Project title:	Development rate of <i>Tuta absoluta</i> under UK glasshouse conditions
Project number:	PE 002
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Report:	Final report, March 2011
Previous report:	N/A
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Date project commenced:	01/04/2010
Date project completed (or expected completion date):	31/03/2011

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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.

Dr Andrew G S Cuthbertson Applied Entomologist, Project Leader The Food and Environment Research Agency

Signature Date

Report authorised by:

Mr Phil Northing Applied Entomology Team Leader The Food and Environment Research Agency

Signature Date

CONTENTS

Grower Summary	1
Headline	1
Background	1
Summary	1
Financial Benefits	3
Science Section	4
Introduction	4
Materials and Methods	4
Data analysis	7
Results	7
Discussion	
Conclusions	15
Knowledge and Technology Transfer	16
References	17

GROWER SUMMARY

Headline

• New information on the development of *Tuta absoluta* will assist in the development of IPM programmes for glasshouse tomatoes

Background

Tuta absoluta is currently the most damaging pest of tomato crops in the Mediterranean basin and has completely destroyed some crops in southern Spain (Jacobson and Morley, 2010a). When it was first found in the UK in 2009, British growers were concerned that they would suffer similar catastrophic losses. At that time, there were no known control measures that were both effective against *T. absoluta* and compatible with the biological control agents used successfully against other tomato pests in the UK. Since then, another HDC project (PC 302) has developed an effective contingency plan (Jacobson and Morley, 2010b). However, that project also identified important gaps in the knowledge of the biology and behavior of *T. absoluta* which were constraining the further development of integrated control strategies. Many of those gaps have been addressed in the present study.

The main concern of British growers now relates to disruption of supplies to customers. If larvae of *T. absoluta* are detected inside tomato fruit by retailers or consumers, 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 (Jacobson, 2010a). 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 per season depending on the time of year that the infestation is first detected (Jacobson, 2010b). Elimination of this risk will require further fine tuning of the IPM programme (Jacobson and Morley, 2010c). This work is progressing in a continuation of HDC project PC 302 but its success will be dependent on the outcome of the present studies.

Summary

The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a major pest of tomato plants in South America. Since 2006 it has been detected in several European countries where much damage to crops has been reported (EPPO, 2008). It was first

recorded in the UK in 2009 where it was subjected to a policy of eradication. *Tuta absoluta* has the potential to cause devastating effects on UK tomato crops should it become established. Information on many aspects of the general biology and population development of *T. absoluta* is lacking. In the current work the population development of *T. absoluta* is lacking. In the current work the population development of *T. absoluta* is lacking. UK glasshouse temperatures.

- The optimum temperature from the current study for Tuta absoluta development ranged from 19 - 23°C. At 19°C, there was 52% survival of T. absoluta from egg to adult.
- As the temperature increases above 23°C the development time of the moth appears to decrease. This may well make T. absoluta better suited for UK glasshouse conditions compared to hotter Mediterranean climates.
- Population development ceases between 7 and 10°C. Only 17% of eggs hatched at 10°C but no larvae developed through to adult moths. However, this does not mean that the population will always die out. No eggs hatched when maintained at 7°C. Data on low temperature survival is essential.
- Under laboratory conditions the total lifespan of the moth was longest (72 days) at 13°C and shortest (35 days) at both 23 and 25°C. The physiological age of insects is known to increase more slowly at lower temperatures. Personal observation noted that moths at lower temperatures were less easily agitated.
- Development from egg to adult took 58 days at 13°C; 37 days at 19°C and 23 days at 25°C.
- High mortality of larval stages occurred under all temperatures tested. Under field conditions the egg stage would also be a highly vulnerable life-stage being readily open to attack from predators.
- Under laboratory conditions, first instar larvae were exposed on the leaf surface for approximately 82 minutes before fully tunneling into the leaf. This was a lot less than what was anticipated from unsubstantiated reports, where as long as 24 hours was thought to be the time first instar larvae spend on the leaf surface.

