Project title:	*Tuta absoluta*: Investigating resistance to key insecticides and seeking alternative IPM compatible products
Project number:	PE 028
Project leader:	Dr R J Jacobson, RJC Ltd, 5 Milnthorpe Garth, Bramham, West.Yorks, LS23 6TH
Report:	Final report, December 2015
Previous report:	Not applicable
Key staff:	Dr C Bass, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ
Location of project:	Rothamsted Research, Harpenden, Hertfordshire RJC Ltd, Bramham, West Yorks Libraries at Fera and Lancaster, Leeds and York Universities
Industry Representative:	Dr P Morley (TGA Technical Officer), Tomato Growers' Association, TGA, Pollards Nursery, Lake Lane, Barnham, West Sussex, PO22 0AD
Date project commenced:	1 July 2015
Date project completed:	31 December 2015

DISCLAIMER

[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.]

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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CONTENTS

GROWER SUMMARY

Headlines

- Significant resistance to spinosad has been confirmed in *Tuta absoluta* populations at two UK nurseries and the existing IPM programme must be modified accordingly.
- Potentially useful additional products are identified for each stage of the IPM programme.

Background

Tuta absoluta arrived in the UK in 2009 and rapidly became the most important pest of home-grown tomatoes. By 2013, HDC projects PC 302 and PE 020, and associated studies, had developed a completely new IPM strategy for use against the pest and this was detailed in HDC Factsheet 02/14. The programme was based on the predator, *Macrolophus pygmaeus*, integrated with the chemical insecticides, spinosad (Conserve), chlorantraniliprole (Coragen) and indoxacarb (Steward). *Macrolophus pygmaeus* was released at the start of the growing season so that it would start to provide some control of the pest by late spring or early summer. When the pest arrived, it was allowed to colonise the crop but population growth was slowed by applying spinosad through the irrigation system before the first generation of caterpillars completed their development. If necessary, a high volume spray of chlorantraniliprole was applied as a second line of defence during the summer to keep the pest and predator populations in balance. If crop monitoring indicated that a clean-up spray was required at the end of the season, then the third insecticide, indoxacarb, was used to reduce the number of *T. absoluta* surviving in the glasshouse to infest the next crop. The IPM programme was very successful and British tomato growers admit that they became complacent about the pest.

The three insecticides used in the IPM programme were from different Insecticide Resistance Action Committee (IRAC) Mode of Action Classification Groups and, together with the biological control agent, should have formed a robust resistance management strategy. Nonetheless, a strict warning about maintaining an effective insecticide resistance management strategy was incorporated in HDC Factsheet 02/14.

In February 2015, a leading British tomato grower reported concern over recent poor results with spinosad against *T. absoluta* on his nursery. There soon followed similar reports from three other British tomato growers in other parts of the country. At about the same time, a

Scandinavian grower reported poor results with spinosad in a *T. absoluta* population recently inherited from a Spanish supplier. There were no such difficulties reported with chlorantraniliprole in the UK but 100 fold resistance to this chemical had been confirmed in a *T. absoluta* population in Italy. Indoxacarb has rarely been used against *T. absoluta* in the UK because the pest population has usually been reduced to an acceptable level by *M. pygmaeus* before the end of the growing season. However, one British grower did experience treatment failures with this product in 2011. An on-site investigation confirmed that the sprays were prepared and applied correctly yet mortality six days post-treatment was only 11-19% for medium-sized larvae and 23-41% for small larvae. This *T. absoluta* population had only recently become established on that nursery and was believed to have arrived on imported produce from Italy. These control failures made it clear that the British tomato industry must take measures to remain one step ahead of this potentially devastating pest. The British Tomato Growers' Association Technical Committee requested the following actions which became the focus of this project:

1. Spinosad and chlorantraniliprole resistance tests be undertaken by the Insecticide Resistance Team at Rothamsted Research (IRT RR) to establish the current status of populations of *T. absoluta* in the UK.
2. A desk study to search for all products used to control *T. absoluta* and other leaf mining caterpillars in the Americas, Africa, southern Europe, Middle East and Far East, and then to categorise them according their potential value within the UK tomato IPM programme.

Summary

Part one: The original objective was to test the sensitivity of four UK strains of *T. absoluta* to spinosad and chlorantraniliprole. However, one of the growers who had reported poor results with spinosad in the early part of 2015 stopped producing tomatoes and no insects were available from that site. That population was replaced with one from Denmark that was associated with spinosad treatment failure in 2015. The Danish population provided added value as one resistance test had already been completed on that strain and it was therefore possible to investigate whether 'tolerance' declined when spinosad selection pressure was removed for 7-8 months. Two IRT RR 'susceptible' laboratory strains were also incorporated in the study to provide a base line. Full-dose response bioassays were performed using the standard leaf-dip bioassay procedure outlined in the IRAC Susceptibility Test Method 22. The LD50s (*i.e.* the amount of insecticide required to kill 50% of the population) were determined for each population and resistance ratios calculated by dividing the LD50 of the test population by the LD50 of the most susceptible laboratory strain.

In summary, the bioassays confirmed that *T. absoluta* populations at two locations in the UK exhibited high levels of resistance to spinosad. The levels of resistance were high enough to seriously compromise control as both strains would show very significant survivorship at the field rate commonly used for spinosad (87-100 mg L^{-1}). No spinosad resistance was detected in the third UK population and other possible causes of treatment failure are being investigated at that site. The original Danish strain showed some tolerance to spinosad but only 8-fold greater than the most susceptible laboratory strain. This had declined to approximately twice that of the most susceptible laboratory strain at the second test. The interim period of 29 weeks equates to 8-9 generations of *T. absoluta* at the usual temperatures in a commercial tomato crop. It would therefore appear that in the absence of spinosad selection pressure the more susceptible individuals in a population have some developmental advantage and gradually become more dominant. This is good news for growers as it indicates that spinosad should still have some value within the IPM programme if treatments are restricted to no more than one application per growing season.

None of the tested populations showed significant levels of resistance to chlorantraniliprole. However, published information from Italy and Greece has confirmed that resistance to this chemical is present within southern Europe. The fact that there is currently unrestricted importation of tomatoes infested with *T. absoluta* from Italy suggests that British growers could inherit this problem at any time.

Part two: The overall aim of this part of the project was to source and collate information on insecticidal control options for *T. absoluta* and other leaf mining Lepidoptera from around the world. A review of the scientific and horticultural literature was carried out and information was acquired from the IRAC worldwide network of technical specialists. Unpublished information was sourced using the authors' international network of collaborators in both academia and industry. Finally, efficacious products were categorised according to their IRAC resistance group, IPM compatibility and physical properties.

The search identified over 40 chemical insecticides that had been used against leaf mining caterpillars around the world as well as several biopesticides, botanical extracts, entomopathogenic nematodes and macro-biocontrols. The main issue with the chemical compounds was that many were already compromised by direct resistance or they were subject to cross resistance arising from another insecticide within the same IRAC Mode of

Action Classification Group. There seemed little to be gained in the long term by pursuing a candidate insecticide if resistance to it or a related compound had already been recorded in another country. As a consequence, the initial screen based on the biochemical mode of action and the likelihood of resistance, selected just seven potentially useful compounds (*i.e.* abamectin, azadirachtin, *Bacillus thuringiensis* (Bt), clorfenapyr, emamectin benzoate, metaflumizone, methoxyfenozide) in addition to the three already used within the UK tomato IPM programme (spinosad, chlorantraniliprole and indoxacarb).

