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# SID 5 Final Project Report

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## Project identification

1. Defra Project code: PH0405

2. Project title: Efficacy tests for Bemisia tabaci on poinsettia plants.

3. Name and address of contractor:
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4. Total Defra project costs: £ 29,433

5. Project:
   - start date ........... 01 September 2005
   - end date ............ 31 March 2006

6. It is Defra's intention to publish this form.
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When preparing SID 5s contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the SID 5 can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and this section completed. NB: only in exceptional circumstances will Defra expect contractors to give a "No" answer.

In all cases, reasons for withholding information must be fully in line with the Freedom of Information Act 2000 and (prior to January 2005) the Code of Practice on Access to Government Information.

If you have answered NO, please explain why the Final report should not be released into public domain

## Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

1. *Bemisia tabaci* remains a serious threat to the UK horticultural industry. It is of specific economic concern because it is an effective vector of over 111 viruses from several groups, particularly geminiviruses.

2. Within the European Community the UK has 'Protected Zone' status to prevent spread of *B. tabaci*. Within this zone the primary concern is that adult whitefly imported on ornamentals such as poinsettia can infect tomatoes with tomato yellow leaf curl virus (TYLCV) and cucumbers with curcubit yellow stunting disorder virus (CYSDV) and cucumber vein yellowing virus (CVYV). These viruses are not currently present in the protected area but have been detected in *B. tabaci* intercepted in the UK.

3. Statutory action is taken to prevent live stages of the pest entering the UK. However, where outbreaks do occur measures must be taken to eradicate the population.

4. Entomopathogenic fungi biocontrol agents offer potential to control *B. tabaci*. Much work has investigated the efficacy of the fungus *Lecanicillium muscarium* against *B. tabaci*. This fungus has a high efficacy against second instar larval stages of *B. tabaci*. Also *L. muscarium* can be successfully integrated with several chemical insecticides commonly used in the UK for controlling *B. tabaci*.

5. In order to support control and eradication approaches, further information is needed both on compatibility of fungi with candidate conventional insecticides that can be used in conjunction with fungal control agents and the efficacy of a wider range of insecticides for the control of *B. tabaci*.

6. In leaf dipping tests, Spray Oil was the only compound to show potential to be used as a control agent against *B. tabaci* eggs, where 81% mortality was obtained. Leaf dipping against

second instar and adult *B. tabaci* proved better for all chemical products. Majestik, Spray Oil and Agri-50E all produced high second instar larval mortality (93, 87 and 85.5% respectively). Spray Oil again proved the best compound tested against adult *B. tabaci* with 100% mortality being obtained.

7. Direct suspension of the fungus (*L. muscarium*) in recommended label dose rates of the chemical products proved that Agri-50E along with Oberon had the potential to be used in tank mixes for application purposes. Majestik, Savona and Spray Oil had spore germination percentages (6.8, 23 and 47.5% respectively) that would appear to be too low to be of practical use.

8. Testing spray application of the chemical products again showed Spray Oil to have the highest efficacy against *B. tabaci* eggs (74% mortality obtained). Higher mortalities across all chemical products were achieved against second instar larvae. Here, most of the products achieved upwards of 80% mortality bar Oberon (60%) and Agri-50E (63%) which were much lower. Some foliage damage was recorded 7 days post application of Agri-50E.

9. The addition of a fungal application (*L. muscarium*) 24 hours after chemical application produced some added effects. The efficacy of Savona, Spray Oil and Majestik were all boosted above 90% mortality for second instars.

10. Within the glasshouse environment promising results testing the IPM strategy in the 'real' environment were obtained. Mortalities of second instar larvae remained constant when applying the chemical products on their own (e.g. Spray Oil obtaining 79% mortality). On treatments following a sequential application of chemical product and fungus high mortality of second instar larvae was obtained. Sequential treatment of Savona and Spray Oil with *L. muscarium* produced 95 and 96% larval mortality respectively. Again, commercially unacceptable foliage damage was recorded 7 days post application of Agri-50E.

11. *Lecanicillium muscarium* has further proved itself to be of potential use in the development of control measures against *B. tabaci*. This work has shown it to have a good compatibility with a wide range of chemical products and in some cases produce a cumulative effect when applied sequentially. The work has further proved its high efficacy against second instar larval stages following spray application.

## Scientific Report

8. The Scientific Report should be no longer than 20 sides of A4 and include:
   - the scientific objectives as set out in the SID 3 (original application form);
   - the extent to which the objectives set out in the SID 3 have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Transfer).

   Published papers, or details of any other outputs (e.g. presentations), should also be annexed to the SID 5, together with other relevant information which cannot be accommodated within the recommended maximum of 20 pages.

