Examensarbete

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Tritrophic interaction between whiteflies, insect pathogenic fungi and host plant

-Biology of Whitefly, *Bemisia afer*

Ethel Cavén

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HANDLEDARE: BARBARA EKBOM, INST. F. EKOLOGI

EXAMINATOR: RICHARD HOPKINS, INST. F. EKOLOGI

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SLU, Institutionen för ekologi
Box 7044, 750 07 Uppsala

Supervisor in Sweden:

Barbara Ekbom
PhD Professor at the Department of Ecology, SLU, PO Box 7044, 750 07 Uppsala, Sweden
Barbara.Ekbom@ekol.slu.se

Examiner:

Richard Hopkins
Docent at the Department of Ecology, SLU, PO Box 7044, 750 07 Uppsala, Sweden
Richard.Hopkins@ekol.slu.se

Supervisor in Peru:

Jürgen Kroschel
Prof. Dr. of Entomology/IPM at CIP Centro Internacional de la Papa, Lima, Peru
J.Kroschel@cgiar.org

Assistant in Peru:

Octavio Zegarra
Biologist, Research assistant at CIP, Centro Internacional de la Papa, Lima, Peru
O.Zegarra@cgiar.org

Author:

Ethel Cavén
Exam student in Biology at the Agronomy program at SLU, the Swedish University of Agricultural Science

ethelcaven@hotmail.com

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PREFACE

This work, more than being a MFS, had its purpose to be the final and masters thesis of my Agronomy programme, specialized on plant and soil science, includes 30 credits within D level in Biology at the Ecology Department at the Swedish University of Agriculture, SLU. The examination seminar was held on 19 October 2007. Supervisor of the thesis was Professor Barbara Ekbom in Sweden helping out with analysing and forming results of the thesis, supervising at CIP was Doctor Jürgen Kroschel and the assistant, who helped me out in all situations both with the trials and in other circumstances at CIP; Octavio Zegarra. The field study was conducted at the International Centre of Potato, CIP in Lima, Peru, one of the CGIAR (Consultative Group on International Agricultural Research) centre in the world. The thesis was written in English and published at the Ecology Department.

During my education I have been focused on tropical agriculture, organic farming, including biological control. I have done an exchange semester in Argentina at the Agronomy Department at University of Buenos Aires, where I among others studied horticulture and plant pathology including weeds and insects in the agro ecosystem. With this project; my final thesis I have been in contact with people who are working with biological control and I have acquired a complementary approach to biological control. I have complemented and increased my knowledge and combined it with my interest for Latin America.

This thesis has also enhanced my knowledge of CIGIAR institutes and their work as well as increased my knowledge in specific problems in agriculture in a developing country i.e. Peru. In this work I also had the opportunity to visit peasants in e.g. the Cañete Valley and Huancayo and I have gained some comprehension of their daily situation and living situation. This specific project has also given me a deeper knowledge about whiteflies, one of the most important pest species worldwide (Byrne and Bellows, 1992) and alternative management methods.

ABSTRACT

Key words: Aleyrodidae, *Bemisia afer*, tritrophic interactions, *Paecilomyces fumosoroseus, Lecanicillium* sp., sweetpotato, tomato, Peruvian pepper, bean, lifecycle, entomopathogens

The study was preformed at the International Potato Centre, CIP, in the capital of Peru, Lima on the west coast of South America and the Ecology Department, SLU, Uppsala, Sweden.

The main purpose of this thesis was to evaluate the tritrophic interaction and efficacy of entomopathogens on the whitefly on different host plants and different species of fungus. Included was a study on the life cycle of the whitefly specie *Bemisia afer* on four host plants.

Greenhouse trials were carried out at CIP and all conditions like temperature, as well as daylight and night were controlled and held at the same level throughout the whole study to obtain as similar a comparison as possible in between the trials.

Each trial was repeated three times and evaluated all together in the end. The leaves from the four host plants, which were used, were always the youngest but fully developed ones. To keep the whiteflies in good condition, they were held on sweetpotato plants and strictly kept under control from predators and other insects. The fungus was cultured during the trial to keep them young and healthy and free from other agents.

The results of the tritrophic interaction study showed no significant differences between different host plants neither between the different fungi nor strain of fungi and their effect when combating the whiteflies. The life cycle showed some differential behaviour between the host plants and results may show certain behaviour of the whitefly in the field. For example, there were differences in development time of *B. afer* on different host plants. Different characters of host plant causing a special microclimate on leaves might have had an influence on the development time and problems during the growth of the nymphs.