The next step in the screening sequence was to consider compatibility with the biological control agents used by UK tomato growers. It is important to stress that this screen had to include the full range of biocontrols used in the whole tomato IPM programme and not just those used against *T. absoluta*. Without this diligence, the project may have resolved the main issue but created other pest control failures. This stage reduced the number of potentially useful additional compounds for use during the UK tomato growing season to three candidates (Bt, azadirachtin, methoxyfenozide) and one additional compound for use as an end of season clean-up treatment (abamectin). This list could be increased to five potentially useful compounds if we included emamectin benzoate, although important questions remain to be answered about the suitability of that compound.

The final step in the selection procedure considered the physical properties of the remaining compounds with emphasis on their ability to penetrate leaves and / or have systemic activity that would allow application via the irrigation system. It is important to understand that young *T. absoluta* caterpillars usually feed on the surface for less than 90 minutes after hatching before they start to burrow into the plant tissue. This results in a very narrow window of opportunity for surface acting insecticides. There may be other opportunities if the caterpillars move to other parts of the plant during their development but such migrations are unpredictable and of very short duration. Repeated applications of surface acting insecticides are required to protect new growth post-application because this is where *T. absoluta* most commonly lay their eggs.

Bt and methoxyfenozide have no translaminar or systemic activity. Despite this, a niche has already been found for Bt within the tomato IPM programme. Under certain exceptional circumstances, which are not yet fully understood, young caterpillars migrate to the tops of the plants where they ‘graze’ more openly in and around the growing points. Bt sprayed repeatedly at 7-10 day intervals has prevented loss of growing points. However, this technique requires a good understanding of the pest’s activity patterns as well as a significant labour input. The moult accelerating compound, methoxyfenozide, could fulfil a similar role within the IPM programme.

It has proved difficult to source irrefutable evidence of translaminar and / or systemic activity of azadirachtin in tomato pants due to the many different extracts and formulations that have been prepared and used in trials. Nonetheless, several papers indicate that the insecticide could have potential as a direct replacement for spinosad in the UK tomato IPM programme. One researcher stated that systemic treatments of azadirachtin-based products were most effective on young tomato plants, which is consistent with current use of spinosad in the UK.

The translaminar activity of both abamectin and emamectin benzoate is well documented. Emamectin benzoate has short persistence on the leaf surface but is rapidly absorbed into plant tissue. It is therefore ideally suited for high volume spray application against *T. absoluta*. It is not thought to be truly systemic but this should be further investigated.

Financial Benefits

Tuta absoluta is currently the most important pest of tomato crops in the UK. For example, at one nursery in 2012, 30% of fruit were damaged by the pest and graded out during June and July causing losses of approximately £50k per hectare to that grower for that period alone. The existing *Macrolophus*-based IPM programme has prevented such damage but the predator must be supported by other control measures. In particular, the loss of spinosad and / or chlorantraniprole through resistance would take the industry back to the 2012 situation. It is vitally important that additional insecticidal products are added to the armoury.

Action Points

The following modifications to the existing IPM programme are suggested:

- *Macrolophus pygmaeus* remains the biological ‘backbone’ to the IPM programme and should continue to be released, with supplementary food, at the start of the crop.
- Spinosad applied via the irrigation should remain the preferred treatment to slow down *T. absoluta* population growth while the *M. pygmaeus* population is becoming established in the crop. To avoid resistance, spinosad should not be used more than once in a six month period. Where resistance has already been confirmed, the product should not be reused unless resistance tests show that the population has reverted to susceptible status. Thereafter, such populations should only be treated with spinosad at intervals greater than 12 months.

- Alternatives to spinosad should be developed as quickly as possible. The most promising candidate is currently azadirachtin. However, further research is required to determine its efficacy via the irrigation system and compatibility with the biological control agents used in the UK tomato IPM programme. The authors' understand that approval is already being sought to use a product containing azadirachtin in UK tomato crops.
- As yet, there is no known resistance to chlorantraniliprole in the UK although it has been confirmed in southern Europe. This should remain the first choice of second line of defence treatment to keep the pest and predator populations in balance during the summer. However, it must not be used twice in succession unless there is an interval of at least six months.
- The entomopathogenic nematodes, *Steinernema feltiae*, provide a useful second line of defence option for growers of organic crops who are not allowed to use synthetic insecticides. However, at least three applications at 7-10 day intervals are probably required to give acceptable levels of control.
- It will be important to further investigate the potential of emamectin benzoate to provide an alternative to chlorantraniliprole. This will require research to determine its compatibility with the biological control agents currently used in UK tomato crops and its systemic activity. Approval will be required for use in UK tomato crops.
- *Bacillus thuringiensis* can provide useful control of *T. absoluta* larvae when the pests are 'grazing' for prolonged periods in the heads of the plants. However, at least three applications at 7-10 day intervals are required to give acceptable levels of control.
- The moult accelerating compound, methoxyfenozide, could provide an alternative to Bt when *T. absoluta* larvae are 'grazing' in the heads of the plants. An EAMU is already being sought for use of this product in UK tomato crops.
- Indoxacarb remains the first choice as an end of season 'clean-up' treatment. Where there have been difficulties obtaining control of *T. absoluta* with this insecticide, then abamectin should provide an acceptable alternative. Neither product should be used during the main growing season when bumblebees and biological control agents are still active in the crop.

SCIENCE SECTION

Introduction

Tuta absoluta arrived in the UK during 2009 and there were 41 outbreaks in tomato crops by 2012 (Cuthbertson *et al.,* 2013). At that time it was considered to be the most serious threat to the future success of the British tomato production industry. Since 2012 the pest has become endemic in the UK. At one UK nursery during June-July 2012, 30% of fruit were damaged by the pest and rendered unmarketable. That represented a loss of approximately £50k per hectare to that grower for that period alone (Jacobson & Howlett, 2013). Over the following three years, HDC projects PC 302 and PE 020, and associated studies, developed a completely new IPM strategy against *T. absoluta* in the UK. The programme was based on the predator, *Macrolophus pygmaeus*, integrated with the chemical insecticides, spinosad (Conserve), chlorantraniliprole (Coragen) and indoxacarb (Steward) (Jacobson & Howlett, 2014). *Macrolophus pygmaeus* was released at the start of the growing season so that it would start to provide some control of the pest by late spring or early summer. When the pest arrived, it was allowed to colonise the crop but population growth was slowed by applying spinosad through the irrigation system before the first generation of caterpillars completed their development. If necessary, a high volume spray of chlorantraniliprole was applied as a second line of defence during the summer to keep the pest and predator populations in balance. If crop monitoring indicated that a clean-up spray was required at the end of the season, then the third insecticide, indoxacarb, was used to reduce the number of *T. absoluta* surviving in the glasshouse to infest the next crop. Indoxacarb is not compatible with *M. pygmaeus* and may disrupt biological pollination under some circumstances, so it could only be used at the very end of the growing season after the predators and bumblebees had completed their work. The programme was very successful and British growers admit that they became complacent about the pest over the following 2-3 years.

The three insecticides used in the IPM programme were from different Insecticide Resistance Action Committee (IRAC) Mode of Action Classification Groups and, together with the biological control agent, should have formed a robust resistance management strategy. Nonetheless, a clear warning about maintaining an effective insecticide resistance management strategy was incorporated in HDC Factsheet 02/14 (Jacobson & Howlett, 2014).