- Adult longevity was highest at 10°C with adult moths living for 40 days (when supplied with a food source) and lowest at 19°C where they survived for 16 days. Again, this could be related to physiological age and less activity at the lower temperatures.
- Our study determined that in general more males than females are produced (although more or less a 1:1 ratio is maintained).

Tuta absoluta has huge potential to establish populations within the UK protected horticulture industry. Understanding the population development of the pest under specific conditions will aid in the formation and application of integrated control strategies against the pest.

Financial Benefits

The British Tomato Growers' Association recognized that this project in isolation will not provide immediate financial benefits. However, the results will form an important part of a bigger package that will ultimately allow growers to minimise losses caused by this pest. The overall control package will also allow them to continue to use their IPM programme and thereby retain a competitive advantage over their competitors in southern Europe.

SCIENCE SECTION

Introduction

The introduction of non-indigenous pests and diseases into a country can impact on both forestry and horticultural industries (McDonald 1999; Cuthbertson *et al.*, 2009), where crop damage and even complete loss may occur. Environmental damage has also been recorded following establishment of non-indigenous organisms (Murchie *et al.*, 2003). The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one such pest, originating from South America, that devastates tomato plants (Barrientos *et al.*, 1998; Miranda *et al.*, 1998; Torres *et al.*, 2001; EPPO, 2004, 2005). Within Europe it was initially detected in the Iberian Peninsula in 2006 (Urbaneja *et al.*, 2007). Since then it has rapidly moved across the Mediterranean area and has been detected in France, Italy and the United Kingdom (UK). In 2009 there were 12 outbreaks of *T. absoluta* in the UK (Fera, unpublished data).

Studies regarding the basic biology and population development of *T. absoluta* are relatively few and are mainly concentrated in South American countries where the environmental conditions are very favourable for the life-cycle of the pest (Miranda *et al.*, 1998). Females of *T. absoluta* usually deposit their eggs on leaves. The four larval instars live in leaf mines in the inside of the leaf between the upper and lower epidermis where they feed and develop. The pupae are principally found in the ground but may also occur on the tomato plant. Damage produced by this pest is focused on the larval galleries made on the leaves, the terminal buds, the flowers and the fruits of the tomato crops (Haji *et al.*, 1988; Lopes-Filho, 1990). It has been reported that this pest can cause reductions in crop yield of up to 90% (Apablaza, 1992). The current study investigates the population development of *T. absoluta* under environmental conditions associated with UK tomato glasshouses. Also the time period that first instar larvae remain on the leaf surface before burrowing into the leaf surface was determined. Knowing this information can enable the correct timing of application of biological control agents against the pest.

Materials and Methods

Source of Tuta absoluta and maintenance of culture stock

Specimens of *T. absoluta* were obtained from an outbreak within a commercial glasshouse in Portugal in 2010. They were imported into the UK under license (as required for all other non-indigenous insects (Marris *et al.*, 2010)) and maintained within the Plant Health Insect Quarantine Unit at Fera, York. Cultures were initiated and maintained within sealed Perspex cages (60 cm x 60 cm x 80 cm) (Figure 1) on tomato plants (*Lycopersicon esculentum* c.v. Moneymaker) similar to those used for other non-indigenous insects (Cuthbertson *et al.*, 2003) at 20°C, 65% r.h. and a 16:8 hr L:D regime.





Figure 1. (A) Perspex cages maintaining *Tuta absoluta* cultures and (B) *Tuta absoluta* moths on tomato foliage within cage (photos Crown copyright Fera).

