APPLIED PLANT RESEARCH

Biocontrol of whitefly using stabilised *Aschersonia* formulations

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Front page photograph: experimental poinsettia, *Euphorbia pulcherrima*, production in glasshouse research facility.

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1. Introduction

Worldwide over 1200 whitefly species have been described, ranging from very selective monophagous insects to polyphagous ones. Among those that have a pest status, greenhouse whitefly, *Trialeurodes vaporariorum*, and tobacco whitefly, *Bemisia tabaci*, are the most common whitefly pests in the world. Recently, silverleaf whitefly, *Bemisia argentifolii*, also referred to as *Bemisia tabaci* biotype B, was added to this list. Greenhouse whitefly predominantly occurs in greenhouses all over the world and can cause damage on a whole range of vegetable and ornamental crops such as cucumber, tomato, eggplant, sweet pepper, poinsettia, Bouvardia, and Gerbera. Tobacco whitefly is a serious pest in field crops such as tomato, melon, and cotton. Silverleaf whitefly has been observed as a damaging pest in above-mentioned field as well as protected crops.

The damage consists of plant growth retardation or distortion by feeding as direct damage and the presence of honeydew and growth of fungi thereon as indirect damage. All these three whitefly species are able to vector several plant pathogenic viruses that can also have a devastating effect on plant growth and production of fruits and flowers. Until recently, chemical control was the main option for control of whitefly pests. However, these conventional pest control agents bear the risc of losing their effectiveness by development of insecticide resistance, thus resulting in a continuous need for new and invariably more expensive replacement compounds. Another aspect is the growing public awareness of the negative side effects of the use and often abuse of chemical pesticides. This concern has resulted in governmental strategies for reduction of pesticide use and enhancement of alternative pest control methods such as integrated pest management (IPM). In IPM several natural enemies against whitefly pests are available belonging to the categories of predators, parasitoids and entomopathogens.

Microbial control by entomopathogens can take place by the use of viruses, protozoa, rickettsiae, bacteria, fungi and nematodes. The group of fungi has many representatives as natural enemies of whitefly (Fransen, 1990a). Some of these are more broad spectrum in their attack on insects such as *Beauveria bassiana* and *Verticillium lecanii*, although also in these species there is a difference in pathogenicity among strains. The fungi of the genus *Aschersonia* are more specifically pathogenic on whitefly (Aleyrodidae) (Figure 1.1) and soft scales (Coccidae) and can be well integrated in a control system using different natural enemies such as the parasitoid *Encarsia formosa* (Fransen & van Lenteren, 1993,1994).

The mode of infecton of entomopathogenic fungi consists of several steps: conidial attachment, germination, penetration through the insect cuticle, vegetative growth within the host, fungal protrusion outside the insect and conidiogenesis. External conditions play an important role for survival of the infective units after application and for the first phase of infection (attachment, germination, penetration).
Several approaches have evolved for the use of entomopathogens, the most important one being the use of inundative releases, also known as the application of mycoinsecticides. This way of introduction involves the mass production of infective units and formulation and application more or less comparable to the technology used for chemical pesticides (Lacey et al., 2001).

Success in applying these infective units of entomopathogenic fungi will depend on protection against solar radiation, appropriate humidity conditions and good coverage of the host and its habitat, in case of whitefly the abaxial leaf surface.
Formulation techniques can contribute to provide protection from UV light, to influence humidity requirements and to obtain good coverage.

Objectives of research project

The overall objective is to introduce safe IPM-compatible and environmentally-acceptable measures of plant protection that will be cost effective and able to compete with the currently-applied conventional pesticides.

Objectives of this Dutch-Israeli research project are photostabilization of the conidia, maintaining humid microenvironment for conidial germination and proper coverage of the lower leaf surface by adding selected compounds and using specific techniques for formulation.
A method of photoprotection involves the co-adsorption of photolabile pest control agents and selected organic chromophores on clay. With respects to humidity requirements of entomopathogenic fungi, various antidesiccants and antievaporants can be added to the formulation.
As possible interactions of host plants with the formulated fungal entomopathogen and the pest may compromise control results, the proposed study will involve various plant species.

Technical work plan of both partners (D+I)

The technical work plan includes a number of steps: growing and handling the entomopathogenic fungus *Aschersonia* (D+I) ; studying the selectivity of *Aschersonia* spp. by testing them on other Hemiptera (Coccidae and Diaspididae) (D), microencapsulation of the conidia being the infective units, using various biopolymers (I); development of formulations using antidesiccants and photostabilizing chromophores (I); selection of formulations by stressing the protected fungus with UV and desiccation regiments (I); evaluating spore germination(D+I) in relation to the selected formulations; conducting bioassays with formulations using *B. argentifolii* and *T. vaporariorum* that will include different plant species (D+I).

Figure 1.1 Nymphs of *Bemisia argentifolii* infected by the fungus *Aschersonia aleyrodis* on Poinsettia

2. Production and storage of *Aschersonia*

2.1 Material and methods

Production

A virulent strain of *Aschersonia aleyrodis* (Aa4) originating in the tropical region of Columbia provided by the Dutch partner, was used in this research. A two step technology was employed for the production of conidia. In the first phase the isolate stored on Microbank ™ porous beads at – 80 °C was cultured on Petri dishes on Potato dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) for 1-3 weeks at 25 °C and 16L:8D photophase. In the second step, spore suspensions obtained from these plates were used to inoculate autoclaved millet. The fungus grew well on millet, as a solid substrate, in 300 ml Erlenmeyer flasks (10 g millet with 25 ml demineralised water). The Erlenmeyer flasks were closed with sterile cotton for aeration. The cultures were incubated at 25 °C and 16L:8D artificial light. Conidia were harvested from three weeks old cultures by rinsing them with sterilised demineralised water containing 0.03% (v/v) Tween 80 (Merck).
In addition plastic mushroom bags containing autoclaved millet were used for inoculation of *Aschersonia aleyrodis.*

Storage

In a previous research project over 40 isolates belonging to the genus *Aschersonia* had been collected from all over the world, including isolates from existing collections as well as isolates from fresh material (infected whitefly nymphs). The majority of isolates were, as far as known, multisporal. In research by Meekes (2001) it was shown that differences in sporulation and germination were present (Table 1.1). The collection was stored on Microbank ™ porous beads at – 80 °C in the second half of 1996. Reisolation of the strains from the beads took place on PDA (Difco) and SDA at the autumn of 1999 after an overall storage period of three years.

Sporulation and germination capacity was checked after growth of the strains on PDA for three weeks after reisolation. Sporulation was visually estimated by using the following categories: - no sporulation; -/+ few colonies forming pycnidia producing spores; + several colonies forming pycnidia producing spores; ++ many colonies forming pycnidia producing spores.
Germination was visually estimated after application of 5 ml of sporesuspension containing 10^7 sp/ml on water agar (15 g/l agar-agar, Merck) by a DeVilbiss spraying device. Incubation took place at 20°C and natural daylight for 90 hrs. Germination was checked on the water agar, in presence of absence of sugar in the form of honeydew, and was evaluated using the following categories: 0 no germination; 1-10% germination; 10-33% germination; 33-66% germination; 66-100% germination. Germination was rated when germ tubes exceeded the width of the conidium. Records of relative lengths of germ tubes were also made by the use of two categories: germ tubes shorter than the length of the conidium and germ tubes longer or much longer than the length of the conidium.

2.2 Results and discussion

Production

Cultures of *Aschersonia* species grow and sporulate on most conventional media. Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA) and Malt Extract Agar (MEA) are good media for growth and sporulation. When comparing more "allround" media such as corn, millet and rice, highest yields were observed using corn and millet.
Production of strain Aa4 on millet amounted to 10^{10} (range 10^{9} –10^{11}) conidiospores per Erlenmeyer flask. Due to a lower availability of air in the mushroom bags, the production of spores was lower than in the Erlenmeyer flasks. By the use of forced aeration of the mushroom bags the spore production can increase. Good availability of air is essential for the production of spores as spore production was absent in Erlenmeyer flasks closed with rubber stops instead of cotton wool. In contrast, mycelial growth also takes place in Erlenmeyer flasks closed with rubber stops.

The optimal temperature for spore production was previously established to be 25°C. When grown at 27°C mycelial growth was more abundant and sporulation was less. Also at 20°C spore production is good but sporulation starts later than at 25°C.

Light is also an important external factor for spore production. Daylight or even artificial light provided by TL lamps apparently is sufficient for stimulation of sporulation. *Aschersonia aleyrodis* being subcultured three times shows a three times higher spore production when kept under 16L:8D compared with spore production when kept at 0L:24D (Figure 2.1) (unpubl. res., Fransen). In constant light spore production takes place, however the fungus seems to become deregulated and also forms abundant mycelium (Figure 2.1). Germination of conidia of *Aschersonia aleyrodis* was not dependent on daylength.

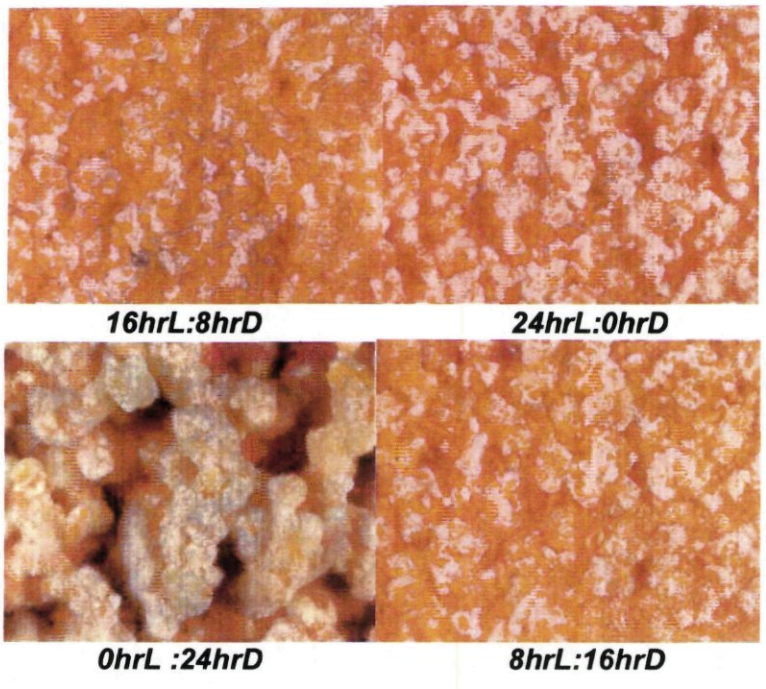

Figure 2.1 *Aschersonia aleyrodis* grown on millet exposed to different artificial light regimes.

Deterioration of cultures can take place after repeated subculturing. This depends on the characteristics of the fungal species, and external influences play a role such as the number of subculturings, the type of medium, and the growing conditions like the provision of light. *Aschersonia aleyrodis* subcultured thirteen times produced 20 times less spores at

16L:8D than *A. aleyrodis* subcultured three times. Being subcultured thirteen *times A. aleyrodis* totally lost the capacity to produce spores in 0L:24D (Fransen, 1987).
In our experiments *A. aleyrodis* was never subcultured more than three times before use.

Culturing fungi in liquid fermentaton processes is generally considered the most efficient production method. Trials in the past with *Aschersonia* spp. in liquid cultures resulted in good production of mycelial structures, whereas infective units were never produced. When tested on whitefly, mycelial structures never resulted in infection of whitefly. Production of infective units is possible with a two-phase fermentation process, in which high amounts of mycelium are produced in liquid culture and thereafter poured out on a solid nutrient surface for production of spores in pycnidia. Solid phase fermentation technology is becoming increasingly common practice, for example conidia of *Beauveria bassiana* are also produced using solid phase fermentation technology (pers. comm. Stefan Jaronsky).

Storage

Differences in sporulation and germination between isolates were apparent (Table 2.1) when evaluated in 1996 and in 1999. Although direct comparison of sporulation in 1996 and 1999 is not possible because of the use of a quantitative method in the former tests and of a qualitative method in the latter tests, the overall capacity of sporulation seems to be well preserved over three years of storage at –80 °C.
Germination generally took more time after three years of storage and final evaluation in 1999 was done after 90 hrs of incubation at 20°C. Some isolates showed a lower number of germinated spores after storage, for example A5, A12, A29 and Aa1. Conidia of most isolates that showed lower germination percentages, also had shorter germination tubes than the conidia of isolates that showed higher germination percentages.
Presence of honeydew influenced germination and the percentages of germination of 1999 as shown in Table 2.1 were found in presence of honeydew. The following isolates showed lower germination rates on water agar without honeydew : A6, A7, A8, A14, A15, A17, A24, A26, A27, A28, and A32. The ability to germinate in absence of nutrients as was present in 1996 when isolates were tested on water agar only, seems to deteriorate when isolates are stored at –80°C.

Table 2.1 Overview of the different islates used for testing germination and sporulation after three years of storage at –80°C.

Code	Original code	Collector	Host	Origin	[a]Sporulation '96	[b]%Germination '96	[c]Sporulation '99	[d]%Germination '99
Aschersonia *sp*								
Al	ARSEF-3014	S.R.Sanchez Pena	Aleyrodidae	Mexico	+	96.3	±	66-100
A2	94008	L.A. Lacey	*Dialeurodes citri*	Brazil	+	94.9	nt	nt
A3	94010	L.A. Lacey	*D. citri*	Brazil	±	97.6	±	66-100
A4	94011	L.A. Lacey	*D. citri*	Brazil	+	98.8	nt	nt
A5	94021	L.A. Lacey	*D. citri*	Brazil	+	95.6	±	10-33
A6	94024	L.A. Lacey	Aleyrodidae	Thailand	+	28.2	++	33-66
A7	94025	L.A. Lacey	Aleyrodidae	Thailand	+	27.6	++	10-33
A8	94026	L.A. Lacey	Aleyrodidae	Malaysia	+++	96.9	++	66-100
A9	94027	L.A. Lacey	Aleyrodidae	Malaysia	+	99.6	nt	nt
A10	ARSEF-3015	S.R.Sanchez Pena	Homoptera	Mexico	++	8.5	++	66-100
A11	I89-490	H.C. Evans	-	Mexico	±/+	3.4	±	1-10
A12	I92-784	H.C. Evans	-	Brazil	±	61.6	±	1-10
A13	I92-787	H.C. Evans	-	Brazil	+	20.6	++	66-100
A14	I92-788	H.C. Evans	-	Madagascar	++	45.2	++	66-100
A15	I93-807	H.C. Evans	-	Guyana	++	94.5	++	66-100
A16	I93-813	H.C. Evans	-	Guyana	++	51.5	+	66-100
A17	I93-815	H.C. Evans	-	Trinidad	++	98.0	++	66-100
A18	I93-856	H.C. Evans	-	Colombia	+++	96.6	nt	nt
A19	I93-858	H.C. Evans	-	Colombia	+	42.0	nt	nt
A20	I93-860	H.C. Evans	-	Colombia	±	99.6	±	66-100
A21	I93-860	H.C. Evans	-	Colombia	-	-	-	-
A22	I93-861	H.C. Evans	-	Colombia	+	24.4	nt	nt
A23	I93-901a	H.C. Evans	-	Ghana	+	97.2	+	66-100
A24	I94-908	H.C. Evans	-	Venezuela	+/++	95.3	++	66-100
A25	I94-910	H.C. Evans	-	Venezuela	+	97.5	nt	nt
A26	-	L.A. Lacey	Aleyrodidae	Malaysia	++	98.2	++	66-100
A27	-	L.A. Lacey	Aleyrodidae	Thailand	+	26.4	++	66-100
A28	-	H.Saito	*Pealius azalea*	Japan	++	96.5	++	66-100

A29	-	S.Balan	*Dialeupora* sp.	India	+	21.6	++	1-10
Code	Original code	Collector	Host	Origin	[a]Sporulation '96	[b]%Germination '96	[c]Sporulation '99	[d]%Germination '99
A30	KV-129	R.A.Samson	-	Thailand	++	98.1	nt	nt
A31	KV-131	R.A.Samson	-	Thailand	+++	95.6	++	66-100
A32	KV-132	R.A.Samson	-	Thailand	++	96.1	++	66-100
A. aleyrodis Webber								
Aa1	ARSEF-430	R.S. Soper	Aleyrodidae	Fla, USA	±/+	98.0	++	33-66
Aa2	ARSEF-992	N. Oho	*D. citri*	Japan	+++	99.5	++	66-100
Aa3	ARSEF-2154	M.C. Rombach	Aleyrodidae	Java	±	99.0	++	66-100
Aa4	ARSEF-2268	W.Gams	Aleyrodidae	Colombia	++	99.3	++	66-100
Aa5	KV-107	W.Gams	Aleyrodidae	Colombia	+++	98.7	nt	nt
Aa6	KV-108	W.Gams	Aleyrodidae	Colombia	+++	98.0	nt	nt
A. goldiana Saccardo & Ellis								
Ag1	ARSEF-431	R.S.Soper	Aleyrodidae	FLA, USA	±	92.2	nt	nt
A. insperata (ined.)								
Ai1	ARSEF-2356	M.C.Rombach	*D.citri*	Philipines	+	29.9	+	33-66
Ai2	ARSEF-2351	M.C.Rombach	*D.citri*	Java	+++	46.4	+	10-33
A. placenta Berkeley & Broome								
Ap1	-	S. Selvakumaran	*D. cardamomi*	India	+++	91.7	++	66-100
Ap2	CBS-917-79	-	-	S.E. Asia	++	95.5	++	66-100
A. turbinata Berkeley								
At1	ARSEF-1030	R.A. Hall	Homoptera	Colombia	±	12.4	nt	nt

[a]: - = no sporulation, ± = less than 5.10^7, + = 5.10^7-5.10^8, ++ =5.10^8-5.10^9, +++ = > 5.10^9 conidia per culture; [b] : average % germination on wateragar; [c]: visual evaluation - = no sporulation, ± = few colonies forming pycnidia producing conidia, + = several colonies forming pycnidia producing spores, ++ many colonies forming pycnidia producing spores; [d]: estimation of % germination in 5 categories:0-1;1-10;10-33;33-66;66-100; nt: not tested

3. Infection of armoured scales (Diaspidae) and soft scales (Coccidae)

3.1 Material and methods

Insects

Leaves of *Ficus benjamini* bearing brown soft scale, *Coccus hesperidum* (Coccidae), or leaves of *Cymbidium* bearing orchid scale, *Diaspis boisduvalii* (Diaspidae), were used in the bioassay tests.
Brown soft scale (Figure 3.1) is a very common polyphagous and virtually world-wide pest of glasshouse ornamentals. Commonly infested hosts include *Ficus, Hibiscus, Hedera,* and *Stephanotis*. This species is viviparous and usually parthenogenetic, each female producing about a thousand nymphs over a 2-3 months period. The young nymphs wander over host plants for a few days before settling down to feed. The complete life cycle from birth to maturity occupies about two months at average glasshouse temperatures. The scales secrete considerable amounts of honeydew, leaves becoming severely covered by moulds which spoils the appearance of the ornamentals (Alford, 1991).
Orchid scale, *Diaspis boisduvalii* (Diaspidae), (Figure 3.2 a, b and c) is a common and world-wide pest of glasshouse-grown orchids, especially *Calanthe, Cattleya, Cymbidium* and *Epidendrum.* Infestations also occur on palms. Eggs are minute, oval and yellow and protected by the female scale. Older nymphs differentiate into female and male scales, the former being flat and oval, yellowish and translucent, the later being elongate with three distinct longitudinal ribs and coated with white waxen threads. Adult females are yellow and the body is protected by a hard, scale-like covering formed from cast-off nymphal skins and wax. Adult males are small orange-yellow single-winged flies.
Damage is restricted to the disfigurement of plants and weakening of plants by the withdrawal of plant juices. The thick masses of white wax associated with male scales are unsightly. Honeydew is not produced by orchid scale (Alford, 1991).

