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Project leaders:	Dr Andrew G S Cuthbertson, The Food and Environment Research Agency (Fera), York YO41 1LZ Dr John H Buxton, ADAS Boxworth, Boxworth, Cambridge CB23 4NN
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Previous report:	N/A
Key staff:	James J Mathers (Fera) Lisa F Blackburn (Fera) Dennis Churchill (ADAS)
Location of project:	Fera, York, and Bordon Hill Nurseries, Stratford-upon-Avon
Industry Representative:	Russell Woodcock, Bordon Hill Nurseries
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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 and Project Leader
The Food and Environment Research Agency

Signature .. Andrew GS Cuthbertson . Date 20/9/11.

Mr Phil Northing
Invertebrate Pest Management Team Leader
The Food and Environment Research Agency

Signature .... Date 20/9/11

Dr John Buxton, Senior Consultant Entomologist, ADAS.

Signature…… John Buxton Date 26/09/2011

Report authorised by:

Dr Rick Mumford
Head of Crop and Food Security Programme
The Food and Environment Research Agency

Signature ..... Date 21/9/11

Mr Peter Bassett
Entomology Sector Manager
ADAS

Signature .. P. Bassett Date 26/09/2011

CONTENTS

GROWER SUMMARY

Headline

Intensive spray programmes using a range of chemically and physically acting insecticides applied four times within the first 21 days after potting poinsettia cuttings showed excellent efficacy against *Bemisia tabaci*. In the same trial the newly approved bio-pesticide 'Naturalis' also proved to be very effective. Crop safety tests of the same programmes on a nursery in the Midlands under commercial conditions, using four varieties of poinsettia, showed no signs of phytotoxicity.

Background

Bemisia tabaci continues to be a major pest of economically important crops worldwide. Within the UK *B. tabaci* remains a notifiable pest subject to a policy of eradication if found on propagators premises and plants moving in trade, and containment/eradication if outbreaks occur at nurseries.

There are numerous 'types' of *Bemisia*, of which two are sometimes associated with poinsettia production. The 'B-biotype' is of specific economic concern because it is an effective vector of over 110 viruses from several families, particularly geminiviruses. The second, 'Q-biotype' is more invasive than the 'B-biotype' and has also shown more resistance to the range of pesticides currently used for whitefly control.

The current work was undertaken following a *Bemisia* outbreak at a commercial nursery during 2009. This population of *Bemisia* proved extremely difficult to eradicate, and was later found to be the 'Q biotype'. Specimens were collected and transported securely and maintained under strict license requirements in Defra's Plant Health Insect Quarantine Unit at Fera. Subsequent work tested the efficacy of different control products applied alone and in sequence in laboratory and semi-field trials against this population of *Bemisia*. Chemical control programmes developed for this type of *Bemisia* should be equally suitable for use against the 'B-biotype'.

To complement the efficacy work in the quarantine facility at Fera, the same sequential spray programmes were tested on a nursery so that any phytotoxic effects could be quantified. The aim of the project was to determine programmes for *B. tabaci* control that were both effective against the pest and safe to the poinsettia crop.

Summary

Poinsettia plants were infested and specimens of whitefly at the desired life-stages for testing were obtained using established methods and data from previous Fera work. In the first trial products were tested using the leaf dip technique against three life stages of *B. tabaci*; eggs, second instar larvae and adults.

All products tested caused some degree of mortality of *B. tabaci* eggs. There was a significant difference in the mortality of eggs after leaf dipping with the different active ingredients. Exposure to Tri-Tek Oil at 2% (product awaiting UK approval at present) produced total mortality of *B. tabaci* eggs. The following products; SB-Plant Invigorator, Gazelle, Dynamec and Spraying Oil at label rates all produced high percentage egg mortalities (96.6, 88.8, 84.1 and 67.8% respectively) that were all significantly higher than the water control. This is extremely promising as the egg stage of *B. tabaci* has always proved difficult to control in the past.

Efficacy of products against the second larval instar stage also produced promising results. The bio-pesticide Naturalis (*Beauveria bassiana*) produced the highest mortality (73.1%). This product has recently been approved for the UK horticultural market. Tri-Tek Oil, Agri 50-E and SB-Plant Invigorator also gave over 70% control of second instar scales.

Naturalis and two of the petroleum oil based products (Tri-Tek, Spraying Oil) gave 100% mortality of the adult stage of *B. tabaci* (see images below).

Adult B. *tabaci* infected with Naturalis and B. *tabaci* adults trapped on Tri-Tek treated leaves.

To determine the compatability of the biological fungal agent (Naturalis) with the chemical products, direct tank-mixing tests were undertaken. Conidia of the fungus (*Beauveria bassiana*) were suspended in insecticide solutions which had been diluted to their recommended rates. After a period of 24 hours, the solution was plated out onto agar and incubated to determine spore survival. Tri-Tek showed the best potential to be used as a tank-mix following 100% germination of the Naturalis spores. Therefore, Naturalis and Tri-Tek could be successfully applied as a tank-mix for whitefly control. Other products, including Addit, Dynamec, Gazelle and Spraying Oil showed no significant reduction in spore germination and so could potentially be tank mixed with Naturalis.

Sequential treatments were also applied as sprays to poinsettia cuttings within the first 21 days after potting. They included a range of physically acting products as well as chemical insecticides, and were intended to overcome insecticide resistant *Bemisia* strains likely to be encountered by UK growers. Trials were started using plants with just the egg stage present, and in a second trial, with only the second instar scale stage present. By counting the number of adults that finally emerged, the success of each treatment could be assessed. The full range of treatments tested is shown in Table 1 below.

In the trial when treatments started at the egg stage, complete control was obtained from all the sequential treatments tested. Adults emerged from the water only control, showing that the experimental technique was valid. The second trial, starting at the second scale instar stage, showed that some larvae survived to reach the 3rd or 4th instar: but no adults emerged, unlike the control where adults readily developed. Sequential treatments of Naturalis also gave excellent control of *Bemisia* eggs and second instars with no adults developing. The sequential treatment programmes are detailed in Table 1 .

Table 1. Sequential application programmes* tested for *Bemisia tabaci* control.

**All the products listed have either label approval or a SOLA for use on ornamentals. Agri 50-E and Spraying Oil are exempt from CRD registration as they act by physical means only. SB-Plant Invigorator is not classed as a pesticide.*

Crop Stage	**3 days after potting**	**7 days after potting**	**14 days after potting**	**20 days after potting**
Likely *Bemisia* life-stage	**Eggs**	**Eggs + 1st Instar scales**	**1st + 2nd Instar scales**	**2nd + 3rd Instars scales**
Programme 1	Water only	Water only	Water only	Water only
Programme 2	Majestik	Oberon + Mycotal + Addit	Spraying Oil	Dynamec + Chess
Programme 3	SB-Plant Invigorator	Oberon + Mycotal + Addit	Oberon + Mycotal + Addit	Spraying Oil
Programme 4	Spraying Oil	Majestik	Savona	Agri 50-E
Programme 5	Savona	Spraying Oil	Dynamec + Chess	Gazelle
Programme 6	SB-Plant Invigorator	Majestik	Dynamec + Chess	Gazelle
Programme 7*	Naturalis	Naturalis	Naturalis	Naturalis

* Naturalis was not included in the phytotoxicity trials as it was not approved at the time the trials were carried out.

The sequential programmes listed in the table above, except programme 7, were applied to newly potted cuttings of the poinsettia varieties ‘Infinity’, ‘Infinity White’, ‘Scandic’ and ‘Champion’ on a nursery, and no detectable phytotoxicity was observed. However, Spraying Oil in particular can cause scorch to poinsettias and so should always be tested on a limited scale before widespread usage. All of the products tested in the current trials have reasonable IPM compatibility. Therefore, biological control agents such as *Encarsia* and *Eretmocerus* parasitoids could be used by growers after the intensive spray programme applied to the cuttings has been completed.

The worst scenario for the poinsettia grower is to have *B. tabaci* identified at a late stage in the crop, possibly when coloured bracts have formed and sprays are likely to damage them, leading to downgrading of the crop and loss of income. Therefore, the early spray programmes tested here, applied when plants are small, are likely to achieve better spray coverage and better control than applications made later, when crop canopy is well developed and under leaf coverage is very difficult to attain. The sequential applications

gave excellent control of *Bemisia*, and so any of the treatment schedules tested could be recommended to poinsettia growers for control/eradication of *B. tabaci.*

Financial Benefits

Current grower estimates suggest there are around 3.5 million poinsettia plants produced in the UK each year with a wholesale value of around £7 million. A further 0.5-1.5 million plants are also currently imported each year, so issues with crop pest contamination and downgrading could lead to product substitution and a greater number of plants being imported.

Direct savings arising from this project are difficult to quantify, but potential financial benefits are considerable. These include:

- Eradication of *B. tabaci* at an early stage prevents any loss of poinsettia crop sales later on and any associated loss of customer confidence.
- Early control negates the need for expensive and often ineffective and potentially damaging clean up sprays (which may be demanded by PHSI) and/or labour to clean up the plants from *B. tabaci* infestation.
- The spray programme is easier to apply when plants are small, resulting in savings in chemical and labour for application.

