 ml product per 100 litres water using the robotic sprayer. The spray was applied to the point of run-off using approximately 2,500 litres of diluted spray per ha. No active mines were found in the crop for two weeks following treatment. Thereafter, the population gradually increased but no further treatments with spinosad were deemed to be necessary in that block until week 19.

Spinosad applied through the irrigation system:

The above results showed that high volume sprays of spinosad would provide the short-term solution to *Tuta* infestations that had been sought by this project. However, there was concern that the treatment may be incompatible with the primary biological control agents, *Macrolophus* and *Nesidiocoris*. One possible solution was to apply spinosad through the irrigation system.

The results of four such treatments were monitored in rockwool-grown crops. In each case, the irrigation was turned off at mid-day to allow the plants to partially dry out the growing medium and the treatment was applied at dusk. The product (Spintor 480SC) was diluted in a spray tank (500 ml per 400 litres water) and pumped through valves in the irrigation manifolds. Following treatment, the pipes were flushed with normal irrigation water. The irrigation system was then turned off until 11.00 am the following day to avoid flushing the product out of the growing

medium. In all cases, there was a rapid decline during the first two weeks post-treatment, which was followed by a period of at least three weeks when there were very few active mines.
These results indicate that this is a potentially useful method of applying spinosad in hydroponic crops. However, the system has yet to be tested in soil grown crops. Furthermore, we know little about the uptake of the chemical and its subsequent transport to the aerial parts of the plant. It is recommended that further work be done to monitor the speed with which uptake occurs, the parts of the plant the chemical reaches and the possible risk of residues being found in fruit.

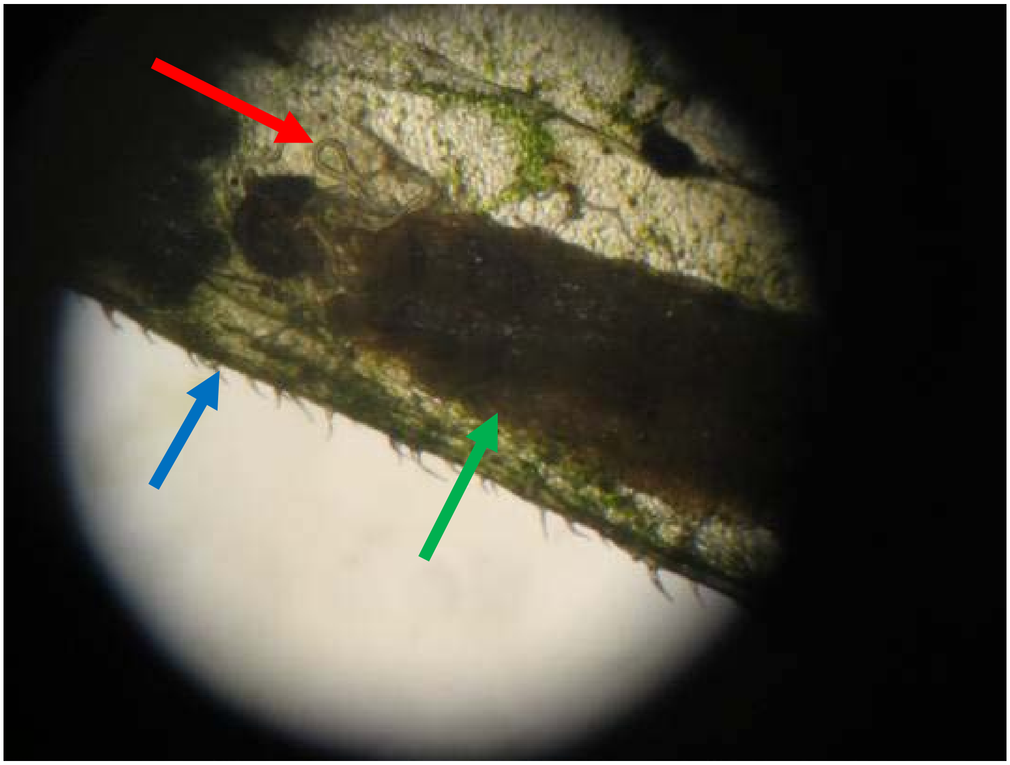
Evaluation of entomopathogenic nematodes against Tuta absoluta:

This study evaluated two species of entomopathogenic nematodes, *Steinernema feltiae* and *S. carpocapsae*, against *Tuta* larvae in tomato leaves*.* The efficacy of the nematodes was compared to an untreated control and to spinosad.

Leaves containing medium sized *Tuta* larvae were sprayed to the point of run-off taking care to cover upper and lower surfaces. Treatments were applied during the last 90 minutes of daylight to slow the rate of drying and allow the nematodes more time to find entry holes to mines. The dry leaflets were then placed in plastic boxes with tissue paper refuges and examined at daily intervals until dead or pupated. On each date, a sample of dead larvae was dissected to determine whether nematodes were present inside the cadaver.

There was very little mortality in the untreated controls, while those treated with spinosad died within six days of application. The first dead *Tuta* larvae were seen in the *Steinernema* treatments two days after application of the spray and all were dead by day 6. 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. A photographic record of the effect of the treatments is shown in the series of images given below. Further studies are required to determine the efficacy and cost effectiveness of the nematodes when used in a commercial cropping situation.

Photograph taken through leaf two days after application of *S. feltiae* showing:



Blue arrow – leaf edge

Green arrow – *Tuta absoluta* larva

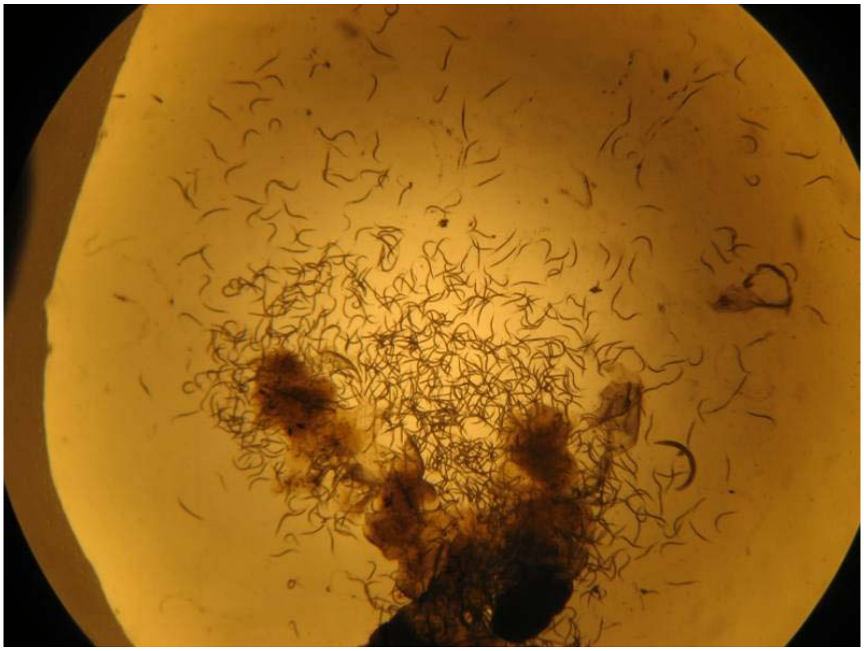
Red arrow – nematode having located larva within mine

Dead *Tuta absoluta* larvae two days after application of nematodes in mine (above) and removed from mines (below)

Nematodes emerging from dissected dead *Tuta absoluta* larva four days after application to leaves on plants. The most advanced were now mature females (red arrow)

Eight days after application of *S. feltiae* to leaflets on plants showing offspring within an adult female nematode

Nematodes spilling out of a dissected dead *Tuta absoluta* larva ten days after treatment



Other biocontrols

While the main objective of this project was to develop a short-term solution that could be implemented immediately against *Tuta*, the authors also took the opportunity to record and collate information relevant to biological control of the pest.

Nesidiocoris is emerging as the most effective biological control agent in the Iberian peninsular. It is a voracious predator feeding on *Tuta* eggs and larvae as well as many other species of pests. *Nesidiocoris* also feed on tomato plants and the authors have first hand experience of the serious damage that can occur to growing points and trusses after invertebrate pests have been controlled. The latter is being addressed in Phase 2 of this project.

Macrolophus pygmaeus is closely related to *Nesidiocoris* and is the more common species in natural vegetation in northern Spain and France. They feed on immature stages of *Tuta* and can provide some control of the pest in the absence of harmful insecticide residues. The related species, *Macrolophus caliginosus*, has become established on many of our tomato nurseries, and currently offers the best option for biological control in the UK.

An egg parasitoid, *Trichogramma achaeae*, is effective against *Tuta* and has out-performed other *Trichogramma* species in Europe and South America. Large numbers of these parasitoids are reported to have controlled the pest in a series of Spanish trials. However, most practitioners would require more substantive data before risking commercial crops.

Several surveys in Mediterranean countries have sought alternative natural enemies with potential against *Tuta.* In addition to the species mentioned above, two parasitoids (*Necremnus artynes* and *Hemiptarsenus zilahisebisi*) have been detected on *Tuta* larvae but it remains to be seen whether they have value as biological control agents. Perhaps most surprisingly, the predatory mite, *Amblyseius swirskii*, has been seen feeding on first instar *Tuta* larvae.

Bacillus thuringiensis (*Bt*) will kill *Tuta* larvae under experimental conditions but its success is dependant upon contact / ingestion by the pest. In theory, the larvae should only collect a lethal dose when outside the mine, so the most vulnerable stage should be the free living first instar. In practice, later instars may be vulnerable if they move between leaves. Nonetheless, the product would have to be applied at regular intervals to have an impact on the pest population and this has led to considerable debate about its cost effectiveness. Although no formal trials were done with *Bt* in this project, the product was used in several blocks and we constantly looked for beneficial effects. The only occasion that cadavers were found with typical symptoms of death by *Bt* were following application of the product in a tank mix with Neem. It is possible that the Neem drove the larvae out of the leaf where they came into contact with the *Bt*. This approach is worthy of further investigation.