## Introduction

The poinsettia strain of the tobacco whitefly, *Bemisia tabaci*, remains a serious threat to the UK horticultural industry[1]. The damaging B-biotype is of specific economic concern because it is an effective vector of over 111 viruses from several groups, particularly geminiviruses[2]. In addition this biotype is more polyphagous, develops faster and is more fecund than other strains. Within the European Community the UK has 'Protected Zone' status to prevent spread of *B. tabaci*. Within this zone the primary concern is that adult whitefly imported on ornamentals such as poinsettia can infect tomatoes with tomato yellow leaf curl virus (TYLCV) and cucumbers with curcubit yellow stunting disorder virus (CYSDV) and cucumber vein yellowing virus (CVYV). These are viruses not currently present in the protected area but which have been detected in *B. tabaci* intercepted in the UK. Direct damage to plants by *B. tabaci* is caused by feeding activity and indirect damage due to contamination of leaves with honeydew, on which black mould develops and intercepts light, thereby reducing photosynthesis[3]. Both sources of damage affect the marketability of the crop.

Statutory action is taken to prevent live stages of the pest entering the UK. However, where outbreaks do occur measures must be taken to eradicate the population. Where crops have a distinct growing season clean up measures can be taken to prevent the pest transferring to subsequent plantings. However, this is particularly difficult when commodities with a longer growing season such as plants for the production of vegetables or cut flowers are planted. It is also difficult to eradicate *B. tabaci* from areas which allow public access as this often constrains the use of chemical insecticides.

Where intensive cropping regimes exist there is always a continuous supply of young plant material for the pest to infest. There are few opportunities for complete glasshouse sterilisation and unless a targeted and synchronised treatment regime is implemented, long term outbreaks can occur. Commercial constraints and regulatory restrictions applied to pesticides mean that few products are available for use in the UK.

Much work has been done on the potential of entomopathogenic biocontrol agents being incorporated into integrated pest management (IPM) strategies against *B. tabaci*, not least as a result of previous Plant Health Division funded work (Research Project: PH0157). Here, an entomopathogenic nematode (*Steinernema feltiae*) and the fungus used in the current study (*Lecanicillium muscarium*) were shown to be most effective against second instar *B. tabaci* larvae[4-6]. Both entomopathogenic control agents also proved potentially suitable for IPM strategies with commonly used chemical insecticides for the control of *B. tabaci* within the UK[7,8] and offer potential control against second instar larvae within the glasshouse environment[9,10]. In support of such control and eradication approaches, further information is needed on compatibility of the fungus with conventional insecticides, the maximum dose rates of insecticides that can be used in conjunction with fungal control agents and the efficacy of a wider range of insecticides for the control of *B. tabaci*.

The aim of the current project was to investigate a range of low toxicity, IPM-compatible insecticide products against all relevant *B. tabaci* life-stages that infest potted poinsettia plants.

**Objective 1: The efficacy of a range of low toxicity, IPM-compatible insecticide products against *B. tabaci*.**

*Dip tests for efficacy of conventional insecticide, soap and spray oil products.*

## Material and Methods

*Products and insect cultures*

Under quarantine conditions *Bemisia tabaci* were cultured in perspex cages (60 x 60 x 80 cm) on poinsettia plants following the method of Cuthbertson *et al.*[5] The entomopathogenic fungus *Lecanicillium muscarium* (reclassified from *Verticillium lecanii*, though still marketed under this product name) was supplied as Mycotal from Koppert Biological Systems Ltd., UK.

The selected insecticidal products to test were as follows (product make-up and dose rate): Majestik (natural plant extract; 2.5ml/100ml water); Agri-50E (alginate/polysaccharide; 300µl/100ml water); Spraying Oil (petroleum oil; 1ml/100ml water); Savona (fatty acids; 2ml/100ml water); Oberon (spiromesifen; 0.05g/100ml water).

The efficacy of the products were tested using the standard dip testing techniques for quarantine organisms evaluated and amended under the recent Defra Plant Health Division project PH0172. All tests were conducted in accordance with CSL Standard Operating Procedures (EFF454: A laboratory leaf-dip test method for investigating the effects of insecticides on quarantine whitefly species; EGBE/004/04: Dilution and application of pesticides for dip tests of leaves or filter paper; EBGE/007/04: Preparing fungal solutions for spray application to foliage).

Three life stages of *B. tabaci* were tested against; eggs, second instar and adult flies. Plants were infested following the methods of Cuthbertson *et al.*[5-10], and the desired life-stages were reared through based on the studies of Butler *et al.*[11], Bethke *et al.*[12] and Wang and Tsai[13]. This allowed all life-stages to be available for experimental use on the same date. Four separate insecticide dilutions were prepared. Two poinsettia leaves, containing eggs and second instar respectively, were dipped for 10 seconds then allowed to dry, before being placed within sealed Petri dishes for each individual dilution of insecticide. For adult studies, leaves were dipped and allowed to dry. Five adult whitefly were then placed on the leaf and held in position by a clip cage modelled on those described by MacGillivray and Anderson[14]. These were then placed within sealed Petri dishes and incubated at 20ºC, 14:10 L:D for 48 hours. In the case of the Mycotal treatments and the egg trials, dishes were incubated for 7 days in order to allow both the fungus to germinate[6] and eggs to potentially hatch[11-13]. Data underwent analysis of variance.