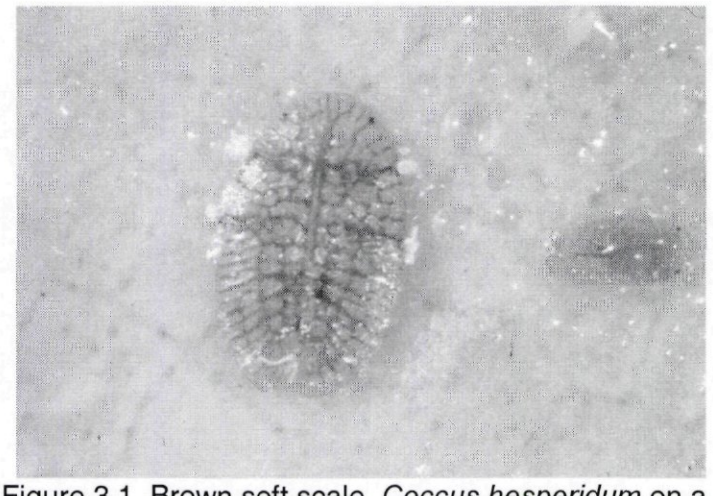

Figure 3.1. Brown soft scale, *Coccus hesperidum* on a multi-coloured *Ficus benjaminii.*

Spore application and evaluation

A leaf of *Ficus benjamini* bearing nymphs and matures of brown soft scale, *Coccus hesperidum* (Coccidae), or a leaf of Cymbidium bearing nymphs and matures of orchid scale, *Diaspis boisduvalii* (Diaspidae), was present on Petri dishes containing a 5 mm layer of water agar (15 g/l agar-agar, Merck). These were treated by spore suspensions of the different *Aschersonia*

isolates and of a *Verticillium lecanii* isolate, originating from the product Mycotal® (Koppert B.V.). Spore germination was also checked and is shown in Table 2.1. The amount of 5 ml sporesuspension containing 10^7 sp/ml was applied per Petri dish by a DeVilbiss spraying device.

Figure 3.2a. Female scales and adult male fly and immature male scale of orchid scale, *Diaspis boisduvalii.*

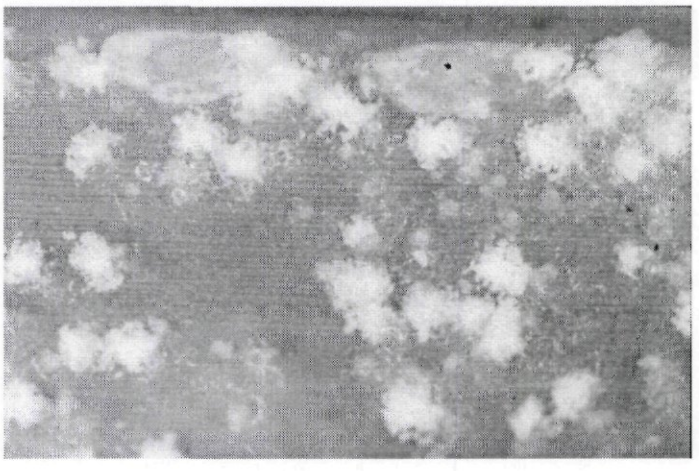

Figure 3.2b. Female scales and male immatures covered by wax threads of orchid scale, *Diaspis boisduvalii.*

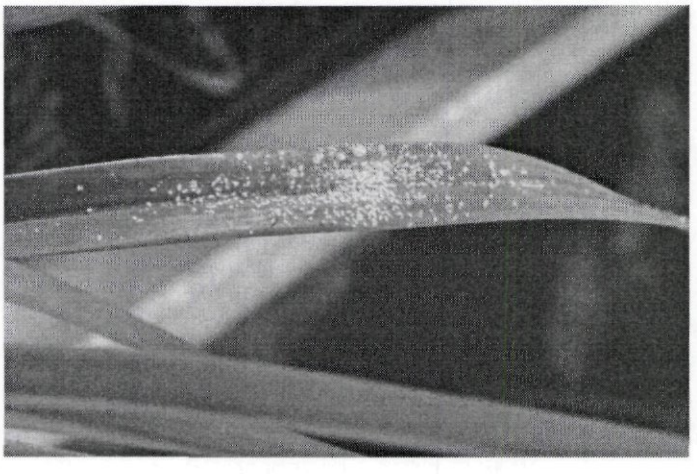

Figure 3.2c. View of orchid scale on Cymbidium.

One Petri dish per isolate and insect species was used in this bioassay test. Incubation took place at 20°C and natural daylight and visual observations for signs of infection by means of a Zeiss stereomicroscope (20-80x) were made four and fourteen days after application.

3.2 Results and discussion

In Table 3.1 results from the bioassay, testing *Aschersonia* isolates for infectivity of *C. hesperidum* and *D. boisduvalli* , are shown. On whitefly, germination of *Aschersonia* spores and penetration of the insect cuticle can be observed using fluorescent techniques and scanning electron microscopy. When using a stereomicroscope, signs of infection can be identified as follows : first stage : opaqueness of the whitefly nymphs; second stage : discolouration of the interior to an orange-opaque or orange-white colour; third stage : protrusion of mycelium from the margins and anal opening in the insect body; fourth stage : sporulation from pycnidia formed on mycelium at the outer border of the insect and/or covering the insect. The third and fourth stage will occur under favourable conditions i.e. high humidity. In case of *Aschersonia aleyrodis* the orange spore masses are very conspicuous (Figure 1.1) (Fransen, 1987, Meekes, 2001).

Exposure of immatures and matures of *C. hesperidum* and *D. boisduvalli* to spores of the different *Aschersonia* isolates did not result in clear symptoms of infection as were found on whitefly hosts. Although circumstances in the Petri dishes were favourable for growth of mycelium and sporulation outside the insect body, these phenomena were not observed on orchid scale and brown soft scale. In Table 3.1 question marks are present referring to infection when isolates A1, A10, A13, A14, A15, A16, A17, A23, A28, Ai1 and Ai2 were applied on brown soft scale and when A24 was applied on orchid scale. Some opaqueness of immatures and/or matures was observed here, but it is uncertain whether this is due to infection or just being a mere artefact. Mycelium of *Verticillium lecanii* (KV-01 from Mycotal) was observed growing on the surface of the cuticle of both *C. hesperidum* and *D. boisduvalli.* It could not be concluded whether the fungus actually penetrated the insect host. Whitefly nymphs infected by *V. lecanii* show white mycelium inside the insect body. This was not observed in *C. hesperidum* and *D. boisduvalli.*

Fungi belonging to the genus *Aschersonia* are specialized pathogens of whiteflies (Homoptera: Aleyrodidae) and/or scale insects (Homoptera: Coccidae) (Petch, 1921, Evans & Hywel-Jones, 1990). Most isolates come from hosts not identified to species (Table 3.1). In several cases no living hosts remain and total destruction of the original host by the fungus is so complete that identification is not possible (Evans & Hywel-Jones, 1990). Petch made a clear distinction between species pathogenic to whiteflies (Aleyrodiicolae) and species that are able to infect scale insects (Lecaniicolae). Morphologically, these two groups differ in presence (Aleyrodiicolae) or absence (Lecaniicolae) of paraphyses in the pycnidium (Petch, 1921). However, recently it has been shown that presence of paraphyses in the pycnidia on the host may be absent when the fungus is grown in pure culture. Thus, this appearance of paraphyses is likely to be a phenotypically determined characteristic and not a reliable taxonomic feature (Evans & Hywel-Jones, 1997). This has also been underlined by the observation of *Aschersonia aleyrodis*, for instance, not only infecting several species of whiteflies (Fransen, 1990), but also being isolated from scale insects (Vargas Sarmiento et al., 1995). When testing isolates of *Aschersonia* species on *C. hesperidum* and *D. boisduvalli* we hypothesized that those isolates not infecting whitefly, may possibly infect insects from the Coccidae family specifically and show no results on insects of the Diaspidae family. However, no apparent infection of insects of either species was observed. Several explanations can be given: (1) the host range of the isolates did not include the used test insects. (2) Bioassay set-up was inappropriate for screening on coccids and diaspids. For instance, it is unknown whether *Cymbidium* or *Ficus* have any influence on survival of *Aschersonia* spores. (3) Attenuation of the isolates due to storage may play a role.
Germination and sporulation of the isolates after three years of storage under –80oC showed good results. However, the isolates, except the Aa4, were not tested in bioassays on whitefly in the 1999 experiment. The Aa4 isolate was used for the formulation bioassays (see Chapter 4) and showed good virulence on greenhouse whitefly and silverleaf whitefly. Not only attenuation during the

three-year storage at our laboratory may have influenced results, but also attenuation may have taken place during previous storage at the provider. Good germination and sporulation are not always positively correlated with virulence (Meekes et al., 2002).

More research has to be directed towards the host range and virulence of *Aschersonia* species and their supposed correlation to taxonomic characteristics.

Table 3.1. Infection of whitefly, brown soft scale and orchid scale by *Aschersonia* isolates and *Verticillium lecanii.*

Code	Original code	Collector	Host	Origin	Infection '96 Whitefly[a]	Infection '99 *Coccus hesperidum*	Infection '99 *Diaspis boisduvalli*
Aschersonia *sp*							
Al	ARSEF-3014	S.R.Sanchez Pena	Aleyrodidae	Mexico	+[b]	?[e]	--[d]
A2	94008	L.A. Lacey	*Dialeurodes citri*	Brazil	+	nt[c]	nt
A3	94010	L.A. Lacey	*D. citri*	Brazil	+	-	-
A4	94011	L.A. Lacey	*D. citri*	Brazil	+	nt	nt
A5	94021	L.A. Lacey	*D. citri*	Brazil	+	--	--
A6	94024	L.A. Lacey	Aleyrodidae	Thailand	+	--	--
A7	94025	L.A. Lacey	Aleyrodidae	Thailand	+	--	--
A8	94026	L.A. Lacey	Aleyrodidae	Malaysia	+	--	--
A9	94027	L.A. Lacey	Aleyrodidae	Malaysia	+	nt	nt
A10	ARSEF-3015	S.R.Sanchez Pena	Homoptera	Mexico	--	?	--
A11	I89-490	H.C. Evans	-	Mexico	+	--	--
A12	I92-784	H.C. Evans	-	Brazil	--	--	--
A13	I92-787	H.C. Evans	-	Brazil	--	?	--
A14	I92-788	H.C. Evans	-	Madagascar	+	?	--
A15	I93-807	H.C. Evans	-	Guyana	+	?	--
A16	I93-813	H.C. Evans	-	Guyana	--	?	--
A17	I93-815	H.C. Evans	-	Trinidad	+	?	--
A18	I93-856	H.C. Evans	-	Colombia	+	nt	nt
A19	I93-858	H.C. Evans	-	Colombia	--	nt	nt
A20	I93-860	H.C. Evans	-	Colombia	--	--	--
A21	I93-860	H.C. Evans	-	Colombia	--	--	--
A22	I93-861	H.C. Evans	-	Colombia	--	nt	nt
A23	I93-901a	H.C. Evans	-	Ghana	+	?	--
A24	I94-908	H.C. Evans	-	Venezuela	+	--	?
A25	I94-910	H.C. Evans	-	Venezuela	--	nt	nt
A26	-	L.A. Lacey	Aleyrodidae	Malaysia	+	--	--
A27	-	L.A. Lacey	Aleyrodidae	Thailand	+	--	--
A28	-	H.Saito	*Pealius azalea*	Japan	+	?	--
A29	-	S.Balan	*Dialeupora* sp.	India	+	--	--

Code	*Original code*	*Collector*	*Host*	*Origin*	*Infection '96 Whitefly*[a]	*Infection '99 Coccus hesperidum*	*Infection '99 Diaspis boisduvalli*
A30	KV-129	R.A.Samson	-	Thailand	+[b]	nt[c]	nt
A31	KV-131	R.A.Samson	-	Thailand	+	--[d]	--
A32	KV-132	R.A.Samson	-	Thailand	+	--	--
A. aleyrodis Webber							
Aa1	ARSEF-430	R.S. Soper	Aleyrodidae	Fla, USA	+	--	--
Aa2	ARSEF-992	N. Oho	*D. citri*	Japan	+	--	--
Aa3	ARSEF-2154	M.C. Rombach	Aleyrodidae	Java	+	--	--
Aa4	ARSEF-2268	W.Gams	Aleyrodidae	Colombia	+	--	--
Aa5	KV-107	W.Gams	Aleyrodidae	Colombia	+	nt	nt
Aa6	KV-108	W.Gams	Aleyrodidae	Colombia	+	nt	nt
A. goldiana Saccardo & Ellis							
Ag1	ARSEF-431	R.S.Soper	Aleyrodidae	FLA, USA	+	nt	nt
A. insperata (ined.)							
Ai1	ARSEF-2356	M.C.Rombach	*D.citri*	Philipines	+	?[e]	--
Ai2	ARSEF-2351	M.C.Rombach	*D.citri*	Java	+	?	--
A. placenta Berkeley & Broome							
Ap1	-	S. Selvakumaran	*D. cardamomi*	India	+	--	--
Ap2	CBS-917-79	-	-	S.E. Asia	+	--	--
A. turbinata Berkeley							
At1	ARSEF-1030	R.A. Hall	Homoptera	Colombia	--	nt	nt
Verticillium lecanii							
	KV-01 (Mycotal)	Koppert	Aleyrodidae	United Kingdom	+	?/+[f]	?/+

[a] both *Bemisia argentifolii* and *Trialeurodes vaporariorum* were examined (after Meekes et al., 2002);[b]:symptoms of infection present; [c]:not tested; [d]:no symptoms observed; [e]:symptoms uncertain; [f]:growth of mycelium over cuticula.

4. Infection of greenhouse whitefly, *Trialeurodes vaporariorum*, and silverleaf whitefly, *Bemisia argentifolii*, by different formulations of *Aschersonia*.

4.1 Material and methods

Plants

Experment 1

Cuttings of Poinsettia, *Euphorbia pulcherrima*, cultivar white princess (unrooted), were ordered in October 1999. Pests on these cuttings were controlled by means of integrated control with no insecticides before being delivered at the experimental site. The cuttings were kept in the greenhouse for about 10 weeks to obtain full grown plants before use in the experiment. The plants had not been topped to obtain side shoots, but they had one main stem. One-stem poinsettia plants are also a commercial product and have the advantage of good handling during spraying and during observations. For experimental purposes the plants were kept in the vegetative state by using daylength prolongaton to 12 hours per day by means of artificial lights from the beginning of October 1999 till end of the evaluation in January 2000. The plants were kept on two ebb and flow tables in a 122 m^2 compartment and the temperature set-point was kept at 22 ($\pm$ 0.3) °C and humidity ranged from 57 to 60% RH by assistance of artificial humidification (Compartment L127; Appendix 1).

Experiment 2

Cuttings of Poinsettia, *Euphorbia pulcherrima*, cultivar white princess (rooted), were ordered in June 2000. Pests on these cuttings were controlled by means of integrated control with no insecticides before being delivered at the experimental site. The cuttings were kept in the greenhouse for two weeks to obtain enough full grown leaves before use in the experiment. The plants had not been topped to obtain side shoots, but they had one main stem as in experiment 1. Daylength prolongation was not necessary to keep the plants in a vegetative state as the experiment was carried out in mid-summer. When light intensity was below 100 Watt additional lights were automatically put on during the day. The plants were kept on two ebb and flow tables in a 122 m^2 compartment and the temperature set-point was kept at 22 ($\pm$ 0.3)°C and relative humidity ranged from 60 to 80% RH by assistance of artificial humidification (Compartment L125, Appendix 2).