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1 INTRODUCTION

Whiteflies (Homoptera: Aleyrodidae) are widely spread around our world and are a problem in many agricultural, greenhouse and ornamental crops. There are many studies done on the most common species of whiteflies e.g. *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood), but fewer on the species *Bemisia afer* (Priesner & Hosny) at least in America, The New World.

The species *B. tabaci*, is a particularly serious pest of sweetpotato (*Ipomoea batatas* Lam.), transmitting viruses and other diseases. Lately, large populations of whiteflies have been reported to be significantly affecting sweetpotato yields in the coastal valley of Peru. In a closer look of what was infecting the sweet potatoes they found *B. afer* which will be studied in this thesis. This species of whiteflies is poorly studied and was first reported from Latin America in Cañete Valley in the central coast of Peru.

Integrated Pest Management (IPM) is the use of practical, economically efficient, and environmentally sound practices to control insect pests. One component of IPM is biological control, the use of natural enemies, pathogens, predators, and parasitoids to combat insect attacks. Biological control using entomopathogens is of great interest as an alternative to pesticides for resource poor farmers in developing countries.

The focus of the International Potato Centre (CIP) is on sustainable agricultural development with environmentally sound management of natural resources and the long-term goal is to contribute to poverty and hunger alleviation and to protect natural resources in developing countries. At CIP, production and utilization of root and tuber crops such as sweet potato and potato are in focus, as well as natural resource management. The research program at CIP includes different projects addressing the most pressing needs of developing countries and one of them deals with IPM. There are several examples of successful, CIP initiated, IPM programs where the use of insect pathogenic fungi has been a key control factor.

Before 1997 and the occurrence of the weather phenomenon El Niño the population densities of the whitefly species were modest and the damage caused moderate in Peru in Cañete Valley (Cisneros & Mujica, 1999). The situation changed dramatically due to the climatic impact and severe whitefly outbreaks occurred with devastating effect on sweet potato yields (Lagnaoui *et al*, 2001). Cañete Valley is situated 150 km south of Lima, and the 23 000 ha area is one of the powerhouses of Peruvian agriculture. On the broad plain, approximately 5000 small farmers grow sweet potato, cotton, tomatoes, cassava, potatoes, cucumber and other crops in an intensive, irrigated production system year round. There are two seasons with different climatic conditions. In summer (Dec. to Mar.) the average temperature is 24 C and in winter (Jun. to Aug.) only 17 C. Precipitation is low year round but there is a small peak in the winter months. The relative humidity is high year round (Åsman, K *et al*. 2007).

2 OBJECTIVES

The use of chemical pesticides, herbicides and fungicides are getting harder and more difficult to handle with increasing resistance from insects, weeds and fungi.
The larger costs for the farmers and sometimes poor distribution of chemicals when needed in field make it harder for the resource poor farmer to combat the growing problems with their crops. The aim with this study is to get a better knowledge of the whitefly species *B. afer* and thereafter be able to design a biological control program for management of whiteflies with insect pathogenic fungi in Peru. By identifying the pathogen species that provides the best result in a particular crop in combination with increased knowledge and understanding about population dynamics, the outcome of biological control with insect pathogenic fungi will be more reliable and thereby make it more attractive for farmers to use. The expectations were that in the long term this will lead to introduction of biological insecticides to resource poor farmers and the replacement of chemical insecticides it will not only contribute to increased yields and benefits but also to a reduced use of chemical insecticides among farmers in developing countries.

In a system where the pest insect has a broad host plant range and where there is variation in host plants both in time and space, it is especially important to have a good control and method of treatment of the pest. Basic knowledge of whitefly population dynamics in different crops is an essential component of an IPM program. The studied system in this case is whiteflies with a special focus on the species *Bemisia afer*, biological control agents - insect pathogenic fungi (i.e. strains of *Paecilomyces fumosoroseus* and *Verticillium lecanii*) and host plants; sweetpotato, tomato, chilli pepper and beans. To study the tritrophic interaction the whitefly species *B. afer* was reared on the different host plants, after infestations were established; different doses of the bio-insecticides were applied to *B. afer* nymphs. The aim was to generate specific deliverables from the study and practical recommendations to vegetable growers in Peru about choice of bio insecticide product, depending on crop and whitefly species.