Action Points

- Poinsettia growers should assume that imported cuttings are infested with *Bemisia* and apply a control programme within the first 4 weeks after potting, when plants are small and under leaf coverage from sprays is likely to be effective.

- A sequential programme from the list in the table should be selected and applied at the suggested timings. Biological control programmes could be planned to follow.

- If using the bio-pesticide Naturalis, tank-mixes with Tri-Tek Oil, Addit, Dynamec and Gazelle are possible without harming spore germination. This could help to improve control of difficult pests such as *Bemisia,* although Naturalis performed well as a stand alone treatment.

SCIENCE SECTION

Introduction

The tobacco whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), is a major pest of economically important crops worldwide (Gerling *et al*., 1980; Nomikou *et al*., 2000). Damage can be caused directly by feeding on phloem sap or indirectly by the large amounts of honeydew produced. *Bemisia tabaci* is also a vector of many plant viruses (Alegbejo, 2000; Simón *et al*., 2003). Within the United Kingdom (UK), *B. tabaci* is a notifiable pest subject to a policy of eradication if found on propagators' premises or on plants moving in trade, and of containment/eradication if outbreaks occur at nurseries (Cuthbertson, 2005; Cuthbertson *et al*., 2011). The UK maintains Protected Zone status against *B. tabaci* and eradication generally involves use of chemical insecticides, though much research has shown the potential of entomopathogens to control *B. tabaci* populations (Cuthbertson *et al*., 2011). There are several active ingredients currently used in the UK for treating *B. tabaci* outbreaks (Buxton and Clarke, 1994; Cannon *et al*., 2005; Cheek and Macdonald, 1994; Sharaf, 1986), but with chemical resistance being shown by *B. tabaci* (Ahmad *et al*., 2002; Cahill *et al*., 1994, 1996; Osborne and Landa, 1992; Prabhaker *et al*., 1985) an integrated strategy using both biological and chemical agents is required.

Since 1987, *B. tabaci* has been intercepted at nurseries in the UK on an extremely wide range of hosts. In 1987, there were 98 interceptions and outbreaks of *B. tabaci* at nurseries, all on poinsettias, predominantly from the Netherlands (Bartlett, 1992). The following year (1988), there were 87 interceptions and outbreaks on growing sites, again predominantly on poinsettias from the Netherlands. Over recent years, *B. tabaci* has continually been intercepted on poinsettia, with this host plant accounting for the majority of outbreaks in every year between 1998-2009 (Cuthbertson *et al.,* 2011). In 2009, 56% of interceptions at growing sites were on *E. pulcherrima* (poinsettia), there were also interceptions on *Lantana* (10%), *Hibiscus* (9%), *Dipladenia* (7%) and *Artemisia dracunculus* (tarragon) (5%). In addition to the interceptions of *B. tabaci* at growing sites, there were also regular import interceptions at ports, distribution centres and retail outlets. In 2009, there were 55 import interceptions of *B. tabaci* including: 14 on *E. pulcherrima, Hibiscus* and *Hypericum* from the Netherlands; 13 on aquatic plants from Singapore and 10 on *Ocimum basilicum* (basil) from Thailand. Plants from the Netherlands have accounted for more interceptions (328) at growing sites than any other source between 1998-2009, followed by plants from Israel (198) (Cuthbertson *et al*., 2011).

The ongoing threat of *B. tabaci* outbreaks coupled with the high volume of outbreaks consisting of insecticide resistant populations highlights a strong need for finding resistance breaking strategies. This will ensure that containment and eradication are possible under the vast majority of scenarios.

The aim of this current work was to evaluate sequential insecticide applications, applied within the first 21 days after potting poinsettia cuttings. This is on the basis that early sprays, applied when the plants are small, are likely to achieve better spray coverage and better pest control than applications made later when the crop canopy is well developed and under leaf coverage is difficult. The efficacy of the chemicals against various *B. tabaci* life-stages was also evaluated as was the potential of the entomopathogenic fungi *Beauveria bassiana* and *Lecanicillium muscarium* to be directly tank-mixed with the various insecticides. In parallel with the efficacy work it was important to investigate the potential phytotoxic effects of the various treatment programmes as poinsettias are very susceptible to phytotoxicity from pesticides.

Materials and methods

A. Efficacy work.

Insects and products used

Specimens of *Bemisia tabaci* were collected from a commercial nursery during the growing season of 2009. The population had proved extremely difficult to control/eradicate. It was assumed to be chemically resistant. The specimens were transported under the required conditions (Marris *et al*., 2010) to the Plant Health Insect Quarantine Unit at Fera. The *B. tabaci* were cultured under quarantine conditions in perspex cages (60 x 60 x 80 cm) on poinsettia (*Euphorbia pulcherrima* c.v. Freedom Red) plants at 23 ± 1°C following the method of Cuthbertson *et al*. (2005a,b, 2008a,b).

The entomopathogenic fungus *Beauveria bassiana* was supplied as Naturalis from Intrachem and *Lecanicillium muscarium* was supplied from Koppert as Mycotal. Table 2 lists the chemical products used and their application rates. All work was undertaken in the insect quarantine unit at Fera. The whitefly underwent molecular testing and was determined to be *B. tabaci* 'Q-biotype'. This biotype is known for its ability to develop insecticide resistance.

Table 2. List of products * tested for efficacy against *Bemisia tabaci*.

**All the products listed have either label approval or a SOLA for use in ornamentals. Agri 50-E and Spraying Oil are exempt from CRD registration as they act by physical means only. SB-Plant Invigorator is not classed as a pesticide.*

Product	Active ingredient	Rate of use (%) or ml or g /100 L water	Comments
Addit	Adjuvant	0.25%	Add to Naturalis or Mycotal in tank
Agri 50-E	Surfactant	300 ml (0.3%)	Physically acting product
Chess	Pymetrozine	60g (0.06%)	Azomethine SOLA rate, tank mix with Dynamec
Dynamec	Abamectin	50 ml (0.05%)	Macrocyclic lactone Tank mix with Chess
Gazelle	Acetamiprid	50g (0.05%)	Neonicotinoid
Majestik	Starch based	2,500 ml (2.5%)	Physically acting product
Naturalis	*Beauveria bassiana*	300g (0.3%)	Insect pathogenic fungus
Mycotal	*Lecanicillim muscarium*	100g (0.1%)	Insect pathogenic fungus
Oberon	Spiromesifen	50ml (0.05%)	Lipid synthesis inhibitor
Savona	Surfactant	1000 ml (1%)	Physically acting product
SB-Plant Invigorator	Surfactant	200 ml (0.2%)	Physically acting product
Tri-Tek	Refined petroleum oil	2000 ml (2%)	Physically acting product **(awaiting UK registration**)
Spraying Oil	Refined petroleum oil	1000 ml (1%)	Physically acting product

***Leaf dipping to test efficacy of control agents against* Bemisia tabaci**

Three life stages of *B. tabaci* were tested against eggs, second instar larvae and adults. Poinsettia plants were infested following the method of Cuthbertson *et al*. (2003) (Figure 1), and cohorts at the desired life-stages were obtained using the methods and data of Butler *et al*. (1983), Bethke *et al*. (1991), Wang and Tsai (1996) and Cuthbertson *et al*. (2003a, 2007). Then following the method of Cuthbertson *et al.*, (2009) four separate insecticide dilutions (all UK recommended dose rates) of each chemical and fungal product were prepared for replication purposes. Poinsettia leaves containing eggs were dipped into each dilution for 10 seconds then allowed to air dry, before being placed within sealed Petri dishes for each individual dilution of each insecticide. This procedure was repeated with

leaves infested with second instar larvae. For adult studies, leaves were dipped and then five adult whitefly were exposed to the leaf surface again using a clip cage while the leaves were still wet (Figure 2) modelled on those described by MacGillivray and Anderson (1957). The adults therefore had space not to sit on the leaf surface should they choose, however, to feed they had to settle on the leaf surface and therefore would come into contact with the product. These were maintained in sealed Petri dishes and replicated five times for each chemical. All Petri dishes were incubated at 20ºC, 14 hrs: 10 hrs Light: Dark for 48 hours. Control samples for each lifestage were also carried out using water.

Figure 1. Clip cages used to infest leaves with *Bemisia tabaci* (adult whitefly on underside of leaf).