Gerbera plants, cultivar Karaoke, were obtained from Terra Nigra, in August 2000. Pests on these plants were controlled by means of biological control. Plants were continuously producing flowers during het experimental period. The plants were kept on two ebb and flow tables in a 122 m^2 compartment and the temperature set-point was kept at 22 ($\pm$ 0.3)°C and relative humidity ranged from 55% to 80% RH by additional supply of artificial humidification (Compartment L125, Appendix 3).

after which period the adults were removed and eggs were given time to develop into first instar stage nymphs. The application of the *Aschersonia* formulations took place two weeks later (23/24

Insects

Experiment 1

The adults from the original population of silverleaf whitefly, *Bemisia argentifolii (Bemisia tabaci* Biotype B*)*, used for the experiment, were reared for many generations on Poinsettia, *Euphorbia pulcherrima*, at the location Aalsmeer. Greenhouse whitefly, *Trialeurodes vaporariorum*, was reared on Gerbera plants (*Gerbera jamesonii* hybrids).

Only the two youngest full-grown leaves of each poinsettia plant were used for infestation of whitefly. Approximately 50 whitefly adults (males and females) were put in small clip cages (2 cm diameter) on the underside of poinsettia leaves for 48 hours (08 to10 December 1999) at 25°C, after which period the adults were removed and eggs were given time to develop into first instar

stage nymphs. The application of the Aschersonia formulations took place two weeks later (23/24 December 1999). The mean number of whitefly per leaf are shown in Table 4.1.

Table 4.1. Mean number of whitefly per leaf at first and second count in experiment 1 and 2.

Whitefly sp.	Mean # whitefly per leaf 1st count	Min-max range 1st count	Mean # whitefly per leaf 2nd count	Min-max range 2nd count
Experiment 1				
Greenhouse whitefly (Poins)	104	23- 203	105	25-197
Silverleaf whitefly (Poins)	180	50-308	182	50-314
Experiment 2				
Greenhouse whitefly (Poins)	111	45-235	116	41-234
Silverleaf whitefly (Poins)	289	62-513	286	79-506
Greenhouse whitefly (Gerbera)	308	54-754	336	49-644
Silverleaf whitefly (Gerbera)	132	28-339	118	30-257

Experiment 2

The whitefly originated from the same mass rearings as mentioned in Experiment 1. Again only the two youngest full-grown leaves of each poinsettia plant were used for infestation of whitefly. Approximately 50 whitefly adults (males and females) were put in small clip cages (2 cm diameter) on the underside of poinsettia leaves for 48 hours (27 to 29 July 2000) at 22°C, after which period the adults were removed and eggs were given time to develop into first instar stage nymphs. The application of the *Aschersonia* formulations took place two weeks after egg laying (09 August 2000). During the experimental period healthy nymphs developed into pupae and later on emerged as adults from the pupal skin which was left on the leaf. As whitefly nymphs are sedentary after a short period as crawler during the first instar stage, the numbers counted at the end of the experiment agree with the numbers of nymphs present at the time of application. Also infected nymphs and pupae as well as those which died by natural causes can be observed on the leaf. The mean number of whitefly (nymphs, pupae, pupal skins) per leaf are shown in Table 4.1. At the end of the experiment, the number of whitefly that had emerged, exceeded 65% of the total present. At that time an increase in infection wil not occur anymore (Table 4.2).

For Gerbera also the youngest full-grown leaves were used and again 50 whitefly males and females per clip cage were used. One clip cage per leaf and two leaves per plant were used. Adult whiteflies were put on the leaves for 48 hours (13 to 15 September 2000) at 22 °C, after which period the adults were removed and eggs were given time to develop into first instar stage nymphs. The application of the *Aschersonia* formulations took place two weeks later (27 September 2000). The mean number of whitefly per leaf is shown in Table 4.1.

Table 4.2. Division in developmental stages in the control treatment without fungus *Aschersonia aleyrodis* at the time of counting

Whitefly sp.	% nymphs alive	%pupae alive	% adults (=empty pupal skins)
Experiment 1			
First count			
Greenhouse whitefly	51	39	0
Silverleaf whitefly	50	30	13
Second count			
Greenhouse whitefly	0	6	67
Silverleaf whitefly	4	3	88
Experiment 2			
First count			
Greenhouse whitefly (Poins)	61	36	0
Greenhouse whitefly (Gerb)	54	42	4
Silverleaf whitefly (Poins)	87	13	0
Silverleaf whitefly (Gerb)	64	34	0
Second count			
Greenhouse whitefly (Poins)	9	9	65
Greenhouse whitefly (Gerb)	0	11	88
Silverleaf whitefly (Poins)	9	13	76
Silverleaf whitefly (Gerb)	0	17	80

Fungal isolate

Aschersonia aleyrodis isolate Aa4 from Colombia was used for experiment 1 and 2. Sporulating colonies on Sabouraud Dextrose Agar (SDA, Difco) or Potato Dextrose Agar (PDA, Difco), originating from spores of the isolate stored on Microbank ™ porous beads at -80 °C, were used to make a spore suspension to inoculate millet cultures (10 g millet with 25 ml demineralised water in 300 ml Erlenmeyer flasks, autoclaved twice before use). The flasks were closed with sterile cotton to provide aeration. Cultures were incubated at 25°C and L16:D8 during three weeks and spores were harvested thereafter by rinsing with sterile demineralised water containing 0.03% Tween 80 (Merck). For the bioassays a concentration was determined by using a haematocytometer. Suspensions contained 1 x 10^7 spores/ml for experiment 1 and 2.4 x 10^7 spores/ml for experiment 2.

Experimental procedure

Different combinations of additives were send to our laboratory from Prof. E. Cohen of the Hebrew University in Jerusalem, Israel. One batch was send in 1999 and this was used for experiment 1 and another batch was send in 2000 and this was used for experiment 2. Both batches with different clay and clay-photostabilizer samples were kept at the refridgerator at 5 °C and were packed in aluminium foil to prevent exposure to artificial or day light. The additives were added to the *Aschersonia* spore suspensions in different concentrations as shown in Table 4.3 and Table 4.4 for experiment 1 and 2, respectively. As control treatments the original spore

suspension was applied as well as demineralised water plus Tween 0.03% without the spores. Application of the different formulations took place by means of a Potter Spray Tower (Burkard Manufacturing, Manchester, UK). Before each application the suspensions were stirred on a 'press-to-mix'mixer (Retsch). Due to clogging of the nozzle there were problems in application of the higher doses of formulations (1.0 and 2.5 % w/v). In experiment 2 the doses were lowered to 0.05 and 0.1% w/v (Table 4.3 and 4.4). The application in experiment 1 took place at 23 and 24 December 1999. The application in experiment 2 took place on 9 August 2000 for poinsettia and on 27 September 2000 for Gerbera.

To determine germination capacity of the *Aschersonia* conidia in the different formulations one ml of conidial suspension was sprayed onto water agar plates (15 g/l agar-agar, Merck) using a Potter Spray Tower (Burkard Manufacturing UK). After incubation for 24 hours at 25°C in artificial light (L16: D 8) percentage spore germination was determined by observing 150-500 spores per plate (n= 2 per formulation and concentration) in experiment 1 and 300 spores per plate (n= 2 per formulation and concentration) in experiment 2. Germination was rated when germ tubes exceeded the width of the conidium.

In the bioassays two ml of conidial suspension, prepared as described above, was sprayed by means of the Potter spray tower onto the abaxial surface of the leaf bearing the whitefly nymphs. For each treatment two plants with two leaves bearing whitefly nymphs were used (n=4). After evaporation of the water, plants were covered with plastic bags for 48 hours to create a condition of 95-100% relative humidity (RH). Plants were kept on tables with an ebb and flow irrigation system and a temperature of 22°C and fluctuating RH (See Appendix 1, 2 and 3). Nymphal and pupal mortality was assessed in a first count two and three weeks after application for greenhouse whitefly and silverleaf whitefly in experiment 1, respectively. The second assessment took place four and five weeks after application for greenhouse whitefly and silverleaf whitefly, respectively. The counts of silverleaf whitefly were carried out later than those of greenhouse whitefly as silverleaf whitefly takes longer to develop than greenhouse whitefly under temperatures lower than 25°C and low light intensity in winter months. In the control treatment without fungal conidia the percentage emergence amounted to 67 and 88% for greenhouse whitefly and silverleaf whitefly, respectively (Table 4.2). In experiment 2 nymphal mortality was assessed two and four weeks after application for both whitefly species and host plants. Adult emergence amounted to 65% for greenhouse whitefly and 76% for silverleaf whitefly at the final count on poinsettia. Adult emergence on Gerbera was 88% for greenhouse whitefly and 80% for silverleaf whitefly at the final count (Table 4.2).
Mortality was divided into mortality caused by the fungus and mortality by other causes. A nymph was considered infected when it became 'cloudy' and later turned orange. A nymph was considered dead by other causes, when the insect was desiccated and no apparent cause of death was visible.

Overall mortality and infection were both used in statistical analysis of variance after logit transformance of data using a binomial distribution.

Table 4.3. Overview of treatments in experiment 1.

Treatment no.	Clay additve	Photostabilizer
1 [a, b, c]	Bentonite	FGFCF
2 [a]	Bentonite	Control
3 [a, b]	Montmorillonite	FGFCF
4 [a, b]	Montmorillonite	Control
5 [a]	Attapulgite	FG
6 [a]	Attapulgite	Control
7 [a]	Montmorillonite	NYS
8 [a]	Montmorillonite	Control
9	Water + 0.03%Tween 80	Control+ Aschersonia
10	Water + 0.03%Tween 80	Control– Aschersonia

[a]0.5% w/v: 0.1 g/20 ml:;[b]1.0% w/v: 0.2 g/20 ml ; [c] 2.5% w/v: 0.5 g/20ml

Table 4.4. Overview of treaments in experiment 2.

Treatment no.[a]	Clay additive	Photostabilizer
1	Kaoline	NYS[b]
2	Kaoline	FG[c]
3	Kaoline	Control
4	Montmorillonite	NYS
5	Montmorillonite	Control
6	Attapulgite	NYS
7	Attapulgite	Control
8	Bentonite	NYS
9	Bentonite	FG
10	Bentonite	Control
11	Water + 0.03% Tween 80	Control +Aschersonia
12	Water + 0.03% Tween 80	Control - Aschersonia

[a]: all treatments in conc: 0.05 and 0.1 % w/v; [b] :Naphthol Yellow S, [c] :Fast Green;

4.2 Results

Germination tests

In experiment 1 the germination of the conidia of *Aschersonia aleyrodis* in the different formulations and concentrations was not different from the germination of the conidia in demineralised water and 0.03% Tween 80 alone (Table 4.5). Treatment 2 with bentonite as clay additive showed some lower germination in one replicate but as bentonite with photostabilizer showed good germination in treatment 1, this may be considered an artefact.
On the water agar plates in several treatments lumps were observed and in general it was difficult to keep the clay particles well suspended before application despite the mixing shortly before. The heavy clay particles tended to sink to the bottom of the vials.
In experiment 2 again spore germination ranged from 94 to 100% and no differences were found in germination between treatments (Table 4.6). Also increase of the concentration of the formulations from 0.05 to 0.1% did not result in any negative influence on germination. In treatment 1 and 10 clusters of spores were observed on the water agar.

Overall the addition of clay products and photostabilizers did not negatively influence the germination of *Aschersonia aleyrodis* conidia. The formulations require improvement considering the inadequate suspending of the additives and the riscs of blockage of spraying nozzles.

Table 4.5. Germination of *Aschersonia aleyrodis* spores in different formulations on water agar at 25°C 24 hrs after application by the Potter Spray Tower (Experiment 1).

Treatment	Concentration	Germinated	Ungerminated	Total	Percentage	Remarks
1	0.5	179	10	189	94.7	Lumps
1	0.5	128	6	134	95.5	
1	1.0	280	14	294	95.2	Lumps
1	1.0	178	9	187	95.2	
1	2.5	239	15	254	94.1	Lumps
1	2.5	278	9	287	96.7	
2	0.5	364	66	430	84.7	Germtubes shorter
2	0.5	30	229	259	11.6	?
3	0.5	192	21	213	90.1	
3	0.5	184	16	200	92.0	
4	0.5	317	25	342	92.7	
4	0.5	279	11	290	96.2	
5	0.5	352	25	377	93.4	Small lumps
5	0.5	420	27	447	94.0	
6	0.5	247	15	262	94.3	Lumps
6	0.5	424	38	462	91.8	Lumps
7	0.5	206	23	229	90.0	Fewer spores
7	0.5	-	-	-	-	Blockage of spray system
8	0.5	546	42	588	92.9	Small lumps
8	0.5	234	15	249	94.0	
9Contr+A	No formulation	440	30	470	93.6	Good spread
9Contr+A	No formulation	493	22	515	95.7	Good spread
10Contr-A	No formulation	0	0	0	0	
10Contr-A	No formulation	0	0	0	0	

Table 4.6. Germination of *Aschersonia aleyrodis* spores in different formulations on water agar at 25°C 24 hrs after application by the Potter Spray Tower (Experiment 2)

Treatment	Concentration	Germinated	Ungerminated	Total	Percentage	Remarks
1	0.05	301	3	304	99.0	Clusters
1	0.05	302	3	305	99.0	Clusters
1	0.10	314	2	316	99.4	Clusters
1	0.10	306	5	311	98.4	Clusters
2	0.05	299	3	302	99.0	
2	0.05	294	8	302	97.4	
2	0.10	331	3	334	99.1	
2	0.10	310	4	314	98.7	
3	0.05	303	1	304	99.7	
3	0.05	300	1	301	99.7	
3	0.10	300	4	304	98.7	
3	0.10	300	0	300	100.0	
4	0.05	293	7	300	97.7	
4	0.05	308	3	311	99.0	
4	0.10	306	9	315	97.1	
4	0.10	327	6	333	98.2	
5	0.05	294	8	302	97.4	
5	0.05	296	7	303	97.7	
5	0.10	294	5	299	98.3	
5	0.10	311	9	320	97.2	
6	0.05	283	17	300	94.3	
6	0.05	296	4	300	98.7	
6	0.10	300	9	309	97.1	
6	0.10	302	4	306	98.7	
7	0.05	293	8	301	97.3	
7	0.05	304	2	306	99.3	
7	0.10	293	6	299	98.0	
7	0.10	312	3	315	99.0	
8	0.05	291	4	295	98.6	
8	0.05	297	7	304	97.7	
8	0.10	300	1	301	99.7	
8	0.10	297	11	308	96.4	
9	0.05	303	2	305	99.3	
9	0.05	300	0	300	100.0	
9	0.10	301	1	302	99.7	Residu
9	0.10	300	0	300	100.0	Residu
10	0.05	301	3	304	99.0	Residu
10	0.05	299	5	304	98.4	Residu
10	0.10	299	6	305	98.0	Clusters
10	0.10	291	12	303	96.0	Clusters

11Control +Aschersonia	No formulation	296	4	300	98.7	Good spread
11Control +Aschersonia	No formulation	299	1	300	99.7	Good spread
12Control -Aschersonia	No formulation	0	0	0	0	
12Control -Aschersonia	No formulation	0	0	0	0	

Bioassays

The percentages shown in figures 4.1-4.4 and 4.6-4.9 are based on calculated means of the percentages mortality and infection over the replicates. The percentages shown in the tables 4.7 and 4.9 are based on estimated means, retransformed from logits which were estimated in the statistical analysis of variance using logit transformation of the data. There are slight differences between these percentages in the tables and the figures.

Experiment 1

Mean overall mortality of greenhouse whitefly two weeks after application was lowest ranging from 8% on the untreated control plants to 9.7% on the plants of treatment 3, montmorillonite + FGFCF. In other treatments the mortality varied between 17.3% in treatment 4, montmorillonite, and 25.1% in treatment 5, attapulgite + FG. Infected nymphs showing symptoms were only observed in low amounts below 10% (Figure 4.1).
In the final assessment (second count), overall mortality of greenhouse whitefly had still not increased above 30% in the control treatment with *Aschersonia aleyrodis.* The natural mortality of greenhouse whitefly on poinsettia is known to be generally high (Meekes, 2002, Fransen, 1990b). The dosage of *Aschersonia* conidia applied in this experiment was expected to cause about 60 to 80% mortality (Meekes, 2002). Final infection percentages, however, were low in all treatments, but still highest in the *Aschersonia* control with 12.2 % (Figure 4.2).
Regarding the statistical analysis overall negative effects on mortality by *Aschersonia* infection by use of the clay formulations could not be observed, except for the combination of bentonite with FGFCF photostabilizer and montmorillonite with FGFCF photostabilizer (Table 4.7a and 4.7 b). These seem to result in lower infection rates than the clays on their own. A contradictory effect was found for montmorillonite alone as it was used in treatment 4 and 8, the result in treatment 4 lacking behind that in treatment 8. The estimated mortality rates in the treatments were, except the above mentioned treatments, not significantly different from the control treatments (Table 4.7a and 4.7b).

For silverleaf whitefly in the first count after application overall mortality varied regarding different treatments (Figure 4.3). Highest mortality of 23% was found in treatment 5, attapulgite + FG, whereas treatment 1, bentonite + FGFCF, and the control without *Aschersonia* resulted in the lowest overall mortality of 7.2% and 7.5 %, respectively. Infection levels also varied with 13.9% infection in treatment 5, attapulgite + FY, being highest and with 0.9% infection in treatment 1, bentonite + FGFCF, being lowest. Again as with greenhouse whitefly, the estimated mortality and infection rates of treaments 1 and 3, bentonite +FGFCF and montmorillonite+FGFCF respectively, were significantly lower than those in the other treatments with additives (Table 4.7c).
In the final assesment five weeks after application, overall mortality as well as infection was highest in treatment 5, attapulgite + FG, with 28.5% and 22.4%, respectively (Figure 4.4.) The natural mortality in the control treatment without fungal conidia stayed low. Infection levels increased until the final count and ranged from 11.2% to 22.4%, except for the levels in treatment 1, bentonite with FGFCF, and 3, montmorillonite with FGFCF, which amounted to only 2.7% and 5.3%, respectively. Statistical analysis of the data in the second count again showed the significantly lower mortality

and infection rates of these treatments. All mortality rates in treatments 1 to 9 were significantly higher than the one in the control without *Aschersonia* (Table 4.7d).