Population development under various temperatures

The rate of development of *T. absoluta* was investigated under the following constant temperatures: 7, 10, 13, 19, 23 and 25°C. Development was also investigated under the fluctuating temperature of 23°C for 16 hours (day) and 18°C for 8 hours (night). This would give approximately an average temperature of around 21°C, similar to conditions in glasshouses. Ten newly laid (all within 24 hours of being laid) eggs were placed on an individual tomato leaf the end of which was then maintained in cotton wool in an eppindorf tube with water. The leaf was then placed in a plastic box, lined with moist tissue, with a mesh lid to allow air movement and maintained in a controlled environment (CE) cabinet at the desired temperature. The tissue paper was moistened daily. The experiment was replicated ten times giving 100 eggs per trial (each temperature) in total. Once eggs had hatched and larvae had entered the leaves, each leaf was placed into an individual Petri dish (15 cm diameter) with fresh leaves added as required. This was then sealed with parafilm and placed in the appropriate CE cabinet. This allowed adult moths emerging from individual leaves to be counted. These moths had no food supply as once leaves rotted there was no way of adding new leaves, as to remove the lid would have meant the

possibility of moths escaping. The following data was recorded for each temperature: time taken for eggs to hatch, percentage egg hatch, time taken to develop from larvae to adult, percentage adult emergence, adult longevity without food, sex of adult moths emerged.

Sexing Tuta absoluta moths

Moths were sexed with certainty by Anastasia Korycinska (Fera). The genitalia were examined through dissection. The hindwing frenulum was also checked (Figure 2). The method of dissection is different depending on the sex of the moth, so it was important to determine this if at all possible before the procedure began (Figure 2). In general male moths have genitalia with 2 valves that can be seen as a slit on the ventral side of the abdomen at the posterior end: gently brushing away scales may help to show this. The frenulum on the anterior edge of the hind wing consists of a single thick bristle. The abdomen is usually slender, often parallel-sided. The antennae may be more plumose than the female, though this is not the case with *T. absoluta*. Female abdomens will taper to a point posteriorly, possibly with a visible ovipositor, but always with no central slit. The frenulum consists of at least two bristles. The abdomen may look more swollen, and is less likely to be parallel-sided.





Larval 'wandering' time on leaf surface

To assess and determine the time first instar larvae spend on the leaf surface after hatching and before they burrow into the leaf surface to continue their lifecycle and development, eggs were brought through to an almost hatching stage at 19°C (determined by visible head

capsules through the egg chorion/shell). An individual larvae as it hatched was observed manually under a light microscope while others were placed under a time lapse camera microscope to try and record larval behaviour. The hatching eggs were observed and the time the larvae spent on the leaf surface following hatching until full submergence into the leaf structure was recorded. This procedure was repeated on three separate occasions for individual larvae.

Adult moth longevity when supplied with food source

The longevity of adult moths at the various temperatures stated above was determined by taking a newly emerged (less than 24 hours old) adult moth and placing it within a ½ litre plastic container containing a honey/water solution as a readily available food source. The experiment was replicated 15 times for each temperature tested. Mortality of moths was assessed daily.

Data analysis

Where appropriate data were analysed. Assuming normality and constant variance, analysis of variance (ANOVA) was used to test any significant difference between different treatments (temperature). Pairwise comparison (with FDR multiple correction) was used to quantify the differences.

Results

Population development under various temperatures

No eggs hatched at 7°C. At 10°C only 17% of eggs hatched (Figure 3), significantly less than all the higher temperatures (P<0.001). Eggs maintained at 13°C had the highest percentage hatch with 92% hatching though this was not significantly more than at 19°C (P=0.47). The remaining temperatures all had hatch rates above 60%.

The highest adult emergence occurred at 19°C with 52% of eggs surviving through to adult moths (Figure 4). Even though eggs hatched at 10°C only 3% of eggs reached the pupal stage, however these died and therefore no adults emerged. Adult emergence rates also declined, after peaking at 19°C, with increasing temperature (Figure 4). There was larval mortality rates of greater than 50% (assuming all eggs that hatched were viable) (Figure 5), for example, at 13°C there was 92% egg hatch but only 40% adult moth emergence.