**Results**

All products apart from Oberon provided some form of control mortality against *B. tabaci* eggs following leaf dipping (Fig.1). Spray Oil was the only treatment to cause significantly more mortality of eggs than the water control (d.f. =1,3, F= 19.1, $P<0.05$).

On leaf dipping of leaves infested with second instar *B. tabaci*, all products tested caused significant mortality. Oberon, which had no effect on eggs, caused significantly higher mortality than the water control (d.f.= 1,3, F= 16.12,$P<0.05$) as did all the other products (Fig. 2).

Dipping of leaves and then testing for resultant efficacy against adult *B. tabaci* generated similar mortality to that for second instar larvae, apart from the treatment of Spray Oil, where total mortality of adult white fly was recorded (Fig. 3).

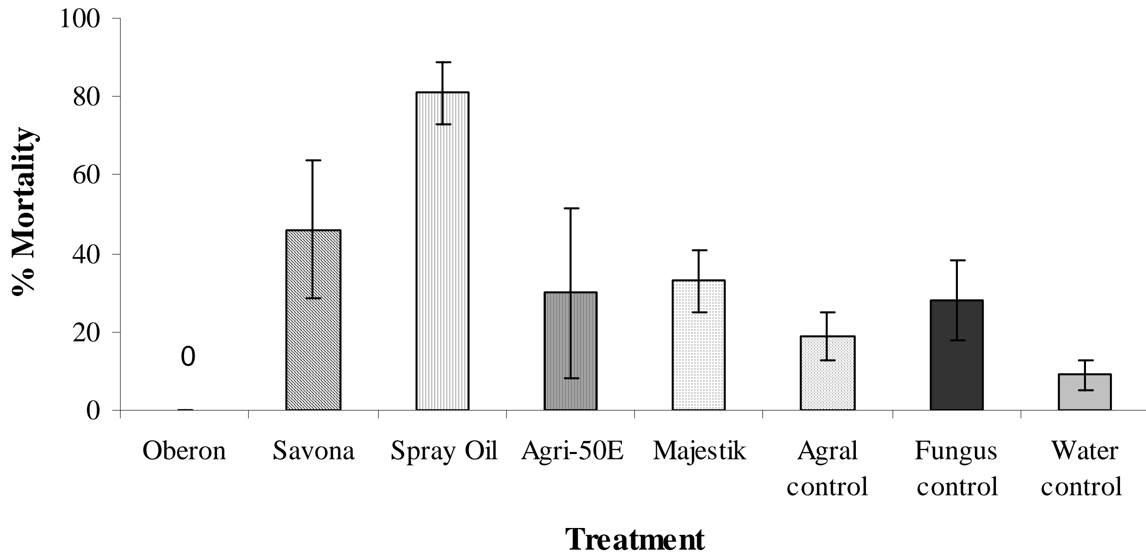


**Fig. 1.** The efficacy of insecticide treatments against *Bemisia tabaci* eggs following leaf dipping. Mortality assessed after 7 days. Bars are standard errors (±) of the mean.

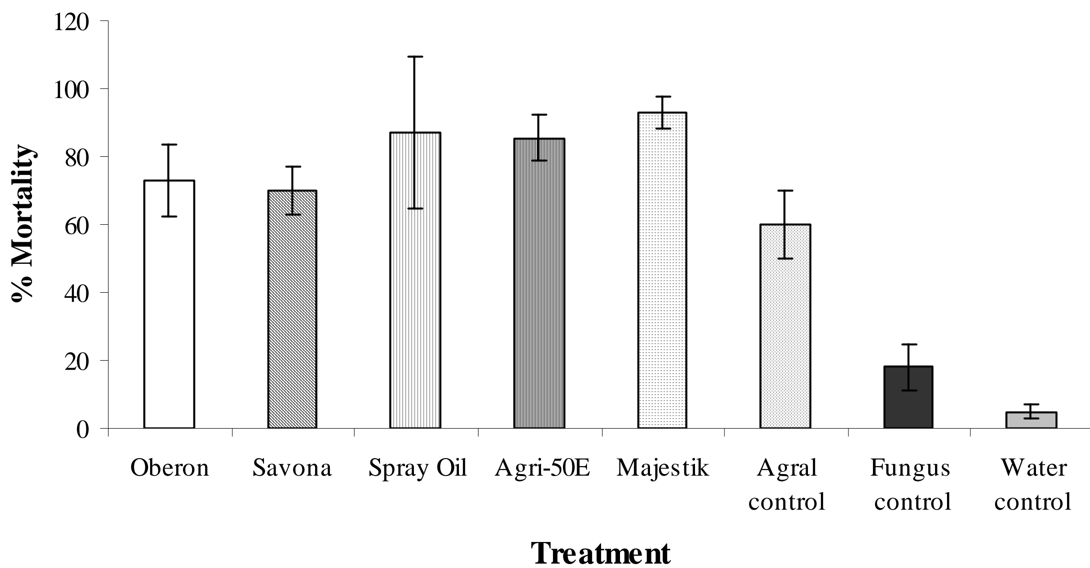


**Fig. 2.** The efficacy of insecticide treatments against *Bemisia tabaci* second instar larvae following leaf dipping. Mortality assessed after 48 hours for all treatments except Mycotal (fungus) which was assessed after 7 days. Bars are standard errors (±) of the mean.