Comparing the results on greenhouse whitefly and silverleaf whitefly, natural mortality of greenhouse whitefly is considerably higher than that of silverleaf whitefly. Overall mortality of greenhouse whitefly was higher than that of silverleaf whitefly, due to this higher natural mortality. However, the amount of infection of larvae and pupae in silverleaf whitefly was somewhat higher than that in greenhouse whitefly. It was noticed that in treatment 5 and 6 with Attapulgite+ FG and Attapulgite, respectively, the highest amount of infected *Bemisia* pupae were found. With greenhouse whitefly the amount of infected pupae was very low (Tables 4.7a-4.7d).

Table 4.7a. Estimated means and significance (P<= 0.05, after logit transformation, binomial distribution) of percentage overall mortality, percentage infected larvea and percentage infected pupae for greenhouse whitefly, *Trialeurodes vaporariorum,* treated by different formulations on poinsettia in experiment 1 two weeks after application.

treatment		estimated % overall mortality*		estimated % larval infection*		estimated % pupal infection*	
1	bentFGFCF	13.98	ab	0.96	A	0.00	*a*
2	bent	19.64	cd	9.22	C	0.00	*a*
3	montFGFCF	9.20	a	0.00	A	0.00	*a*
4	mont	16.67	bc	3.43	B	0.00	*a*
5	attapFG	26.09	e	5.63	BC	0.26	*a*
6	attap	20.00	cd	4.05	B	0.00	*a*
7	montNYS	20.88	cde	6.40	BC	0.00	*a*
8	mont	23.72	de	5.98	BC	0.00	*a*
9	contr+A	18.31	bc	3.74	B	0.00	*a*
10	contr-A	8.99	a	0.00	A	0.00	*a*

*significant differences (P<=0.05) are indicated by different letters of the same type being either in lower or upper case or italics

Table 4.7 b. Estimated means and significance (P<= 0.05, after logit transformation, binomial distribution) of percentage overall mortality, percentage infected larvea and percentage infected pupae for greenhouse whitefly, *Trialeurodes vaporariorum,* treated by different formulations on poinsettia in experiment 1 four weeks after application.

treatment		estimated % overall mortality*		estimated % larval infection*		estimated % pupal infection*	
1	bentFGFCF	21.01	abc	1.45	A	0.00	*a*
2	bent	27.91	d	10.84	D	0.60	*a*
3	montFGFCF	20.14	abc	0.00	A	0.00	*a*
4	mont	17.11	a	4.16	B	0.00	*a*
5	attapFG	21.52	abc	5.12	BC	0.82	*a*
6	attap	25.45	cd	6.62	BC	0.00	*a*
7	montNYS	25.17	bcd	5.52	BC	0.00	*a*
8	mont	29.59	d	6.05	BC	0.86	*a*
9	contr+A	29.84	d	7.86	CD	4.03	*b*
10	contr-A	26.24	cd	0.00	A	0.00	*a*

*significant differences (P<=0.05) are indicated by different letters of the same type being either in lower or upper case or italics

Table 4.7 c. Estimated means and significance (P<= 0.05, after logit transformation, binomial distribution) of percentage overall mortality, percentage infected larvea and percentage infected pupae for silverleaf whitefly, *Bemisia argentifolii,* treated by different formulations on poinsettia in experiment 1 three weeks after application.

treatment		estimated % overall mortality*		estimated % larval infection*		estimated % pupal infection*	
1	bentFGFCF	6.08	a	0.79	A	0.68	*ab*
2	bent	17.21	fg	4.30	CD	4.30	*de*
3	montFGFCF	9.55	bc	2.12	B	0.66	*ab*
4	mont	10.36	cd	2.32	BC	1.79	*bc*
5	attapFG	22.61	g	8.88	E	4.72	*de*
6	attap	18.79	fg	5.91	D	6.06	*e*
7	montNYS	18.94	fg	11.99	E	1.44	*bc*
8	mont	15.14	ef	5.39	D	3.35	*cd*
9	contr+A	11.94	de	5.27	D	2.81	*cd*
10	contr-A	7.71	ab	0.00	A	0.00	*a*

*significant differences (P<=0.05) are indicated by different letters of the same type being either in lower or upper case or italics

Table 4.7 d. Estimated means and significance (P<= 0.05, after logit transformation, binomial distribution) of percentage overall mortality, percentage infected larvea and percentage infected pupae for silverleaf whitefly, *Bemisia argentifolii,* treated by different formulations on poinsettia in experiment 1 five weeks after application.

treatment		estimated % overall mortality*		estimated % larval infection*		estimated % pupal infection*	
1	bentFGFCF	10.19	b	1.01	A	2.46	*c*
2	bent	26.07	fg	7.33	D	6.52	*ef*
3	montFGFCF	12.93	bc	3.17	B	0.79	*b*
4	mont	19.26	de	6.71	CD	4.24	*de*
5	attapFG	28.18	g	6.37	CD	15.58	*g*
6	attap	22.93	ef	7.84	D	7.39	*f*
7	montNYS	22.83	ef	11.42	E	2.54	*cd*
8	mont	21.89	ef	8.01	D	2.86	*cd*
9	contr+A	15.65	cd	4.56	BC	8.06	*f*
10	contr-A	5.11	a	0.30	A	0.00	*a*

*significant differences (P<=0.05) are indicated by different letters of the same type being either in lower or upper case or italics

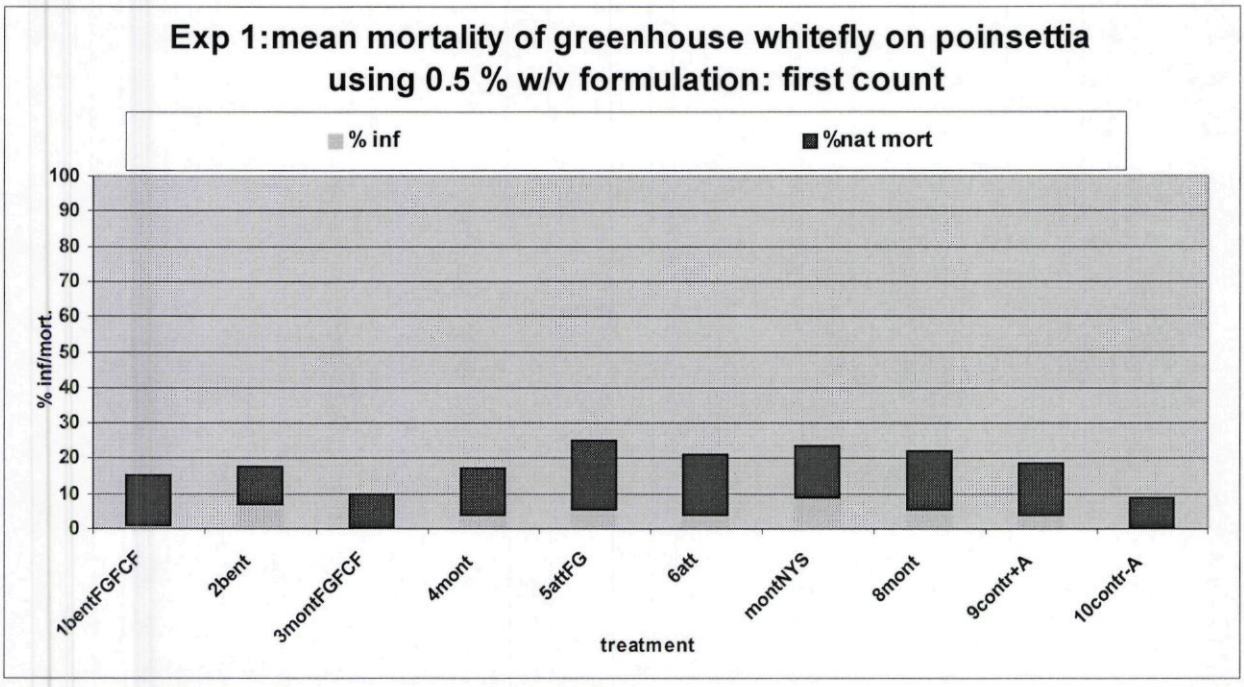

Figure 4.1 Overall mean mortality of greenhouse whitefly using 0.5% w/v additives and *Aschersonia aleyrodis* conidia on poinsettia two weeks after application (first count) in experiment 1.

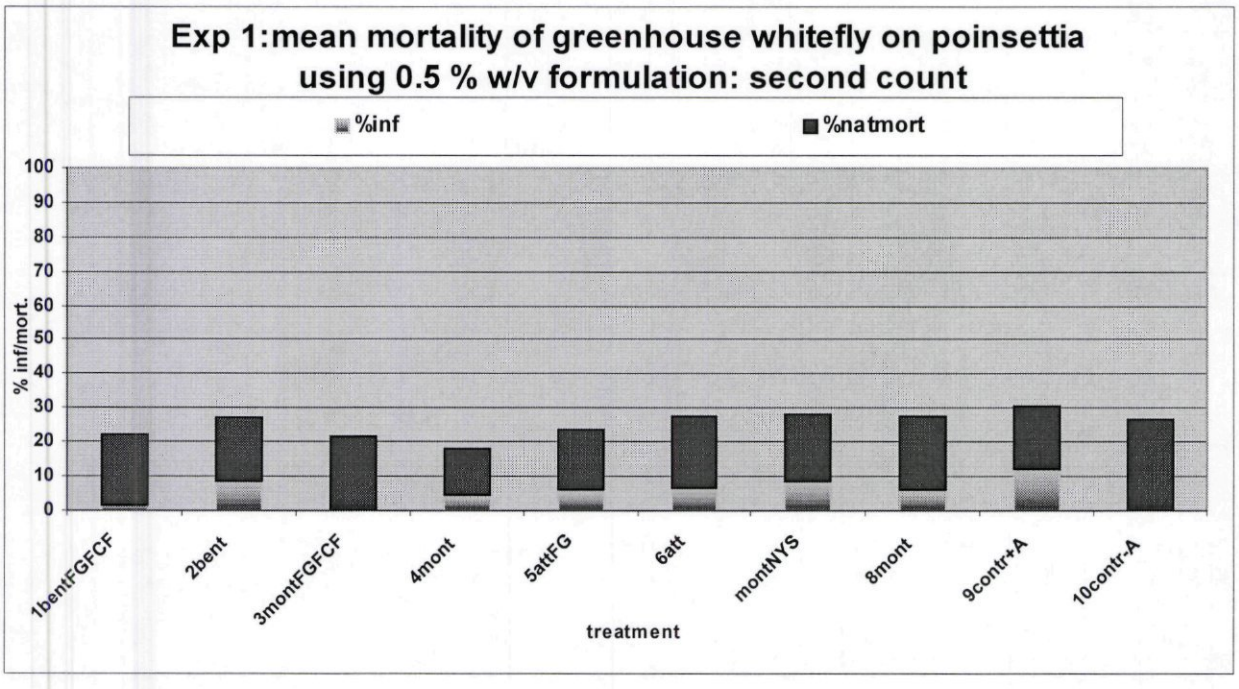

Figure 4.2 Overall mean mortality of greenhouse whitefly using 0.5% w/v additives and *Aschersonia aleyrodis* conidia on poinsettia four weeks after application (second count) in experiment 1.

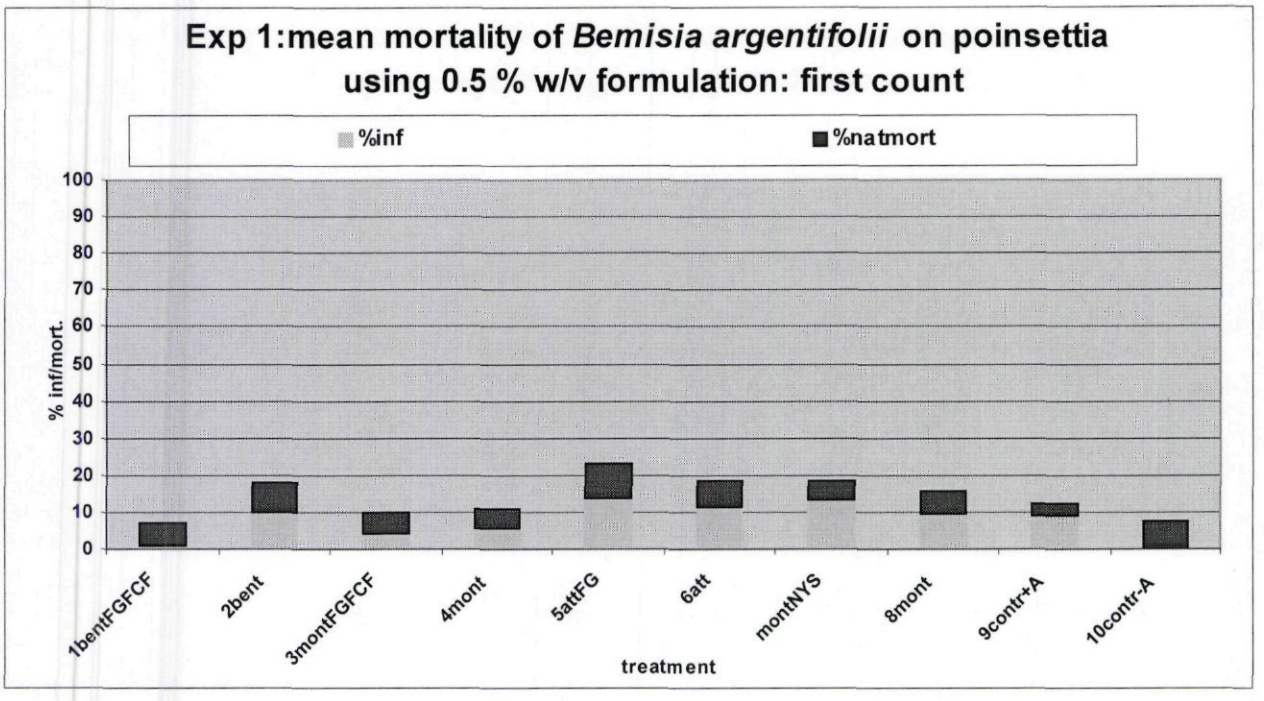

Figure 4.3 Overall mean mortality of silverleaf whitefly using 0.5% w/v additives and *Aschersonia aleyrodis* conidia on poinsettia three weeks after application (first count) in experiment 1.

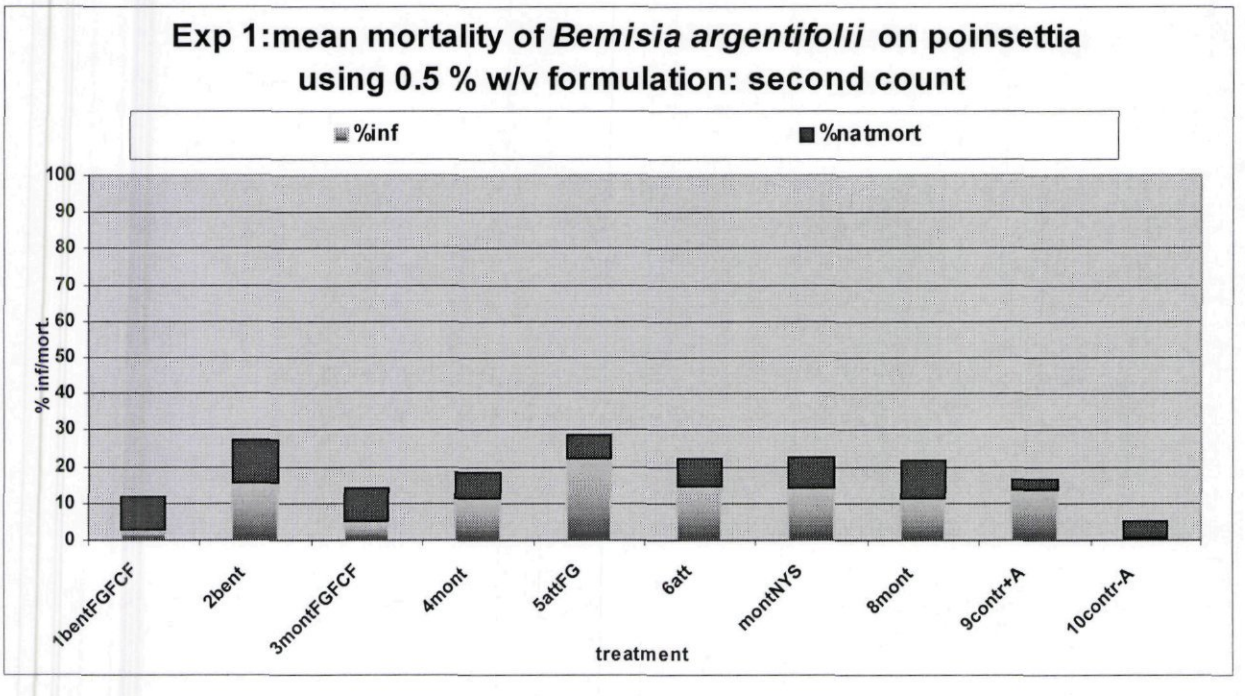

Figure 4.4 Overall mean mortality of silverleaf whitefly using 0.5% w/v additives and *Aschersonia aleyrodis* conidia on poinsettia five weeks after application (second count) in experiment 1.

For both whiteflies the results of treatments 1 and 3 indicate a negative effect of the photostabilizer FGFCF on infection.