Figure 3. Percentage of *Tuta absoluta* eggs hatching at various temperatures.



Figure 4. Percentage emergence of *Tuta absoluta* adults at various temperatures.



Figure 5. Percentage larval mortality at various temperatures

More male moths were observed to develop in the trials than female, though results were not significantly different (Table 1). Eggs of *T. absoluta* took longest to hatch at 10°C (21 days) and least time (3 days) with the fluctuating temperature of 23/18°C (average 21°C) (Table 2; Figure 6). However at 10°C no adult moths developed through. Larvae remained developing in the leaves longest at 13°C (51 days) (Figure 6) and consequently had the longest life-span of 72 days. The adults all lived for between 10-15 days with no food source available (Table 2).

	Male	Female	Total
7°C	0	0	0
10°C	0	0	0
13°C	23	17	40
19°C	27	25	52
23°C	25	19	44
25°C	17	12	29
23/18°C	18	31	49
Total	110	104	214

Table 1. Number of male and female adult *Tuta absoluta* emerging at various temperatures

Temperature (°C)	Egg hatch	Larvae	Adult	Total life-span
7	-	-	-	-
10	21	27*	-	-
13	7	51	14	72
19	5	32	14	52
23	4	16	15	35
25	4	19	12	35
23/18	3	31	10	44

Table 2. Time-span in days of *Tuta absoluta* life-stages developing at the various temperatures tested (no food supplied for adult moths)

*Did not survive to adult



Figure 6. Time-span for *Tuta absoluta* egg and larvae development at various temperatures.

Adult moth longevity when supplied with food source

Newly emerged adult *T. absoluta* survived longest at 10° C (Figure 7). Here the adults lived for 40 days being offered a honey/water solution as a food source. Shorter survival rates were noted for 19° C (16 days) and 7 and 23° C (both 17 days respectively).



Figure 7. Adult Tuta absoluta longevity at various temperatures (°C).

Larval 'wandering' time on leaf surface

First instar larvae were manually observed to take approximately 18 minutes to hatch out of the shell once hatching began (Figure 8i-iii). Once hatched the larvae wandered around the leaf surface for an average of 12 minutes and approximately 15 mm from its egg shell (Figure 8iv) before starting to graze on the leaf surface. From this moment onwards the larvae started to burrow under the leaf surface and after a further 70 minutes was totally encased inside the leaf (Figure 8v). Recording under the camera microscope did not prove as useful as anticipated. Footage was obtained of larvae developing within the shell and then hatching out, but then the larvae simply wandered out of the lens field of vision (camera had to zoom in closely to get the small eggs in focus). This happened upon trying several times.







Discussion

Tuta absoluta is well able to develop under temperatures commonly experienced in UK glasshouses. The current work has determined that between 19 and 23°C is the most favourable temperature for moth development. Temperatures of 10°C and below proved fatal for moth development. It cannot be confirmed that a population would die out if glasshouse temperatures were lowered to this temperature. However, this could form the basis of a control programme for sterilizing the glasshouse following an outbreak of moths. Eggs did hatch at 10°C but the lifecycle never completed, several first instar larvae were noted dead on the leaf surface having failed to burrow into the leaf. It is assumed that full

mortality was caused in the larval stage. No eggs hatched at all when held at 7°C, they simply rotted. From this work we can determine that 10°C is at the lower end of the temperature range for *T. absoluta* development. Temperatures of 23°C and above appeared to have a detrimental effect on *T. absoluta* development and life-span. This would suggest that UK glasshouse conditions will be very favorable for this pest to develop compared to hotter (for example Mediterranean) climates. This conclusion fits with a species that originates from the foothills of the Andes in South America. These findings are also consistent with reports from growers in southern Italy who have reported that population growth of *T. absoluta* is greatest in spring/early summer and again in late summer/autumn with a period of respite in mid-summer (Dr Rob Jacobson, *pers. comm.*).