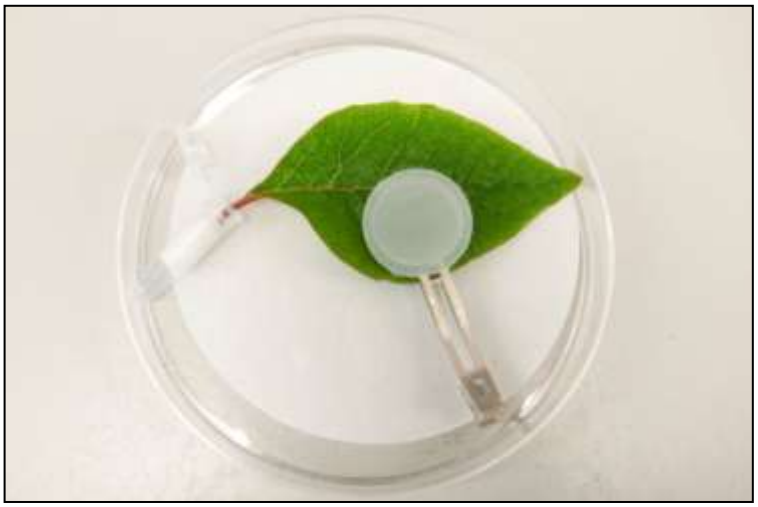

Figure 2. *Bemisia tabaci* adults exposed to chemically treated leaf surface in Petri dish (adult whitefly placed on underside of leaf).

The effect of direct exposure of *Beauveria bassiana* and *Lecanicillium muscarium* to conventional insecticides

Following the protocol of Cuthbertson *et al*. (2005b) the effect of direct suspension of the fungal spores in insecticide solutions was investigated. All products were tested for their direct compatibility with *B. bassiana*. Several selected products were also tested against *L. muscarium* to add to the knowledge base of direct compatibility of chemicals with this fungus for whitefly control (Cuthbertson *et al*., 2005b, 2008b).

Lecanicillium muscarium and *Beauveria bassiana* conidia were suspended (approx. 10^7 conidia/ml) in solutions of the insecticide products. All insecticides were diluted to recommended rates for application to protected ornamentals in the UK. The suspensions were transferred to beakers, sealed with parafilm and incubated in the dark at 20°C for 24 h after which 10 µl of each mixture was pipetted onto a sterile Petri dish containing 10% non-bacterial agar. The dishes were sealed with parafilm and again incubated in the dark for a further 24 h at 20°C before viability of conidia (germinated spores) from a total of 200 randomly chosen conidia were assessed under the microscope.

Each experiment (insecticide solution) consisted of two replicates each from three different batches of fresh dilution in order to replicate the work over time and space (six replicates in total). The above procedure was repeated using all the chemical products.

Sequential treatment efficacy

Plants received the treatments as outlined in Table 3. Four individual leaves on each of four plants were infested with eggs of *B. tabaci* as outlined above. They were then subjected to an individual spraying regime after 3, 7, 14 and 20 days. The plants were sprayed to run-off using a Hozelock Polyspray 2 hand-held sprayer with a cone nozzle. The leaves were allowed to dry before being returned to the conditions defined for infestation with *B. tabaci.* The procedure was repeated for each spray programme listed in Table 3. Control trials were undertaken using water (treatment 1) or the fungus *B. bassiana* (Naturalis) (treatment 7). The procedure was repeated using second instar larvae.

Table 3. Sequential applications tested for *Bemisia tabaci* control.

Crop Stage	3 days after potting	7 days after potting	14 days after potting	20 days after potting
Likely *Bemisia* life-stage	**Eggs**	**Eggs + 1st Instar scales**	**1st + 2nd Instar scales**	**2nd + 3rd Instars scales**
Programme 1	Water only	Water only	Water only	Water only
Programme 2	Majestik	Oberon + Mycotal + Addit	Spraying Oil	Dynamec + Chess
Programme 3	SB-Plant Invigorator	Oberon + Mycotal + Addit	Oberon + Mycotal + Addit	Spraying Oil
Programme 4	Spraying Oil	Majestik	Savona	Agri 50-E
Programme 5	Savona	Spraying Oil	Dynamec + Chess	Gazelle
Programme 6	SB-Plant Invigorator	Majestik	Dynamec + Chess	Gazelle
Programme 7 *	Naturalis	Naturalis	Naturalis	Naturalis

* Programme 7 was not tested in the phytotoxicity trials, as Naturalis had not been approved at the time the work was carried out.

Analysis of data

In all trials *B. tabaci* was recorded as dead or alive. Following chemical insecticide treatment numbers of live and dead *B. tabaci* adults and larvae were recorded after 48 h. In the case of all the fungal treatments and all the *B. tabaci* egg trials, dishes were incubated for 7 days to allow the fungus to germinate and eggs to potentially hatch. Treated eggs were noted as live (hatched larvae) or dead (unhatched). The data underwent non-parametric method testing (Kruskal-Wallis rank sum test and Wilcoxon test) to determine the effect of treatments.

B. Phytotoxicity work

This was carried out at Bordon Hill Nurseries, Stratford-upon-Avon CV37 9RY, using poinsettias in 1 litre pots, selected from a newly-potted crop which was maintained on rolling benches in a modern glasshouse at the nursery. The trial took place between July 27th and August 21st 2010, when environmental conditions were as follows : minimum temperature 19C, no supplementary lighting, watering via overhead gantry system to capillary matting on the benches, so plants took water up from beneath.

Observations were made on the following poinsettia varieties:

- Infinity red

- Infinity white
- Scandic
- Champion

Plants were arranged in a randomized block design on the benches, with four replicates per treatment. Each plot comprised five plants grouped together. The treatments were exactly as shown in Table 3 above, except that Naturalis was not included, as this product had not been approved at the time the phytotoxicity work was carried out. The general layout of the trial is shown in Figure 3 below.

Sprays were applied using a Killaspray knapsack sprayer which was maintained at maximum pressure and applied to the point of run off to the trial plants. A shield was used between replicates to avoid drift. Calculations showed that the water volume used was equivalent to 2,000 l/ha.

Treatments were applied as sequential programmes as shown in Tables 2 and 3, except that the complete Naturalis programme (treatment 7) was not included.

Figure 3. Phytotoxicity trial at Bordon Hill Nurseries.

After treatment, phytotoxicity was assessed using a scoring system according to the EPPO guideline PP 1(135/2), where a score of 1 indicated no damage compared to the water-only control, and 5 indicated severe plant damage.

In addition, the type of damage was classified into the following categories: necrosis, leaf paling, distortion and stunting. Scores for phytotoxicity were made at intervals of 3, 10, 17 and 25 days after the first spray was applied, by visually checking all five plants in each replicate and assigning them to one of the scoring categories 1-5.

The sprays and phytotoxicity scores were carried out on the following dates (Table 4):

Table 4. Timing of sprays and phytotoxicity assessments.

Spray application date	Plant growth stage at time of treatment	Phytotoxicity score timing
27/07/10	Just potted	30/07/10 (3 days after first spray)
30/07/10	At 3-4 leaf stage	06/08/10 (10 days after first spray)
06/08/10	At 4-6 leaf stage	13/08/10 (17 days after first spray)
13/08/10	Newly pinched	21/08/10 (25 days after first spray)

Results

A. Efficacy trials.

Mortality of Bemisia tabaci *eggs following leaf dipping*

All products tested caused some mortality of *B. tabaci* eggs. There was a significant difference in the mortality of eggs after leaf dipping with the different active ingredients ($P < 0.02$). Exposure to Tri-Tek, SB-Plant Invigorator, Gazelle, Dynamec and Spraying Oil was followed by egg mortalities (100, 96.6, 88.8, 84.1 and 67.8% respectively) that were all significantly higher than the water control (Figure 4).

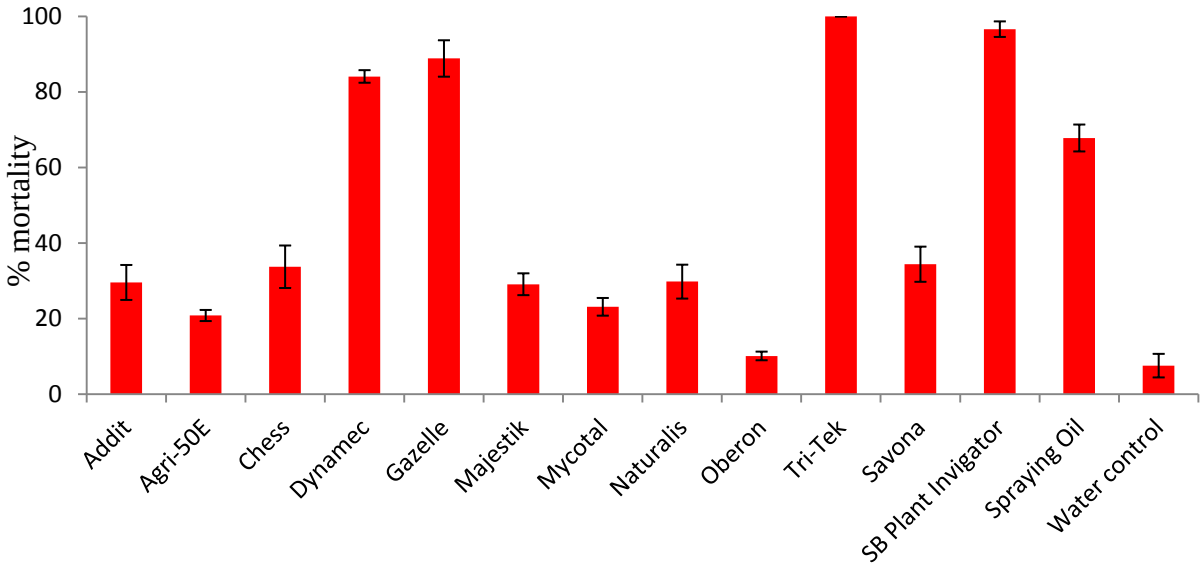

Figure 4. Efficacy of products against *Bemisia tabaci* eggs on poinsettia leaves. Mortality recorded after 7 days. Bars represent standard errors of the means (±SEM).