At the start of the experiment formulations with clay additives were prepared to be applied in several concentrations, but due to clogging of the nozzle most of these applications were disgarded (Table 4.3.). Treatment 1 was carried out in three different concentrations of the clay+photostabilizer complex and the results show that overall mortality did not increase with concentration (Table 4.8.). However, the fungal infection level did decrease with increase of the concentration. Again with treatment 3 the effect of increase in the concentration was similar to that in treatment 1. These results confirm the negative effect this combination of clay + FGFCF has on the infection by *Aschersonia*.

In treatment 4 there is an increase in overall mortality by increasing the concentration from 0.5 to 1.0% w/v of the clay montmorillonite in the suspension, but the infection levels did not show the same trend (Table 4.8). The overall effect of increase of the concentration on the percentage mortality was significant for greenhouse whitefly but not for silverleaf whitefly.

Table 4.8 Mean percentage overall mortality and infection for greenhouse whitefly, *Trialeurodes vaporariorum*, and silverleaf whitefly, *Bemisia argentifolii*, related to different concentrations of additives in the suspensions containing *Aschersonia* conidia.

treatment	% overall mortality count 1		% infection count 1		% overall mortality count 2		% infection count 2	
	Tvap	Bem	Tvap	Bem	Tvap	Bem	Tvap	Bem
1[a] bentFGFCF	15.5	7.2	1.05	0.92	21.9	11.6	1.38	2.68
1[b]	15.1	11.2	0.57	0.86	20.3	8.9	0.13	3.04
1[c]	15.2	7.6	0.0	0.52	24.9	11.0	10.2	1.91
3[a] montFGFCF	9.7	9.7	0.0	4.11	21.5	14.2	0.0	5.33
3[b]	15.3	11.6	0.38	1.08	22.3	15.7	1.0	2.69
4[a] mont	17.3	11.1	3.86	5.80	18.3	18.5	4.5	11.42
4[b]	37.0	11.5	15.52	5.62	35.3	22.2	17.79	9.90

[a] 0.5% w/v: 0.1 g/20 ml:;[b]1.0% w/v: 0.2 g/20 ml ; [c] 2.5% w/v: 0.5 g/20ml

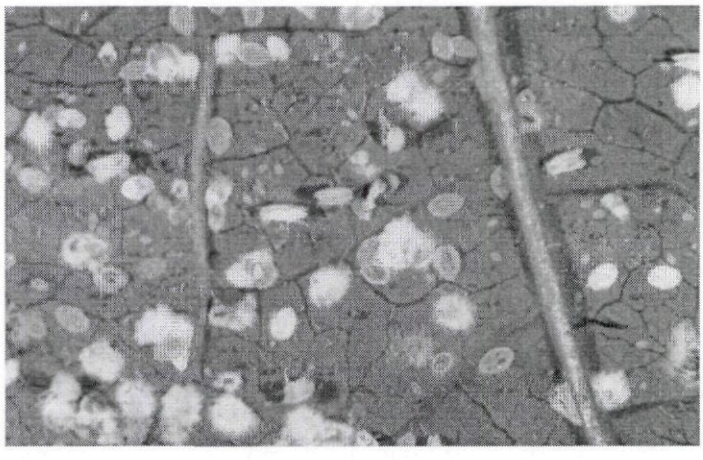

Figure 4.5 Silverleaf whitefly, being infected by *Aschersonia aleyrodis*, empty pupal skins ffrom which adults emerged on a Poinsettia leaf in experiment 2.

Experiment 2
Trialeurodes vaporariorum

In the second experiment overall mortality of greenhouse whitefly, *Trialeurodes vaporariorum,* was already high on Poinsettia two weeks after applicaton ranging from over 90% for kaoline+NYS 0.05% to 51% for attapulgite 0.05% (Figure 4.6). Levels of mortality in the treatments with clays with and without photostabilizers treatments were more or less similar to the mortality in the control treatment with *Aschersonia* conidia only.Thus, any negative effect of formulations on the level of infection was not obvious. However, the treatment containing attapulgite + NYS 0.1% w/v and attapulgite 0.05% w/v showed deviating results as infection level stayed low.
An increase or decrease in overall mortality related to an increase in concentration of the formulation from 0.05%w/v to 0.1% w/v was not found except for Attapulgite + NYS and attapulgite alone, showing a negative effect and a positive effect, respectively, of an increase in concentration on the overall mortality.

At five weeks after application overall mortality had increased up to over 90% for most treatments except for the formulations of attapulgite+ NYS 0.1% w/v and attapulgite 0.05% w/v, which mortality was significantly lower than that in the other treatments (Figure 4.7, Table 4.9). Natural mortality in the formulation treatments was low, whereas the natural mortality of greenhouse whitefly in the control without conidia amounted to 16%. However, in the attapulgite +NYS 0.05%w/v a high natural mortality level of 22% was also noticed (Figure 4.7).
The overall concentration effect was significantly highest with 0.05% w/v treatments, being 92.3% mortality, compared to 90.3% mortality with 0.1% w/v treatments.

On Gerbera the overall mortality data of the first count were disgarded as some discrepancies occurred between the numbers in the first and second count. This is due to the difficulty in finding all young larvae, which are more transparent and more scattered on Gerbera leaves than on Poinsettia leaves. Also, Gerbera leaves are larger and bear more hairs that complicate the observations. At the final count most whiteflies have reached the pupal or adult stage. The pupal cases and pupae are easier to observe and those counts are being considered to be the most accurate.

Again infection levels were high and varied from over 90% in several treatments to 58 and 65% for the attapulgite +NYS 0.05% and 0.1% w/v, respectively, being the lowest (Figure 4.10). The mortality percentages showed a wider range on Gerbera than on Poinsettia and more treatments showed a significantly lower percentage mortality than the control on Gerbera (Table 4.9). Natural mortality of greenhouse whitefly was considerably lower on Gerbera than on Poinsettia. Also greenhouse whitefly lay much more eggs on Gerbera than on Poinsettia. Natural mortality in the clay treatments was neglectable and therefore not shown in Figure 4.10.
A significant overall concentration effect was not observed.

Bemisia argentifolii

Two weeks after treatment of silverleaf whitefly, *Bemisia argentifolii*, on Poinsettia, overall mortality ranged from 53% in treament 2, kaoline + FG 0.05% w/v, to 85% in treatment 3, kaoline 0.05% (Figure 4.8). Natural mortality in the control was very low.

In the final evaluation all infection levels were above 70% (Figure 4.9). Increasing the concentration of the additives from 0.05 to 0.1% w/v had a small but significant negative effect on the infection levels of silverleaf whitefly lowering the overall mortality from 86.4% to 84.6%.
The overall mortality of silverleaf whitefly showed a lower level and varied more than that of greenhouse whitefly (Table 4.9). This may be related to the higher natural mortality of greenhouse whitefly on Poinsettia. Again the treatments with attapulgite showed low infection rates, and the treatment with attapulgite + NYS showed relatively more natural mortality compared to the other treatments, which is in agreement with the results on greenhouse whitefly.

On Gerbera only the second count was considered for the same reasons as mentioned above for greenhouse whitefly. The overall mortality of silverleaf whitefly on Gerbera in the different

treatments showed similar trends as the mortality of greenhouse whitefly on Gerbera (Figure 4.11). Again the attapulgite +NYS and attapulgite showed some of the lowest mortality as for greenhouse whitefly on Gerbera. (Table 4.9).

Increasing the concentration of the additives from 0.05 to 0.1% w/v had a small but significant negative effect on the infection levels of silverleaf whitefly on Gerbera lowering the overall mortality from 82.9% to 80.6%.

Comparing results of greenhouse whitefly and silverleaf whitefly on Poinsettia and Gerbera, all kaoline treatments show good results, montmorillonite + NYS and bentonite + NYS seem to result in lower mortality rates than the control, especially on Gerbera, and attapulgite formulations give inconsistent results, showing agian on Gerbera the lowest mortality rates (Table 4.9)

Table 4.9. . Estimated means and significance (P<= 0.05, after logit transformation, binomial distribution) of percentage overall mortality for greenhouse whitefly, *Trialeurodes vaporariorum*,and silverleaf whitefly, *Bemisia argentidfolii,* treated by different formulations on poinsettia in experiment 2 four weeks after application.

treatment	% overall mortality Poinsettia*		% overall mortality Gerbera*	
	T.vaporariorum	*B.argentifolii*	*T.vaporariorum*	*B.argentifolii*
1 [a]kaolNYS	94.4 fgh	93.7 **i**	92.0 *m*	90.3 ***k***
1 [b]kaolNYS	98.2 j	96.3 **j**	74.9 *d*	90.4 ***k***
2 kaolFG	90.5 de	85.1 **def**	91.7 *lm*	95.2 ***l***
2 kaolFG	94.7 efgh	88.7 **gh**	82.9 *gh*	90.2 ***k***
3 kaol	94.6 fgh	96.4 **j**	92.0 *lm*	93.9 ***kl***
3 kaol	96.4 ghij	87.8 **fgh**	90.7 *lm*	81.2 ***hij***
4 montNYS	95.2 fghi	84.7 **de**	75.3 *d*	77.5 ***ghi***
4 montNYS	90.7 de	86.2 **efg**	88.2 *jk*	73.1 ***efg***
5 mont	92.8 efg	93.8 **i**	87.2 *jk*	91.8 ***k***
5 mont	94.3 efg	86.9 **efg**	84.5 *hi*	93.0 ***kl***
6 attNYS	91.8 def	89.9 **h**	53.3 *b*	63.8 ***cd***
6 attNYS	66.8 b	87.6 **efgh**	65.5 *c*	50.0 ***b***
7 att	75.1 c	73.5 **b**	80.2 *fg*	58.6 ***c***
7 att	90.9 de	70.0 **b**	75.6 *de*	72.6 ***efg***
8 bentNYS	92.6 efg	81.1 **c**	78.5 *ef*	76.7 ***fgh***
8 bentNYS	90.8 de	86.8 **efg**	84.9 *hi*	71.2 ***ef***
9 bentFG	97.0 hij	88.0 **fgh**	75.2 *d*	68.8 ***de***
9 bentFG	93.2 efg	72.4 **b**	64.4 *c*	91.7 ***k***
10 bent	98.0 ij	82.4 **cd**	86.7 *ij*	92.1 ***k***
10 bent	88.8 d	80.1 **c**	91.4 *lm*	82.0 ***ij***
11 contr+A	91.9 def	93.0 **i**	89.9 *kl*	83.3 ***j***
12 contr-A	16.2 a	2.5 **a**	1.1 *a*	2.6 ***a***

*significant differences (P<=0.05) are indicated by different letters of the same type being either in lower case, bold, italics or bold-italics. [a]: 0.05%w/v ; [b]: 0.1% w/v

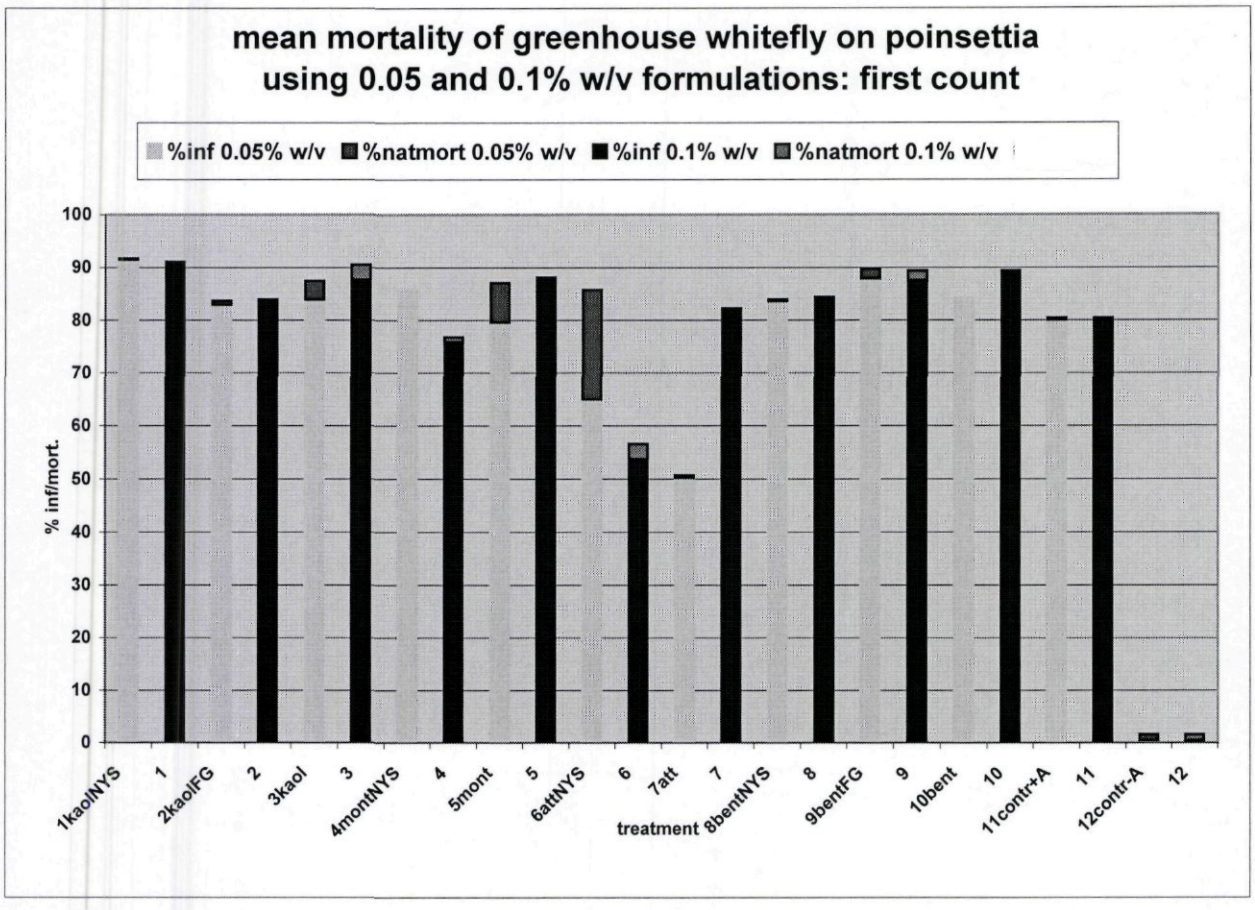

Figure 4.6 Overall mean mortality of greenhouse whitefly using 0.1% and 0.05% w/v additives and *Aschersonia aleyrodis* conidia on poinsettia two weeks after application (first count) in experiment 2.

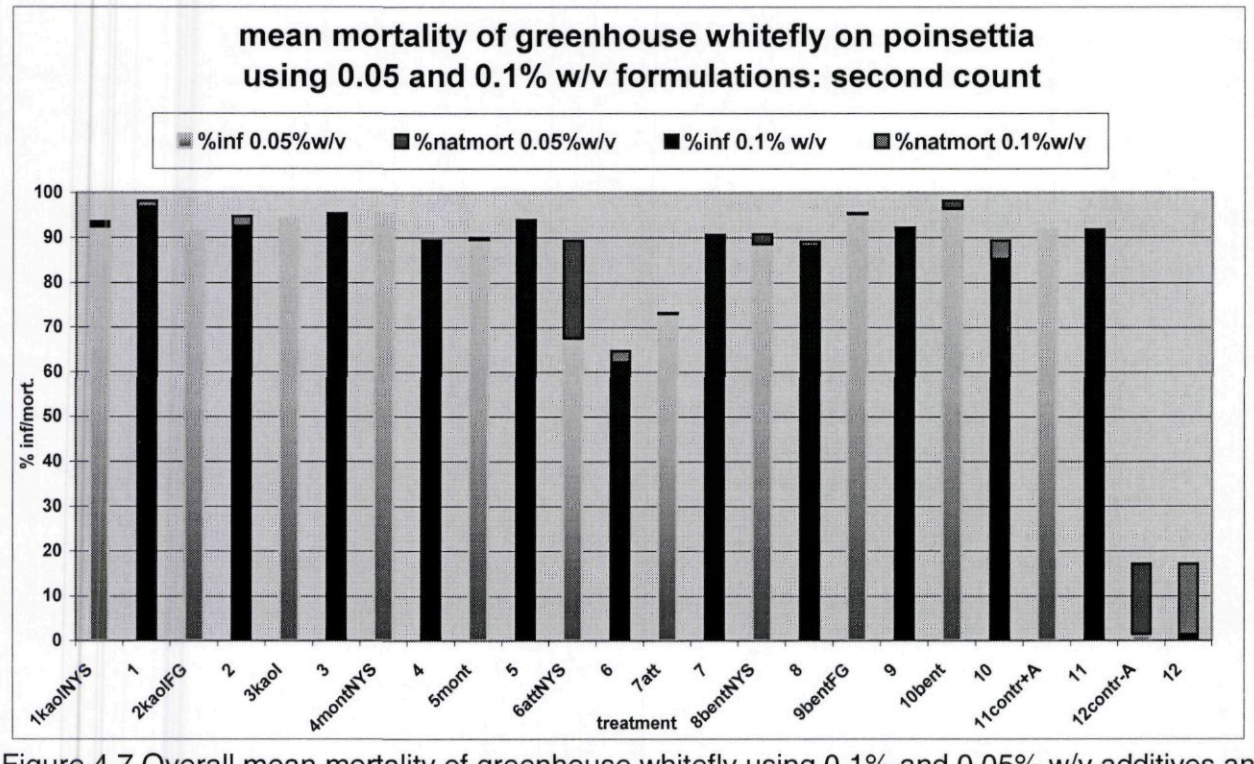

Figure 4.7 Overall mean mortality of greenhouse whitefly using 0.1% and 0.05% w/v additives and *Aschersonia aleyrodis* conidia on poinsettia five weeks after application (second count) in experiment 2.