Tuta absoluta shows high natural mortality. During its life cycle the larval stage is known to be the most critical (Miranda *et al.*, 1998). It has been suggested that third-instar mortality could be due to the dispersal of the caterpillars as they grow, which would increase their exposure to predators in the field. On the other hand, larvae of first and second instars remain in the leaf where oviposition takes place. When they reach the third instar there is more competition for food and therefore the larvae need to spread over the tomato plant (Price, 1984). During the fourth instar most of the caterpillars are in different leaves, but they feed in the same mine throughout this instar. High mortality in instars could also be due to the fact that early instars are closer together and therefore more vulnerable to predators in the field. Under laboratory conditions in the current study presence of natural enemies was not a problem. The study therefore determines that *T. absoluta* has naturally high larval mortality.

High egg mortality is not a rare characteristic among insect species (Price, 1984). Poorly protected eggs on the leaf surface are an easy target for predation and parasitism, but the oviposition of large numbers of eggs, many of which may not be viable, may minimise the impact of these mortality factors on the pest population. This may also be an explanation for the low numbers of adult moths developing through in our laboratory experiments compared to the starting number of eggs. Our experiments showed that in general more males than females developed through in all the trials except the fluctuating temperature trial. However, it was basically a 1:1 ratio. This is the reverse of that found by Fernandez and Montagne (1990) who observed more females than males in general. However, they also still obtained a 1:1 ratio. Explanations for this reversal in gender numbers is unknown.

Tuta absoluta is multivoltine, that is, it produces several broods in a single year, and population parameters suggest that it is what is described as an r selected species (a term

used to describe species that produce many brood but of which few survive) (Pereyra and Sánchez, 2006). The duration of the developmental cycle greatly depends on environmental conditions, with average development time of 76.3 days at 14°C, 39.8 days at 19.7°C and 23.8 days at 27.1°C (Barrientos *et al.* 1998). These figures differ from those obtained within the current study. We found total development from egg to adult only took 58 days at 13°C; 37 days to develop at 19°C and 23 days at 25°C. *Tuta absoluta* would have appeared to have developed faster from egg to adult in our studies that the times recorded in Barrientos *et al.* (1998) study. Vercher *et al.* (2010) stated that they were able to maintain and keep *T. absoluta* larvae alive during several weeks at 4°C, however, in our study no larvae survived 10°C when maintained at this temperature with ample fresh tomato foliage supplied. We also had no egg hatch at 7°C, all simply rotted after being maintained at this temperature for up to 66 days.

Desneux *et al.* (2010) cite information from a web pdf document of work by Estay (2000) (though the author of the current report has not been able to trace this pdf document on the web) that adult *T. absoluta* lifespan ranges between 10 and 15 days for females and 6–7 days for males. How valid this information is cannot be confirmed. However, it is totally dismissed by the current study. Though the adult *T. absoluta* were not sexed in our longevity study they lived much longer than 15 days. In the life stage development trials undertaken (where adult moths had no food supplied) they lived for up to 15 days at 23°C. In the longevity trials where adult moths were supplied with a food source of honey/water they survived for 40 days at 10°C. The physiological age of insects is known to increase at lower temperatures (Curry *et al.*, 1978).

Egg laying by female moths was not recorded in the current study but is stated by Uchoa-Fernandes *et al.* (1995) that females mate only once a day and are able to mate up to six times during their lifespan, with a single mating bout lasting 4–5 h. The most prolific oviposition period is 7 days after first mating, and females lay 76% of their eggs at that time, with a maximum lifetime fecundity of 260 eggs per female.