Efficacy of the products against the second larval instar stage also produced promising results. Here the fungal product Naturalis (*Beauveria bassiana*) produced the highest mortality of all the products against *B. tabaci* (73%). The control given by Agri 50-E, Tri-Tek, and SB-Plant Invigorator (all physically acting products) was also over 70% (Figure 5).

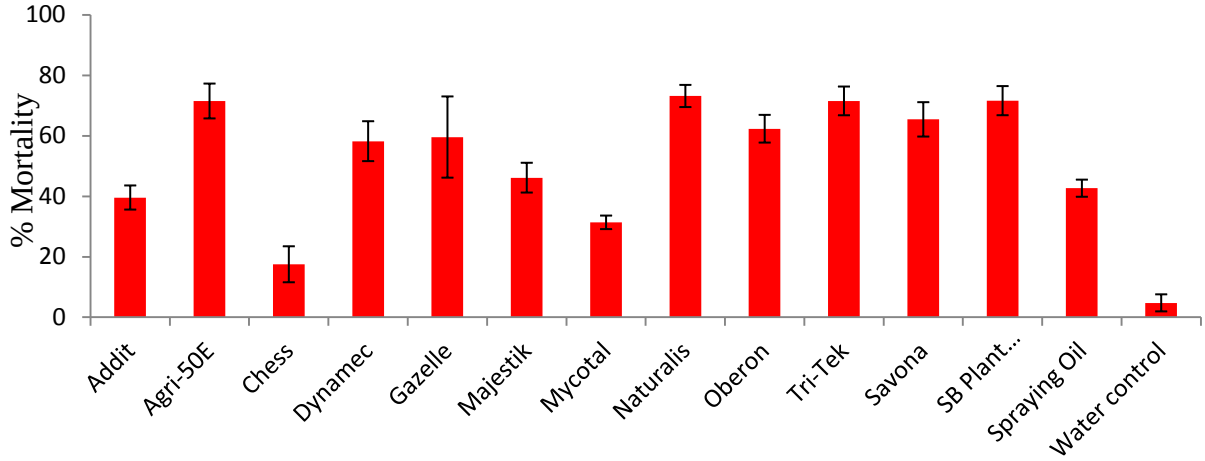

Figure 5. Efficacy of products against *Bemisia tabaci* second instars. Mortality recorded after 2 days following treatment for chemicals and after 7 days following fungal treatment. Bars represent standard errors of the means (±SEM).

Naturalis and several of the petroleum oil based products also gave excellent control of adult *B. tabaci* with total mortality being obtained (Figure 6). The fungus readily grew on the *B. tabaci* adults (Figure 7) and the oil based products (for example Tri-Tek) trapped the adults and they simply died in the treatment (Figure 8).

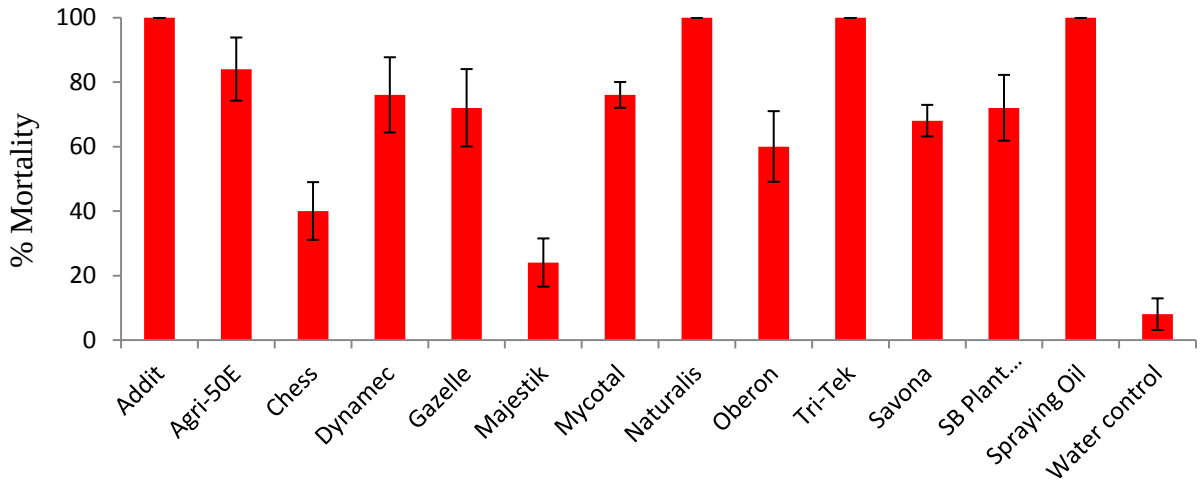

Figure 6. Efficacy of products against *Bemisia tabaci* adults. Mortality recorded after 2 days following treatment for chemicals and after 7 days following fungal treatment. Bars are standard errors of the means (±SEM).

Figure 7. Adult *Bemisia tabaci* infected by Naturalis (*Beauveria bassiana*).

Figure 8. *Bemisia tabaci* adults trapped on Tri-Tek treated leaves.

Tank-mixing entomopathogenic fungi with chemicals

Majestic, Oberon, Savona and SB-Plant Invigorator significantly reduced germination of *Beauveria* bassiana spores and so could not be recommended as tank-mixes with Naturalis. Other products, including Tri-Tek Oil, Spraying Oil, Addit, Dynamec and Gazelle, showed the best potential to be used as a tank-mix with over 90% *B.bassiana* spore germination following exposure to the test products for 24 hours (Figure 9). These products therefore have potential for tank mixing with Naturalis.

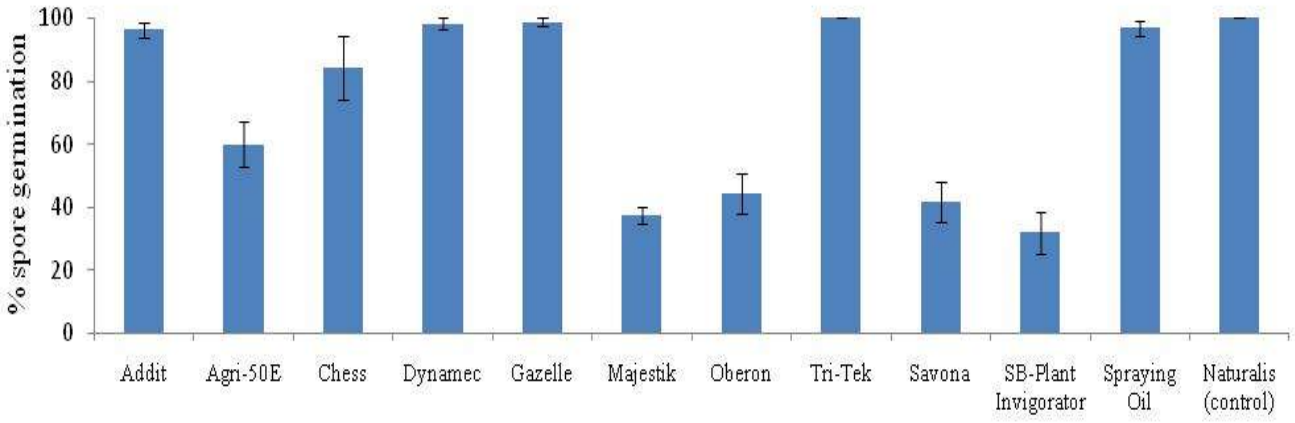

Figure 9. Naturalis (*Beauveria bassiana*) spore germination following 24 h exposure of the entomopathogenic fungus to a range of chemical products. Bars represent standard errors of the mean (±SEM).

Products that had not already been tested in previous Defra funded research (Cuthbertson *et al.,* 2005b, 2008a) for compatibility with Mycotal (*L. muscarium*) were also investigated. Here, direct mixing with Tri-Tek still allowed full fungal spore germination (Figure 10).