It was, at least in part, due to the existence of this IPM programme that Defra changed the quarantine status of *T. absoluta* and stopped inspecting imports for the presence of the pest. The British Tomato Growers' Association Technical Committee (TGA TC) had warned Defra that a possible consequence of stopping those inspections was the unrestricted importation of *T. absoluta* which had already become insecticide resistant due to overuse / misuse of those three important insecticides in the country of origin (TGA TC responses to the UK Plant Health Risk Group on the future control of *Tuta absoluta*, 6 March & 24 July, 2014) (IRAC, 2015).

At the TGA TC meeting on 18 February 2015, a leading British tomato grower reported concern over recent poor results with spinosad against *T. absoluta* on his nursery (Item 14, TC Minutes, 18 February 2015). There soon followed similar reports from three other British tomato growers. At about the same time, a Scandinavian grower reported poor results with spinosad in a *T. absoluta* population inherited from a Spanish supplier (Grotek aps, unpublished information, 2015).

There were no such difficulties reported with chlorantraniliprole in the UK but 100 fold resistance to this chemical had been confirmed in a *T. absoluta* population in Italy as well as lower levels of resistance in Greece (Rodiakis *et al*., 2015).

Indoxacarb has rarely been used against *T. absoluta* in the UK because the pest population has usually been reduced to an acceptable level by *M. pygmaeus* before the end of the growing season. However, one British grower did experience treatment failures with this product in 2011. An on-site investigation confirmed that the sprays were prepared and applied correctly yet mortality six days post-treatment was only 11-19% for medium-sized larvae and 23-41% for small larvae (Holt & Jacobson, unpublished information, 2011). This *T. absoluta* population had only recently become established on that nursery and was believed to have arrived on imported produce from Italy. The trial results indicated that the pest population had an inherent level of tolerance to indoxacarb which was assumed to be due to excessive and / or inappropriate use of that product in the country of origin.

These control failures made it clear that the British tomato industry must remain one step ahead of this potentially devastating pest. As a consequence, Dr Jacobson was asked to liaise with the Insecticide Resistance Team at Rothamsted Research (IRT RR) and undertake a series of studies to strengthen the industry's long-term management of *T. absoluta* (TGA TC Minutes, 3 June 2015). In particular, the TGA TC requested the following tasks which became the focus of this project:

- That spinosad and chlorantraniliprole resistance tests be done to establish the current status of populations of *T. absoluta* in the UK.
- A desk study to search for all products used to control *T. absoluta* and other leaf mining caterpillars in the Americas, Africa, southern Europe, Middle East and Far East, and then to categorise them according their potential value within the UK tomato IPM programme.

Materials and Methods

Part one: The original objective was to test the sensitivity of four UK strains of *T. absoluta* to spinosad and chlorantraniliprole. However, one of the growers who had reported poor results with spinosad in the early part of 2015 stopped producing tomatoes and no insects were available from that site. That population was replaced with one from Denmark that was associated with spinosad treatment failure in 2015. The Danish population provided added value as one resistance test had already been completed on that strain and it was therefore possible to investigate whether 'tolerance' declined when spinosad selection pressure was removed for 7-8 months. Two IRT RR 'susceptible' laboratory strains were also incorporated in the study to provide a base line. The six tested populations are detailed in Table 1. All were reared on tomato plants under conditions of 26°C temperature and 16 hr light in the IRT RR insectary between receipt of the samples and the time of the resistance test.

Table 1. Insect strains used in this study

Strain reference	Geographical source	Date sampled
A	Worcestershire, England	25 June 2015
B	Lancashire, England	25 June 2015
C	Hampshire, England	25 June 2105
D1	Odense, Denmark	2 March 2015
D2	Odense, Denmark	21 September 2015
TA1	Rothamsted Research (originally Spain)	2010
TA3	Rothamsted Research (originally Italy)	2010

Full-dose response bioassays were performed using the leaf-dip bioassay procedure outlined in the IRAC Susceptibility Test Method 22 (IRAC, 2015a). Insecticidal compounds (spinosad and chlorantraniliprole) were diluted in acetone + water containing 0.01% agral to make final concentrations between 1 and 1000 mg L^{-1}. Tomato leaflets (four per concentration) were dipped in beakers of insecticide for 10 seconds and then left to dry on paper towels for 30 minutes. Leaves dipped in 0.01% agral only were used to quantify control mortality. Each leaf was placed in a Petri dish on top of a piece of filter paper moistened with 200 µl water and moistened cotton wool was wrapped around the base of the leaf stem. Eight second instar larvae were placed on each leaf using a fine paint brush. After 72 hrs, the numbers of live and dead larvae were counted using a lamp and dissecting microscope.

GenStat 15th Edition (Payne, 2011) was used to calculate LD50s (the amount of insecticide required to kill 50% of the population). Probit analysis was performed with control mortality estimated for each population. The transformation link for the proportion of insects dead was logit and logs to base 10 were taken of the concentration. The dispersion parameter was fixed with a value of 1.

Results were compared with that of susceptible laboratory strains held at IRT RR and resistance ratios (RRs) were calculated by dividing the LD50 of the resistant strain by that of the susceptible strain.

Part two: The overall aim of this part of the project was to source and collate information on chemical control options for *T. absoluta* and other leaf mining Lepidoptera from around the world. A review of the scientific literature was carried out using Web of Knowledge. Information was also sourced from IRAC that works as a worldwide specialist technical group of the industry association ‘CropLife’ providing a coordinated industry response to prevent or delay the development of resistance in insect and mite pests. Unpublished information was sourced using the network of collaborators of both of the authors in academia and industry. Finally, efficacious products were categorised according to their IRAC resistance group, physical properties and potential compatibility with other components on the whole UK tomato IPM programme.

Results and Discussion

Part one: Bioassays were completed successfully for all strains. In the case of chlorantraniliprole laboratory susceptible strains had never been previously tested, therefore a total of six strains (including the two lab strains TA1 and TA3) were tested against this compound. Full results showing calculated LD50s are provided in Table 2. During testing with spinosad, bioassays had to be repeated with an expanded dose range as it was clear that at least two of the strains from the UK exhibited significant levels of resistance. This was confirmed in subsequent bioassays which revealed that strains A and B had extremely high levels of resistance to spinosad which was 478- and 83-fold greater than the most susceptible laboratory strain respectively (see Table 3). No spinosad resistance was detected in strain C and other possible causes of treatment failure are being investigated at that site. The original Danish strain (D1) showed some tolerance to spinosad but only 8-fold greater than the most susceptible laboratory strain. This had declined to approximately twice that of the most susceptible laboratory strain at the second test. The interim period of 29 weeks equates to 8-9 generations of *T. absoluta* at the usual temperatures in a commercial tomato crop. It would therefore appear that in the absence of spinosad selection pressure the more susceptible individuals in a population have some developmental advantage and gradually become more dominant. This is good news for growers as it indicates that spinosad should still have some value within the IPM programme if treatments are restricted to no more than one application per growing season.

In summary, these bioassays have confirmed that *T. absoluta* populations at two locations in the UK exhibit high levels of resistance to spinosad. The levels of resistance shown by these strains is high enough to seriously compromise control as both strains would show very significant (if not complete) survivorship at the field rate commonly used for spinosad (87-100 mg L^{-1}). However, that level of resistance may decline over several generations if the selection pressure from spinosad applications is eliminated.