ACTION POINTS FOR GROWERS

- High volume sprays of spinosad provide a short-term solution to *Tuta absoluta* infestations in organic tomato crops. Trials in Portugal used Spintor 480SC sprayed to run-off at 25 ml per litre. Two products containing spinosad are available in the UK; Conserve (120 g/litre) and Tracer (480 g/litre). Conserve has approval for tomato (MAPP 12058).
- The following control measures have potential and should be investigated in more detail:
    - Application of spinosad through the irrigation system.
    - Application of spinosad as a tank mix with natural pyrethrins.

- Efficacy and cost-effectiveness of *Steinernema feltiae* and *S. carpocapsae* on a commercial crop scale.
- Efficacy and cost-effectiveness of the egg parasitoid, *Trichogramma achaeae*.
- Use of *Bacillus thuringiensis* in tank mixes with other products which encourage the *Tuta* larvae to emerge from mines.

FINANCIAL BENEFITS

If larvae of *Tuta* are detected inside tomato fruit by retailers, then the produce will be rejected and it is highly likely that further supplies from that source will be put on hold until the grower can provide assurance that the infestation has been completely controlled. It will be very difficult for the grower to find another outlet for that produce at short notice and this could result in very large quantities of produce being dumped. The financial loss could be over £300k per hectare depending on the time of year that the infestation is first detected.

Effective control measures will minimise such losses in organic tomato crops. Furthermore, new IPM compatible control measures will have knock-on benefits to conventional tomato production, particularly to those growers attempting ‘pesticide free’, and will therefore be advantageous to the whole UK tomato industry.

SCIENCE SECTION

1. Monitoring *Tuta absoluta* populations

Background

Female *Tuta absoluta* produce a sex attractant pheromone which has been synthesised and utilised as a very effective lure in traps (Salas, 2004). Even at low population densities and often before there is any other evidence of this pest in the crop, male moths may be caught in sticky traps fitted with pheromone lures (Figure 1). Water traps fitted with lures (Figure 2) are also effective but it is more difficult to sort the catch.

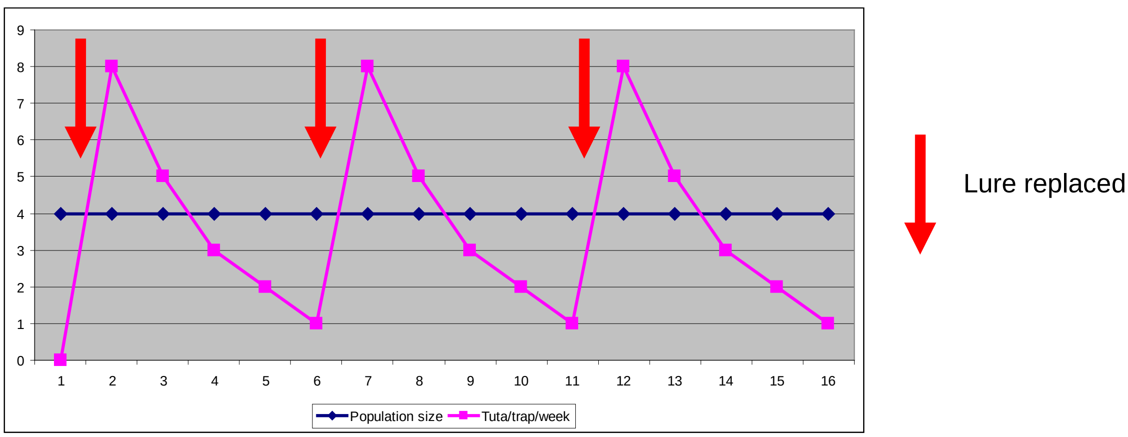
Figure 1. Typical sticky trap and lure containing sex pheromone

Figure 2. Typical water trap with pheromone lure

The lures have a limited life but the rate of release of the pheromone depends on temperature. In the UK, we may expect a lure to continue to release pheromone for 4-6 weeks while this is more likely to be 3-4 weeks in Mediterranean countries (Alzaidi, Russell IPM, pers.com., Oct

2009). However, the release rate is not even over that period. Figure 3 shows a hypothetical scenario over a 16 week period with the lure changed at five week intervals. The initial release is relatively large and this is reflected in the size of the catch. Thereafter, the release declines progressively and the size of the catch may be expected to follow the same pattern. Each time the lure is replaced there will be a surge in both the quantity of pheromone released from the trap and the size of the catch even if the size of the insect population remains constant. As a consequence, the data collected from the traps can be misleading, particularly if it is being used to monitor population trends and the effects of insecticidal treatments. Our solution to this problem was to have multiple traps in any given area, replace them in sequence and then use an average of the counts from all the traps.

Figure 3. Hypothetical fluctuations in trap catches with a constant *Tuta absoluta* population (latter expressed in arbitrary units)



Although the pheromones are highly selective, it can not be assumed that all the moths caught in traps are *Tuta absoluta*. At least three other species of moths have been found in traps; *i.e. Blastobasis lignea* and two species of *Brytropha* spp. (Korycinska, Fera, pers.com. August 2009). While none of these species are pests, it is difficult to separate them from *Tuta absoluta* and their presence could result in misleading monitoring results.

Considerable research has been carried out in southern Europe on methods of monitoring the pest with pheromone baited traps (Jacobson & Morley, 2010). However, the results have been varied and much more work is required to establish reliable treatment thresholds. In particular, researchers must become more consistent in their reporting of pheromone dose rates so that comparisons can be drawn between the results of separate studies.

The sensitivity of the male moth to the pheromone means that there is potential to control the pest using mating disruption techniques. However, relatively large quantities of pheromone are needed to cause sexual confusion and it is very expensive to synthesise. In a definitive presentation on the subject, Marti (2010) stated that "there is a need to obtain the synthetic sexual pheromone at much lower cost in order to obtain a commercial product that meets the agro-economic needs of the end-user".

Al-Zaidi (2010) described a new 'lure and kill' technique based on the combined use of the pheromone sex attractant and cypermethrin. This system is said to be effective in suppressing the pest population and reducing damage but, at the time of writing, it was still to be fully tested in large-scale commercial production units. Al-Zaidi also described a new patented mass trapping device which incorporated the sex pheromone to attract males and a solar powered light to attract females. The device was said to more than double the rate of capture of normal pheromone traps. This trap, which will be marketed by Russell IPM as Ferolite, was not available for testing during the course of this project.

Outside the glasshouses

Four traps were positioned around each of two sets of glasshouse blocks (named 'Old Site' and 'New Site') just before the new tomato crops were planted at Horticilha (Cilha Queimada, Alcochete, Portugal) in week 35 2009. They were examined weekly until the end of the growing season (week 23 2010). Lures were sourced from Russell IPM and Koppert bv depending on price and availability. With the benefit of the knowledge gained during the project, we now realise that we should have maintained a consistent supply of a similar product. This will be addressed in any future trials.

Figures 4 and 5 show that *Tuta absoluta* were consistently caught on traps positioned around the outside of both New and Old sites between weeks 35 2009 and 23 2010. The numbers caught appeared to be influenced by infestations in local potato crops and by the weather conditions (the latter will affect both pheromone distribution and flight of male moths) during the trapping period; for example, up to week 3 2010, low catches occurred during periods of wet weather and high catches coincided with local potato crops being harvested. The gradual increase from week 13 onwards was probably due to the population building in local vegetation through late spring and into early summer.

The results clearly demonstrated that the crops were under constant invasion pressure and all the control measures reported in this document were given a very severe test.

Figure 4. Numbers of male *Tuta absoluta* caught per trap per week outside the New Site from week 35 2009 to week 23 2010 (mean of 4 traps)

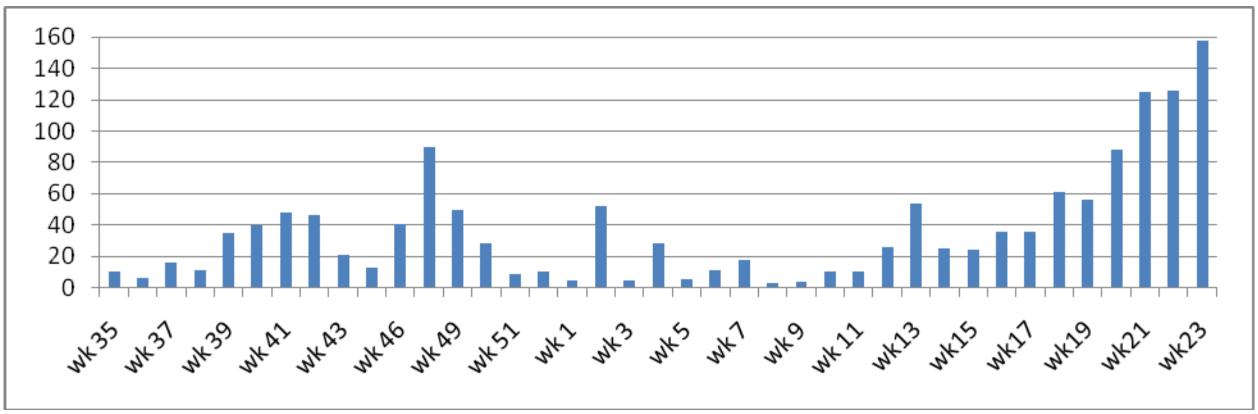
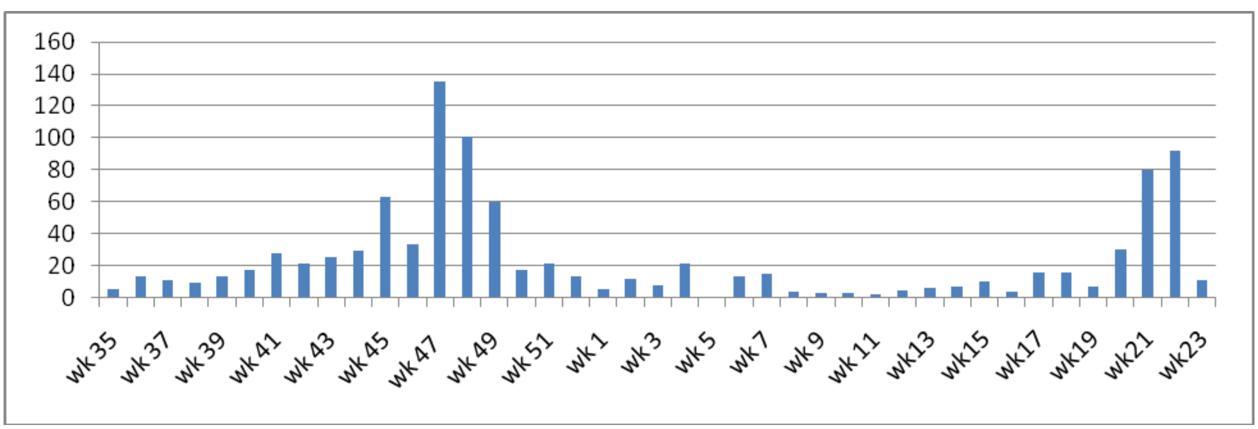