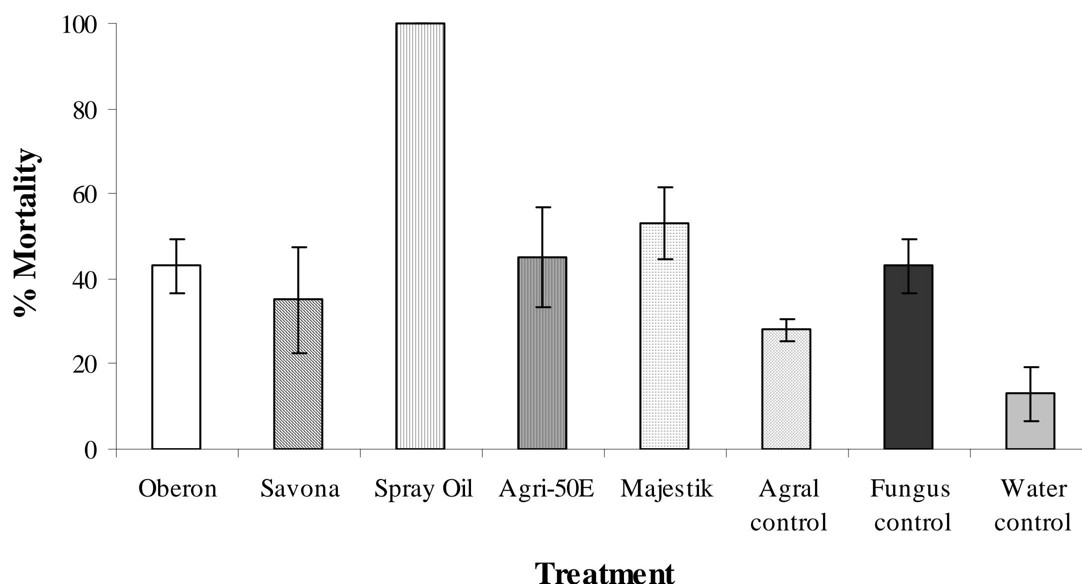


**Fig. 3.** The efficacy of insecticide treatments against *Bemisia tabaci* adults following leaf dipping. Mortality assessed after 48 hours for all treatments bar Mycotal (fungus) which was assessed after 7 days. Bars are standard errors (±) of the mean.

**Objective 2: Compatibility of selected conventional insecticide, soap and spray oil products with entomopathogenic fungi.**

*Direct suspension of fungus in chemical products*

**Materials and Methods**

Following the method of Cuthbertson *et al.*[8] the fungus was suspended in label recommended dilutions of each of the candidate products. A suspension of fungus in water acted as control. Three different batches of insecticide and fungus suspensions were made up in order to replicate the work over time and space. The suspensions were transferred to beakers and sealed with parafilm and incubated in the dark at 20°C. After 24 h 10 µl of each mixture was pipetted onto a sterile Petri dish containing 10% non-bacterial agar. The dishes were sealed with parafilm and incubated in the dark for a further 24 h at 20°C before percentage viability of conidia (germinated spores) from a total of 200 randomly chosen conidia were assessed under the microscope. Data underwent analysis of variance.

**Results**

There was a significant difference in the percentage spore germination of *L. muscarium* following direct exposure to the different active ingredients (Fig. 4). Only Agri-50E proved not significantly detrimental to the growth of the fungus compared to the water control (d.f.=1,2, F=5.61, P=0.14). Majestik, Savona and Spray Oil had spore germination percentages (6.8, 23 and 47.5% respectively) which would appear to be too low for practical use.