The hypothesis that the mortality and infection of three Peruvian insect pathogenic fungi is dependent on which host plant species the whitefly nymph is feeding on was tested in climate chambers at the temperature of 20ºC.The life cycle of the species *B. afer* was studied as well; on four important crops in this region of Peru.

3 BACKGROUND

3.1 Whitefly

The whiteflies are polyphagous pests affecting a broad range of cultivated hosts both in field and greenhouses. They are included in the order of Homoptera and the family Aleyrodidae and there are many different species around the world. The spread of the whitefly in general and *B. afer* in particular has been aided by its broad host range, high reproductive potential, and migration ability, so knowledge about its biology and population dynamics is critical to developing a whitefly management program. Main factors regulating whitefly biology and population dynamics are climate (temperature, rainfall and relative humidity), host plant suitability, natural enemies, and management practices. Temperature and host-plant effects have been identified as important factors affecting development, mortality, and fecundity rates in whitefly population modelling efforts (Nava-Camberos, U. 2001).

Whiteflies feed by piercing the plant tissue. The plant phloem contains no micro organisms capable of causing diseases in the insect and therefore only pathogens with the ability to penetrate the insect cuticle have potential as microbial control agents. Of the various groups of entomopathogens only fungi possess this ability (Inglis *et al* 2001). Under certain conditions natural epidemics can suppress whitefly populations. In general, the natural occurrence of these fungi cannot be relied upon for control. Their appearance is highly unpredictable and an epizootic requires a favourable environment interacting with host and pathogen in many intricate ways (Faria and Wraight, 2001). Whitefly control with entomopathogenic fungi must therefore rely on direct application with infective propagules.

3.1.1 Life cycle of Aleyrodidae

Whiteflies have a hemi-metabolitic development, an incomplete metamorphosis from egg to adult. There are four different nymph stages without going through a pupa stage, even if the fourth instar is very similar to a pupa. The different life stages are; egg, crawler or first instars, second instars, third instars, fourth instars and adult, and the period of the whole cycle is dependent on the temperature, the relative humidity and host plant.
The eggs and nymphs are localized mostly on the underside of the leaves but *B. afer* is one of the species that lays egg on the topside of the leaves as well. Further information about the specific host plants and the *B. afer* behaviour on them will be discussed later on.

3.1.2 Problems on host plants

The Whitefly is one of the most important pests of world agriculture (Oliveira *et al*. 2001). Several species of whiteflies are spread throughout the tropical world and are also a serious problem in greenhouse production in temperate areas. Whiteflies damage the host plant and reduce yield by extracting phloem sap and by secreting honeydew, which serves as a substrate for sooty mould fungi. The whitefly also transmits a number of devastating plant viruses (Byrnes & Bellows 1992). Some common whitefly species in Peru are *Bemisia afer*, *B. tabaci* and *Trialeurodes vaporariorum*. In 2000 there was a severe outbreak of whiteflies in Cañete Valley in Peru. P. Anderson (CIAT) and Ing. José M. Valencia of the Cañete Experimental Station found out by collecting nymphs on sweet potato and after taxonimic verification that the outbreak species was *B. afer sens. lat. Bemisia afer* is considered a common and widespread pest in

Africa, on for example cassava. However, this was the first outbreak in an agricultural situation in the Americas and it seems to be mainly a problem in sweet potato production but, for example, chilli peppers, tomatoes, and beans are also considered as host plants (Andersson *et al.* 2001). There are very few studies done on the biology and population dynamics or biological control of *B. afer*.

3.1.3 Management strategies, and control of whitefly

Current management strategies for whiteflies on the majority of crops are dominated by the use of insecticides. Growers consider chemicals as insurance against the possibility of a devastating crop loss and excessive reliance has resulted not only in highly resistant insect populations but also systematic destruction of natural enemies, creation of new secondary pests and health problems among growers. The enormous yield losses and the serious health and environmental problems connected with the extensive use of insecticides have triggered a growing interest in and an urgent need for new and alternative methods.

Biological control of whiteflies with pathogens, parasitoids or predators represents a key but still little exploited control strategy. Natural enemies alone are unlikely to provide adequate control but through careful integration with other tactics, as in IPM programs, they could make an important contribution (Naranjo 2001). Predators and parasitoids work best if used in a preventive manor when populations are low whereas entomopathogens possess the ability to act curatively and cause high rapid mortality. They are actually most efficient when populations are high, which is desirable when it comes to whiteflies.