Biological control represents one of the main strategies for the integrated management of this pest. Besides applied biological control it is necessary to consider natural biological control through the conservation of natural enemies. This can be achieved through the adoption of cultural practices and the use of selective insecticides. However, biological control is not an easy option if the pest in question does not remain in a position that is open to attack by the predator. The majority of the lifecycle of *T. absoluta* is spent inside the leaf. Eggs of insects are known to be difficult to control and so unless the natural enemy can

pierce the egg shell no control will be afforded. In the case of T. absoluta vulnerable life stages are the larval stages. The current work has determined that T. absoluta first instar larvae only spend approximately 82 minutes on the leaf surface, from hatching out until they burrow into the leaf and become fully enclosed. This time period is a lot less than what was originally anticipated from unsubstantiated reports from Mediterranean workers (Dr Rob Jacobson pers. comm.), where the idea was that T. absoluta first instar larvae could spend up to 24 hours on the leaf surface. The much shorter time period recorded in the present study does not permit a large window of opportunity for natural enemies to have an impact. As a result, the larval predator approach may not be effective. However, egg predators, predatory bugs such as Macrolophus and Nesidicocoris along with entomopathogenic control agents such as nematodes may offer better potential. The T. absoluta larvae produce tunnels with large entry holes to the galleries that could be easily used by the nematodes to penetrate and avoid desiccation and ultraviolet light and then finally infect the larvae. Work assessing the potential of the predatory bugs and in particular the use of nematodes is recorded in a previous HDC report (PC 302, July 2010). Further trials need to be conducted to assess the nematodes ability to potentially control the caterpillars.

Conclusions

- The optimum temperature from the current study for *T. absoluta* development ranged from 19 23°C. At 19°C, there was 52% survival of *T. absoluta* from egg to adult.
- As temperature increases (23°C and above) development time of the moth would appear to decrease. This may well make *T. absoluta* better suited for UK glasshouse conditions compared to hotter Mediterranean climates.
- Population development ceases between 7 and 10°C. Only 17% of eggs hatched at 10°C but no larvae developed through to adult moths. However, this does not mean that the population will always die out. No eggs hatched when maintained at 7°C. Data on low temperature survival is essential.
- Under laboratory conditions the total lifespan of the moth was longest (72 days) at 13°C and shortest (35 days) at both 23 and 25°C. The physiological age of insects is known to increase more slowly at lower temperatures. It was noted that moths at lower temperatures were less easily agitated.

- Development from egg to adult took 58 days at 13°C; 37 days at 19°C and 23 days at 25°C.
- High mortality of larval stages occurred under all temperatures tested. Under field conditions the egg stage would also be a highly vulnerable life-stage being readily open to attack from predators.
- Under laboratory conditions, first instar larvae were exposed on the leaf surface for approximately 82 minutes before becoming fully submerged inside the leaf. This was a lot less than what was anticipated from unsubstantiated reports, where up to 24 hours was thought to be the time first instar larvae spend on the leaf surface.
- Adult longevity was highest at 10°C with adult moths living for 40 days (when supplied with a food source) and lowest at 19°C where they survived for 16 days. Again, this could be related to physiological age and less activity at the lower temperatures.
- Our study determined that in general more males than females are produced (although more or less a 1:1 ratio is maintained).
- Tuta absoluta has huge potential to establish populations within the UK protected horticulture industry. Understanding the population development of the pest under specific conditions will aid in the formation and application of integrated control strategies against the pest.

Knowledge and Technology Transfer

Cuthbertson, A.G.S. (2010). Tuta absoluta development. HDC News, November. 168:27.

Cuthbertson, A.G.S., Mathers, J.J., Blackburn, L.F., Korycinska, A., Luo, W., Jacobson, R.J. and Northing, P. (201-). Population development of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under various environmental conditions in UK glasshouses. *International Journal of Environmental Science and Technology*, in draft.

References

Apablaza, J. (1992). La polilla del tomate y su manejo. Tattersal 79:12–13.