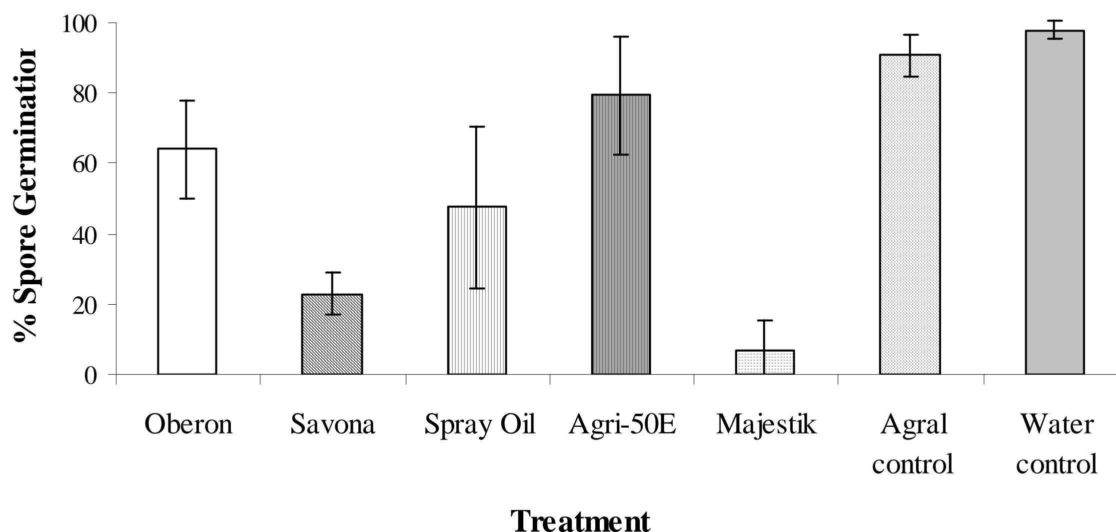


**Fig. 4.** *Lecanicillium muscarium* spore germination following 24 h exposure of the entomopathogenic fungus to chemical insecticides; Oberon (0.05g/100ml water), Savona (2ml/100ml water), Spraying Oil (1ml/100ml water), Agri-50E (300µl/100ml water), Majestik (2.5ml/100ml water), Agral control (200µl/1L water) or water control. Bars are standard errors (±) of the mean.

*Responses of entomopathogenic fungus to dry insecticide residues*

Again, following the protocol of Cuthbertson *et al.*[8] 28 poinsettia plants were each infested with four individual clip cages containing two male and five female *B. tabaci* adults. The plants were then incubated for the set period of time to achieve eggs (as out lined above). Plants were then divided into 2 groups, treatment and control, each with 14 plants. Within each group, two plants were then assigned for each product treatment. A combination of two sequential treatments as outlined in Cuthbertson *et al.*[8] were applied to each plant. The first application consisted of either an insecticide applied at the recommended dose rate for application or a water control. The plants were sprayed to run-off and the leaves allowed to dry before returning to the conditions defined for infestation of *B. tabaci*. The second treatment was applied 24 h later and consisted of either a suspension of $10^7$ conidia/ml with 0.02% of the non-ionic wetting agent Agral® (Syngenta Crop Protection Ltd., Cambridge, UK; active ingredient: alkyl phenol ethylene oxide) or a water control, each sprayed until run off using a Hozelock® Polyspray 2 hand held sprayer with a cone nozzle (Hozelock Ltd., Aylesbury, UK). There were approximately 1.5 x $10^5$ conidia per $cm^2$ of leaf surface. Following the second treatment, the plants were returned to the environmental chamber while still wet and maintained at 20 ± 1 °C, 85% r.h. and a 12:12 Dark:Light (D:L) period (treatments were treated at the start of the dark period). Mortality of eggs was assessed after seven days. Data underwent analysis of variance.

The above procedure was repeated for testing efficacy against second instar larvae.

**Results**

*Mortality of* B. tabaci *eggs.*

After spray application, all products tested caused some mortality of *B. tabaci* eggs. Mortality was even recorded for Oberon (13%) which previously caused no mortality to eggs after leaf dipping. Sequential application of chemical followed 24 h later with *L. muscarium* in most cases caused increased mortality (Fig. 5) for example, with Oberon, which caused significant increased mortality (d.f.=1,3, F=48, P<0.05). Sequential application of Agri-50E, Spray Oil and Majestik

with the fungus all caused increased mortality, however none produced significant increases. Agri-50E was noted to cause some foliage damage (yellowing and curling of leaves) to plant material.

*Mortality of second instar* B. tabaci.

Higher mortalities were recorded for all products tested against second instars than for eggs. All chemical products produced over 80% mortality (Fig. 6) except Oberon and Agri-50E (60 and 63% respectively). The addition of the fungus in sequential treatments in some cases seemed to give added effects. Sequential applications of fungus with Savona, Spray Oil and Majestik all produced mortalities above 90%. However, none of the sequential applications caused significantly higher mortalities than when applying the chemicals individually.