The most important pathogen and biocontrol agents of Aleyrodidae, whiteflies in general and in this case specifically, *B. afer,* are fungi that are found naturally in these areas. By invading actively through the cuticle the fungi can be used to control the whitefly. Several entomopathogenic fungi are important combating the whitefly, including *Paecilomyces fumosoroseus* (Wize) Brown & Smith, *Verticillium lecanii* (Zimmermann) Viégas (now re-classified as *Lecanicillium muscarium* and *Lecanicillium longisporum* (Petch) Zare & Gams (Zare and Gams, 2001)) and *Beauveria bassiana* (Balsamo) Vuill.

3.2 Entomopathogens

3.2.1 Fungi imperfecti

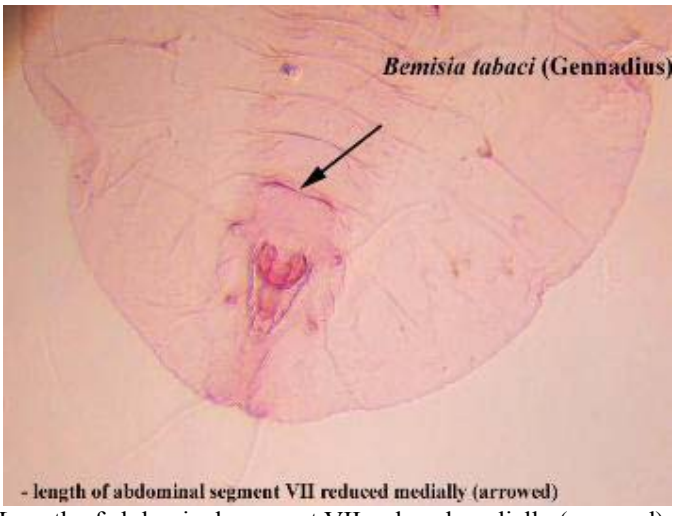
Fungi from many different taxonomic groups infect whiteflies and the most interesting, from a biological control prospective, belong to *Fungi imperfecti*. Species in this group are easy to culture and mass-produce with low inputs. They are also considered to be relatively safe for non-target insects, plants, animals, and humans (Hall, 1993). Fungi, within this class that are considered as whitefly pathogens are *Aschersonia aleyrodis, Verticillium lecanii, and Paecilomyces fumosoroseus.* Commercial products are available of all fungi entomopathogenic species except *A. aleyrodis.*
Of these fungi especially *P. fumosoroseus* seems to be an interesting candidate to use in a management strategy against whitefly populations. *Paecilomyces fumosoroseus* has a rather wide insect host range including whiteflies. The species seems adapted to lowland tropical niches (Hall, 1993) and was observed to cause a natural epizootic in Cañete Valley Peru 1998 (Åsman, K. 2007). At CIP in Peru several projects have dealt with IPM and insect pathogenic fungi. Naturally occurring strains of insect pathogenic fungi have been collected from different sites in Peru and in a recent master thesis one strain of *Paecilomyces fumosoroseus* was very pathogenic towards the whitefly species *Bemisia tabaci* (Böttger, 2006).

3.2.2 Tritrophic interaction between host plants, pest insect, and fungal insect pathogens

In a recent study at CIP three insect pathogenic strains were tested as biological control agents against the whitefly species *B. tabaci*, one *V. lecanii* strain, one commercial *P. fumosoroseus* strain (produced and sold in Peru) and a *P. fumosoroseus* strain collected previously at CIP. All three strains were pathogenic to *B. tabaci*. However, in a laboratory experiment the allelochemicals, i.e. plant substances Tomatine, Capsaicin and Caffeic acid had a negative impact on the spore germination of the commercial *P. fumosoroseus* fungi strain while the *P. fumosoroseus* strain collected by CIP was negatively affected by Tomatine and Capsaicin but not by Caffeic acid (Åsman *et al*. 2007).