Barrientos, Z.R., Apablaza, H.J., Norero, S.A. and Estay, P.P. (1998). Threshold temperature and thermal constant for development of the South American tomato moth, *Tuta absoluta* (Lepidoptera, Gelechiidae). Ciencia e Investigacion Agraria 25:133-137 (in Spanish).

Curry, G.L., Feldman, R.M. & Smith, K.C. (1978). A stochastic model of a temperaturedependent population. Theoretical Population Biology 13:197-213.

Cuthbertson, A.G.S., Head, J., Walters, K.F.A. & Gregory, S.A. (2003). The efficacy of the entomopathogenic nematode *Steinernema feltiae*, against instars of *Bemisia tabaci*. Journal of Invertebrate Pathology 83:267-269.

Cuthbertson, A.G.S., Blackburn, L.F., Northing, P., Mathers, J.J., Luo, W. and Walters, K.F.A., (2009). Environmental evaluation of hot water treatments to control *Liriomyza huidobrensis* infesting plant material in transit. International Journal of Environmental Science and Technology 6:167-174.

Desneux,N., Wajnberg, E., Wyckhuys, K.A.G., Burgio, G., Arpaia, S., Narva´ez-Vasquez, C.A. Gonza´lez-Cabrera, J., Catala´n Ruescas, D., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T. and Urbaneja, A. (2010). Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. Journal of Pest Science 83:197-215.

EPPO (2004). Data sheets on pests recommended for regulation as quarantine pests: *Tuta absoluta*. 3pp.

EPPO (2005). Data sheets on quarantine pests: *Tuta absoluta*. OEPP/EPPO Bulletin 35:434-435.

EPPO (2008). First report of *Tuta absoluta* in Spain. 2008/001. 1pp.

Estay P (2000) Polilla del Tomate *Tuta absoluta* (Meyrick) URL http://alerce.inia.cl/docs/Informativos/ Informativo09.pdf. (have not been able to access this).

Fernandez, S. and Montagne, A. (1990). Tomato leafminer biology, *Scrobipalpula absoluta* (*Meyrick*) (*Lepidoptera: Gelechiidae*). Bulletin of Entomology for Venezuela 5:89-99.

Haji, F.N.P., Parra, J.R.P., Silva, J.P. and Batista, J.G.D. (1988). Biology of the tomato leafminer under laboratory conditions. Pesquisa Agropecuaria Brasileira 23:107-110.

Lopes-Filho, F. (1990). Tomate industrial no submedio Săo Francisco e as pragas que limitam sua producao. *Pesquisa Agropecuaria Brasileira* 25:283-288.

Jacobson, R.J. (2010a). *Tuta absoluta*: Biology and Control. *Tomato Pest and Disease Seminar. Stoneleigh, UK. 14 January 2010.* 6pp.

Jacobson, R.J. (2010b). *Tuta absoluta*: One year on. In: *Abstracts, HDC/HRA 2010 Tomato Conference, Coventry, 30 September 2010* (Ed. Adams, S.), 7-19.

Jacobson, R.J. and Morley, P. (2010a). Research catching up with *Tuta. HDC News*, May 2010. 163:18-19.

Jacobson, R.J. and Morley, P. (2010b). Organic tomato: Phase 1 of contingency plans for the control of *Tuta absoluta* and *Nesidiocoris tenuis*. *Report of contract work undertaken for HDC*, July 2010, 51 pp.

Jacobson, R.J. and Morley, P. (2010c). Tuta meets its match. HDC News, 168:26-27

Marris, G., Cuthbertson, A.G.S., Mathers, J.J. and Blackburn, L.F. (2010). Containing the Small Hive Beetle for research purposes. *Bee Craft*, October 2010:17-21.

McDonald, J.R., Bale, J.S. and Walters, K.F.A. (1999).Temperature development and establishment potential of *Thrips palmi* in the UK. European Journal of Entomology 95:169-173.