None of the tested strains showed significant levels of resistance to chlorantraniliprole with 95% confidence limits of LC50s of the A, B, C and D strains overlapping those of susceptible TA1 and TA3 strains. However, published information from Italy and Greece (Rodiakis *et al*., 2015) confirms that resistance to this chemical is present within southern Europe. The fact that there is currently unrestricted importation of tomatoes infested with *T. absoluta* from Italy suggests that British growers may inherit this problem at any time.

Table 2. Sensitivity of six strains of *Tuta absoluta* to spinosad and chlorantraniliprole. Results are expressed as LD50s (the amount of insecticide required to kill 50% of the population). Biological variation is expressed as 95% confidence limits.

Sample reference number	**Spinosad**		**Chlorantraniliprole**	
	LD50 (ppm / mg L^{-1})	**95% confidence limits**	**LD50 (ppm / mg L^{-1})**	**95% confidence limits**
A	860	484-2114	0.12	0.005-1.0
B	149	31-538	4.75	1.7-12.8
C	8.6	5.3-13.9	4.75	1.7-12.8
D1	15.0	10.2-27.5	-	-
D2	3.9	1.6-6.8	3.40	1.9-5.9
TA1	5.2	3.1-7.3	1.23	0.09-5.5
TA3	1.8	1.0-2.8	0.93	0.17-2.0

Table 3. Sensitivity of six *Tuta absoluta* strains to spinosad and chlorantraniliprole, compared to laboratory susceptible strains. Results are expressed as resistance ratios and indicate the relative sensitivity the susceptible strains. Results with a * are resistant.

Sample reference number	**Resistance ratio** (LD50 of the resistant strain divided by that of the most susceptible strain)	
	Spinosad	**Cholrantraniliprole**
A	478*	0.1
B	83*	5.1
C	4.7	5.1
D1	8.3	-
D2	2.1	3.7
TA1	2.9	1.3
TA3	1.0	1.0

Part two: *Possible candidates for the control of* Tuta absoluta *in UK tomato crops*

As a starting point, it is useful to consider the global distribution of *T. absoluta* and determine when the pest first became a problem in each of those countries (Figure 1) because we may expect the knowledge accrued about control measures to correlate to the length of time the pest has been present.

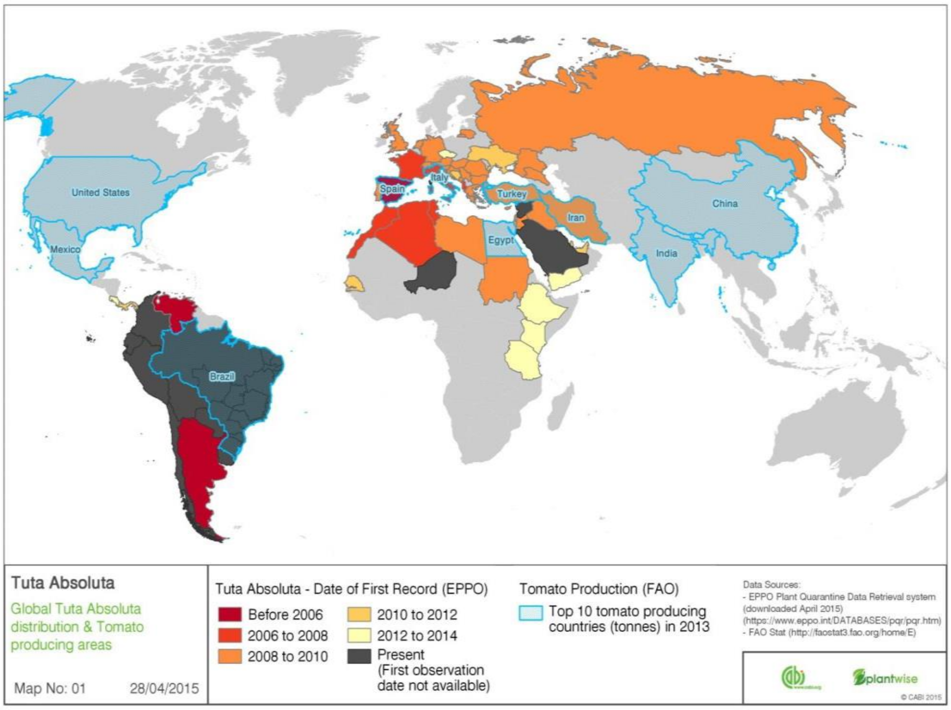

Figure 1. Map of *Tuta absoluta* distribution and tomato producing areas. (Copyright CABI 2015. Reprinted with permission from CABI).

Tuta absoluta is native to Central America but has been present in South America for more than four decades (Razuri & Gargas, 1975). Researchers and practitioners in Brazil have gained considerable practical experience of managing the pest and they have proved to be a particularly valuable source of published and unpublished information about the efficacy of chemical insecticides. *Tuta absoluta* was first detected in Europe (Spain) in 2006 and by 2008 had spread to Italy, France and North Africa (Morocco and Algeria). This triggered the start of research and the development of control measures in the northern hemisphere. In 2009 it was detected in the Netherlands, Portugal, Tunisia, Libya, Germany, Switzerland, Greece, Romania, Cyprus, Turkey, Albania, Bahrain, Kuwait, Malta and the UK. By the end of 2011 it had invaded 35 countries in Europe, North Africa and Asia (Desneux, 2011).
A comprehensive list of insecticidal compounds registered throughout the world for the control of *T. absoluta* has been sourced from the Insecticide Resistance Action Committee

(IRAC) and is provided in Table 4. This includes compounds from thirteen separate classes of chemicals.

Table 4. List of compounds registered for control of *Tuta absoluta* (source IRAC).

Chemical Class	Compounds
Organophosphates	Chlorpyrifos, Methamidophos
Pyrethroids	Bifenthrin, Cyfluthrin, beta-Cyfluthrin, gamma-Cyhalothrin, lambda-Cyhalothrin, Cypermethrin, alpha-Cypermethrin, beta-Cypermethrin, zeta-Cypermethrin, Delthamethrin, Esfenvalerate, Etofenprox, tau-Fluvalinate, Fenpropathrin, Permethrin
Spinosyns	Spinetoram, Spinosad
Avermectins,	Abamectin, Emamectin benzoate
Biopesticide	*Bacillus thuringiensis aizawai, Bacillus thuringiensis kurstaki*
Pyrroles	Chlorfenapyr
Nereistoxin analogues	Cartap
Benzoylureas	Diflubenzuron, Flufenoxuron, Lufenuron, Novaluron, Noviflumuron, Teflubenzuron, Triflumuron
Diacylhydrazines	Chromafenozide, Methoxyfenozide, Tebufenozide
Oxadiazine	Indoxacarb
Semi-carbazone	Metaflumizone
Diamides	Chlorantraniliprole, Flubendiamide
Tetranortriterpenoid	Azadirachtin

The most efficacious insecticides in South America are the diamides, spinosyns and chlorfenapyr – all of which are registered for use on tomato in Brazil. Metaflumizone shows excellent activity but is not yet registered. In addition, abamectin is often used in combination with mineral oil, milbemectin and Bt (Siquera, Universidade Federal Rural de Pernambuco, Brazil, Pers. Comm., 2015).