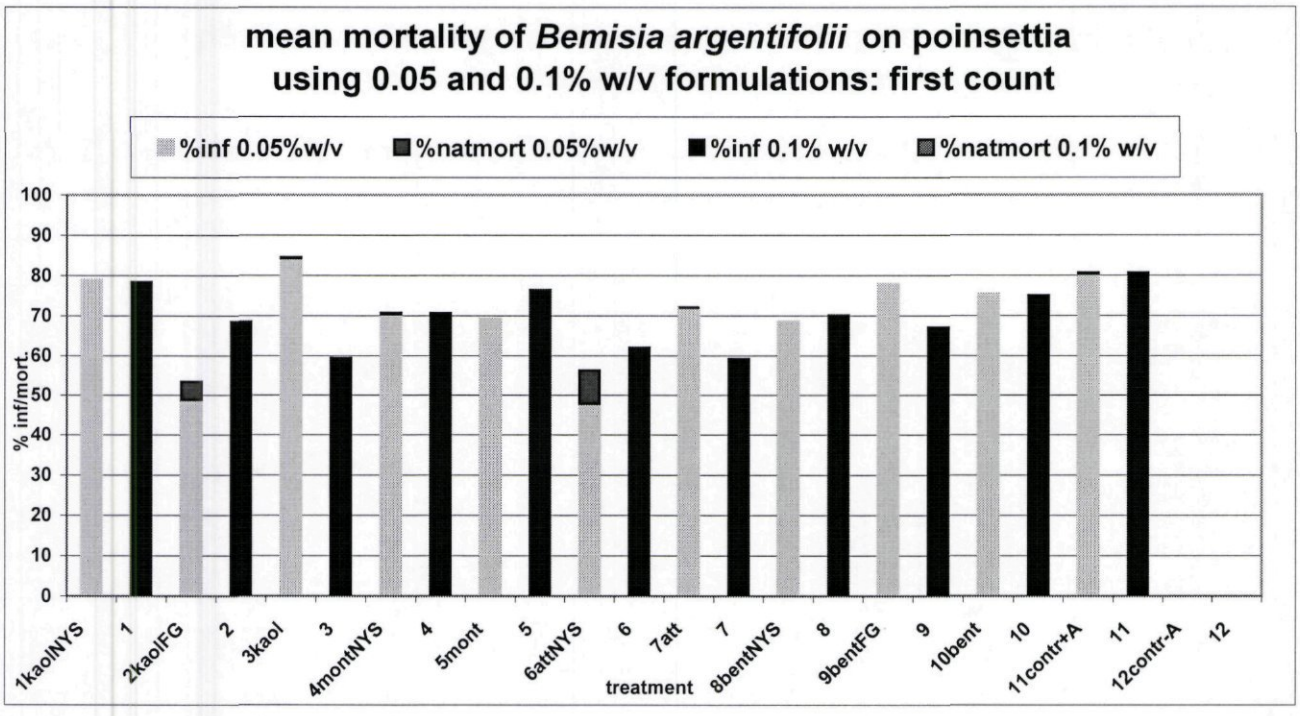

Figure 4.8 Overall mean mortality of silverleaf whitefly using 0.1% and 0.05% w/v additives and *Aschersonia aleyrodis* conidia on poinsettia two weeks after application (first count) in experiment 2.

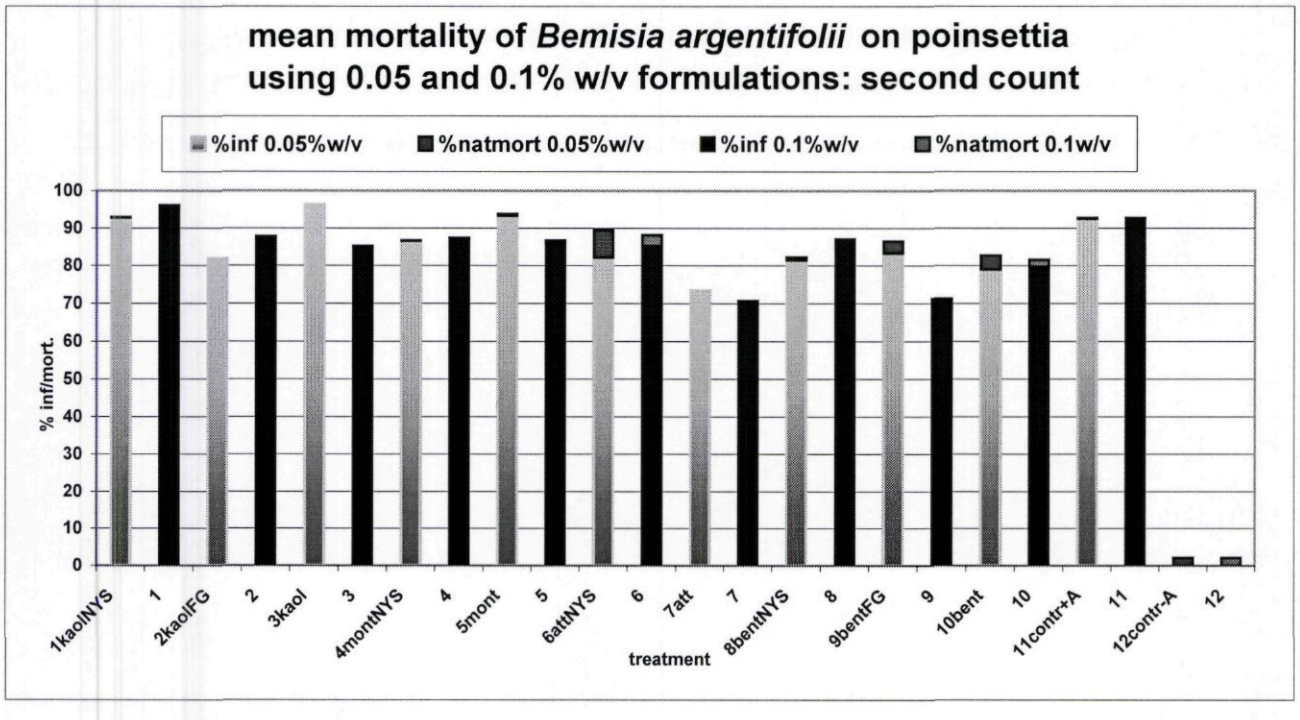

Figure 4.9 Overall mean mortality of silverleaf whitefly using 0.1% and 0.05% w/v additives and *Aschersonia aleyrodis* conidia on poinsettia five weeks after application (second count) in experiment 2.

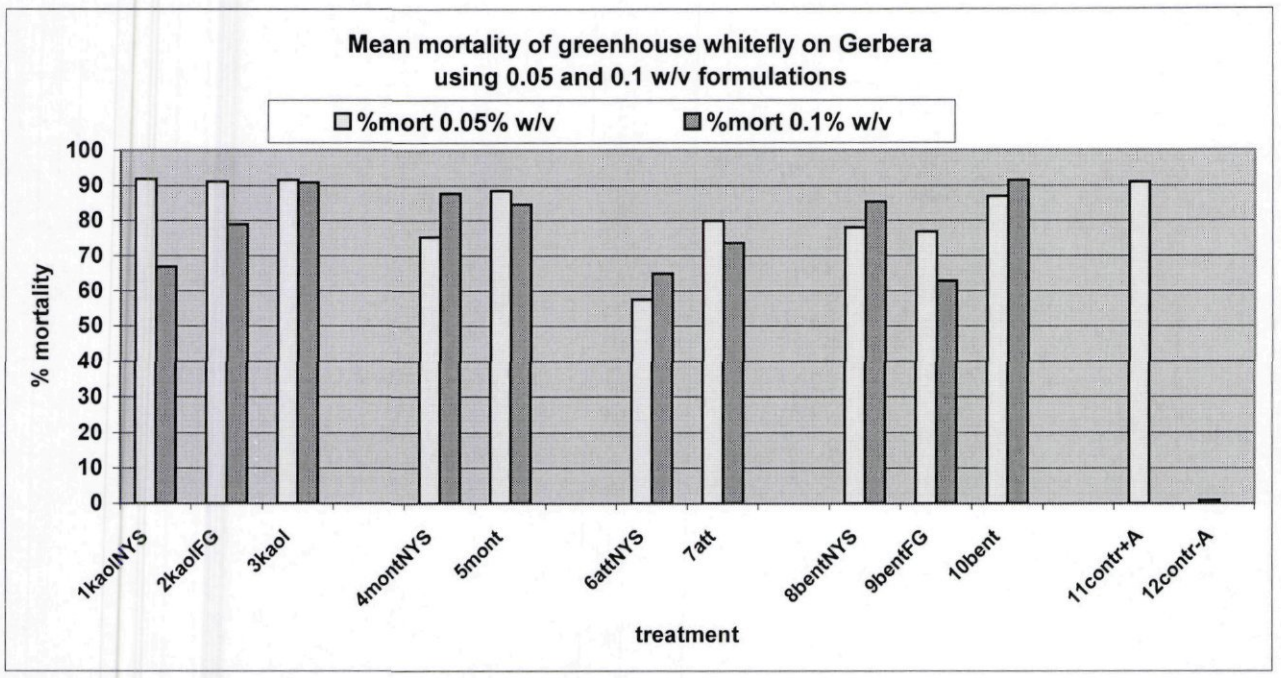

Figure 4.10 Overall mean mortality of greenhouse whitefly using 0.1% and 0.05% w/v additives and *Aschersonia aleyrodis* conidia on Gerbera five weeks after application (second count) in experiment 2.

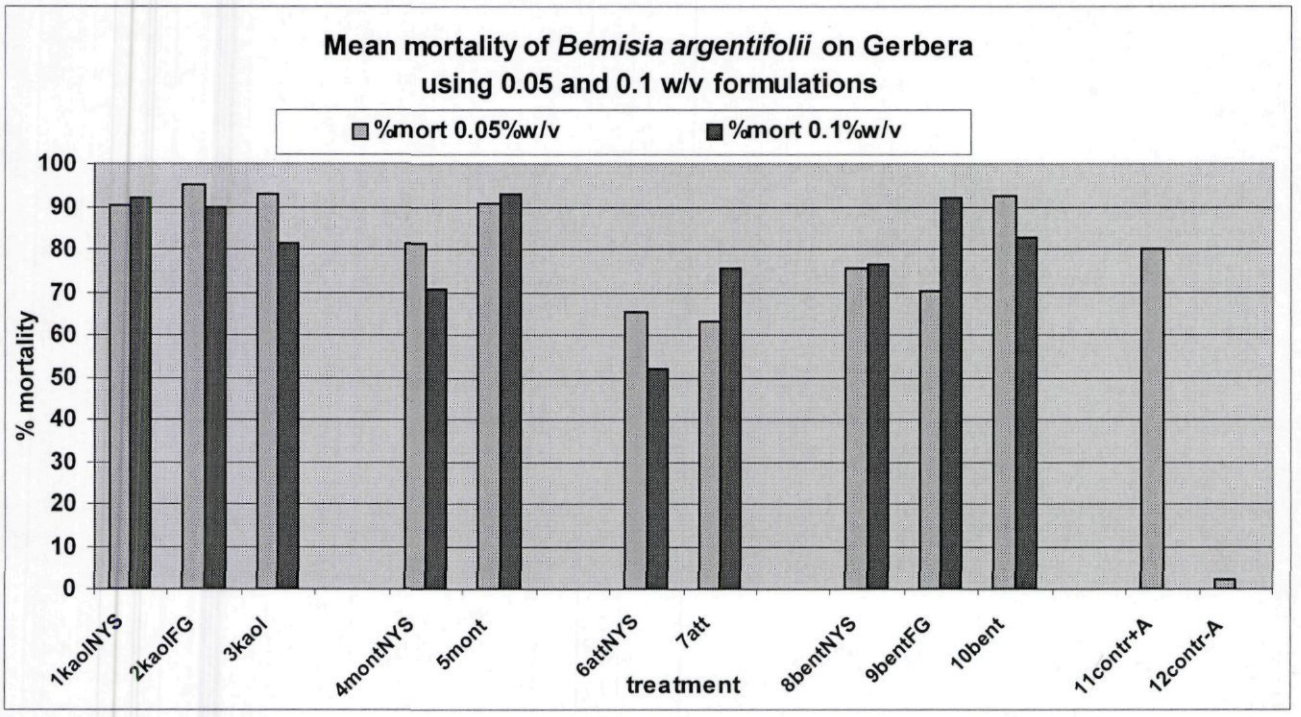

Figure 4.11 Overall mean mortality of silverleaf whitefly using 0.1% and 0.05% w/v additives and *Aschersonia aleyrodis* conidia on Gerbera five weeks after application (second count) in experiment 2.

4.3 Discussion

Addition of clays and photostabilizers

The formulations with clays and clays + photostabilizers did not influence the germination of conidia of *Aschersonia aleyrodis* in both experiments.

The mortality of whitefly in the formulation treatments used in both experiments could show levels higher, similar or lower than the mortality oin the control treatment with *Aschersonia* conidia only. In experiment 1 some negative effects were found when using the photostabilizer FGFCF in combination with the clays bentonite and montmorillonite (Fig. 4.1 to 4.4). The clay attapulgite alone and in combination with the photostabilizers FG or NYS showed variable results indicating in some instances a negative effect on whitefly mortality levels. Uniform overall positive effects on whitefly mortality levels due to clays and clay + photostabilizer combinations compared to the control, have not been observed for a particular treatment. However, on the whole the treatments with kaoline, bentonite and montmorillonite alone and in combination with photostabilizers NYS and FG showed levels of mortality comparable to those in the control (Fig. 4.1 to 4.4 and 4.6 to 4.11).

Aschersonia aleyrodis produces conidia in mucus and natural spread takes place by means of rain and by splash dispersal (Fransen, 1990a), in contrast to conidia of for instance *Metharhizium anisopliae*, which are dispersed by air. Clay formulations are being used for powders and dusts, such as a kaolin-based powder formulation of *Metarhizium anisopliae* for application against stink bugs in soy-bean fields (Sosa-Gómez and Moscardi, 1998). Formulation of *A. aleyrodis* is related to spraying with water or oil as a carrier referring to the way conidia are spread in a natural habitat and also referring to their host insect, the whiteflies being present at the underside of leaves. In that case a spray formulation with stickers and spreaders will give better results than dusts.

When clays are added to a spray formulation, good suspension of those heavy particles can only be obtained when adding other specific additives.

Host plant effects

There are differences in suitability of the host plants for greenhouse and silverleaf whitefly. On Poinsettia, silverleaf whitefly lays more eggs than greenhouse whitefly when about 50 whitefly per cage have been given the opportunity to lay eggs: the average number of silverleaf whitefly per leaf was finally 182 and 289 in experiment 1 and 2, respectively, whereas the number of greenhouse whitefly was 105 and 116, respectively (Table 4.1). The differences between the numbers in experiment 1 and 2, especially for *B. argentifolii*, can be related to seasonal influences as it was observed in previous experiments that *Bemisia argentifolii* is also influenced by light intensity with regard to development and egg laying (Fransen, unpubl. res.).

In addition, the natural mortality of greenhouse whitefly with 26.8% was much higher than that of silverleaf whitefly with 5.1% in experiment 1 (Figure 4.2 and 4.4) and in experiment 2 the natural mortality of greenhouse whitefly is still higher with 17.2% than the natural mortality of silverleaf whitefly with 2.1% (Figure 4.7 and 4.9). This difference in suitability in terms of less preference for egg laying and lower survival rate did not contribute to differences in infection levels by *Aschersonia aleyrodis* for *B. argentifolli* and *T. vaporariorum* and did not interfere with the different formulation treatments. Regarding developmental time this is longer for *Bemisia argentifolii* on Poinsettia than for *Trialeurodes vaporariorum* at temperatures below 25°C (Fransen, 1990b, Fransen, 1994, Fransen, unpubl.res.). At temperatures of 25°C and over *T. vaporariorum* takes longer to develop than *B. argentifolii*. For the experimental temperature of 22°C *B. argentifolii* is taking somewhat longer to develop and would be more exposed to infection by the fungus than *T. vaporariorum*. Also this phenomenon did not result in higher infection percentages of *B. argentifolii*, as was also found by Meekes et al. (2002)

For Gerbera it was found that *T. vaporariorum* showed higher preference for egg laying than *B. argentifolii* as an average of 336 eggs per leaf were laid when about 50 greenhouse whitefly per cage had been given the opportunity to lay eggs and an average of 118 eggs per leaf, laid when

about 50 silverleaf whitefly per cage had been given the opportunity to lay eggs (Table 4.1). Nevertheless, only a small difference in natural mortality of both species on Gerbera was found, average natural mortality in the control-*Aschersonia* treatment being 0.9% for *T. vaporariorum* and 2.2% for *B. argentifolii* (Figure 4.10 and 4.11). The developmental period of *T. vaporariorum* on Gerbera is again somewhat shorter at 22°C than that of *B. argentifolii* (Fransen, unpubl. res.), but this did not influence the levels of mortality and infection as those were comparable (Figure 4.10 and 4.11).

Influence of climate

Infection levels stayed low in experiment 1. It was expected that a level of about 60 to 80% infection could be obtained comparable to results of Meekes (2001) when 48 hours of high humidity by using plastic bags was maintained. She found this infection level at ambient relative humidity (RH) of 45% as well as at ambient RH of 85%. The results in experiment 1 were too low compared to this information. At the ambient rather constant RH of 55-60% (See Appendix 1) in experiment 1 the whitefly mortality did not exceed 30%, whereas at the ambient fluctuating RH of 60-80% (See Appendix 2) in experiment 2 this resulted in very successful infection rates. Supposedly not only RH but also activity of the plant such as photosynthesis and consequently, evaporation may influence the success of the infection process. A plant actively evaporating may create better humidity conditions for germination of conidia in the phyllosphere environment. During the days following application of the formulations and *Aschersonia* in experiment 1 radiation levels were very low varying from 20 to 200 W/m^2, outdoors, being winter gloomy wheather, and in the glasshouse radiation levels were varying from 100-200 W/m^2 due to additional lights. However, during the days following applications in experiment 2 radiation levels were high varying from 600-800 W/m^2, being in the middle of summer. Consequently during these winter days in experiment 1 the photosynthetic activity of the poinsettia plants would have been low. The ambient temperature was kept at 22°C in both cases.
A protective effect of a formulation in case of a negative influence of relative humidity on germination and infection of *Aschersonia aleyrodis* could not be identified.
Comparing RH regimes in the trials with Gerbera and poinsettia it is shown that about the same fluctuating RH of 60-80% was present (Appendix 2 and 3). Infection levels were comparable on both host plants.