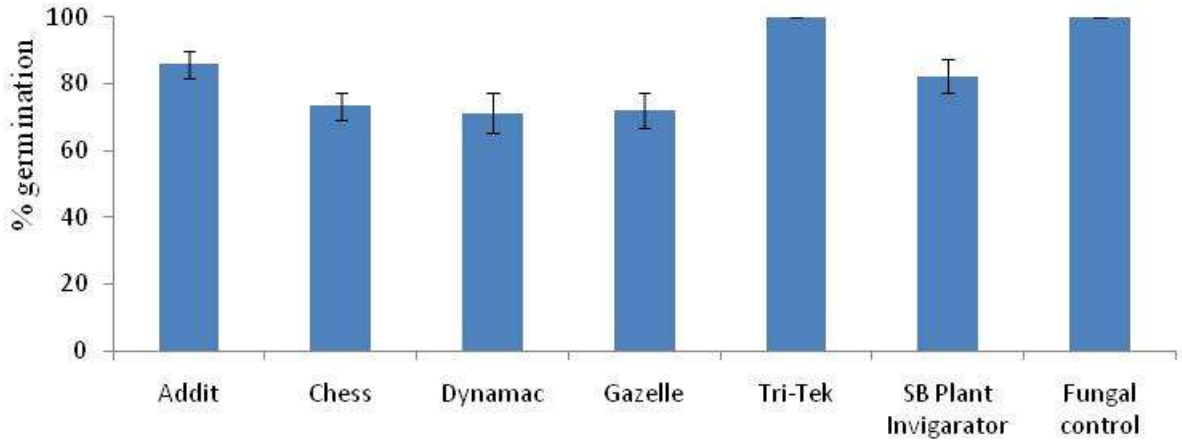

Figure 10. Mycotal (*Lecanicillium muscarium*) spore germination following 24 h exposure of the entomopathogenic fungus to a range of chemical products. Bars represent standard errors of the mean (±SEM).

Sequential treatment efficacy

Bemisia tabaci was eradicated in all treatment programmes tested (apart from the water control) in Table 3.

When starting with the egg stage, some instars developed during the process in treatment programmes 2 and 5 but nothing survived through to adult. This result correlates with the lower efficacy of these first treatment chemicals (Majestik and Savona) recorded against the eggs in the efficacy trials. However, with the continued treatments the instars were efficiently controlled with nothing surviving through to adult.

When beginning the trials with the 2nd instar life-stage again some developed through to 3/4th instar after 14 and 20 days in all treatment programmes but no adults emerged, unlike the control where adults readily developed. Either the larvae were all killed or their development was severely reduced, however, after maintaining the treated plants for a week under favourable conditions (23°C) following the final treatment no adults developed. Therefore, control must have been complete.

B. Phytotoxicity trials

The results of the phytotoxicity trials at Bordon Hill Nurseries, using the sequential programmes listed in Table 5, are shown in Tables 6 to 9 below.

Table 5. Sequential applications tested for phytotoxicity on a commercial nursery.

Crop Stage	3 days after potting	7 days after potting	14 days after potting	20 days after potting
Programme 1	Water only	Water only	Water only	Water only
Programme 2	Majestik	Oberon + Mycotal + Addit	Spraying Oil	Dynamec + Chess
Programme 3	SB-Plant Invigorator	Oberon + Mycotal + Addit	Oberon + Mycotal + Addit	Spraying Oil
Programme 4	Spraying Oil	Majestik	Savona	Agri 50-E
Programme 5	Savona	Spraying Oil	Dynamec + Chess	Gazelle
Programme 6	SB-Plant Invigorator	Majestik	Dynamec + Chess	Gazelle

Table 6. Mean scores for phytotoxicity on the variety **Infinity Red** according to EPPO standard PP1 (135/2). (Scale 1-5 where 1=no damage and 5=severe plant damage).

Programme number	30th July (3 days after spray 1)	6th August (10 days after spray 1)	13th August (17 days after spray 1)	21st August (25 days after spray 1)
1 (water only)	1.3*	1	1	1
2	1.3	1.3	1	1
3	1	1	1	1
4	1.3	1	1	1
5	1	1	1	1
6	1.3	1.3	1	1

- The first assessments showed a very minor amount of leaf marking, even in the water only treatment, and inspection showed that this was due to onion thrips (*Thrips tabaci*) feeding on the leaves.

Table 7. Mean score for phytotoxicity on the variety **Champion** according to EPPO standard PP1 (135/2). (Scale 1-5 where 1=no damage and 5=severe plant damage).

Programme number	30th July	6th August	13th August	20th August
1 (water only)	1	1.3	1	1
2	1.3	2 *	1	1
3	1.3	2	1	1
4	1	1	1	1
5	1	1	1	1
6	1.3	1	1	1

* Small brown spots showed on the leaves due to the feeding of onion thrips (*Thrips tabaci*).

Table 8. Mean scores for phytotoxicity on the variety **Scandic** according to EPPO standard PP1 (135/2). (Scale 1-5 where 1=no damage and 5=severe plant damage).

Programme number	30th July	6th August	13th August	20th August
1 (water only)	1.3 *	1.5	1.3	1
2	1.3	1.3	1.5	1
3	1.3	1.5	1.5	1
4	1.3	1.5	1	1
5	1.4	1.3	1.3	1
6	1.3	1	1.3	1

* Small brown spots showed on the leaves due to the feeding of onion thrips (*Thrips tabaci*).

Table 9. Mean scores for phytotoxicity on the variety **Infinity white** according to EPPO standard PP1 (135/2). (Scale 1-5 where 1=no damage and 5=severe plant damage)**.**

Programme number	30th July *	6th August	13th August	20th August
1 (water only)	1	1	1	1
2	1	1	1	1
3	1	1	1	1
4	1	1	1	1
5	1.3	1	1	1
6	1	1	1	1

*Infinity white was the least affected by onion thrips damage overall.

Yellow sticky traps placed within the trial showed that a large number of onion thrips had immigrated into the glasshouse and had caused feeding damage to the poinsettia leaves. The insecticides applied in the sequential programmes shown in Table 5 above did give some control of thrips, and this affected the phytotoxicity scores in the tables above. However, no direct damage from the insecticide treatments was seen at any time. On the 1st September, 18 days after the final sequential spray had been applied, the whole trial was scored for thrips damage. The results are shown in Table 9 below.

Table 9. Mean score for thrips damage. (Scale 1-5 where 1=no damage and 5=severe leaf distortion/necrosis).

Treatment	Infinity red	Champion	Scandic	Infinity white
1 (water only)	4.0	4.3	5.0	3.3
2	2.5	2.5	3.0	2.3
3	2.8	2.8	3.8	2.5
4	2.8	2.5	3.3	2.5
5	1.0	1.5	1.5	1.0
6	1.5	1.3	2.0	1.0

This assessment showed that Infinity White was the least affected variety, and Scandic the most affected by onion thrips. The damage was most obvious in the water-only treatment, while treatment 5 (Savona/Spraying Oil/Dynamec+ Chess/Gazelle) and treatment 6 (SB-Plant Invigorator/Majestik/Dynamec+ Chess/Gazelle) had the lowest damage (Table 9). This was assumed to be the result of more effective control of thrips by these treatments. Figure 11 shows the variety Scandic with severe leaf distortion typically seen as a result of onion thrips feeding damage, while Figure 12 shows the plants from treatment 5, which were much less affected.

Figure 11. Severe damage from onion thrips in variety Scandic, plants treated with water only.

Figure 12. Plants treated with the programme of Savona/Spraying Oil/Dynamec + Chess/Gazelle.

Discussion

To develop effective integrated control programmes for insect pests of crops which include fungal biopesticides, clarification of the effects of chemical insecticides on the fungal product in question is necessary. However, there have been few in vitro tests (Olmert *et al*., 1974; Anderson *et al*., 1983; Wang Xiu-Fang and Cheng-Fa, 2004). Different biopesticides based on *L. muscarium* (formerly *V. lecanii*) are utilised on greenhouse crops to manage pests such as greenhouse whitefly, aphids and thrips in various European countries (Faria and Wraight, 2001). Recent advances in production, formulation and application of insect pathogenic fungi have resulted in improvements in longstanding whitefly mycoinsecticide products based on *L. muscarium*, and the development and registration of several new products based on *Paecilomyces fumosoroseus* (Wise) Bronn & Smith and *Beauveria bassiana* (Balsamo) Vuillemin (Cross *et al*., 1999). These products have the capacity to suppress and, in some instances, to provide good control of whiteflies in both greenhouse and field crops. However, numerous factors continue to impede the commercial development of fungi as whitefly biological control agents. These include: slow action, poor adulticidal activity, potentially negative interactions with insecticides, relatively high cost, limited shelf life and dependence on favourable environmental conditions (Cross *et al.*, 1999). Therefore, there is a need to investigate the potential of combining fungi with other biocontrol agents and chemical insecticides to form components of IPM strategies for key pests.

Cross *et al.* (1999) suggest that a key limiting factor to the effectiveness of fungi in IPM programmes is the requirement for high humidities and moderate temperatures for spore

germination and development, but these can be readily achieved under glasshouse conditions.

For the successful introduction of an IPM programme, information is needed on susceptibility of the pest species to the control product. Targeting treatment at certain life-stages has been shown to substantially improve pest population control (Cuthbertson *et al*., 2003; Williams and Walters, 2000; Candy, 2003).