Figure 5. Numbers of male *Tuta absoluta* caught per trap per week outside the Old Site from week 35 2009 to week 23 2010 (mean of 4 traps)



Pest exclusion from glasshouses

All the organic crops (*i.e.* blocks 1 and 5 Old Site and all New Site) were fitted with screened roof ventilators and double entrance doors (Figure 6) in an attempt to prevent pest invasion. The conventional crops (*i.e.* blocks 2, 3, 4 and 6 Old Site) had normal unscreened roof ventilators and doors. Figures 7 and 8 show the numbers of male *Tuta absoluta* caught per trap per week inside the glasshouses of the Old Site between week 37 2009 and the end of the crops in 2010.

Figure 6. Examples of screened roof ventilators and double entrance doors fitted to blocks growing organic crops. Note the outward facing fans which help to prevent insects flying into the central compartment when the outer door is open.

Figure 7. Numbers of male *Tuta absoluta* caught per trap per week in the unscreened conventional blocks of the Old Site between week 37 2009 and week 21 2010 (mean of 3-6 traps per block).

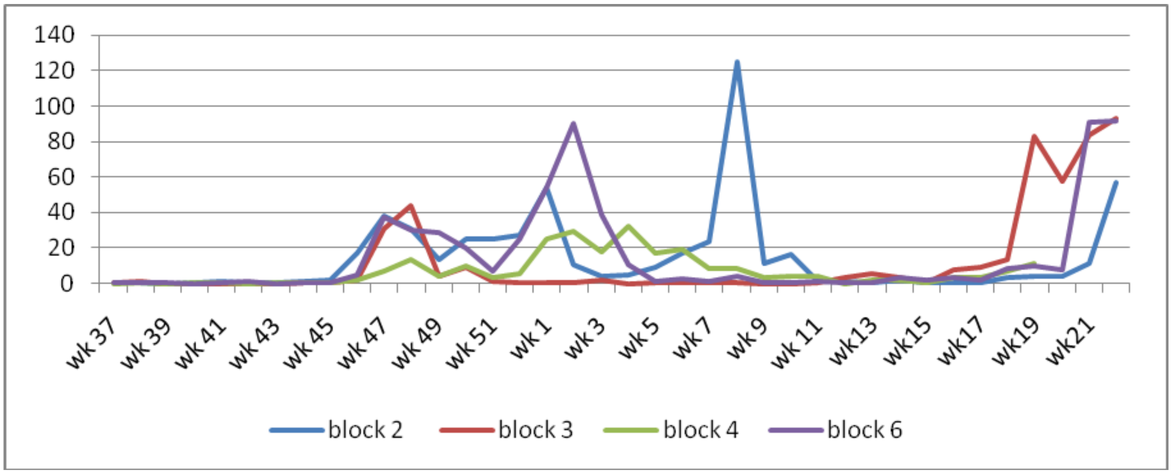
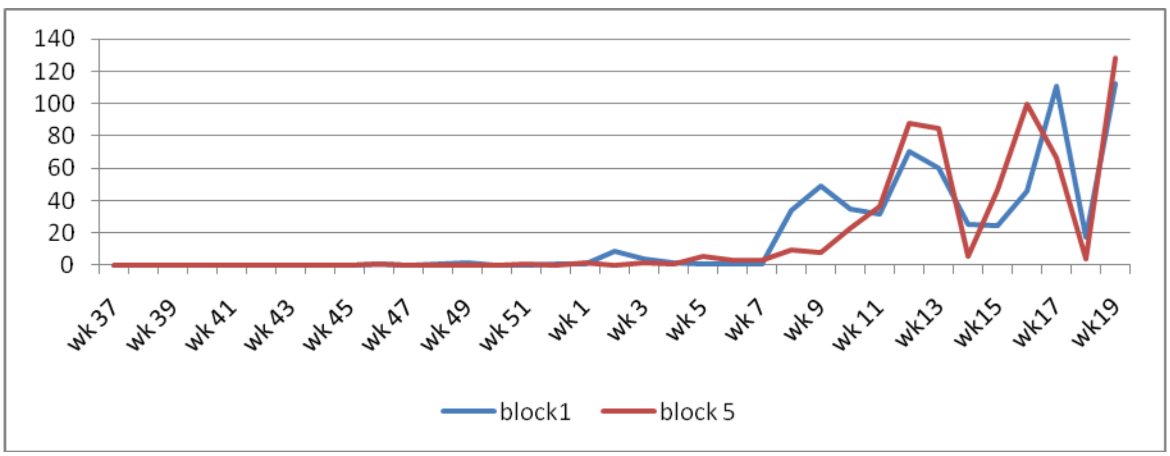


Figure 8. Numbers of male *Tuta absoluta* caught per trap per week in the screened organic blocks of the Old Site between week 37 2009 and week 19 2010 (mean of 3-6 traps per block).



The benefits of screening the glasshouses to exclude *Tuta absoluta* are immediately apparent. Significant numbers of moths were caught within the unscreened glasshouse from week 46 2009 and the first treatments were applied in week 48 2009. In contrast, very few moths were caught in the screened glasshouses until week 8 2010 and no treatments were applied against *Tuta absoluta* before week 10.

General observations about *Tuta absoluta* development and habits:

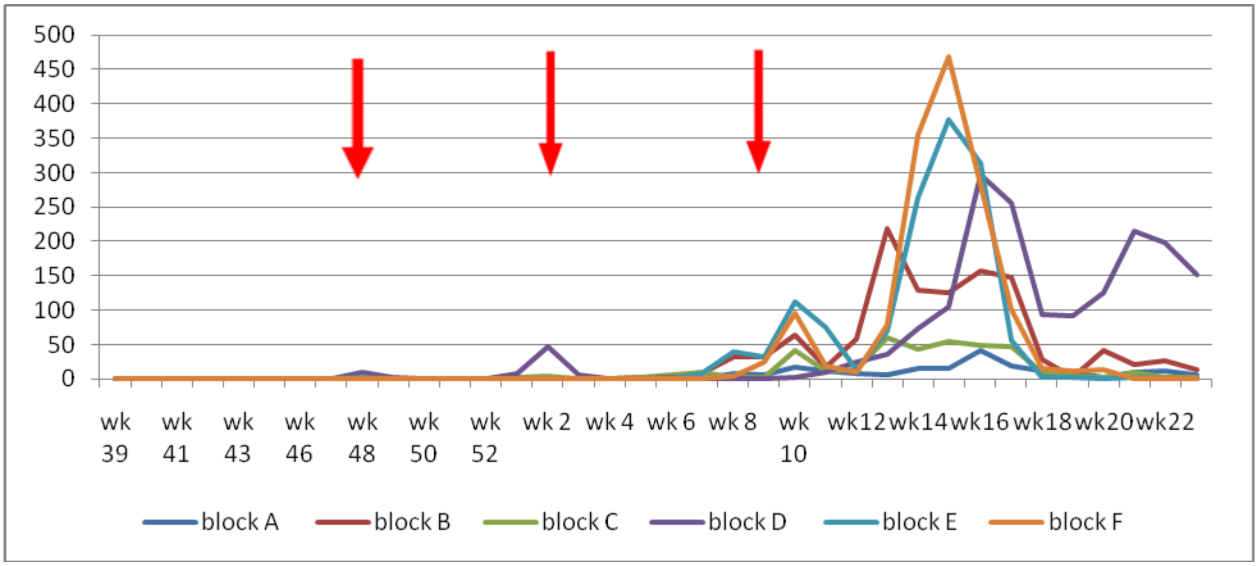
Life cycle times

At the start of this project, three key documents had been produced in English which each summarised available information about *Tuta absoluta* (*i.e.* European and Mediterranean Plant Protection Organization, 2006; Korycinska & Moran, 2009; van der Straten, 2009). Much of the knowledge was based on observations / trials data generated in South America and, to a lesser extent, Mediterranean countries. There was little irrefutable information which was directly relevant to the UK. By extracting and collating data from those three documents we estimated the following life cycle times for autumn - winter conditions in central Portugal, which are broadly similar to spring – early summer conditions in the UK:

- Adults live 6-22 days)
- Eggs hatch in 4-8 days) Hence the life cycle could
- Larvae feed for 11-17 days) take from 3 to 6 weeks
- Pupal stage 7-9 days)

The trap monitoring data from New Site between week 39 2009 and week 10 2010 appears to support these data (Figure 9). These glasshouses are screened and are not subject to mass invasion from outside. Therefore, moths that are caught on the traps are likely to have developed from a very small number that survived within the glasshouse from the previous crop. The monitoring results appear to show peaks in the numbers of adult moths at about 5 – 6 week intervals. Thereafter, the generations lose synchrony and population growth becomes further confused by the application of control treatments.

Figure 9. *Tuta absoluta* population trends measured by pheromone baited sticky traps in the six screened organic crops at New Site. The red arrows show possible population peaks at about 5 – 6 week intervals (mean of 2-4 traps per block).