Growers in the Iberian peninsula mainly use diamides (chlorantranilprole and flumbendiamide), emamectin benzoate and spinosad. They also use Bt-based insecticides, but "they are only effective against very early stages (L1) and with repetitive applications". (Bielza, Universidad Politecnica de Cartagena, Spain, Pers. Comm., 2015).

In south eastern Europe, a number of compounds are registered for control, including methomyl, spinosad, emamectin benzoate, abamectin, Bt, indoxacarb, metaflumizone, chlorantraniliprole, flubendiamide and two mixtures, chlorantraniliprole with abamectin (Voliam Targo 063 SC, Syngenta) and chlorantraniliprole with lambda-cyhalothrin (VoliamTargo 063 SC, Syngenta). (Roditakis, Plant Protection Institute of Heraklion, Greece, Pers. Comm., 2015).

No chemical insecticides other than those listed in Table 4 were identified for the control of other leaf mining Lepidoptera except *Phyllocnistis citrella* (citrus leaf miner). In this case neonicotinoids such as imidacloprid have provided excellent control. However, this class of insecticides has shown poor efficacy against *T. absoluta* in laboratory trials at IRT RR and also has a poor profile against natural enemies (Bass, Pers. Comm., 2015).

Apart from the chemical insecticides listed above, a few biopesticides have been trialled and used against *T. absoluta*. These options are described below:

Bacillus thuringiensis (Bt) is a naturally occurring bacterium of the family Bacillaceae. Different varieties and strains of this bacterium kill different insects with *Bt kurstaki* and *Bt aizawai* being most effective against Lepidopteran larvae. Within a particular variety, several strains can be distinguished on the basis of the crystalline proteins they produce. Over the last 40 years, *Bt kurstaki* has been used against many species of caterpillars on many different crops in many different countries of the world (Malais & Ravensberg, 2003). In laboratory experiments there was significantly less *T. absoluta* leaf damage in tomato plants treated with a Bt formulation than in untreated controls. First instar larvae were the most sensitive with just 1% of the area of leaves damaged when the larvae were exposed to Bt, whereas 77% of the area of control leaves was mined (González-Cabrera *et al.*, 2011). In glasshouse experiments, no damaged fruit was obtained in areas sprayed with Bt compared to an average of three fruits per plant damaged in the control crops. In open-field conditions significantly fewer infested leaflets and infested fruits were recorded in Bt-treated plots compared to non-treated (González-Cabrera *et al.*, 2011). The use of Bt early in the growing season has been combined with the release of the predatory bug *Nesidiocoris tenuis* to provide effective control of *T. absoluta*. Once *N. tenuis* has established Bt sprays are no longer required to keep *T. absoluta* under control. Plants treated with Bt once a week for two months in combination with a single release of *N. tenuis* had no fruit damage and higher yields than control plants and half of the fruit from untreated tomatoes were infested with *T. absoluta* (Molla, 2011). Experience in both the UK and Portugal has shown that Bt can provide useful control of *T. absoluta* larvae when they are 'grazing' in the head of the plant (Jacobson & Howlett, 2014). However, at least three applications at 7-10 day intervals were required to give adequate control.

Bacillus thuringiensis Vip3Aa16 protein may show good efficacy against *T. absoluta* (Sellami, *et al.*, 2014). Purified Vip3Aa16 showed lower LC50s against third instar larvae (Toxin/tomato leaf) (335+/-17 ng/cm^2) compared to that of *B. thuringiensis kurstaki* HD1 δ-endotoxins (955+/-4 ng/cm^2) ($P<0.05$). However, to the authors' knowledge, the use of Vip3Aa16 is not yet registered for *T. absoluta* control in any country.

Extracts of the neem plant have been used against *T. absoluta* in many different formulations and with varying degrees of success. The less well refined formulations contain numerous possible active ingredients and synergists, many of which are poorly documented. It is highly unlikely that such extracts would receive approval for use in UK food crops and have not been considered in more depth in this report. One particular extract, azadirachtin, has been refined and formulated into a product (NeemAzal), which is now registered for use in several countries but not the UK. In one study, azadirachtin was claimed to have good efficacy against *T. absoluta* (Tomé, 2013). However, in another study, only the combination of azadirachtin and Bt was found to reduce yield loss to a level comparable to that of synthetic insecticide control treatment (Bue *et al.,* 2012). Practical experience in organic tomato crops in Portugal suggests that weekly applications of azadirachtin and Bt to the heads of the plants are required to suppress *T. absoluta* population growth (Alves, Horticilha, Portugal, Pers. Com., 2014). However, it was not possible from these observations to determine which of the two components of the sprays was having the greatest effect.

A number of researchers are currently studying the properties and efficacy against *T. absoluta* of essential oils extracted from a wide range of plants; for example from *Mentha longiflora* (Malekmohammadi & Jafaripoordaragahi, 2015), *Eucalyptus globulus* and *Achillea millefolium* (Saber & Razzaghi, 2015) and *Syzygium aromaticum* (Doumandii *et al*., 2015). Most of this research is still laboratory-based where it is focusing on extraction methods and the optimisation of formulations. It is too early to predict how well these and other essential oils will perform within an IPM programme in crops. However, this is an interesting area of research which the UK industry should keep under close observation.

The entomopathogenic nematodes, *Steinernema feltiae* (as Nemasys), have provided 40-50% control of medium-sized *T. absoluta* larvae in commercial crops when applied to the point of foliar run-off at the rate of 1 million infective juveniles per litre (Jacobson & Martin, 2011). The sprays must be applied late in the evening so that the leaves remain wet for as long as possible and thereby allow the nematodes time to find entry points into the mines. It is probably necessary to apply at least 2-3 applications at 7-10 day intervals to provide adequate control. Nonetheless, this could be a useful option for growers of organic crops who are not allowed to use synthetic insecticides (Jacobson & Howlett, 2014).

Apart from the biopesticides and entomopathogenic nematodes described above, there are many 'macro-biologicals' which have been reported to attack *T. absoluta*. For example, natural enemies from 15 genera and 9 different families have been found attacking *T. absoluta* in the Mediterranean basin (Urbaneja *et al.*, 2012).

The benefits of releasing the mirid bug, *Macrolophus pygmaeus*, in tomato crops are well known to British tomato growers and this predator already forms the backbone of the UK IPM programme (Jacobson & Howlett, 2014). Several other mirid bugs are known to attack *T. absoluta* in other parts of the world; eg *Nesidiocoris tenuis*, *Macrolophus basicornis, Campyloneuropsis infumatus* and *Engytatus varians*. However, none of these species are indigenous to the UK and it is highly unlikely that a licence would be obtained to introduce such generalist predators due to the risk of unforeseen effects on the UK's natural fauna.

Parasitic wasps in the genus *Trichogramma* are natural parasites of *T. absoluta* eggs in both South America and Europe, and *T. achaeae* is commercially available to control *T. absoluta* in some countries. However, extremely large numbers of *T. achaeae* must be released every week for successful parasitism (Chailleux *et al.,* 2012) and the economics of this control measure are questionable. At best, *T. achaeae* may make a contribution to an IPM programme if it is compatible with the primary biological control agent and the second line of defence insecticides.