The current work has shown that several products are better than others for targeting different life-stages of *B. tabaci*. For treating the egg stage Dynamec, Gazelle, Tri-Tek and SB-Plant Invigorator all proved excellent.

None of the products gave total control of second instar larvae. However, Agri-50E, Naturalis, Tri-Tek and SB-Plant Invigorator all gave over 71% mortality. For adult control, Naturalis and the oil based products (Addit, Tri-Tek and Spraying Oil) all produced 100% mortality of *B. tabaci*. The current work has also demonstrated that Naturalis offers better control of *B. tabaci* than that of Mycotal.

In successfully dealing with a pest species either direct tank-mixing or simultaneous use of insecticides and biocontrol agents may be required. For an insecticide to be compatible with a biocontrol agent and be cost effective within an IPM system it is necessary for the mortality of the target organism to be increased when both insecticide and control agent are used over either treatment alone. In the current study sequential application of treatments based on the efficacy treatments and previous Fera research were applied to poinsettia cuttings within the first 21 days after potting. When starting with the egg stage some instars developed during the process, but nothing survived through to adult. When beginning the trials with the second instar life-stage again some developed through to 3/4th instar but no adults were produced, unlike the control where adults readily developed. Either the larvae were all killed or their development was reduced, however, after maintaining the treated plants for a week under favourable conditions following the final treatment still no adults developed. Sequential treatments of Naturalis also gave excellent control of *B. tabaci* eggs and second instars with no adults developing.

The current work has demonstrated that Tri-Tek, Gazelle, Spraying Oil, Dynamec and Addit offer great potential for application as tank-mixes with Naturalis for the control of *B. tabaci*. All of the products tested in the current trials are deemed IPM compatible. Therefore, other biological control agents, such as *Encarsia* and *Eretmocerus* parasitoids could be used

safely after the intensive spray programme applied to the cuttings has been completed. This would help to insure the plant material is *B. tabaci* free. The worst scenario for the poinsettia grower is to have this pest identified at a late stage in the crop, possibly when bracts have formed and sprays are likely to damage the bracts, leading to downgrading of the crop and loss of income. Therefore, early sprays, applied when plants are small, are likely to achieve better spray coverage and better control than applications made later, when crop canopy is well developed and under leaf coverage is very difficult.

One of the concerns of growers is that intensive treatment with insecticides at an early crop stage could cause serious phytotoxicity. It is well known that Spraying Oil can be very damaging to some varieties of poinsettia (HDC project PC 254, 2007). The crop safety trials undertaken at Bordon Hill nursery, using the sequential programmes on four varieties of poinsettia showed no signs of phytotoxicity. This is encouraging, as it means that the sequential programmes can be both effective and safe to these varieties**. However, it is important for growers to test other varieties for crop safety on a small scale before widespread use, as sprayer types and environmental conditions may vary between sites**.

The sequential applications of the products tested produced excellent control/eradication of *B. tabaci* under controlled laboratory conditions. As it stands any of the treatment schedules in Table 3 could be recommended to poinsettia growers for control/eradication of *B. tabaci.*

Conclusions

1) The population from the commercial nursery proved to be the 'Q' biotype, known for its resistance to a range of chemicals. This was the first confirmed record of this biotype within the UK. However, few outbreaks have been tested so many of the interceptions/outbreaks could be Q-biotype.

2) Any of the sequential treatments tested and listed in Table 3 can be recommended to poinsettia growers as all gave full control of *B. tabaci* eggs and second instar larvae under controlled conditions. The sequential treatments have proved more effective than 'one-off' applications tested in previous Fera work (Cuthbertson *et al*., 2005b, 2008a).

3) Under the conditions in the nursery where the trials were conducted, there was no phytotoxicity from any of the sequential programmes to the poinsettia varieties Infinity, Scandic, Infinity White and Champion.

4) Full spray coverage of underside of leaf surfaces is essential to obtain best control from the products tested, especially the physically acting products. This will be best accomplished in the first 4-6 weeks after potting poinsettias.

Knowledge and Technology Transfer

1) Cuthbertson, A.G.S. Visit to the commercial nursery where samples were originally collected from to update the growers on project findings. 15th November 2010.

2). Buxton, J.H. and Cuthbertson, A.G.S (2010). 'Initial findings from project PO 003'. Poinsettia growers BPOA meeting, Oakheart Nursery Leics, 16th November 2010.

3) Buxton, J.H. and Cuthbertson, A.G.S. (2011). 'Safe and effective whitefly control'. British Protected Ornamental Association's technical seminar. 16th February, Hellidon Lakes, Daventry.

4) Cuthbertson, A.G.S. and Buxton, J.H. (2011). Answers found to *Bemisia*. *HDC News*, **172:** 24-25.

5) Cuthbertson, A.G.S., Buxton, J.H., Blackburn, L.F., Robinson, K.A., Bell, H.A., Powell, M.E., Fleming, D.A. & Northing, P. (2011). Screening products for the early eradication of tobacco whitefly on poinsettia crops. *Proceedings of the 4th European Whitefly Symposium*, Rehovot, Israel, 11-16th September 2011, pp. 49.

6) Cuthbertson, A.G.S., Blackburn, L.F., Robinson, K.A., Powell, M.E., Luo, W., Buxton, J.H., Bell, H.A., & Northing, P. (2011). Environmental screening of products and sequential applications for control of *Bemisia tabaci* Q biotype. *International Journal of Environmental Science and Technology* (in draft).

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APPENDICES

Data analysis for direct toxicity testing

Method: Without imposing the normality and constant variance assumption for the count data, non-parametric methods (Kruskal-Wallis rank sum test and Wilcoxon test) were used to determine the effect of treatment.

Efficacy of products against *Bemisia tabaci* eggs leaf.

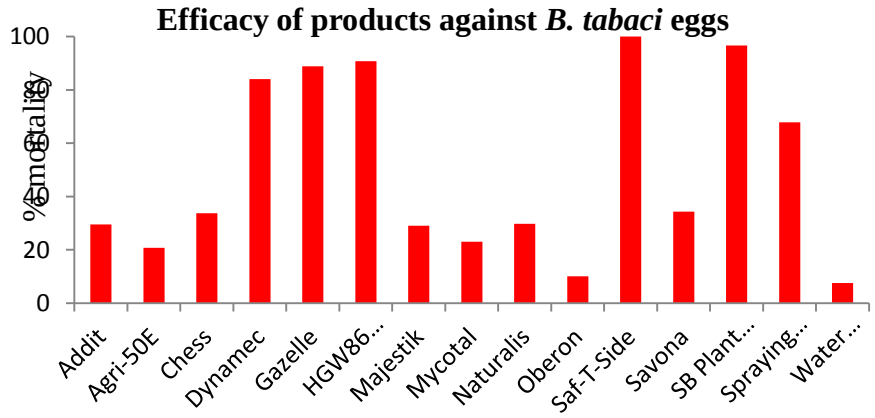

Figure 1

There was significant difference in mortality of eggs after leaf dipping with different active ingredients (Figure 1).

```
        Kruskal-Wallis rank sum test

data:  Mortality by treat
Kruskal-Wallis chi-squared = 5.3333, df = 1, p-value = 0.02092
```

Pairwise comparison

	Addit	Agri-50E	Chess	Dynamec	Gazelle	HGW86 1000	Majestik	Mycotal	Naturalis	Oberon	Saf-T-Side	Savona	SB Plant Invigator	Spraying Oil
Agri-50E	0.309	-	-	-	-	-	-	-	-	-	-	-	-	-
Chess	0.384	0.243	-	-	-	-	-	-	-	-	-	-	-	-
Dynamec	0.029	0.029	0.029	-	-	-	-	-	-	-	-	-	-	-
Gazelle	0.029	0.029	0.029	0.343	-	-	-	-	-	-	-	-	-	-
HGW86 1000	0.029	0.029	0.029	0.114	0.772	-	-	-	-	-	-	-	-	-
Majestik	0.686	0.059	0.343	0.029	0.029	0.029	-	-	-	-	-	-	-	-
Mycotal	0.343	0.552	0.245	0.029	0.029	0.029	0.114	-	-	-	-	-	-	-
Naturalis	1.000	0.191	0.686	0.029	0.029	0.029	1.000	0.486	-	-	-	-	-	-
Oberon	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	-	-	-	-	-
Saf-T-Side	0.021	0.020	0.021	0.021	0.069	0.021	0.021	0.021	0.021	0.021	-	-	-	-
Savona	0.364	0.059	1.000	0.029	0.029	0.029	0.343	0.114	0.561	0.029	0.021	-	-	-
SB Plant Invigator	0.029	0.028	0.029	0.029	0.183	0.191	0.029	0.029	0.029	0.029	0.186	0.029	-	-
Spraying Oil	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.021	0.029	0.029	-
Water control	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.772	0.021	0.029	0.029	0.029