This situation may be comparable to UK tomato crops from planting in December to late March when it is highly unlikely that any moths would fly into the greenhouse from outside due to ambient weather conditions. Nonetheless, it was recognised that there were many gaps in our knowledge of the basic biology of *Tuta absoluta* and a spin-off project (HDC Project PE002) was encouraged to generate life cycle data that would be directly relevant to UK growing conditions.

Position of mines on plants in crop canopy

Up to week 11, it was noted that there were rarely any mines in the top 0.7 m - 0.8 m of the plant canopy, which represented about 2-3 weeks of new growth (Figure 10). It is possible that eggs were being laid on the upper leaves but, due to the time to hatch, the mines were not seen until those leaves were lower down the plant.

Figure 10. Example of recently deleafed crop (block 3 – week 4 2010). This image illustrates where the active mines were commonly found in the crop canopy and how a proportion were removed when the crop was aggressively deleafed.

Horticilha had an aggressive deleafing policy resulting in only about 5 - 6 weeks of leaf growth remaining on the plant (Figure 10). This removed a proportion of mines in which *Tuta absoluta* larvae had not yet completed their development. There was little doubt that this practice slowed the pest's population growth and assisted the overall control programme.

If aggressive deleafing is to be adopted as part of an IPM strategy against this pest, then it is important that the removed leaves are taken from the glasshouse and destroyed as soon as possible. The consequences of failing to do so were clearly illustrated in block 2 between weeks 6 and 8 2010. There had been an area of several bays where there had been a significant number of mines on plants in weeks 4 to 6. Many of those leaves were removed as part of the aggressive deleafing policy but were left on the floor. Between weeks 6 and 8, adult moths could be found sheltering within this material having recently emerged from pupae. They could also be found in the lower crop canopy and this practice was probably responsible for the peak of adult moth activity recorded on sticky traps in block 2 in week 8 (Figure 5).

Growers should also be reminded that such an aggressive deleafing strategy could remove parasitised whitefly scales before adult *Encarsia* spp. have emerged and thereby have implications on biological control of that pest.

Behaviour of Tuta absoluta following treatment with pesticides:

It was difficult to interpret the results of several plant scale experiments with *Tuta absoluta* because the larvae evacuated the leaves following treatment and it was not possible to determine whether they had survived or died. The worst possible scenario was that the treatment had driven the larvae from the leaves and they had moved into fruit, thus increasing the risk of damaged produce reaching the retail customer. Techniques are described in the remainder of this report to overcome this experimental difficulty.

2. Evaluation of spinosad against *Tuta absoluta*

Introduction

Preliminary desk studies and small-scale trials at WSG's nursery in Spain in 2009 indicated that high volume sprays of spinosad could control *Tuta absoluta* larvae within the leaf. This insecticide is derived from naturally-occurring soil fungi and is therefore allowed in organic growing systems. In the past, there has been difficulty interpreting the results of some plant scale experiments with *Tuta absoluta* because the larvae evacuated the leaves following treatment and it was impossible to determine whether they were alive or dead. In this trial, we applied sprays to infested leaves both on the plant (Part 1) and removed from the plant in lined trays (Part 2). In all cases, the treated leaves were removed from the original position one day after treatment and stored in plastic boxes during the assessment period.

Materials and methods

The study was based in a mature organic tomato crop (cv Dimple [mini-plum]) which had been planted in block 5 in August 2009 at Horticilha, Cilha Queimada, Alcochete, Portugal.

There were two treatments:

- Spinosad (as Spintor 480SC) applied at the rate of 25 ml per 100 litres water

- Untreated control

The sprays were applied to the point of run-off using a manual Matabi pneumatic sprayer with a 15 litre tank and fitted with a standard lance / nozzle.

Part 1

For each treatment, 40 leaflets were selected which each contained a medium sized larvae (Figure 11). The petioles were marked with coloured tape so that they could be easily identified post-treatment (Figure 12). The marked leaves were sprayed and examined the following morning (*i.e.* approximately 18-20 hours later) when the larvae were classified as:

- Alive in mine
- Dead in mine
- On surface of leaf
- Missing from leaf

The dry leaflets were collected from the crop, placed in plastic boxes with tissue paper refuges and incubated at 21 ^{o}C +/- 3 ^{o}C. They were examined at daily intervals until dead or pupated.

Figure 11. Typical mine selected for each treatment

Figure 12. Treated plot showing the 40 marked leaves

Part 2

80 leaflets infested with medium sized *Tuta absoluta* larvae were collected from the crop 6 hours prior to treatment and stored at 11°C until the time of use. They were divided into two sets of 40 and each set placed in a lined plastic tray (Figure 13). One treatment was applied to each tray using the Matabi pneumatic sprayer. The leaflets were left in situ in the trays until the following morning. Each leaflet was then examined and the larvae were classified as described in Part 1. Leaflets still containing live larvae were placed in plastic boxes with tissue paper refuges and incubated at 21 °C +/- 3 °C. They were examined at daily intervals until dead or pupated.

Figure 13. An example of the experimental arenas used in Part 2

Results and Discussion:

Table 1 shows the proportion of *Tuta absoluta* larvae that were i) still in the mine, ii) on the surface of the leaf or iii) missing from each of the four treatments the day after the sprays were applied.

A greater number of *Tuta absoluta* larvae evacuated the leaves following the application of the three treatments, which was consistent with previous observations. Where those leaves remained on the plants, the larvae were lost and had to be discounted from subsequent assessments. However, where the leaves were in trays, the larvae were recovered and progressed to the next stage of the experiment.

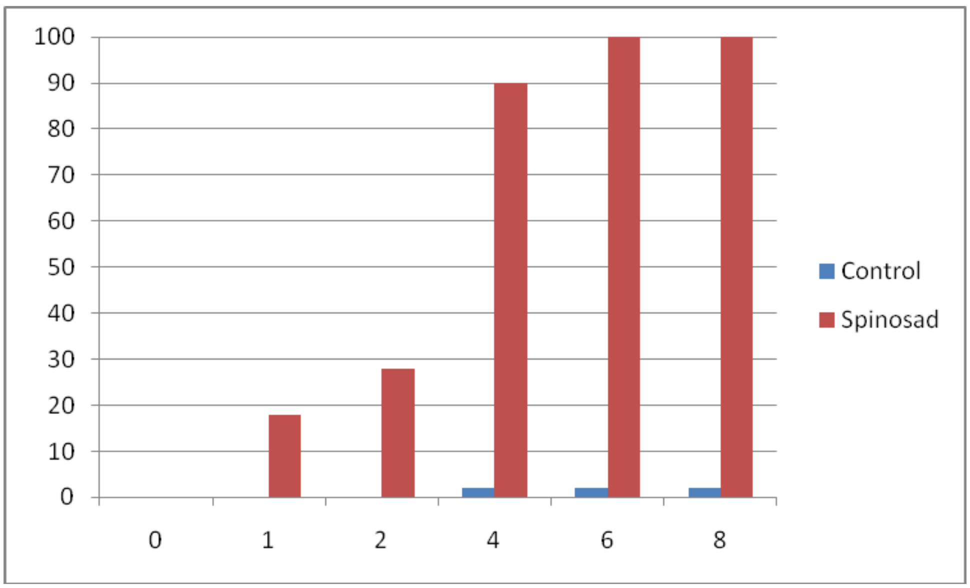
Table 1. *Tuta absoluta* larvae recorded as in mine, on leaf surface or missing one day after application of treatments

Treatment	Tray or plant	*Tuta absoluta* larvae:		
		in mine	on leaf surface	missing
Control	Tray	38	2	0
	Plant	39	1	0
Spinosad	Tray	32	8	0
	Plant	33	0	7

Part 1

The percentage mortality of *Tuta absoluta* larvae recorded 1, 2, 4, 6 and 8 days after application of the two treatments to leaves on plants are shown in Figure 14. There was very little mortality in the untreated controls. By day four, most of the larvae had moved out of the untreated mines and were present on the surface of the leaves. They began to pupate 6 days post-treatment and by 8 days most had entered this life cycle stage.

Figure 14. Percentage mortality of *Tuta absoluta* larvae over eight days following treatment of leaves on plants with spinosad.



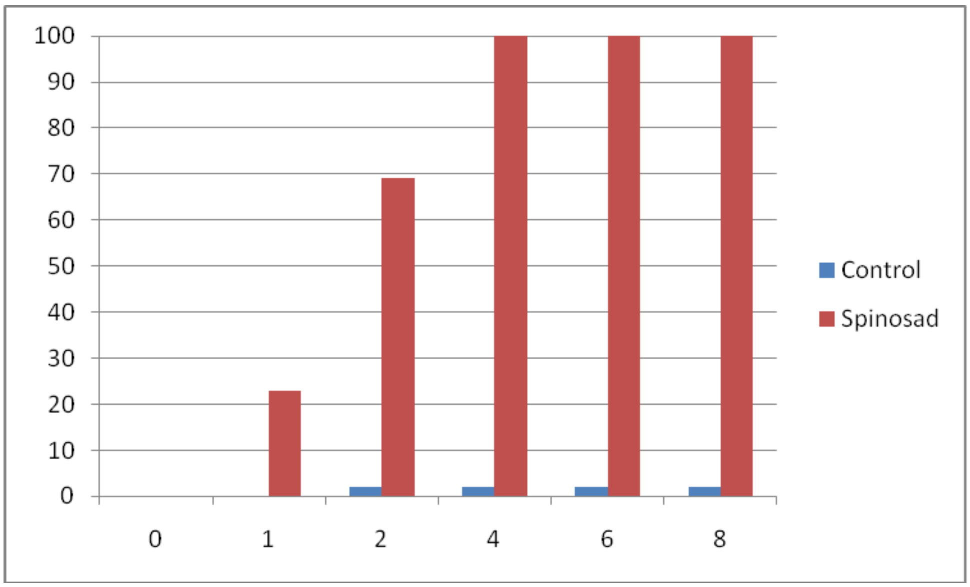
In the spinosad treatment, some mortality was noted the day after the leaves were sprayed and many other larvae appeared to be unhealthy. However, the majority succumbed to the chemical 2 to 4 days post application. Approximately half died within the leaves while the rest had moved out into the plastic container. All were dead by day 6. The treatment effect was so clear that statistical analysis was deemed to be unnecessary.