Selection of candidates with the greatest potential

In addition to efficacy, the potential of each candidate control measure must be evaluated according to the following criteria:

- Mode of action and insecticide resistance status
- Compatibility with biological control agents utilised in the UK tomato IPM programme.
- Systemic and / or translaminar activity
- Availability / approval in other countries

The issue with the chemical compounds detailed above is that many are compromised by resistance. This is illustrated in Table 5 which provides information on the mode of action and the IRAC resistance group of each chemical class highlighted in Table 4. In addition, the occurrence of known resistance is identified by a 'Yes' or 'No' entry and a score out of ten is used to indicate how widespread / severe is the resistance. There is little to be gained by pursuing a candidate insecticide if resistance has already been reported in another country.

A comparison of resistance between field populations collected in Brazil in the late 1990s found resistance ratios of 7 for permethrin, 9 for abamectin, 4 for methamidophos and 22 for cartap. A significant positive correlation between the number of sprays of a particular insecticide at a given location and the resistance of *T. absoluta* in that location to that insecticide was found for abamectin, cartap and permethrin. However, this correlation was not observed for the organophosphate methamidophos (Siqueira *et al*., 2000). Two glasshouse populations of *T. absoluta* (Bella vista and Rosario) collected in 2000 in Argentina showed resistance to deltamethrin and abamectin but there was no resistance to methamidophos. The resistance ratio to abamectin was extremely low; 2.5 and 3.6 in Rosario and Bella Vista respectively. The resistance to deltamethrin in both populations was so high that most larvae were alive at the highest dose tested, close to the solubility limit of the insecticide, so the exact resistance ratio could not be determined (Lietti *et al.*, 2005). A more recent study of Brazilian populations of *T. absoluta* published in 2011, found significant resistance in at least one population to six classes of insecticide: avermectins, spinosyns, pyrethroids, oxadiozines, benzoylureas and *B. thuringiensis* (Silva *et al.*, 2011). It is important to note that significant resistance means that there is variability between populations, but doesn't necessarily equate with control failure in the field. Therefore, the authors predicted the likelihood of control failure by estimating the percentage mortality of insects treated with the recommended label rate of insecticide. Mortality was predicted to be significantly lower than 80% in at least one population for the insecticides bifenthrin, indoxacarb, permethrin, diflubenzuron, teflubenzuron, triflumoron and *B. thuringiensis*. In contrast, all populations had 100% estimated mortality at the label rate of abamectin and spinosad (Silva *et al.*, 2011).

Table 5. IRAC Grouping, mode of action and existence of resistance in *Tuta absoluta* for each class of chemical insecticides described in Table 4 (Source IRAC) The severity of resistance is indicated by a 1-10 score where 10 is the most resistant.

Chemical Class	**IRAC Group**	**Mode of Action**	**Known resistance and severity (1-10)**
Organophosphates	1B	Acetylcholinesterase (AChE) inhibitors	Yes (7)
Pyrethroids	3A	Sodium channel modulators	Yes (10)
Spinosyns	5	Nicotinic acetylcholine receptor allosteric modulators	Yes (5)
Avermectins, Milbemycins	6	Chloride channel activators	Yes (3)
Pyrroles	13	Uncouplers of oxidative phosphorylation via disruption of the proton gradient	No
Nereistoxin analogues	14	Nicotinic acetylcholine receptor channel blockers	Yes (7)
Benzoylureas	15	Inhibitors of chitin biosynthesis, type .	Yes (6)
Diacylhydrazines	18	Ecdysone receptor agonists	Yes (6)
Oxadiazine	22A	Voltage-dependent sodium channel blockers	Yes (2)
Semi-carbazone	22B	Voltage-dependent sodium channel blockers	No
Diamides	28	Ryanodine receptor modulators	Yes (1)
Tetranortriterpenoid	UN	Compounds of unknown or uncertain MoA	No

Studies at IRT RR have shown that pyrethroids are essentially completely ineffective against *T. absoluta* populations worldwide due to target-site resistance (Haddi *et al.*, 2012). IRT RR has also detected target-site resistance to organophosphates and, in the present study, resistance to spinosad. Resistance to spinosad also appears to be a growing problem in South America (Campos *et al.,* 2014).

Phthalic and anthranilic diamides comprise a relatively new insecticide class for *T. absoluta* control. These insecticides currently show high efficacy against South American populations of *T. absoluta* (Campos *et al.,* 2015). However, resistance to this class of insecticide has recently been recorded, with over 1000-fold resistance described in populations originating from Sicily (Rodiakis *et al*., 2015).

The most serious resistance issues in Brazil occur with organophosphates, pyrethroids, IGRs (including ecdisteroids) and cartap (Siquera, Universidade Federal Rural de Pernambuco, Brazil, Pers. Comm., 2015). Some populations are already showing some reduced efficacy of spinosyns and diamides, but resistance is currently low-moderate. Depending on geographical location, there is some resistance to indoxacarb but, so far, this has not been cross-linked to metaflumizone which is in a closely related IRAC Group.

Spinetoram may be more effective against susceptible *T. absoluta* than the related compound, spinosad, and its use as an alternative to that insecticide could delay the development of further resistance to the spinosyn class of insecticidal compounds. However, spinetoram's use in tomatoes in the UK would require a completely new approval at considerable expense. This would not be supported by Dow AgroSciences due to the likelihood of cross resistance (Harris, Dow AgroSciences Pers. Com., 2015). As a consequence, spinetoram has been eliminated at this stage of the screening procedure.

In addition to the above references to abamectin resistance, the authors' have received reports of the failure of abamectin treatments against *T. absoluta* in Portugal (Townshend, Horticilha, Portugal, Pers. Com., 2012). However, after a gap of approximately 12 months, a single abamectin treatment was once again effective at the population level. This indicated that the abamectin susceptible individuals had some other advantage over the more tolerant individuals which allowed them to dominate in the absence of the insecticide. Nonetheless, the delayed abamectin treatment re-selected the more tolerant individuals and the subsequent treatment failed. These observations indicate that abamectin may have a useful role if used only once per year. The authors' have not seen confirmed reports or heard rumours of treatment failures arising from the use of the related compound, emamectin benzoate, against *T. absoluta*.

Of the 40 insecticides identified above, the initial screen based on the biochemical mode of action and the likelihood of resistance, selected just seven potentially useful compounds (*i.e.* abamectin, azadirachtin, *Bacillus thuringiensis*, clorfenapyr, emamectin benzoate, metaflumizone, methoxyfenozide) in addition to the three already used within the UK tomato IPM programme (spinosad, chlorantraniliprole and indoxacarb).

The next step in the screening sequence is to consider compatibility with the biological control agents used by UK tomato growers. It is important to stress that this screen must include the full range of biocontrols used in the whole IPM programme and not just those used against *T. absoluta*. Without this diligence, the project may resolve the main issue but create other pest control failures. Much of the information used in this stage of the screen originates from the International Organisation for Biological Control (IOBC) database and has been accessed via the 'Side Effects' section of the Koppert B.V. website.