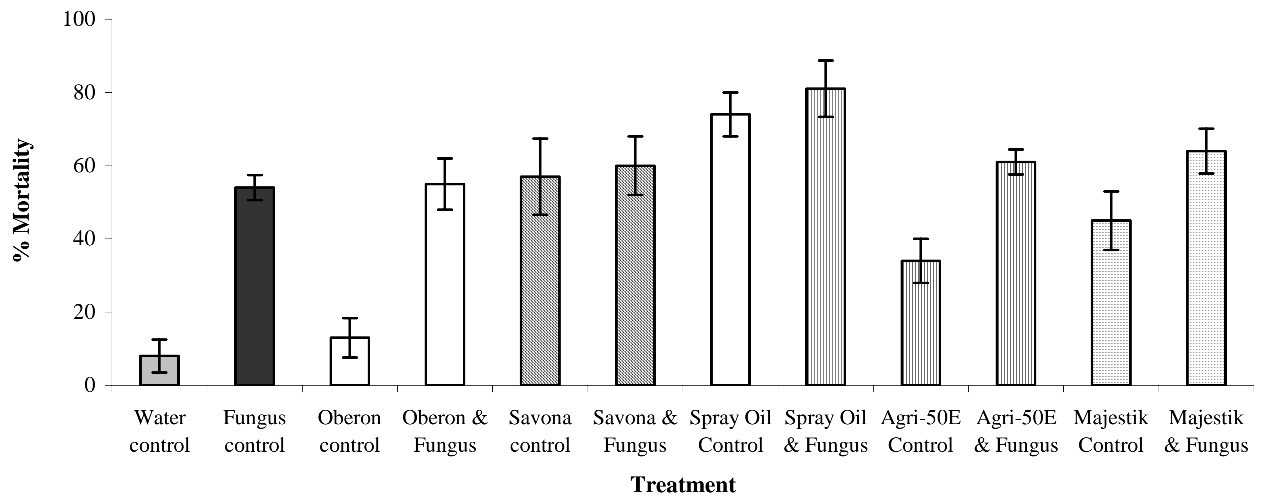


**Fig. 5** The effect of chemical residues on poinsettia plants on the infectivity of *Lecanicillium muscarium* (ca. 1.5 x $10^5$ spores per $cm^2$ of leaf area) against *Bemisia tabaci* eggs. The second treatment application was applied 24 h following the first treatment. Bars are standard error (±) of the mean.

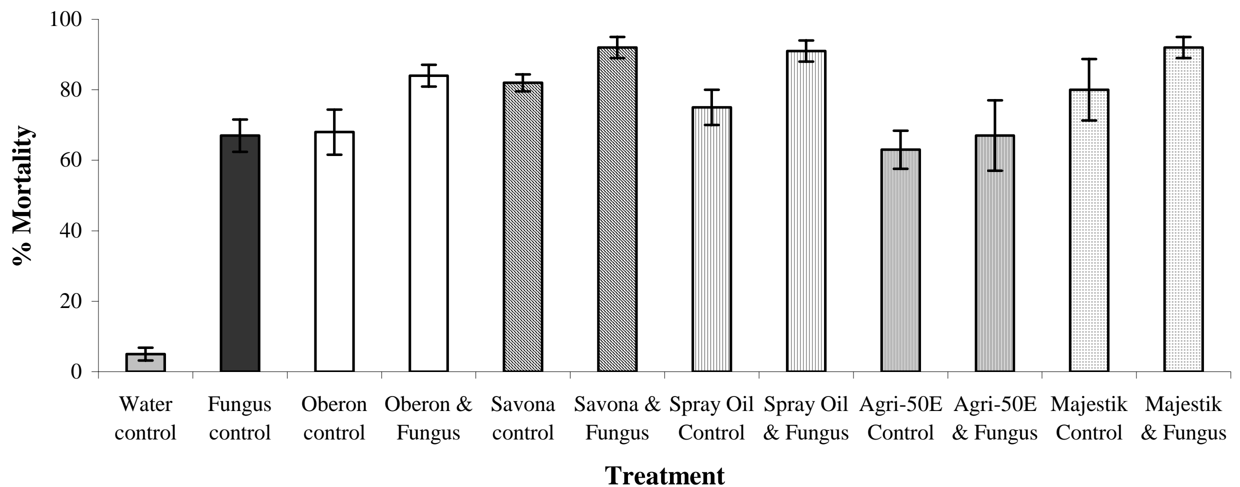


**Fig. 6.** The effect of chemical residues on poinsettia plants on the infectivity of *Lecanicillium muscarium* (ca. 1.5 x $10^5$ spores per $cm^2$ of leaf area) against second instar *Bemisia tabaci* larvae. The second treatment application was applied 24 h following the first treatment. Bars are standard error (±) of the mean.

**Objective 3: The effect of dose rate of products and compatibility with entomopathogenic fungi.**

Due to the findings of experiments carried out under Objectives 1 & 2 it was deemed uneconomic to test different dose rates of the chemical insecticides against the efficacy of *L. muscarium*. Objectives 1 & 2 proved that the normal recommended label dose rate of each insecticide did not give total mortality of *B. tabaci* eggs or larval stages. This decision was taken after consultation with the Defra Project Manager and the Project Steering Group.

**Objective 4: Field-use protocols and conducting glasshouse testing/validation of two selected products and one product combination.**

As objective 3 was not undertaken it was agreed with the Project Steering Group that Objective 4 would be lengthened. As all the chemicals showed some potential in being incorporated into IPM strategies against *B. tabaci* it was decided to test all five chemical products within the glasshouse environment.

Following the method of Cuthbertson and Walters[9] three glasshouse trials were undertaken. Each trial tested two different chemicals, both on their own and in combination with *L. muscarium* in sequential treatment. Designated quarantine glasshouse cubicles were used for experimental purposes.

For each trial 30 plants were infested with *B. tabaci* as outlined previously and following the methods of Cuthbertson *et al*[8,9]. After egg laying had occurred and the adults had been removed, the infested plants were transferred in sealed boxes to the designated glasshouse cubicle. The plants (treatments) were arranged randomly throughout the cubicle and conditions maintained at 25 °C for a further 12 days to allow the second instar to develop. After 12 days the plants were divided into six groups, each containing five plants. 10 plants received a treatment of Savona, 10 a treatment of Spray Oil, 10 a treatment of water as control. These were then left for 24 h after which: five of the Savona treated plants received an application of *L. muscarium* as did five of the Spray oil treated plants. Five of the water treated plants also received an application of *L. muscarium*. The remaining fifteen plants received an application of water. Both the pesticides and the fungus were applied using a hand held Hozelock® Polyspray hand held sprayers with cone nozzles. Data underwent analysis of variance.