Part 2

The percentage mortality of *Tuta absoluta* larvae 1, 2, 4, 6 and 8 days after application of the two treatments to excised leaves are shown in Figure 15. As in Part 1, there was very little mortality in the untreated controls. By day four, most of the larvae had moved out of the untreated mines and were present on the surface of the leaves. They began to pupate 6-8 days post-treatment.

The effect of spinosad on *Tuta absoluta* larvae was more rapid on these excised leaflets than when the leaflets remained on the plants during treatment. This slightly enhanced effect was probably due to more spray remaining on the leaves and a greater quantity of the chemical penetrating the plant tissue. As in Part 1, the treatment effect was so clear that statistical analysis was deemed to be unnecessary.

Figure 15. Percentage mortality of *Tuta absoluta* larvae over eight days following treatment of excised leaves in trays with spinosad.



3. Observations on commercial crop scale spinosad treatments

Introduction

The experiment reported previously showed that a foliar application of spinosad would kill *Tuta absoluta* that were present in mines at the time of treatment but they took up to six days to die. In addition to that formal study, we took every opportunity to gain more information about the use of spinosad on a commercial crop scale and the impact that the treatment would have on *Tuta absoluta* at the population level. This involved using up to four different methods of monitoring the pest population before and after application of the treatment. This approach also provided valuable information about the robustness of those monitoring procedures.

We monitored the effect of high volume spray applications of spinosad in a logical progression from the smaller scale experiment and the results showed that spinosad did have the potential to control the *Tuta absoluta* population on a commercial crop scale. However, there was concern that it may be incompatible with the primary biological control agents, *Macrolophus caliginosus* and *Nesidiocoris tenuis*. In a previous trial in 2009, numbers of *Nesidiocoris tenuis* were reduced by 30% following treatment with spinosad (Verissimo, Horticilha, pers.com., March 2009). Other reports suggested that the effects on populations of both predators could be even more devastating (Knight, Koppert, pers.com., November 2009). One possible solution to this problem was to apply spinosad through the irrigation system. The results of four such treatments are reported below.

It must be borne in mind that all these spinosad treatments were superimposed upon normal agronomic practice and upon an integrated control programme for all the other pests and diseases that attacked the crops. As a consequence, there were additional factors which could have contributed to the suppression of *Tuta absoluta* post-treatment:

- The plants were aggressively deleafed, thus reducing the proportion of *Tuta absoluta* larvae that could complete their development on the plants.
- Soft soaps (as Savalim) and *Bacillus thuringiensis* (as Bactil) were applied as necessary against other pests.
- Numbers of *Nesidiocoris tenuis* were very low throughout both sites towards the end of 2009 but typically rose to about 0.5 / plant in week 3 and about 5 / plant between weeks 10 and

13. Thereafter, the population was culled as necessary with natural pyrethrins (as Pyrethrum 5EC).

Pest monitoring procedures

Pest populations are routinely monitored at Horticilha using techniques which were originally developed for the management of whiteflies. This project was able to draw on the results of those routine pest monitoring practices:

- **Presence / Absence:** This technique is used to monitor the early stages of pest establishment. There are about 48 sample points in each hectare block and each comprises the length of row between support posts (*i.e.* approximately 36 plants). All plants are scanned weekly and the presence of pests is recorded. This technique is particularly useful for monitoring low levels of pest infestation.
- **Population trends:** This technique is used to monitor the population trends of established pests. There are seven sample stations per hectare block and each comprises six plants. At weekly intervals, the plants are examined and all pest / beneficial species are counted and the numbers entered onto charts for ease of interpretation. This technique is particularly useful for measuring population growth and the impact of pest control actions.

In addition, two new techniques were introduced to specifically monitor *Tuta absoluta*, populations:

- **Trap counts:** Weekly counts of *Tuta absoluta* on 2 - 6 pheromone baited sticky traps per block. The traps were changed in sequence at four week intervals. Mean numbers of male moths per trap per week per block were calculated.
- **Marked leaves:** Five leaves which each contained a medium sized *Tuta absoluta* larvae (*eg* Figure 11) were selected in each of 8 crop rows giving a total of 40 assessment points. The petioles were marked with coloured tape so that they could be easily identified post-treatment (*i.e.* similar to Figure 12). The marked leaves were checked daily over the following 7 days recording whether the insects were alive, dead or missing.

Robustness of monitoring procedures

While pheromone baited sticky traps are an excellent means of detecting adult moths at very low levels of infestation, we have not found them to be particularly helpful when monitoring population trends before and after insecticidal treatments. One possible reason be uneven release of pheromone from lures over time. The trap catches may also be influenced by immigration of moths from outside the glasshouse and / or by emergence of moths from pupae on the floor or other unsprayed parts of the glasshouse. Whatever the reason, the trap catches did not consistently reflect our observations of pest activity on plants.

The “marked leaf” technique may have been expected to be the most robust method of monitoring the effect of an insecticidal treatment. However, this technique did not provide conclusive results. Over all the large scale trials that used this technique, we only found dead bodies within about 3% of the marked leaves. Typically, between 65% and 88% of the mines would be empty by day 4 and the remaining larvae would successfully complete their development. The results of the smaller scale experiment would indicate that the missing larvae would have died but we couldn’t make any assumptions about their fate.

As a consequence, our assessment of the high volume and irrigation treatments was largely based on the nursery’s own routine pest monitoring procedures.

Spinosad high volume spray treatment

In week 10 2010, all plants in block 5 of the Old Site were treated with Spintor 480SC at 25 ml product per 100 litres water using the robotic sprayer (Figure 16). The spray was applied to the point of run-off using the equivalent of approximately 2,500 litres of diluted spray per hectare.

Overall, the mean number of *Tuta absoluta* mines per plant was quite small (0.4 / plant) prior to treatment. However, the spray was applied because numbers of active mines were greater (2.5 / plant) in a localised area of the crop and some fruit damage had been seen.

No active mines were found in the crop for two weeks following treatment. Thereafter, the population gradually increased but no further treatments with spinosad were deemed to be necessary in this block until week 19. This was considered to be a successful treatment.

Figure 16. Robotic sprayer as used for high volume applications of spinosad

Spinosad applied through the irrigation system

Spinosad was applied through the irrigation systems in blocks 3, 6, 2 and 4 in weeks 48 2009, 51 2009, 3 2010 and 3 2010 respectively. In each case, the irrigation was turned off at mid-day to allow the plants to partially dry out the rockwool growing medium and the treatment was applied at dusk. The product (Spintor 480SC) was diluted in a spray tank (500 ml per 400 litres water). The irrigation system in each hectare block was divided into four sections, with each having a separate manifold. 100 litres of diluted product was applied through each manifold (Figure 17). There were 2.7 drippers per m^2 and so, assuming even application, there should have been 14.8 ml of diluted product applied through each dripper. Following treatment, the system was flushed with normal irrigation water. The irrigation system was then turned off until 11.00 am the following day to avoid flushing the product out of the growing medium.

The mean number of male moths caught / trap / week / block and the percentage of monitoring sites with active mines during the three week period prior to and the five week period after treatment with spinosad via the irrigation system are shown in Table 2.

Figure 17. Showing (left to right) the spray tank / pump and the attachment to the irrigation manifold.

There was no correlation between the size of the trap catches and the infestations on the plants. Furthermore, there was no consistent response in numbers of moths caught on traps after the spinosad treatments; in some cases numbers suddenly declined while in other cases they increased.

However, the percentage of monitored sites with active mines present was both consistent and explicable (Table 2). In all cases, there was a steady increase leading up to the treatment, a rapid decline during the first two weeks post-treatment, followed by a period with very few mines for at least three weeks.

These results indicate that this is a potentially useful method of applying spinosad in conventional hydroponic crops. However, the system has yet to be tested in soil grown organic crops. Furthermore, we know very little about the uptake of the chemical through the roots and its subsequent transport to the aerial parts of the plant. It is recommended that further work be done to monitor the speed with which the uptake occurs, the parts of the plant the chemical reaches and the possible risk of residues being found in fruit.

Table 2. The mean number of male moths caught / trap / week / block and the percentage of monitoring sites with active mines during the three week period prior to and the five week period after treatment with spinosad via the irrigation system.

Week number relative to treatment	Treatment 1 (block 3 – wk 48)		Treatment 2 (block 6 – wk 51)		Treatment 3 (block 2 – wk 3)		Treatment 4 (block 4 – wk 2)	
	Mean trap count	% monitored sites with infestation	Mean trap count	% monitored sites with infestation	Mean trap count	% monitored sites with infestation	Mean trap count	% monitored sites with infestation
-3	-	-	30	29	27	10	6	-
-2	1	6	29	66	54	2	25	17
-1	31	15	20	70	10	30	29	44
0	44	19	7	79	4	88	18	70
+1	4	6	25	14	5	58	32	15
+2	9	2	54	0	9	0	17	2
+3	1	0	90	0	17	0	19	2
+4	1	0	39	0	23	0	8	-
+5	0.5	0	10	0	125	0	8	-

Combined treatment with spinosad and natural pyrethrins

Towards the end of the growing season, a tank mix of spinosad (as Spintor 480SC at 25 ml / 100 litres water) and natural pyrethrins (as Pyrethrum 5EC at 2.4 / l00 litres water) was applied to all plants in block F of the New site in an attempt to reduce numbers of both *Tuta absoluta* and *Nesidiocoris tenuis*. The sprays were applied with the robotic sprayer (Figure 16) to the point of run-off delivering 2,991 litres / hectare. Similarly infested plants in block D provided an untreated control. Following treatment, it was noted that the panels of the sprayer were covered with many dead bodies of *Tuta absoluta* adults (Figure 18). This had not been seen following high volume treatments of Spintor 480SC alone and we had previously assumed that the product was not particularly effective against the adult moths. However, the present observation suggests that Pyrethrum EC alone, or in combination with Spintor 480SC, will provide very rapid knockdown of *Tuta absoluta* adults.