Bt, azadirachtin and methoxyfenozide have the most acceptable side effect profiles. In particular, Bt applied as a high volume spray, has minimal effect on the biocontrols used in the UK tomato IPM programme. It is recommended that bumblebee hives are covered during spraying but there is reported to be no residual effect (Koppert, 2015). While the data sourced for azadirachtin is encouraging, it is somewhat confused by the many different extracts and formulations that have been prepared from the seed kernals of the tropical neem tree, *Azadirachta indica*. The IOBC database states that high volume sprays of azadiractin are harmless to *Phytoseiulus persimilis* and *Orius laevigatus*, slight-moderately harmful to *Macrolophus caliginosus* and *Encarsia formosa*, and moderately harmful to *Diglyphus isaea*. As with Bt, it is recommended that bumblebee hives are covered during spraying but there is reported to be no residual effect (Koppert, 2015). When applied through the irrigation system, neem-based products were reported to have only a marginal effect on the whitefly parasitoid, *Eretmocerus warrae* (Kumar *et al*., 2008). Similar systemic applications have been successfully integrated with the predatory mites, *Amblyseius cucumeris* and *Hypoaspis aculeifer*, (Thoeming & Poehling, 2006a) although these species are only indirectly relevant to the tomato IPM programme. Methoxyfenozide is reported to be harmless to *E. formosa, P. persimilis* and adult *M. caliginosus*, and only slightly harmful to *M. caliginosus* nymphs. There is no information about the effect of this compound on *D.*

isaea or *Dacnusa sibirica*. As with Bt and azadirachtin, it is recommended that bumblebee hives are covered during spraying but there is no residual effect (Koppert, 2015).

Abamectin is already available to UK tomato growers but rarely used during the cropping season because it is very harmful to most of the biological control agents used in the IPM programme (Koppert, 2015). It is particularly harmful and persistent towards *Macrolophus* spp. In addition, there are restrictions on its use during flowering due to the presence of bumblebees in the crop. This essentially limits its use to end of season 'clean-up' treatments. There is limited side effect information available for the related compound, emamectin benzoate. As it has a similar biochemical mode of action to abamectin, it may also be expected to have similar impact on natural enemies. However, in one study which evaluated lethal effects of emamectin benzoate on *M. pygmaeus* through three routes of exposure (direct, residual and oral), it caused less than 25% mortality which would be considered harmless by the IOBC rating scheme (Martinou *et al.,* 2014). Unpublished information from a Spanish researcher stated that "a fresh residue of emamectin benzoate is moderately toxic [to predatory bugs] but after a week is quite safe." (Bielza, Universidad Politecnica de Cartagena, Spain, Pers .Com, 2015). This requires further investigation and specific compatibility studies for the biological control agents currently used in UK tomato crops.

Chlorfenapyr and metaflumizone can both be eliminated at this stage due very harmful effects on a wide range biocontrols and bumblebees (Koppert, 2015). This information is reinforced by other studies which have shown both compounds to cause over 75% mortality to *M. pygmaeus* and other predatory bugs (Martinou *et al.,* 2014; Arno´ & Gabarra, 2011).

The second screen in the selection process has therefore reduced the number of potentially useful <u>additional</u> compounds for use during the UK tomato growing season to three candidates (Bt, azadirachtin, methoxyfenozide) and one additional compound for use as an end of season clean-up treatment (abamectin). This list can be increased to five potentially useful compounds if we include emamectin benzoate, although important questions remain to be answered about the suitability of this compound. The next stage in the selection procedure considered the physical properties of the remaining compounds with emphasis on their ability to penetrate leaves and / or have systemic activity that would allow application via the irrigation system.

Both *Bt kurstaki* and *Bt aizawai* are only effective when ingested by the target organism. There is no translaminar or systemic activity, so larvae are only vulnerable to Bt when feeding on the surface of the plant. The bacterium produce spores and protein crystals, the latter being ultimately responsible for the death of the caterpillars. The bacterial spores enter the body cavity of the insect and multiply, feeding ceases and the caterpillars die within 2-5 days. Young caterpillars are usually better controlled than their larger counterparts as they have to ingest less material. In most situations the young *T. absoluta* caterpillars feed on the surface for less than 90 minutes after hatching before they start to burrow into the plant tissue (Cuthbertson, 2011) resulting in a very narrow window of opportunity for this control measure. There may be other opportunities if the caterpillars move to other parts of the plant during their development but such migrations are unpredictable and of very short duration. In order to suppress *T. absoluta* population growth with Bt, sprays must be applied at 7-10 days intervals and be directed at the part of the crop canopy where the adult moths are laying eggs. This clearly requires a good understanding of the insect's activity patterns as well as a significant labour input.

The moult accelerating compound, methoxyfenozide, is primarily active by ingestion but also has some contact action. It has no translaminar or systemic activity. Dow AgroScience product information states that applications against leafmining caterpillars should be made prior to the insects moving into the plant – which would mean a very narrow window of opportunity for the control of *T. absoluta*. The product information also states that applications at label rates should provide 14-21 days of residual control on treated surfaces. However, in rapidly growing crops, such as protected 'high-wire' tomato, repeated applications would be required to protect new growth post-application because this is where *T.absoluta* most commonly lay their eggs.

It has proved difficult to source irrefutable evidence of translaminar and / or systemic activity of azadirachtin in tomato pants. Figure 2 illustrates the transportation of the azadirachtin active ingredient into leaf tissue two hours after foliar application. This diagrammatic representation was provided by Trifolio-M GmbH, who market an azadirachtin-based product in Europe, and was produced to illustrate the need to repeat the application if rain falls within two hours of treatment. It must be stressed that this diagram was not produced to illustrate translaminar activity nor does it specifically refer to tomato plants. Nonetheless, it indicates that 38% of the applied active ingredient may be expected to penetrate the leaf within two hours of treatment which would be a useful property when combating leaf mining caterpillars.

Various formulations of azadirachtin have been shown to have systemic activity on plants such as green bean (*Phaseoulus vulgaris*) (Theoming *et al*., 2003; Theoming *et al*., 2006) and tomato (Kumar *et al*., 2005; Kumar & Poehling, 2006; Thoeming & Poehling, 2006; Winkler *et al.*, 2015).

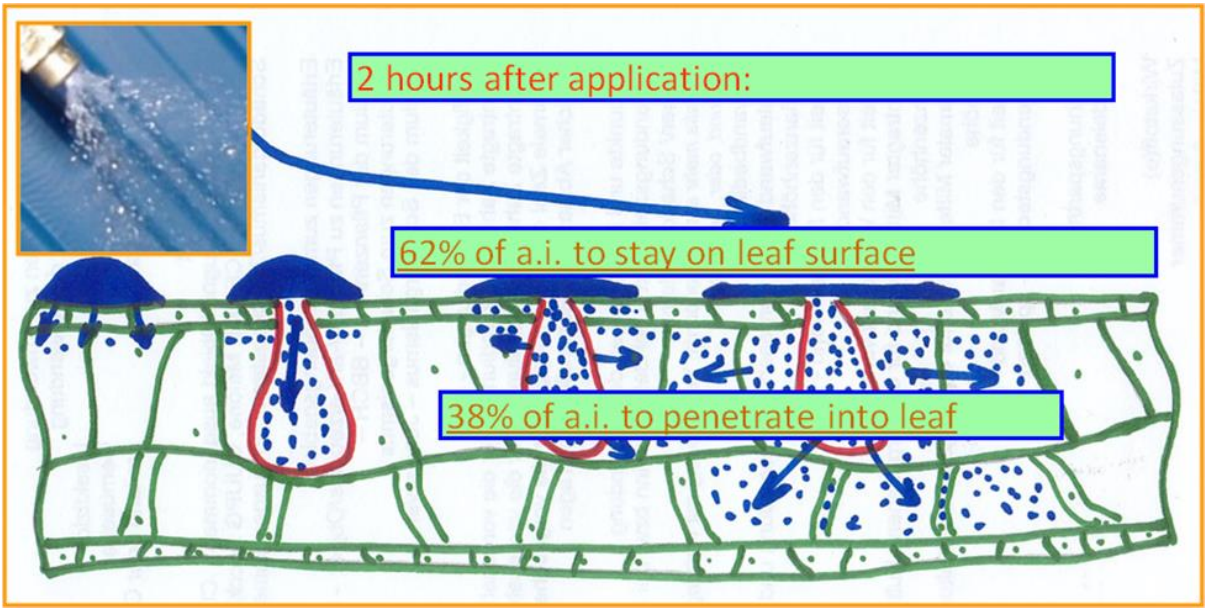

Figure 2. Transportation of the azadirachtin active ingredient into a leaf two hours after foliar application (Trifolio-M GmbH, unpublished information, 2015).