The above procedure was repeated for each trial to test the other chemical products.

**Results**

None of the treatments of chemical followed by fungus gave any significantly better control of whitefly compare to the chemical being used on its own (Fig. 7a-c). Higher mortality was recorded following application of *L. muscarium* on foliage previously treated with Spray Oil but it was not significantly better than the chemical control (d.f.= 1,19, F=3.13, P=0.09), the same phenomena was recorded for Oberon (d.f.= 1,19, F= 2.14, P = 0.15) and Agri-50E (d.f. =1,19, F=1.46, P=0.24). However, on assessment of plants 7 days post application of Agri-50E commercially unacceptable foliage damage was recorded (Plate 1a,b). Some Agri-50E treated leaves had died and fallen off the plants.

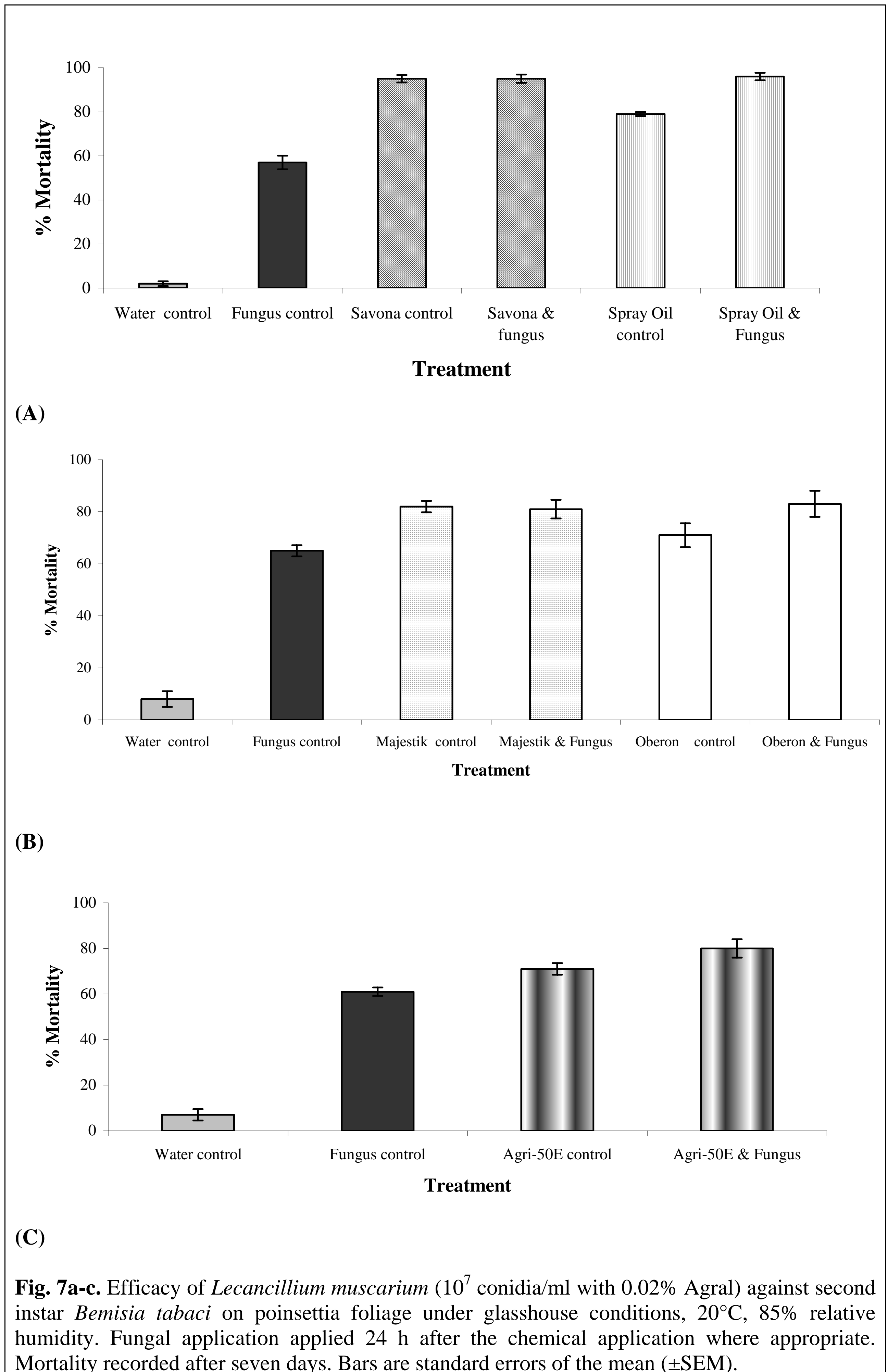


**Fig. 7a-c.** Efficacy of *Lecancillium muscarium* ($10^7$ conidia/ml with 0.02% Agral) against second instar *Bemisia tabaci* on poinsettia foliage under glasshouse conditions, 20°C, 85% relative humidity. Fungal application applied 24 h after the chemical application where appropriate. Mortality recorded after seven days. Bars are standard errors of the mean (±SEM).