Miranda, M.M.M., Picanco, M., Zanuncio, J.C. and Guedes, R.N.C. (1998). Ecological life table of *Tuta absoluta* (Meyrick) (Lepidoptera: gelechiidae). Biocontrol Science and Technology 8:597-606.

Murchie, A.K., Moore, J.P., Walters, K.F.A. and Blackshaw, R.P. (2003). Invasion of agricultural land by the earthworm predator, *Arthurdendyus triangulata* (Deny). Pedobiologia 47:920-923 (2003).

Pereyra, P.C. and Sánchez, N.E. (2006). Effect of two Solanaceous plants on development and population parameters of the Tomato Leaf Miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Neotropical Entomology 35:671-676.

Price, P.W. (1984). *Insect Ecology*, 2nd Edn. John Wiley & Sons, New York.

Torres, J.B., Faria, C.A., Evangelista, W.A. and Pratissol, D. (2001). Within-plant distribution of the leaf miner *Tuta absoluta* (Meyrick) immatures in processing tomatoes, with notes on plant phenology. International Journal of Pest Management 47:173-178.

Uchoa-Fernandes, M.A., Della Lucia, T.M.C. and Vilela, E.F. (1995) Mating, oviposition and pupation of Scrobipalpula absoluta (Meyrick) (Lepidoptera: Gelechiidae). Anais da Sociedade Entomologica do Brasil 24:159–164.

Urbaneja, A., Vercher, R., Navarro, V., Garcı'a Marı' F. and Porcuna, J.L. (2007). La polilla del tomate, *Tuta absoluta*. Phytoma España 194:16–23 (in Spanish).

Vercher, R., Calabuig, A. and Felipe, C. (2010). Ecología, muestreos y umbrales de *Tuta absoluta* (Meyrick). Phytoma España 217:23–26.

Appendices

Data analysis

Method: With assuming normality and constant variance, analysis of variance (ANOVA) was used to test any significant difference between different treatments (temperature). Pairewise comparison (with FDR multiple correction) was used to quantify the difference for every two treatment.



1. Egg hatch data

Summary of ANOVA result

```
Analysis of Variance Table

Response: y

Df Sum Sq Mean Sq F value Pr(>F)

treat 6 890.17 148.362 84.054 < 2.2e-16 ***

Residuals 63 111.20 1.765

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Signif. codes: 0 `***' 0.001 `**' 0.01 `*' 0.05 `.' 0.1 ` ' 1
```

There was significant difference in eggs hatching rate with temperature.

Pairewise comparison

	T07	T10	T13	T19	T23	T25
T10	0.00497	-	-	-	-	-
T13	< 2e-16	< 2e-16	-	-	-	-
T19	< 2e-16	< 2e-16	0.47044	-	-	-
T23	< 2e-16	2.5e-16	0.17697	0.55624	-	-
T25	1.0e-14	1.6e-09	1.1e-06	2.3e-05	0.00022	-
TC23/18	< 2e-16	< 2e-16	0.64614	0.73753	0.39089	7.1e-06

1. Adult emergence data



Summary of ANOVA result

Analysis of Variance Table

Response: y Df Sum Sq Mean Sq F value Pr(>F) treat 6 275.97 45.995 17.691 6.859e-12 *** Residuals 63 163.80 2.600 ---Signif. codes: 0 `***' 0.001 `**' 0.01 `*' 0.05 `.' 0.1 ` ' 1

There was significant difference in adult emergence rate with temperature.

Pairewise comparison

	T07	T10	T13	T19	T23	T25
T10	1.00000	-	-	-	-	-
T13	2.2e-05	2.2e-05	-	-	-	-
T19	9.0e-09	9.0e-09	0.03476	-	-	-
T23	2.5e-07	2.5e-07	0.28432	0.33535	-	-
T25	0.00033	0.00033	0.45153	0.00424	0.06238	-
TC23/18	1.3e-07	1.3e-07	0.18502	0.45153	0.82154	0.03476

2. Adult moth longevity data