Kumar *et al*. (2005) studied three different neem treatment methods (seed, soil and foliar) and two different commercial neem products (NeemAzal T/S 1% azadirachtin and NeemAzalU 17% azadirachtin) against *Bemisia tabaci* on tomato plants in cages in air-conditioned cultivation rooms. All three methods of neem treatments resulted in reduced colonisation and oviposition by *B. tabaci*. Overall oviposition intensity was significantly reduced (44%) by the treatment of tomato seeds but an even higher reduction (74%) was achieved through soil drenching both with 3.0 g/l NeemAzal-U and foliar spraying (82%) with 10 ml/l of NeemAzal-TS compared with control treatments. The mortality among immatures increased in relation to azadirachtin concentrations with young larvae being the most sensitive. Foliar treatment was the most efficient, with 100% mortality for all three larval stages at high concentrations (10 ml/l of NeemAzal T/S) compared with 78–87% mortality with soil treatment (at 3.0 g/l NeemAzalU).

In a subsequent study, Kumar & Poehling (2006) explored the persistence of neem-based products against *B. tabaci* in rearing rooms and tropical netted greenhouses. Two commercial neem products, NeemAzal-T/S and NeemAzal-U, were used. Foliar application, under room conditions at dose-rates of 7 and 10 ml NeemAzal-T/S, induced an immature mortality of 32 and 44% respectively, whereas 7 days post-application, under greenhouse conditions, mortality rates declined to 5 and 7%, respectively. This result indicated rapid dissipation of the active ingredients. However, systemic application by soil drenching resulted in more stable effects under both laboratory and greenhouse conditions. After soil drenching with solutions of 3.0 g NeemAzal-U, immature mortality declined from 88% for the first day to almost half (45%) by day 7 in the greenhouse and from 90% on first day to 64% by day 7 under laboratory conditions. Similar response trends for *B. tabaci* were obtained for other parameters such as adult colonisation, egg deposition, and egg hatch. The loss of efficiency of the neem products was considered to be closely related to dose-rate, methods of application, and environment (*i.e.* temperature and UV light). Soil application was claimed to be a convenient approach to achieve high efficiency and persistence with neem products under the conditions in tropical greenhouse environments for whitefly management.

The effects of soil-applied neem products have also been investigated for the control of *Ceratothripoides claratris*, an important thrips pest on tomatoes cultivated under tropical conditions in greenhouses in Thailand (Thoeming & Poehling, 2006a). NeemAzal-U soil applications resulted in strong systemic effects against *C. claratris* on young tomato plants when high azadirachtin concentrations (400 mg/l) were repeatedly applied. Different application schedules (every second week, weekly, twice a week) as well as organic matter content of typical growing substrates resulted in no significant influence on thrips control. However, plant age did have an influence with stronger effects in young plants.

Winkler *et al.* (2015) placed 'sticks' impregnated with azadirachtin (as NeemAzal technical) in soil around plants and then demonstrated slow release into the soil as well as subsequent uptake and transport into the leaves of tomato and paprika plants. This treatment controlled *T. absoluta* and other pests with the full effect becoming apparent after seven days. They concluded that the technique had "very good possibilities" but required further development.

The described results for systemic applications of neem-based products in tomato suggest that azadirachtin could have potential as a direct replacement for spinosad against *T. absoluta* in the UK tomato IPM programme. In the UK, spinosad is applied via the drip irrigation system during the first few weeks of the growing season to slow down *T. absoluta* population growth while the predatory bugs, *M. pygmaeus*, become established. Thoeming & Poehling (2006) stated that the systemic treatments of neem-based products were most effective on young tomato plants, which is entirely consistent with the UK IPM strategy.

Furthermore, the systemic application of neem-based products has been successfully integrated with predators in other situations (Thoeming & Poehling, 2006a) albeit these were predatory mites rather than predatory bugs.

Emamectin benzoate has short persistence on the leaf surface but is rapidly absorbed into plant tissue and is translaminar. It is therefore ideally suited for high volume spray application against *T. absoluta*. It is not thought to be truly systemic but this should be further investigated.

Conclusions

A significant level of resistance to spinosad has been confirmed in *T. absoluta* populations at two UK nurseries and the existing IPM programme must be modified accordingly.

- *Macrolophus pygmaeus* remains the biological 'backbone' to the IPM programme and should continue to be released, with supplementary food, at the start of the crop.
- Spinosad applied via the irrigation should remain the preferred treatment to slow down *T. absoluta* population growth while the *M. pygmaeus* population is becoming established in the crop. To avoid resistance, spinosad should not be used more than once in a six month period. Where resistance has already been confirmed, the product should not be reused unless resistance tests show that the population has reverted to susceptible status. Thereafter, such populations should only be treated with spinosad at intervals greater than 12 months.
- Alternatives to spinosad should be developed as quickly as possible. The most promising candidate is currently azadirachtin. However, further research is required to determine its efficacy via the irrigation system and compatibility with the biological control agents used in the UK tomato IPM programme. The authors' understand that approval is being sought to use a product containing this compound in UK tomato crops.
- As yet, there is no known resistance to chlorantraniliprole in the UK although it has been confirmed in southern Europe. This should remain the first choice of second line of defence treatment to support *M. pygmaeus* during mid-season if conditions favour the pest over the predator. However, it must not be used twice in succession unless there is an interval of at least six months.
- The entomopathogenic nematodes, *Steinernema feltiae*, provide a useful second line of defence option for growers of organic crops who are not allowed to use synthetic insecticides. However, at least three applications at 7-10 day intervals are probably required to give acceptable levels of control.

- It will be important to further investigate the potential of emamectin benzoate to provide an alternative to chlorantraniliprole in the UK. This will require research to determine its compatibility with the biological control agents currently used in UK tomato crops and its systemic activity. Approval will be required for use in UK tomato crops.
- Experience in both the UK and Portugal has shown that *Bacillus thuringiensis* can provide useful control of *T. absoluta* larvae when the pests are 'grazing' for prolonged periods in the heads of the plants. However, at least three applications at 7-10 day intervals are probably required to give acceptable levels of control.
- The moult accelerating compound, methoxyfenozide, could provide an alternative to Bt when *T. absoluta* larvae are 'grazing' in the heads of the plants. It is anticipated that repeated treatments would be required to protect new plant growth. An EAMU is being sought for use of this product in UK tomato crops.
- Indoxacarb remains the first choice as an end of season 'clean-up' treatment. Where there have been difficulties obtaining control of *T. absoluta* with this insecticide, then abamectin should provide an acceptable alternative. Neither product should be used during the main growing season when bumblebees and biological control agents are still active in the crop.

Knowledge and Technology Transfer

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