Figure 18. Dead *Tuta absoluta and Nesidiocoris tenuis* on body of the robotic sprayer after application of Spintor 480SC and Pyrethrum 5EC

.

Approximately 12 hours after the tank mix treatment, it was also noted that the majority of partially developed *Tuta absoluta* mines contained very unhealthy or recently killed caterpillars. This was in sharp contrast to previous trials with Spintor 480SC alone, where usually more than half of the *Tuta absoluta* larvae had evacuated the mines overnight.

Previous studies have shown that *Tuta absoluta* larvae take a few days to die following an application of spinosad. However, it would appear that when this product is used in a tank mix with Pyrethrum 5EC, the larvae become incapacitated before they can exit the mine. It is possible that this is due to the rapid knockdown effect of Pyrethrum 5EC. Natural pyrethrins in the Pyrethrum 5EC formulation are not normally considered to penetrate leaves but must do in this case. This could be a consequence of the pest activity weakening the leaf surface membrane and / or causing multiple entry holes. Alternatively, it could be due to a component of the Spintor 480SC formulation aiding uptake by the leaf.

These observations suggest that there could be several advantages to applying Pyrethrum 5EC in a tank mix with Spintor 480SC against *Tuta absoluta*. It is recommended that these factors be investigated in more detail.

4. A preliminary evaluation of entomopathogenic nematodes against T*uta absoluta*

Introduction

This study evaluated two species of entomopathogenic nematodes, *Steinernema feltiae* and *S. carpocapsae*, against *Tuta absoluta* larvae*.* The efficacy of the nematodes was compared to an untreated control and to a standard chemical pesticide (spinosad).

Nematodes were applied to infested leaves both on the plant (Part 1) and removed from the plant in lined trays (Part 2). In all cases, the treated leaves were removed from the original position one day after treatment and stored in plastic boxes during the assessment period.

Materials and methods

The study was based in a mature organic tomato crop (cv Dimple [mini-plum]) which had been planted in August 2009 at Horticilha, Cilha Queimada, Alcochete, Portugal.

There were four treatments:

- Untreated control
- Positive control – spinosad (as Spintor 480SC) applied at the rate of 25 ml per 100 litres water.
- *Steinernema feltiae* supplied by Becker Underwood in a pack of 25 million and diluted to give the rate of 10 million per litre.
- *Steinernema carpocapsae* were prepared as described for *S. feltiae*.

All spray mixtures were prepared during the day so they could be applied quickly during the last 90 minutes of daylight (*i.e.* between 17.00 and 18.30 hrs). The aim was to avoid sprays drying too quickly as this would restrict the movement of the nematodes on the leaf surface and reduce the time for them to find entry holes to mines. All sprays were applied to the point of run off with a manual Matabi pneumatic sprayer with a 15 litre tank and fitted with a standard lance / nozzle.

Part 1

For each treatment, 40 leaflets were selected which each contained a medium sized larvae (as shown in Figure 11). The petioles were marked with coloured tape so that they could be easily identified post-treatment (as shown in Figure 12).

The marked leaves were sprayed with the appropriate treatment taking care to cover both surfaces. They were examined the following morning, *i.e.* approximately 18-20 hours later. The larvae were classified as:

- Alive in mine
- Dead in mine
- On surface of leaf
- Missing from leave

The dry leaflets were collected from the crop, placed in plastic boxes with tissue paper refuges and incubated at 21 ^{o}C +/- 3 ^{o}C. They were examined at daily intervals until dead or pupated. On each date, a sample of dead larvae from each of the nematode treatments was dissected to determine whether nematodes were present inside the cadaver. The remainder were further incubated to determine whether second generation nematodes would be produced.

Part 2

160 leaflets infested with medium sized *Tuta absoluta* larvae were collected from the crop 6 hours prior to treatment and stored at 11^{o}C until the time of use. They were divided into sets of 40 and each set placed in a lined plastic tray (as shown in Figure 13). One of each of the four treatments was applied to each tray using the Matabi pneumatic sprayer. The leaflets were then turned so that the opposite side could also be treated. The leaflets were left in situ in the trays until the following morning. Each leaflet was then examined and the larvae were classified as described in Part 1. Leaflets still containing live larvae were placed in plastic boxes with tissue paper refuges and incubated at 21 ^{o}C +/- 3 ^{o}C. They were examined at daily intervals until dead or pupated. Dead larvae from the nematode treatments were incubated separately to determine whether second generation nematodes were produced.

Results

The day after treatment, there was no discernable difference between the proportion of *Tuta absoluta* larvae that were still in the mine in the untreated leaflets which remained on the plants and those which were removed and placed in the tray (*i.e* approximately 3-5% in each case). This indicated that *Tuta absoluta* larvae do not immediately evacuate leaves when those leaves are removed from plants and the movement of plant sap stops. This was useful information for future trials.

Overall, 22% of *Tuta absoluta* larvae evacuated the leaves following the application of the three spray treatments, which was broadly consistent with previous observations. Where those leaves remained on the plants, the larvae were lost and had to be discounted from subsequent assessments. However, where the leaves were in trays, the larvae were recovered and progressed to the next stage of the experiment.

Part 1

The percentage mortality of *Tuta absoluta* larvae recorded 1, 2, 4, 6 and 8 days after application of the four treatments to leaves on plants are shown in Figure 19. There was very little mortality in the untreated controls. By day four, most of the larvae had moved out of the untreated mines and were present on the surface of the leaves. They began to pupate 6 days post-treatment and by 8 days most had entered this life cycle stage.

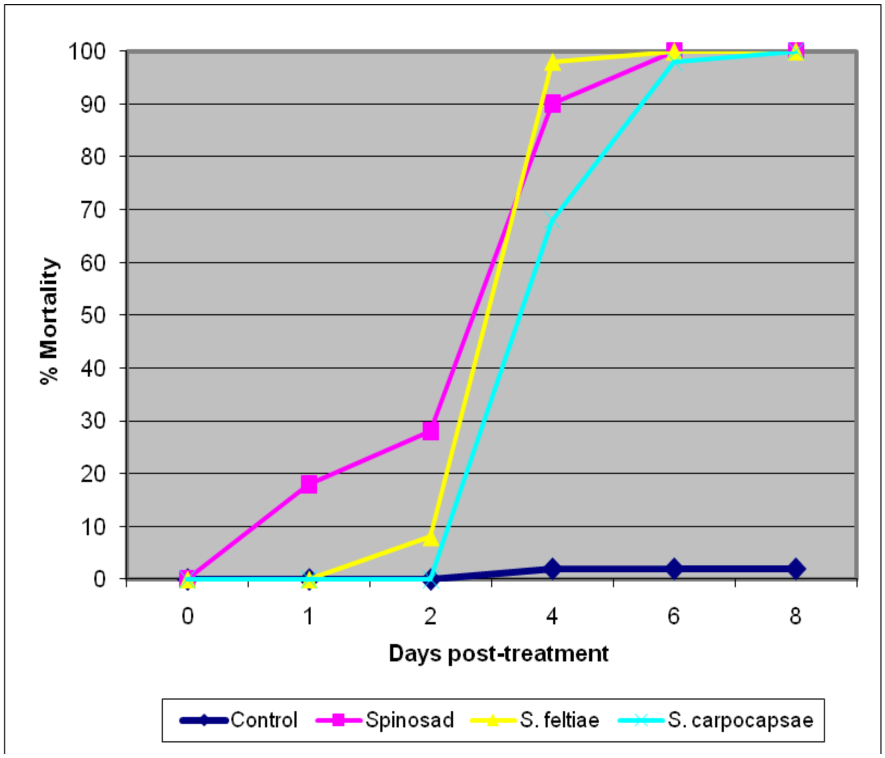
As described in Section 2, *Tuta absoluta* larvae in leaves treated with spinosad died within six days of application.

The first dead *Tuta absoluta* larvae were seen in the *S. feltiae* treatment two days after application of the spray (Figure 22). However, more than half of the remainder appeared to be unhealthy. By day 4 only one larva remained alive and by day 6 it had died. Approximately half of the larvae died within the mines while the rest had moved out into the plastic box. Dead larvae dissected on day 4 contained nematodes at a range of development stages with the most advanced being adult females (Figure 23). By day 8, there was evidence of offspring within dead female nematodes (Figure 24) and these had been released into the cadaver by day 10 (Figure 25).

The mortality of *Tuta absoluta* larvae following application of *S. carpocapsae* was broadly similar to the *S. feltiae* treatment. Dead *Tuta absoluta* larvae dissected four days post treatment contained numbers and life cycle stages of nematodes that were comparable to those seen in the *S. feltiae* treatment.

Further aspects of the development of both species of nematodes are provided in Section 4.

Figure 19. Mortality of *Tuta absoluta* larvae over eight days following treatment of leaves on plants with two species of entomopathogenic nematodes or spinosad.