**(a)**

**(b)**

**Plate 1a,b.** Foliage damage recorded on poinsettia plants 7 days after spray application of Agri-50E (alginate/polysaccharide; 300µl/100ml water) within the glasshouse environment.

**Discussion**

For the successful introduction of an IPM programme, information is not only needed on the biology of the control agent in question but also on its' potential to be mixed with other control agents, namely chemicals. Clarification of the effects of chemical insecticides on the wide variety of entomopathogenic fungi is necessary. However, to date there have been few *in vitro* tests. Different biopesticides based on *L. muscarium* (formerly *V. lecanii*[15]) are utilised on greenhouse crops to manage pests such as greenhouse whitefly, aphids and thrips in various European countries[16].

This study investigated the potential of using both a standard leaf dipping technique and spray application to test the compatibility of *L. muscarium* with low toxicity, IPM-compatible insecticide products commonly used within the UK for the control of *B. tabaci*. All of the insecticides tested were used at the highest legal application rates; lower rates of pesticide use might have lower impacts on the activity of *L. muscarium* and less detrimental effect on plant foliage.

The leaf dipping trials were investigated as a possible means of development of a disinfestation protocol for poinsettia cuttings in order to ensure eradication of the pest, not for integrated pest management purposes. Oberon, which has been stated not to be particularly ovicidal[17], produced no mortality of *B. tabaci* eggs following leaf dipping. Indeed all chemicals with the exception of Spray Oil produced low egg mortalities. The results from this study further confirm the difficulty of eradicating egg stages. Leaf dipping against second instar and adult life stages produced higher mortality. Here, Spray Oil showed great potential as total mortality of adult *B. tabaci* was achieved.

Direct exposure of *L. muscarium* for 24 hours to Majestik, Spray Oil and Savona resulted in very low spore germination, unsuitable for commercial use. However, the chemicals Oberon and Agri-50E produced acceptable spore germination. This study has shown that direct mixing of *L. muscarium* with these two chemicals is a viable IPM option.

The implementation of an IPM scheme may require sequential rather than simultaneous applications of insecticides and entomopathogenic fungi. Apart from the study of Cuthbertson *et al.* (2005)[8] few previous studies have tested the effect of dry insecticide residue on fungal activity. In the current study, when *L. muscarium* was applied to plants sprayed 24 h earlier with a standard application of one of five contact insecticides, no significant reduction in infectivity (mycelial growth) was detected in any cases. Therefore, *L. muscarium* could be applied sequentially with Oberon, Savona, Spray Oil, Agri-50E and Majestik for the control of *B. tabaci*, with second instars again proving highly susceptible to fungal attack, as found by Cuthbertson *et al.* (2005)[6]. However, after application of Agri-50E commercially unacceptable foliage damage was recorded. It is therefore unlikely that this product would be a candidate for further research on poinsettia plants. Other plant species may however prove to be more tolerant of this product.

This study has identified several further approaches to the successful integration of chemical insecticides with the entomopathogenic fungus, *L. muscarium*. Firstly, Oberon and Agri-50E can be applied simultaneously with *L. muscarium*. Secondly, high levels of control of *B. tabaci* can be achieved by application of the fungus to foliage previously treated with Oberon, Savona, Spray Oil, Agri-50E and Majestik. Overall levels of control of all *B. tabaci* lifestages have been encouraging, especially against second instar larvae. The trials undertaken have demonstrated the efficacy of the chemicals and the fungus against the range of *B. tabaci* lifestages. Further work could involve applying the fungus followed by insecticides at increasing time intervals to investigate potential protection of the fungus with increasing time before application of an insecticide. Testing for Agri-50E, which in several cases produced high levels of *B. tabaci* mortality and showed a high potential for direct mixing with *L. muscarium*, phytotoxic effects on other plant species could prove useful in the development of IPM strategies.

## References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material relating to or generated by this project.

**References to cited material**


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**Scientific papers planned to arise from this current project:**

1) Cuthbertson *et al*., To investigate the efficacy of a range of insecticidal products to control *Bemisa tabaci* by leaf dipping. In draft – potentially for *Journal of Applied Entomology*.

2) Cuthbertson *et al*., - Further investigations into the compatibility of *Lecanicillium muscarium* and insecticidal products for the eradication of *Bemisia tabaci.* In prep. – potentially for *Mycopathologia*.

3) Cuthbertson *et al*., - Further investigations into the use of *Lecanicillium muscarium* to control *Bemisia tabaci* under glasshouse conditions. In prep. – potentially for *Mycopathologia*.

**Technology transfer/presentations:**

Cuthbertson, A.G.S. (2006). *Quarantine Entomology Research*. Plant Health and Seeds Inspectorate, Technical Refresher Course, Central Science Laboratory, Sand Hutton, York. 27th - 29th March 2006.