Efficiacy of products against *Bemisia tabaci* second instar larvae

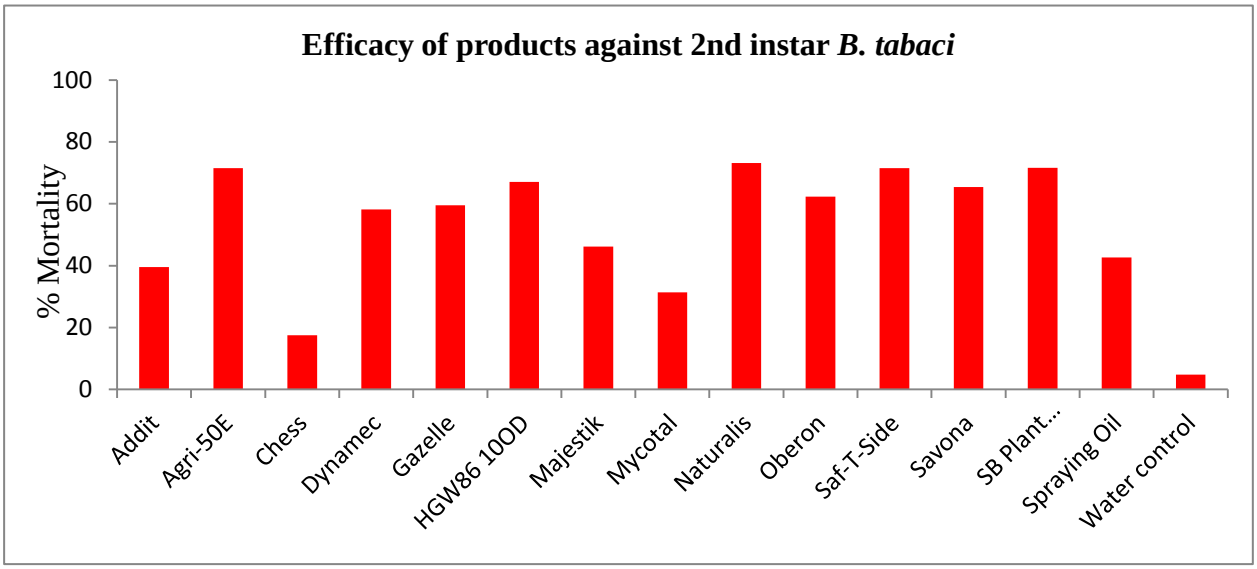

Figure 2

There was significant difference in mortality of 2nd instar after leaf dipping with different active ingredients (Figure 2).

```
        Kruskal-Wallis rank sum test

data:  Mortality by treat
Kruskal-Wallis chi-squared = 44.0923, df = 14, p-value = 5.72e-05
```

Pairwise comparison

	Addit	Agri-50E	Chess	Dynamec	Gazelle	HGW86 10OD	Majestik	Mycotal	Naturalis	Oberon	Saf-T-Side	Savona	SB Plant Invigator	Spraying Oil
Agri-50E	0.029	-	-	-	-	-	-	-	-	-	-	-	-	-
Chess	0.029	0.029	-	-	-	-	-	-	-	-	-	-	-	-
Dynamec	0.081	0.114	0.029	-	-	-	-	-	-	-	-	-	-	-
Gazelle	0.384	0.686	0.029	0.772	-	-	-	-	-	-	-	-	-	-
HGW86 10OD	0.041	0.657	0.029	0.304	1.000	-	-	-	-	-	-	-	-	-
Majestik	0.486	0.057	0.029	0.200	0.486	0.059	-	-	-	-	-	-	-	-
Mycotal	0.200	0.029	0.055	0.029	0.200	0.029	0.029	-	-	-	-	-	-	-
Naturalis	0.029	0.772	0.029	0.114	0.886	0.301	0.029	0.029	-	-	-	-	-	-
Oberon	0.029	0.384	0.029	0.686	1.000	0.657	0.081	0.029	0.081	-	-	-	-	-
Saf-T-Side	0.029	1.000	0.029	0.343	0.772	0.862	0.029	0.029	1.000	0.146	-	-	-	-
Savona	0.042	0.245	0.029	0.384	1.000	0.559	0.057	0.029	0.200	0.886	0.486	-	-	-
SB Plant Invigator	0.029	0.886	0.029	0.486	0.686	1.000	0.029	0.029	0.886	0.200	0.772	0.686	-	-
Spraying Oil	0.661	0.029	0.029	0.081	0.384	0.041	0.686	0.029	0.029	0.029	0.029	0.042	0.029	-
Water control	0.029	0.029	0.100	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029

Efficacy of products against *Bemisia tabaci* adults

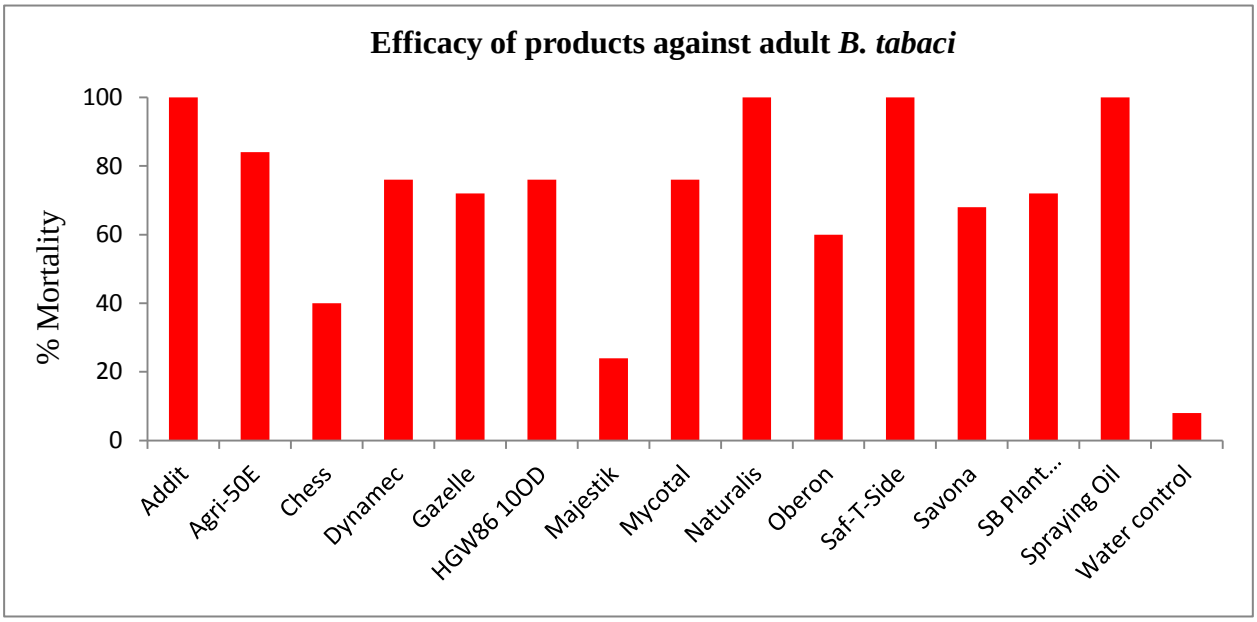

Figure 3

There was significant difference in mortality of adult after leaf dipping with different active ingredients (Figure 3).

```
        Kruskal-Wallis rank sum test

data:  Mortality by treat
Kruskal-Wallis chi-squared = 53.1167, df = 14, p-value = 1.817e-06
```

Pairwise comparison

	Addit	Agri-50E	Chess	Dynamec	Gazelle	HGW86 10OD	Majestik	Mycotal	Naturalis	Oberon	Saf-T-Side	Savona	SB Plant Invigator	Spraying Oil
Agri-50E	0.1770	-	-	-	-	-	-	-	-	-	-	-	-	-
Chess	0.0071	0.0285	-	-	-	-	-	-	-	-	-	-	-	-
Dynamec	0.0720	0.6513	0.0696	-	-	-	-	-	-	-	-	-	-	-
Gazelle	0.0707	0.4884	0.1026	0.9125	-	-	-	-	-	-	-	-	-	-
HGW86 10OD	0.0243	0.5762	0.0301	1.0000	0.8266	-	-	-	-	-	-	-	-	-
Majestik	0.0071	0.0104	0.2733	0.0192	0.0189	0.0112	-	-	-	-	-	-	-	-
Mycotal	0.0056	0.5047	0.0160	0.9107	0.8266	1.0000	0.0092	-	-	-	-	-	-	-
Naturalis	-	0.1770	0.0071	0.0720	0.0707	0.0243	0.0071	0.0056	-	-	-	-	-	-
Oberon	0.0243	0.1461	0.2733	0.3855	0.5047	0.1313	0.0101	0.1887	0.0243	-	-	-	-	-
Saf-T-Side	-	0.1770	0.0071	0.0720	0.0707	0.0243	0.0071	0.0056	-	0.0243	-	-	-	-
Savona	0.0065	0.2568	0.0434	0.5829	1.0000	0.4884	0.0104	0.2703	0.0065	0.3494	0.0065	-	-	-
SB Plant Invigator	0.0248	0.4432	0.0696	0.8294	1.0000	0.9128	0.0192	0.9060	0.0248	0.4504	0.0248	0.7352	-	-
Spraying Oil	-	0.1770	0.0071	0.0720	0.0707	0.0243	0.0071	0.0056	-	0.0243	-	0.0065	0.0248	-
Water control	0.0065	0.0097	0.0285	0.0107	0.0104	0.0104	0.1461	0.0086	0.0065	0.0104	0.0065	0.0097	0.0107	0.0065