Part 2

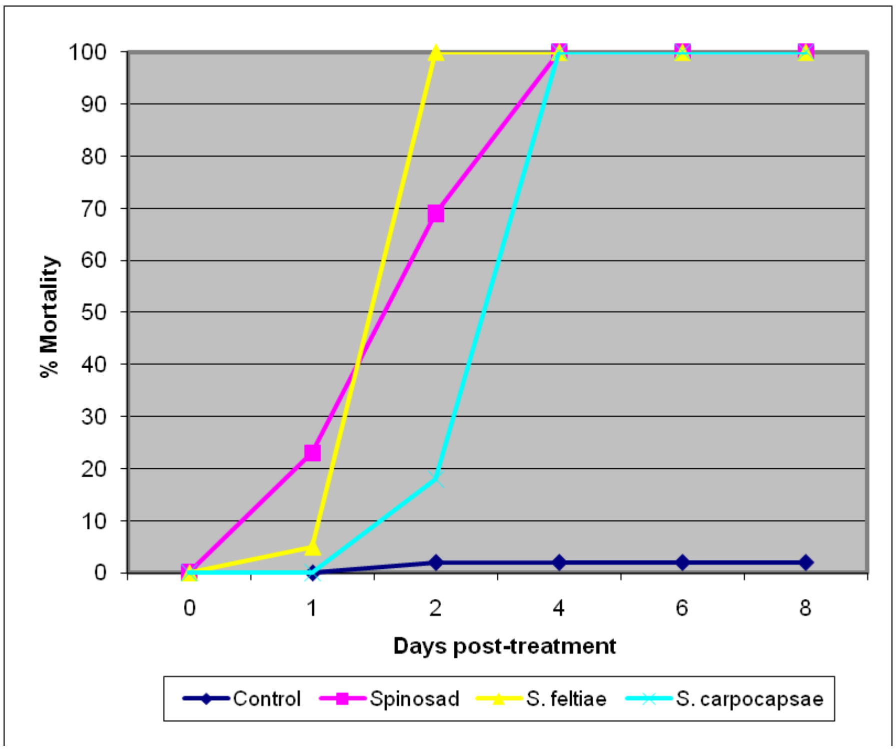
The percentage mortality of *Tuta absoluta* larvae 1, 2, 4, 6 and 8 days after application of the four treatments to excised leaves are shown in Figure 20. As in Part 1, there was very little mortality in the untreated controls. By day four, most of the larvae had moved out of the untreated mines and were present on the surface of the leaves. They began to pupate 6-8 days post-treatment.

The effect of spinosad on *Tuta absoluta* larvae was broadly comparable to Part 1.

By day 2, all the *Tuta absoluta* larvae were dead in the *Steinernema feltiae* treatment. Approximately half died within the mines while the remainder were on the leaf surfaces.

Photographs taken on day 2 illustrate nematodes inside the mine next to a larva (Figure 21) and dead caterpillars (Figure 22). It is assumed that the near optimum conditions during the first night allowed very large numbers of nematodes to penetrate the mines and find the caterpillars resulting in the rapid kill. By day 4, the first adult females were seen and by day 6 there were very large numbers. At day 8, the first offspring were confirmed within adult female nematodes (*eg* Figure 24) and these had been released into the cadaver by day 10 (Figure 25).

Figure 20. Mortality of *Tuta absoluta* larvae over eight days following treatment of excised leaves in trays with two species of entomopathogenic nematodes or spinosad.



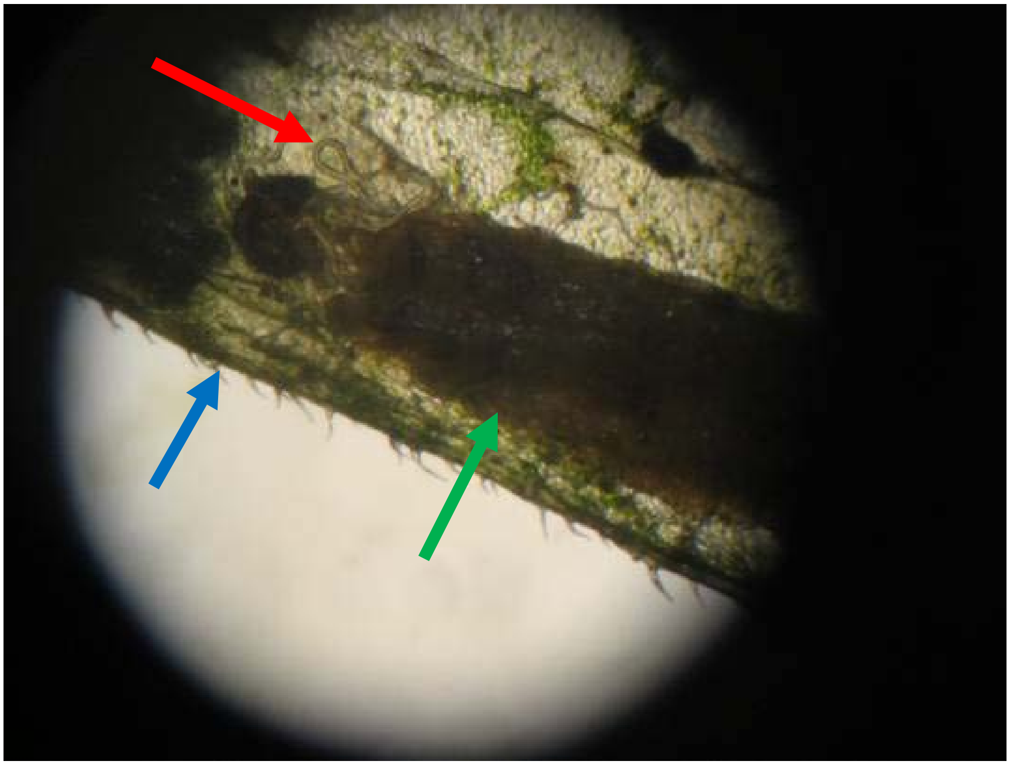
Steinernema carpocapsae were a little slower to act than *S. feltiae* but there were some dead bodies by day 2 and total mortality by day 6. Dissection of cadavers on day 6 revealed smaller numbers of nematodes than in the *S. feltiae* treatment but the most advanced were adult females (*eg* Figure 23). There was evidence of production of offspring within females by day 8 (*eg* Figure 24) and these were released into the cadaver in vast numbers by day 10 (Figure 25).

This trial was a very successful proof of concept demonstrating that both *Steinernema feltiae* and *S. carpocapsae* were capable of killing and reproducing in *Tuta absoluta* larvae. However, the nematode application rates were quite high and unlikely to be economically acceptable in commercial crops (*i.e.* the treatments were 5-7 times more expensive than a typical spinosad treatment). Further work is therefore required to determine the efficacy of the nematodes at lower application rates.

Entomopathogenic nematode invasion and development in *Tuta absoluta*

The authors hold a full photographic record of the development of *Steinernema feltiae* and *S. carpocapsae* in *Tuta absoluta* larvae. The following series of images provide a concise summary which is relevant to both species.

Figure 21. Photograph taken through leaf two days after application of *S. feltiae* showing:



Blue arrow – leaf edge

Green arrow – *Tuta absoluta* larva

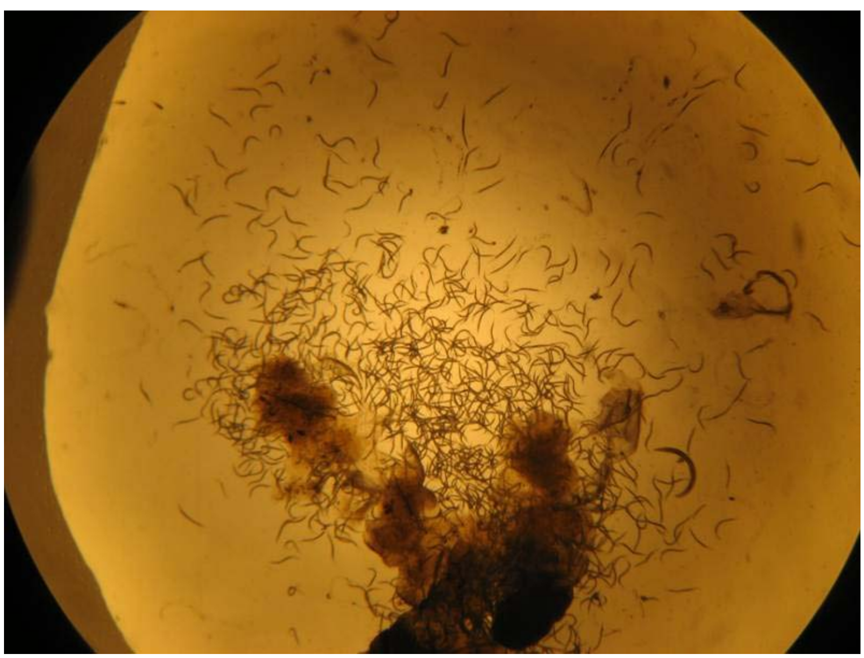
Red arrow – nematode having located larva within mine

Figure 22. Dead *Tuta absoluta* larvae two days after application of nematodes in mine (above) and removed from mines (below)

Figure 23. Nematodes emerging from dissected dead *Tuta absoluta* larva four days after application to leaves on plants. The most advanced were now mature females (red arrow)

Figure 24. Eight days after application of *S. feltiae* to leaflets on plants showing offspring within an adult female nematode

Figure 25. Nematodes spilling out of a dissected dead *Tuta absoluta* larva ten days after treatment



Key points from this study

- *Tuta absoluta* larvae do not immediately evacuate leaves when movement of plant sap stops.

- Both *Steinernema feltiae* and *S. carpocapsae* are capable of killing *Tuta absoluta* larvae within 2-6 days of application. The time to kill is probably influenced by the number of nematodes that successfully enter the mine.

- Both *Steinernema feltiae* and *S. carpocapsae* are capable of producing offspring within dead *Tuta absoluta* larvae.

- Further studies are required to determine the efficacy of the nematodes when used in a normal tomato cropping situation. Such studies should also take into account the cost-effectiveness of application rates.

5. Opportunities for control of *Tuta absoluta* with other biological control agents

Introduction

The main objective of this project was to develop short-term solutions that could be implemented immediately against *Tuta absoluta*. However, the authors have also taken the opportunity to record and collate information relevant to biological control of the pest. This knowledge will pave the way towards longer-term more sustainable control measures that may be used within IPM programmes.

Control of *Tuta absoluta* with *Nesidiocoris tenuis* and *Macrolophus* spp.