Conclusions

- The addition of clays and clays + photostabilizers did not have a negative effect on germination of conidia of *Aschersonia aleyrodis.*
- The addition of clays and clays + photostabilizers in the suspension resulted in difficulties of obtaining an evenly distributed suspension
- The photostabilizer FGFCF in combination with clays montmorillonite and bentonite showed a negative influence on infection and mortality of whitefly when treated with conidia of *Aschersonia aleyrodis.*
- Addition of most clays and clays + photostabilizers in 0.05%w/v, 0.1%w/v and 0.5%w/v did not negatively influence the infection/mortality levels of both greenhouse whitefly, *Trialeurodes vaporariorum*, and silverleaf whitefly, *Bemisia argentifolii*, compared to the levels of infection/mortality in the control treatment with *Aschersonia* conidia only.
- When the clay attapulgite and attapulgite + photostabilizers FY and NYS were applied, inconsistent mortality rates were shown.
- The different whitefly species showed differences in preference of host plants Gerbera and poinsettia, but this did not result in differences between mortality levels except for greenhouse whitefly which showed higher natural mortality on poinsettia than silverleaf whitefly.
- Differences in climatic conditions (RH and radiation) in experiment 1 and 2 may have contributed to the differences in mortality rates of experiment 1 and 2.

5. Projectverslag (Dutch summary)

Titel: Biocontrol of whiteflies using stabilised *Aschersonia* formulations (DIARP 97/18)
Looptijd: 1998-2000
Projectleider: dr.ir.J.J.Fransen

Beoogde resultaten:
(Nederlandse partner)
-opzetten kweek van entomopathogene schimmels van het geslacht *Aschersonia*
-opleiden van Israelische medewerkster in omgaan met *Aschersonia* spp.
-uitvoeren van bioassays met door Israelische partner aangeleverde formuleringen
-bestudering van sporendistributie op bladoppervlakken.

Behaalde resultaten:
- Een veertigtal isolaten van entomopathogene schimmels van het geslacht *Aschersonia* zijn na drie jaar opslag (in olie op kraal bij –80°C) op schaal gezet. Het overgrote gedeelte der isolaten groeide weer uit en vertoonde sporulatie. Tevens trad goede kieming van de nieuw geproduceerde sporen op.
- De isolaten van *Aschersonia* spp. werden gescreend op infectie van dopluis en schildluis. Er werd geen duidelijke infectie van deze gastheren gevonden (uitvoering in combinatie met project 1673).
- Kweken van *Aschersonia* isolaten werden opgezet op gierst. Materiaal werd naar Israël verstuurd.
- Experimentele toevoegingen, ontvangen uit Israël, zijn in suspensie gebracht met *Aschersonia* sporen en getoetst in het laboratorium op sporekieming op wateragar. Negative effecten op sporekieming zijn niet gevonden.
- Er werden bioassays uitgevoerd op poinsettia en Gerbera met kaswittevlieg, *Trialeurodes vaporariorum*, en tabakswittevlieg, *Bemisia argentifolii* als toetsorganismen onder kasomstandigheden. De toegezonden formuleringen waren gebaseerd op fijne kleideeltjes met toevoegingen en bleken snel uit te zakken. Bij toediening in de Potter spuittoren trad bij hogere dichtheden van de toevoegingen verstopping op.
- De toevoegingen hadden over het algemeen geen sterk negatief noch positief effect op de infectie van wittevlieg. In enkele behandelingen met de toevoeging van FGFCF werden significant lagere infectieniveau's gevonden in vergelijking met de controle. De kleisoort attapulgite al of niet in combinatie met licht-stabiliserende kleurstoffen, gaf geen eenduidige resultaten.
- De toevoegingen gaven geen bescherming tegen effecten van lagere luchtvochtigheid.
- De mortaliteit en infectie van kaswittevlieg en Bemisia waren vergelijkbaar.
- Er traden geen verschillen op in niveau van infectie/mortaliteit tussen de waardplant poinsettia en Gerbera, behalve dat kaswittevlieg een hogere natuurlijke mortaliteit op poinsettia vertoont.
- Er is gewerkt aan de ontwikkeling van een laboratoriumtoets voor het vergelijken van verspreiding van sporen van entomopathogene schimmels op bladoppervlakken van verschillende waardplanten. Er is grote variatie aan bedekking binnen dezelfde behandeling. Duidelijke verschillen treden op in druppelvorming op verschillende waardplanten. De kleuring van sporen met diverse kleurstoffen bleek weinig succesvol voor standaardisatie. Fluorescerende stoffen zoals calcufluor en uvitex, geven het beste resultaat voor directe waarnemingen.

Activiteiten

- Bezoek van Tamar Joseph, assistente van Prof. Cohen, die werd opgeleid in het isoleren van entomopathogenen van insecten, het kweken van *Aschersonia* spp. en het uitvoeren van bioassays.
- Bezoek van Prof. Cohen aangaande afstemming project.
- Bezoek aan International Symposium Biological Control Agents in Crop and Animal Protection, 24-28 August, University of Wales, Swansea, U.K. waarbij Prof. Cohen aan diverse collega's in het veld van onderzoek werd geïntroduceerd.
- J.J.Fransen fungeerde als subcoordinator van een werkgroep 'Natural enemies of whiteflies' (coordinator Prof. D. Gerling, Israël) in de Concerted Action : "European Whiteflies Studies Network". In dit kader werd de eerste bijeenkomst bijgewoond van EWSN op het John Innes Research Centre, Norwich, UK van 3-7 mei, 1999.
- Wetenschappelijke artikelen zijn opgesteld betreffende EG en PT gefinancieerd onderzoek dat in voorgaande jaren op het PBG heeft plaatsgevonden en sterk gerelateerd is aan dit project evenals aan project 1663.
- De projectleider was copromotor van S.Sütterlin aangaande onderzoek aan wittevlieg op Gerbera uitgevoerd in samenwerking met Prof. J.C. van Lenteren WU en gefinancieerd door PT van 1989 -1994.

Afwijkingen van oorspronkelijke planning:

- De uitwisseling van materiaal tussen Israël en Nederland ging uiterst moeizaam. Het te testen isolaat van *Aschersonia* van Prof. Cohen is nooit op het PBG in Nederland ontvangen.
- De labtoetsontwikkeling voor verspreiding van sporen of formuleringen op bladoppervlakten is niet afgerond vanwege tegenvallende resultaten.
- De rapportage van het Nederlandse gedeelte is uitgesteld naar 2003 vanwege langdurige ziekte van de projectleider.
- Rapportage van Israelisch gedeelte van onderzoek is nooit door Nederlandse partner ontvangen.

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6. Summary

Forty isolates of entomopathogenic fungi of the genus *Aschersonia* were tested for viability after three years of storage (oil-based on beads at –80 °C). Most of the isolates showed growth on PDA and SDA and produced conidiospores. Also newly produced conidiospores germinated. Production of *Aschersonia aleyrodis* was set up and cultures were send to the Israeli partner.

The isolates of *Aschersonia* spp. were tested for infectivity of armoured orchid scale, *Diaspis boisduvalii*, on *Cymbidium* and brown soft scale, *Coccus hesperidum*, on *Ficus*. Clear symptoms related to infection by entomopathogenic fungi were not found in this experiment.

Experimental clay additives and photostabilizers were received from Israel in 1999 and 2000. These were tested in combination with spore suspensions of *Aschersonia aleyrodis* for germination on water agar. Negative effects of the additives on spore germination were not found.

Bioassays were carried out to test the experimental formulations on greenhouse whitefly, *Trialeurodes vaporariorum*, and silverleaf whitefly, *Bemisia argentifolii*, with Poinsettia, *Euphorbia pulcherrima*, and *Gerbera jamesonii* as host plants.The formulations were based on small clay particles which could not be kept suspended without stirring the suspension. At concentration levels of 1.0 and 2.5 % w/v the nozzle of the Potter Spray Tower was clogged in some treatments. Addition of most clays and clays + photostabilizers in 0.05%w/v, 0.1%w/v and 0.5%w/v did not negatively influence the infection/mortality levels of both greenhouse whitefly, *Trialeurodes vaporariorum*, and silverleaf whitefly, *Bemisia argentifolii*, compared to the levels of infection/mortality in the control treatment with *Aschersonia* conidia only. However, the photostabilizer FGFCF in combination with clays montmorillonite and bentonite showed a negative influence on infection and mortality of whitefly when treated with conidia of *Aschersonia aleyrodis*. When the clay attapulgite and attapulgite + photostabilizers FY and NYS were applied, inconsistent mortality rates were shown.
The different whitefly species showed differences in preference of host plants Gerbera and poinsettia, but this did not result in differences between mortality levels except for greenhouse whitefly which showed higher natural mortality on poinsettia than silverleaf whitefly.

7. Literature

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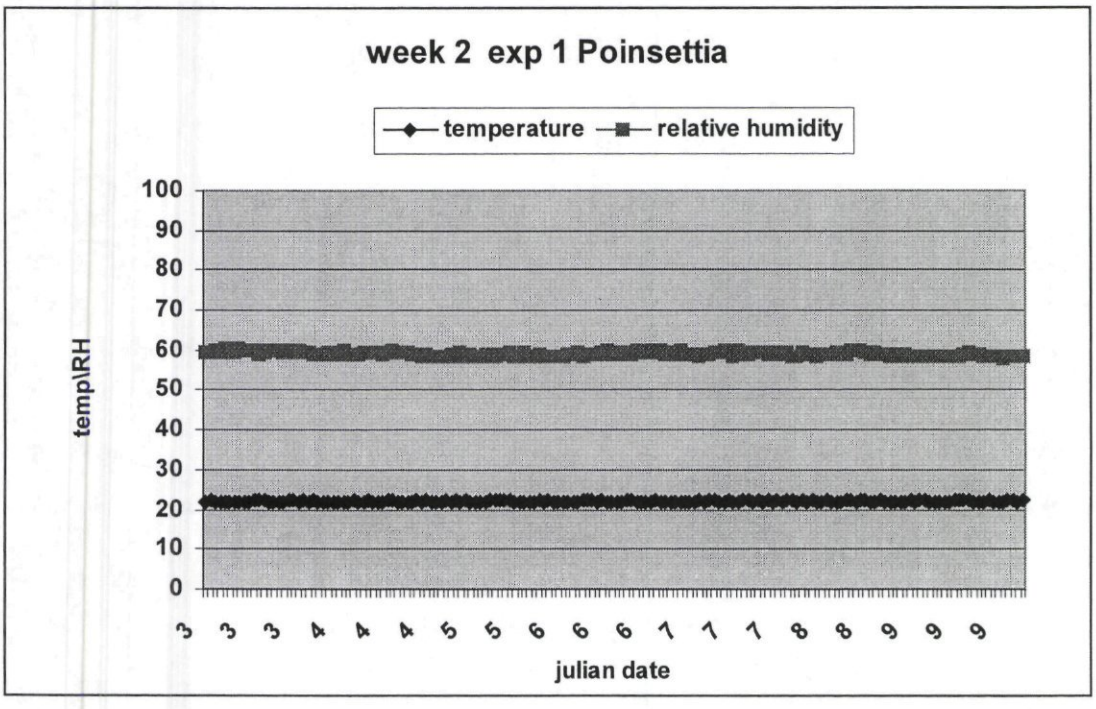
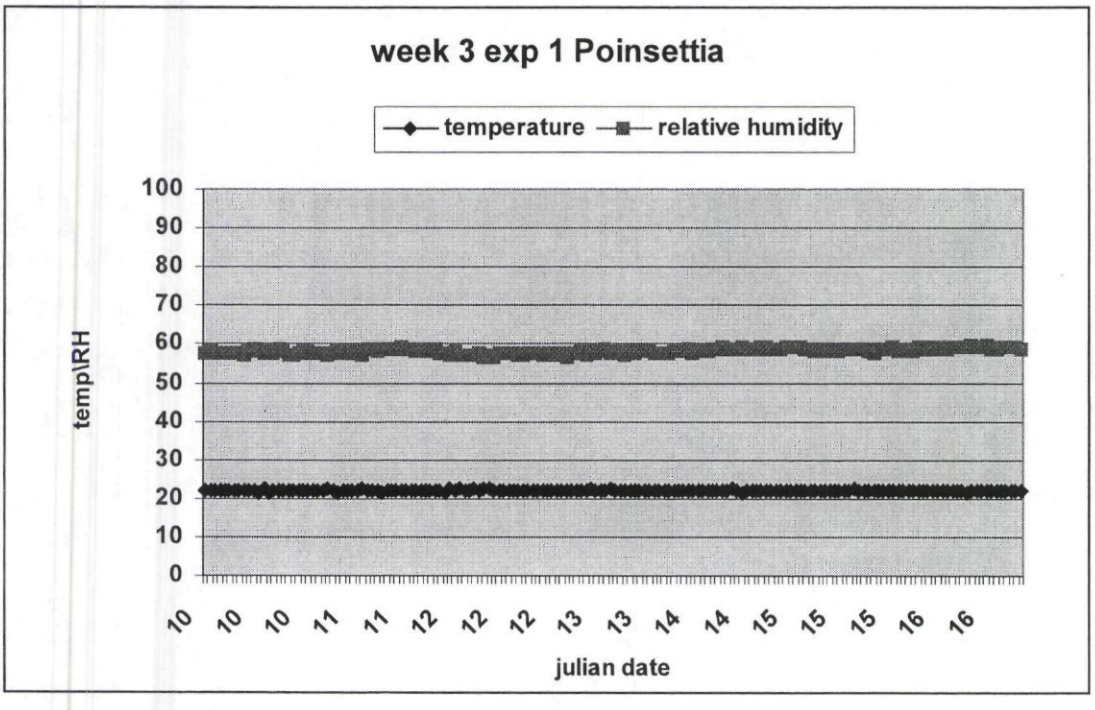
Appendix 1

Climate Data of Experiment 1 Poinsettia
Hourly means of 10 minute interval measurements.

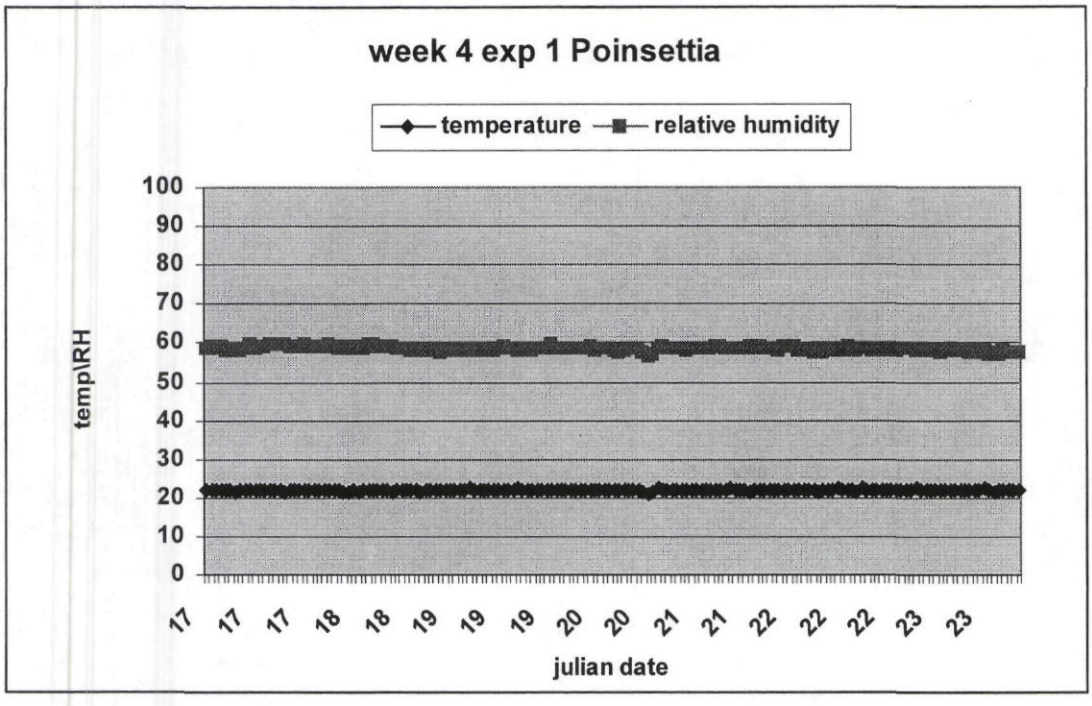
mean RH: 59% Range 58.3-60.2% mean temp: 22±0.3°C