Direct compatibility of Naturalis (*Beauveria bassiana)* with a range of insectidal products

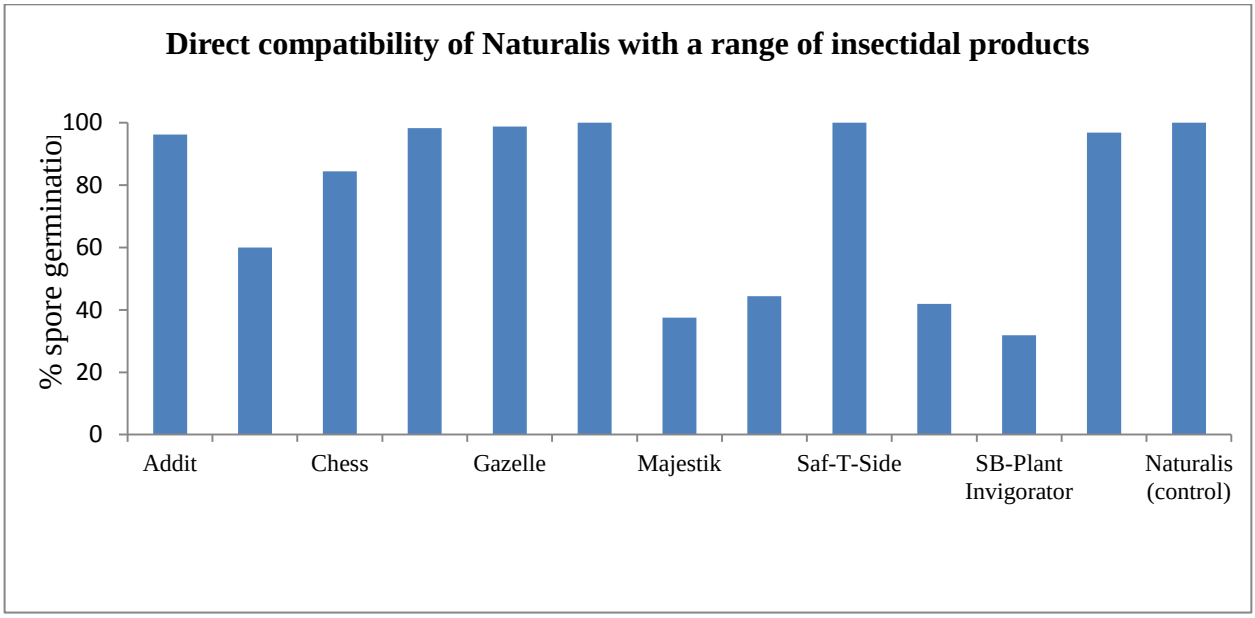

Figure 4

There was significant difference in % of spore germination of Naturalis between a range of insectidal products (Figure 4)

```
        Kruskal-Wallis rank sum test

data:  Germinate by treat
Kruskal-Wallis chi-squared = 43.3744, df = 12, p-value = 1.951e-05
```

Pairwise comparison

	Addit	Agri-SOE	Chess	Dynamec	Gazelle	HGW86 1000	Majestik	Oberon	Saf-T-Side	Savona	SB-Plant Invigorator	Spraying Oil
Agri-SOE	0.029	-	-	-	-	-	-	-	-	-	-	-
Chess	0.372	0.200	-	-	-	-	-	-	-	-	-	-
Dynamec	0.620	0.027	0.219	-	-	-	-	-	-	-	-	-
Gazelle	0.505	0.027	0.163	1.000	-	-	-	-	-	-	-	-
HGW86 1000	0.186	0.021	0.069	0.453	0.453	-	-	-	-	-	-	-
Majestik	0.029	0.001	0.029	0.027	0.027	0.021	-	-	-	-	-	-
Oberon	0.029	0.200	0.057	0.027	0.027	0.021	0.306	-	-	-	-	-
Saf-T-Side	0.186	0.021	0.069	0.453	0.453	-	0.021	0.021	-	-	-	-
Savona	0.029	0.146	0.042	0.027	0.027	0.021	0.561	0.886	0.021	-	-	-
SB-Plant Invigorator	0.029	0.057	0.029	0.027	0.027	0.021	0.661	0.306	0.021	0.343	-	-
Spraying Oil	1.000	0.029	0.301	0.620	0.620	0.186	0.029	0.029	0.186	0.029	0.029	-
Naturalis (control)	0.186	0.021	0.069	0.453	0.453	-	0.021	0.021	-	0.021	0.021	0.186

Direct compatibility of Mycotal (*Lecanicillium muscarium*) with range of chemicals

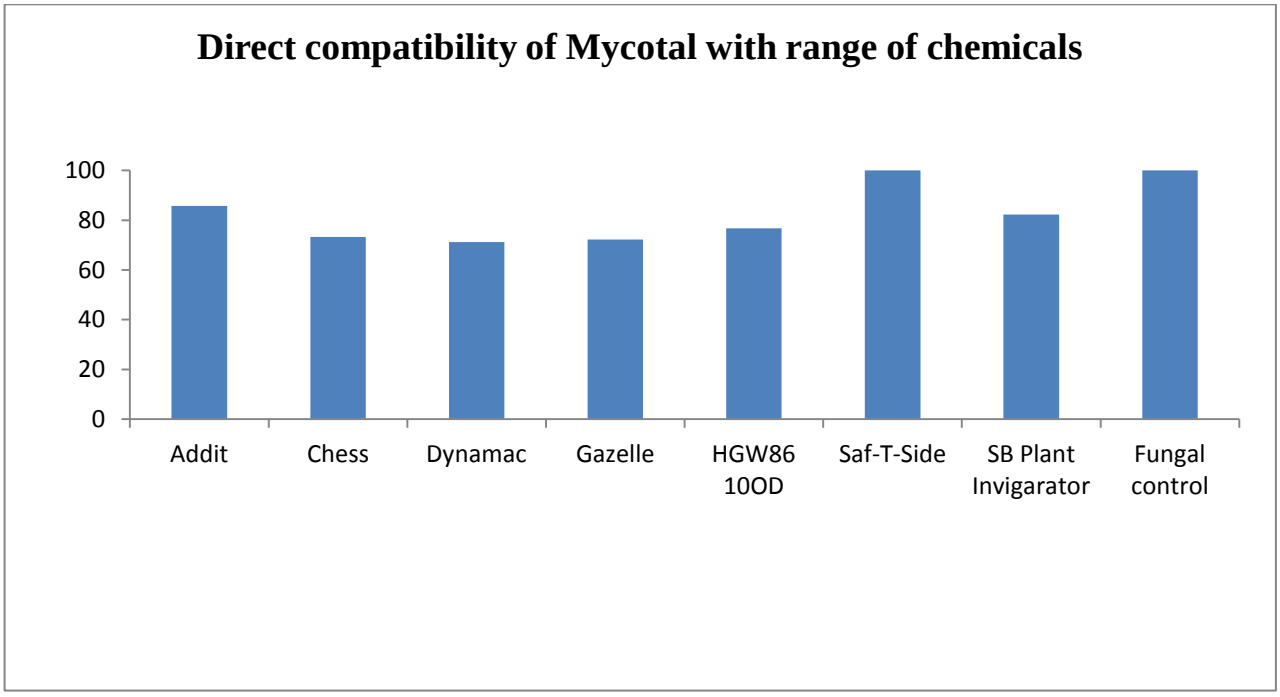

Figure 5

There was significant difference in % of spore germination of Mycotal between a range of insectidal products (Figure 5)

```
        Kruskal-Wallis rank sum test

data:  Germinate by treat
Kruskal-Wallis chi-squared = 21.6488, df = 7, p-value = 0.002920
```

Pairwise comparison

	Addit	Chess	Dynamac	Gazelle	HGW86 10OD	Saf-T-Side	SB Plant Invigarator
Chess	0.081	-	-	-	-	-	-
Dynamac	0.114	1.000	-	-	-	-	-
Gazelle	0.114	1.000	1.000	-	-	-	-
HGW86 10OD	0.384	0.661	0.686	0.686	-	-	-
Saf-T-Side	0.021	0.021	0.021	0.021	0.021	-	-
SB Plant Invigarator	0.661	0.200	0.200	0.200	0.561	0.021	-
Fungal control	0.021	0.021	0.021	0.021	0.021	-	0.021