At the start of this project, the authors believed that the primary biological agents for *Tuta absoluta* would be the predatory bugs, *Nesidiocoris tenuis* and *Macrolophus* spp. (Jacobson, 2009). This was based on experience gained from working with these species against other leaf miners (*eg. Liriomyza* spp.). At that time there was little published information to support the assumption. However, a number of researchers in southern Europe are now studying this predator / prey interaction and useful papers are being published which confirm the potential of both species as control agents of *Tuta absoluta* (*eg* Arno *et al.,* Molla *et al*., 2009; Urbaneja *et al*. 2009).

Nesidiocoris occurs naturally throughout the Iberian peninsular and will colonise tomato crops in the absence of harmful chemicals. At a conference on *Tuta absoluta* in Valencia in March 2010 (Jacobson & Morley, 2010), speakers from Murcia and Catalonia explained the role that this predator can play in the overall pest control programme and described the importance of avoiding the use of harmful broad spectrum insecticides. It seemed that the majority of those researchers sought to harness natural populations rather than purchase *Nesidiocoris tenuis* products reared by biocontrol companies.

Nesidiocoris tenuis also feed on tomato plants and the authors have first hand experience of the serious damage that can occur to growing points and trusses after invertebrate pests have been controlled. This aspect of *Nesidiocoris tenuis* behaviour was rarely mentioned during the Valencia conference, probably because the predators are usually eliminated with one of a wide range of broad spectrum chemicals before the populations reach damaging levels.

At Horticilha in 2009/10, numbers of *Nesidiocoris tenuis* were very low throughout both sites towards the end of 2009 but typically rose to about 0.5 / plant by week 3 and to about 5 / plant between weeks 10 and 13. Thereafter, the population was culled as necessary with natural pyrethrins (as Pyrethrum 5EC). By week 20, numbers of *Nesidiocoris tenuis* were peaking at about 7 individuals per plant in some blocks.

During an assessment around week 20, most motile life cycle stages of *Nesidiocoris tenuis* were seen feeding on *Tuta absoluta* larvae. Furthermore, many small to medium size *Tuta absoluta* were found dead inside partially completed mines (Figure 26). These larvae appeared to be freshly killed and, in most situations, the only possible causal agent was *Nesidiocoris tenuis*.

Figure 26. Examples of dead *Tuta* larvae - most probably killed by *Nesidiocoris tenuis* in week 20

Macrolophus pygmaeus is closely related to *Nesidiocoris tenuis* and is the more common species in natural vegetation in northern Spain and France. They feed on immature stages of *Tuta absoluta* and can also provide good control of the pest in the absence of harmful insecticide residues. Following laboratory studies, Nannini (2009) recommended that the potential of this species be 'accurately estimated in the field'. The related species, *Macrolophus caliginosus*, which is sold by biological control suppliers in northern Europe, is now established on many UK tomato nurseries, and it currently offers the best option for biological control of *Tuta absoluta* in northern Europe.

Impact of *Bacillus thuringiensis*

Bacillus thuringiensis has been shown to kill larvae of *Tuta absoluta* under experimental conditions (Gonzalez-Cabera *et al.*, 2010) but its success is dependant upon contact with, or ingestion by, the pest. In theory, the larvae should only collect a lethal dose when outside the mine and so the most vulnerable life cycle stage should be the free living first instar. In practice, later instars may be vulnerable if they move between leaves. Nonetheless, the product would have to be applied at regular (perhaps even weekly) intervals to have an impact on the *Tuta absoluta* population. As a consequence, there has been considerable debate about the cost benefit of *Bacillus thuringiensis* treatments in commercial tomato crops.

Although no formal trials were done with *Bacillus thuringiensis* in this project, the product was used in several blocks and we constantly looked for beneficial effects from the treatments. The only occasion that cadavers were found which showed typical symptoms of death by *Bacillus thuringiensis* (Figure 27) were following a trial application of the product in a tank mix with Neem. It is possible that the Neem drove the larvae out of the leaf (see Section 1) and they then came into contact with the *Bacillus thuringiensis*. This is worthy of further investigation.

Figure 27. Larva of *Tuta absoluta* killed by *Bacillus thuringiensis*

***Trichogramma* spp.**

Trichogramma achaeae (Figure 28) is an egg parasite which was collected in Spain in 2007 in tomato plants growing in greenhouses located in areas with high temperatures. It is a cosmopolitan species and has since been identified in many countries (*eg.* China, India, Cape Verde, Argentina, Barbados, Chile, Trinidad & Tobago, USA and Russia) but not in the UK.

In 2008, a collaborative venture between the University of Almeria and Agrobio began to evaluate the potential of *Trichogramma archaeae* against *Tuta absoluta* with laboratory studies and semi-field trials using plants placed in cages (Kabiri *et al*, 2009). This was followed by a promising field trial in an experimental greenhouse in 2009. Production of *Trichogramma archaeae* was then scaled up so that larger trials could be conducted in commercial crops. Kabiri *et al*. (2009) and Cabello *et al*. (2009 and 2010) describe trials in which large numbers of the parasitoids (over 1 million *Trichogramma* / ha / week) were released prophylactically as soon as crops were planted and they reported complete control in 90% of 20 commercial crops. However, this positive view was not shared by all delegates at the Valencia conference in March 2010 (Jacobson and Morley, 2010). Several delegates questioned the economics of the high releases and most practitioners would require more substantive data before risking their commercial crops.

Figure 28. *Trichogramma* spp. probing a *Tuta absoluta* egg

Other parasitoids and predators

Several surveys in Mediterranean countries have sought alternative natural enemies with potential for use against *Tuta absoluta.* In addition to the species mentioned above, two larval parasitoids have been detected on *Tuta absoluta*; *i.e. Necremnus artynes* and *Hemiptarsenus zilahisebisi*. It remains to be seen whether they will have any value as biological control agents. Perhaps most surprisingly, the predatory mite, *Amblyseius swirskii*, has been seen feeding on first instar *Tuta absoluta* larvae.

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*'.
- Presentation to Tomato Pest and Disease Seminar (14 January 2010, Stoneleigh); 'Tuta absoluta: Biology and control'.
- HDC Fact Sheet (03/10) Tomato leafminers. January 2010.
- HDC Poster. Tomato Leafminers. March 2010
- 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*'.

REFERENCES

- Al-Zaidi, .S (2010). Manejo de *Tuta absoluta* mediante feromonas. In: *Abstracts, Phytoma Conference “La polilla del tomate, un problema en plena expansion*”. Valencia 23-24 March 2010.
- Arno, J., Sorribas, R., Prat, M., Matas, M., Pozo, C, Rodriguez, D., Garreta, A., Gomez, A. & Gabarra, R (2009). *Tuta absoluta*, a new pest in IPM of tomatoes in the northeast of Spain. IOBC WPRS Bulletin 49, 203-208
- Cabello, T., Gallego, J.R., Vila, E., Soler, A, del Pino, M., Carnero, A., Hernandez-Suarez, E. & Polasek, A. (2009). Biological control of the South American Tomato Pinworm, *Tuta absoluta* (Lep.: Gelechiidae), with releases of *Trichogramma achaeae* (Hym.: Trichogrammatidae) in tomato greenhouses of Spain. IOBC WPRS Bulletin 49, 225-230
- Cabello, T., Gallego, J.R., Fernandez, F.J., Vila, E., Soler, A and Parra, A. (2010). Applicacion de parasitoides de huevos en el control de *Tuta absoluta* en Espana. In: *Abstracts, Phytoma Conference “La polilla del tomate, un problema en plena expansion*”. Valencia 23-24 March 2010. 53-59.
- European and Mediterranean Plant Protection Organization (2006). Data sheets on quarantine pests, *Tuta absoluta*. Web version 2006-03. 4pp
- Gonzalez-Cabera, J., Molla, O, Monton, H & Urbaeja, A. (2010). Control biologico de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) con *Bacillus thuringiensis* (Berliner). In: *Abstracts, Phytoma Conference “La polilla del tomate, un problema en plena expansion*”. Valencia 23-24 March 2010. 69-73.
- Jacobson, R. (2009). Two more threats: *Tuta absoluta* and *Nesidiocoris tenuis*. In: Abstracts, *Tomato Conference 2009*, Coventry 24 September 2009. 9-18.
- Jacobson, R.J. & Morley, P. (2010). Research catching up with Tuta. *HDC News*, May 2010. Vol 163. 18-19
- Kabirii, F., Vila, E. & Cabello, T. (2010). *Trichogramma achaeae*: an excellent biocontrol agent against *Tuta absoluta*. Sting (IOBC Newsletter on Biological Control) 33. 5-6.
- Korycinska, A. & Moran, H. (2009). South American tomato moth *Tuta absoluta*. Plant Pest Notice No 56. The Food and Environment Agency. April 2009. 4pp.
- Marti, S. (2010). Uso de feromonas para el-control de *Tuta absoluta* primera experiencia en campo. In: *Abstracts, Phytoma Conference “La polilla del tomate, un problema en plena expansion*”. Valencia 23-24 March 2010.

- Molla, O., Monton, H, Vanaclocha, P., Beitia, F. & Urbaneja, A. (2009). *Predation by the mirids*, Nesidiocoris tenuis *and* Macrolophus pygmaeus *on the tomato borer* Tuta absoluta. IOBC WPRS Bulletin 49, 209-214.
- Nannini, M. (2009). Preliminary evaluation of *Macrolophus pygmaeus* potential for the control of *Tuta absoluta*. IOBC WPRS Bulletin 49, 215-218
- Salas, J. (2004). Capture of *Tuta absoluta* in traps baited with its sex pheromone (in Spanish). Revista Columbiana de Entomogia 20, 75-78.
- Urbaneja, A., Monton, H, & Molla., O (2009). *Suitability of the tomato borer* Tuta absoluta *as prey for* Macrolophus caliginosus *and* Nesidiocoris tenuis*.* Journal of Applied Entomology, 133, 292-296.
- Van der Straten, M. (2009). Tuta absoluta (Lepidoptera, Gelechiidae) tomato leafminer. National Reference Laboratory, Plant Protection Service, The Netherlands, April 2009. 3pp