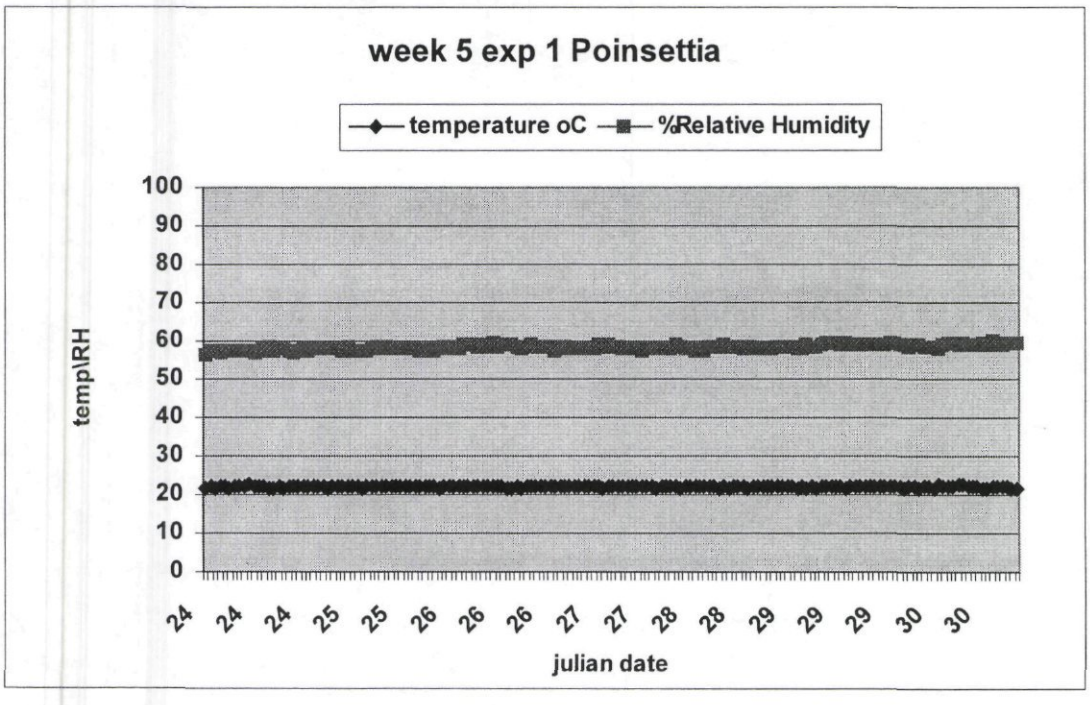
mean RH: 58% Range 56.5-59.2% mean temp: 22±0.4°C



mean RH: 58.6% Range 57.2-59.7% mean temp: 22±0.4°C



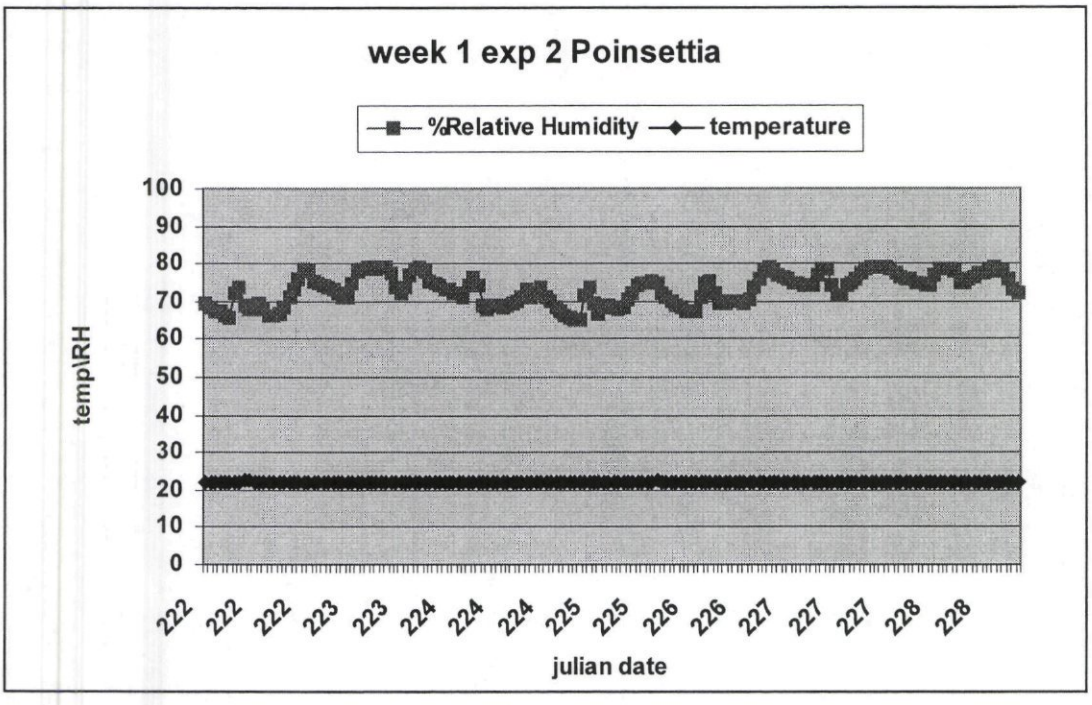
mean RH: 58.2% Range 57.0-60.0% mean temp: 22±0.4°C



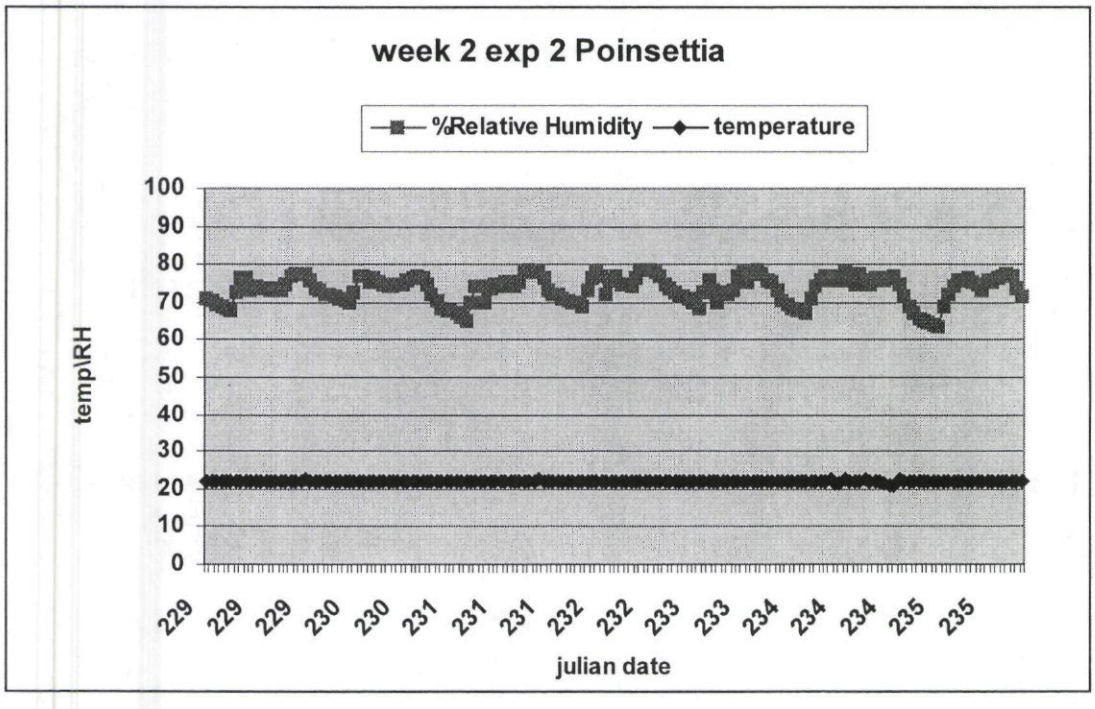
Appendix 2

Climate Data of experiment 2 Poinsettia

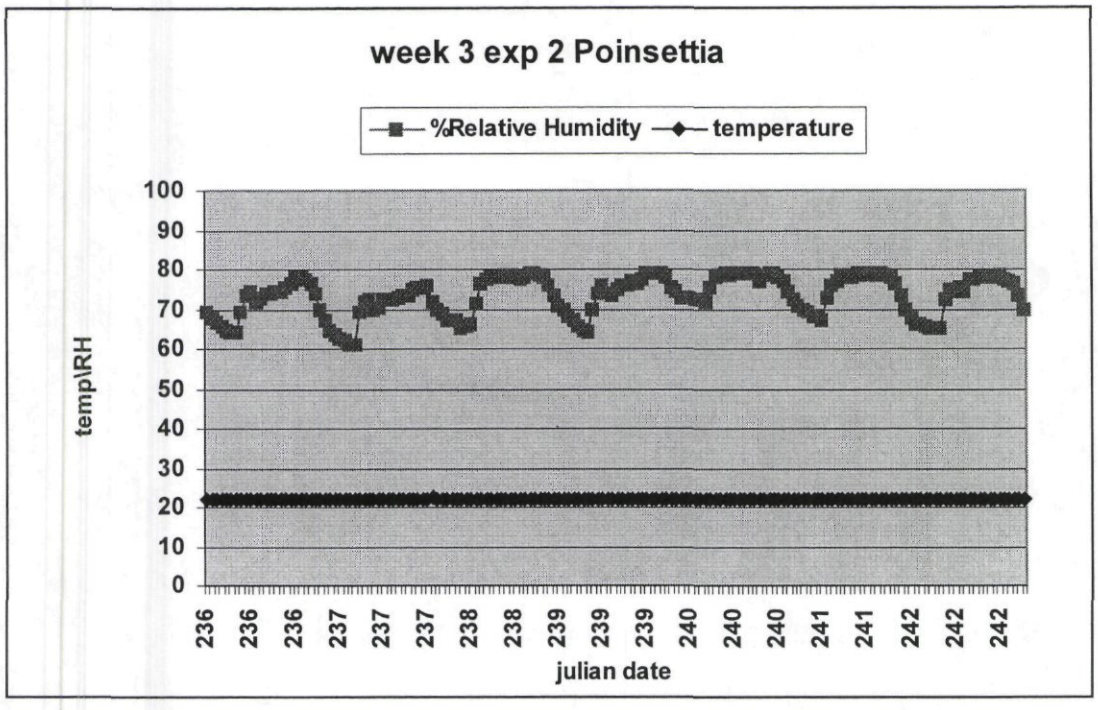
mean RH: 73.1% Range 66.0-79.0% mean temp: 22±0.3°C



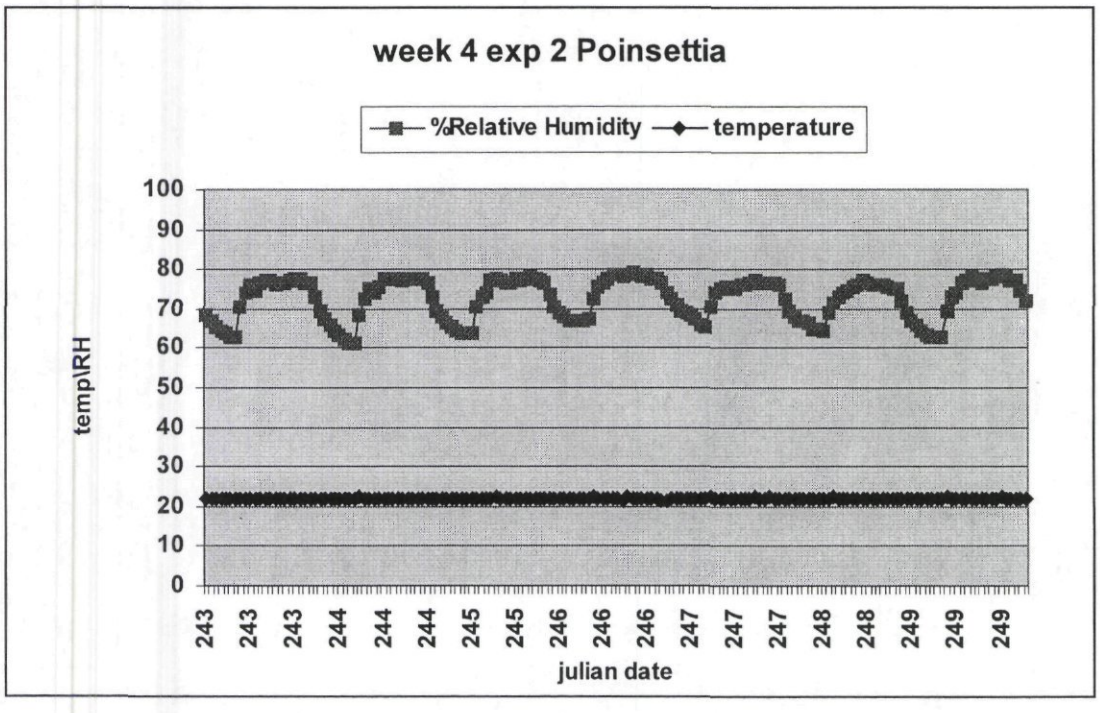
mean RH: 73.5% Range 63.5-78.7% mean temp: 22±0.3°C



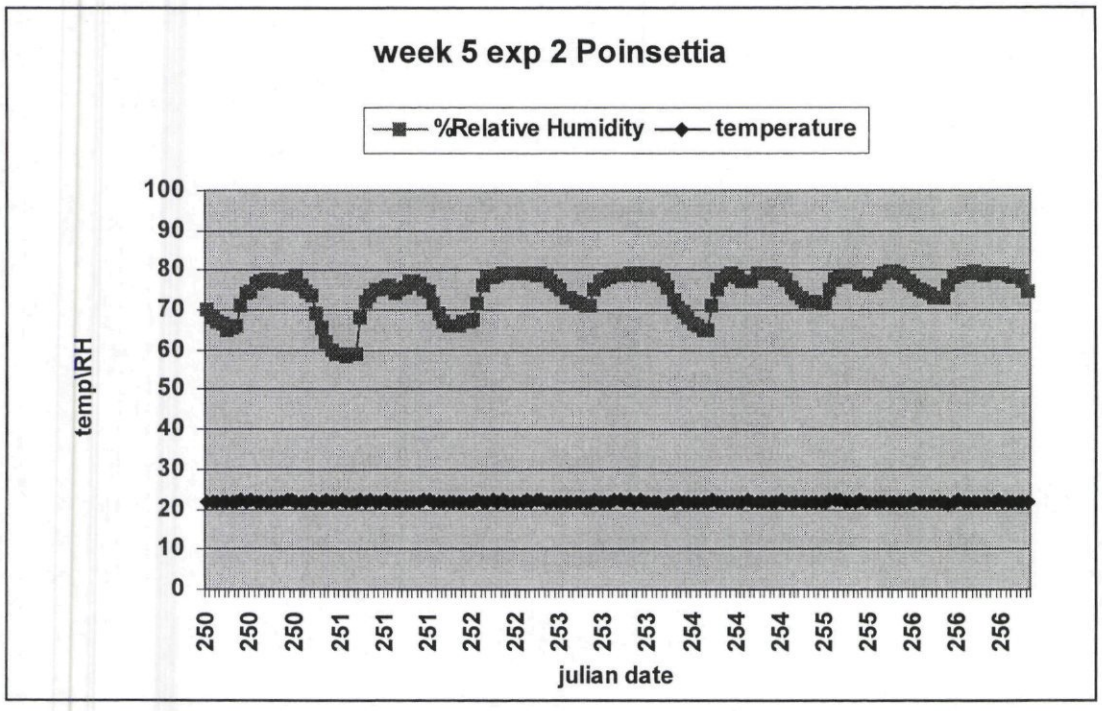
mean RH: 73.4% Range 61.0-79.0% mean temp: 22±0.3°C



mean RH: 72.6% Range 61.2-79.0% mean temp: 22±0.3°C



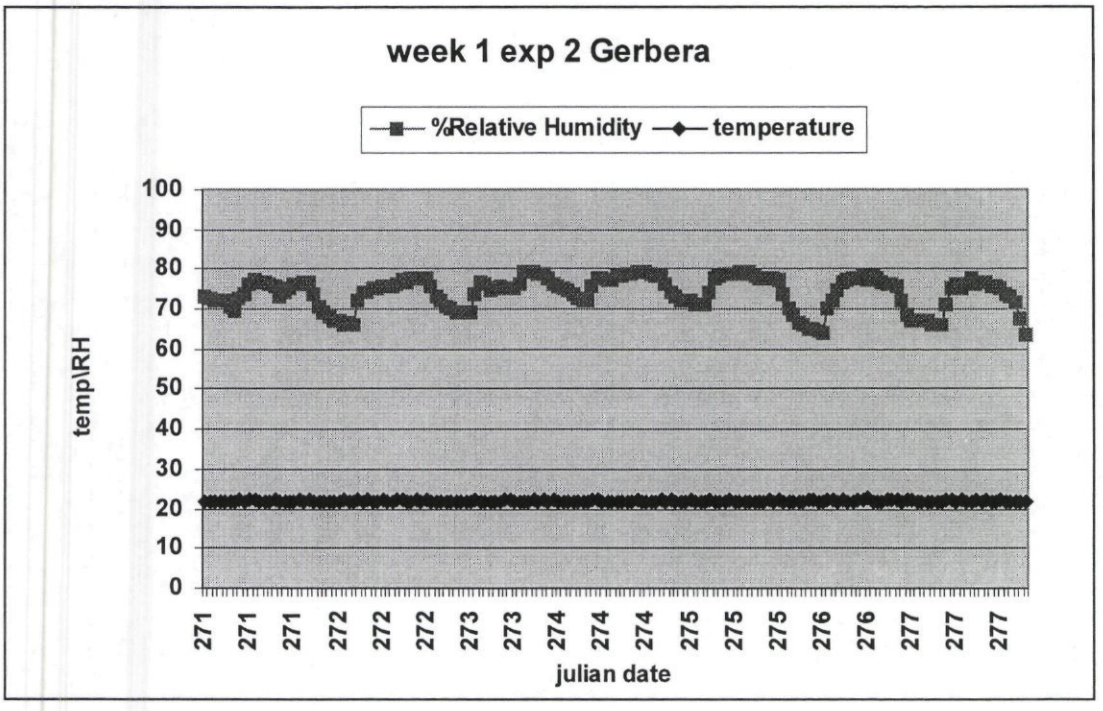
mean RH: 74.5% Range 58.5-79.7% mean temp: 22±0.3°C



Appendix 3

Climate Data of experiment 2 on Gerbera

mean RH: 74.1% Range 63.3-79.0% mean temp: 22±0.3°C



mean RH: 67.7% Range 55.0-76.7% mean temp: 22±0.3°C