3.3 Bemisia afer

Bemisia afer, was identified and first found in August of 2000, when P. Anderson (CIAT), Cristina Foneseca (CIP) and Ing. Jose M. Valencia (Cañete Experimental Station) made a field visit to the Cañete Valley, approximately 150 km south of Lima. Nymphs were collected from sweet potato for taxonomic verification and identified by P. Hernandez (CIAT, Colombia) and verified as *Bemisia afer sens. lat*., by J. Martin at the Natural History Museum in London, UK (BMNH) (Andersson *et al.* 2001).
Diagnostic Characters of two species of whitefly:
B. tabaci - Caudal setae always stout, usually at least as long as vasiform orifice. Vasiform orifice inset from margin of pupal case by less than its own length (ie. vasiform orifice length > caudal furrow), sides of orifice almost straight.
B. afer - Caudal setae usually less than half length of vasiform orifice. Vasiform orifice usually inset from posterior margin of pupal case by at least its own length, its sides distinctly concave. Occasionally with distinct stippling on median area of venter as well as on thoracic and/or caudal tracheal fold (Martin, J. 1987).



Length of abdominal segment VII reduced medially (arrowed)

Caudal setae less than half length of vasiform orifice
Sides of vasiform orifice distinctly concave.
Photos by Kathryn Sparks, AQIS, Victoria

Except for these diagnostic characters there are no visible differences between the whiteflies *Bemisia tabaci* and *Bemisia afer* when adult, an experienced eye can see the difference between them in the nymphal stage.

4 MATERIAL AND METHODS

4.1 Material used for the tritrophic interaction trial with entomopathogens

4.1.1 Whitefly

The whitefly, *Bemisia afer* (Aleyrodidae), population originated from the Cañete Valley in Peru (150km south of Lima, 50 m asl.) and had been maintained in a chamber with sweetpotato as a host plant at CIP for several generations.

4.1.2 Host plants

Plants cultivated at CIP and grown in the greenhouse during the experiment at CIP were: tomato (*Solanum lycopersicum* c.v. Rio grande), chilli pepper (*Capsicum baccatum* c.v. Escabeche, from gene bank of CIP), sweet potato (*Ipomoea battatas* L., Poir c.v. Costanera, (INIA-100) from gene bank of CIP) and beans (*Phaseolus vulgaris* "Canario peruano" Canary beans).
The beans were about 1-7 weeks when they were used in this trial. Bean seeds were planted in pots and trials were done on all the host plants on the youngest fully developed leaves. The chilli peppers were about 9-12 weeks when they were used. For the first trial we used chilli pepper that we bought from the University Garden of UNALM (Universidad Nacional Agraria La Molina).
The tomato plants used in the first and second trial were 5-7 weeks old. The tomato plants used in third trial were 3 weeks old. Tomatoes and chilli pepper were seeded in Jiffy 7 peat pellets and then transplanted to larger pots and grown in greenhouse for 1-7 weeks. Stems of the sweetpotato plant were directly transplanted and renewed every now and then and never older than 3-5 weeks.

4.1.3 Fungus

The three Peruvian isolates of fungus *P. fumosoroseus* strain CIPWF24, one commercial *P. fumosoroseus* strain CCB-LE818 used in Peru and *V. lecanii* strain CCB-LE502 were used see table 1.

Table 1 Origin of the isolates

Species	Strain	Local	Host species	Crop
P. fumosoroseus	CIPWF24	Cañete, Peru	Whitefly *B.* sp	Sweetpotato
P. fumosoroseus	CCB-LE818	Cañete, Peru	Whitefly *B. tabaci*	Cotton
V. lecanii	CCB-LE502	Huaral	Whitefly *B. tabaci*	Broccoli

The P. fumosoroseus strain CIPWF24 from CIP, Centro International de la Papa, Peru, *P. fumosoroseus* strain CCB-LE818 (commercial strain) and *V. lecanii* (now re-classified as *Lecanicillium* sp.) from SENASA collection (Ministerio Agricultura Servicio Nacional de Sanidad Agraria, Lima, Peru)

Cultures of fungi were grown on potato dextrose agar (PDA; Difco, Detroit, MI) and after 12-14 days harvested into 0,05 % Triton X and centrifuged 8 min at 10 000 rpm. The supernatant was discarded; the

pellet was resuspended in 0,05 % Triton X solution. The conidia solution was adjusted to a concentration of 1 x 10^8 conidia/ml.
Streptomycin sulfate FW 1457.4 (745 units/mg) was used (10µg/ml) (antibacterial antibiotic) in the water agar used when nymphs were placed on agar to see if fungal growth could be detected.

4.2 Methods for the tritrophic interaction trial with entomopathogens

Three trials were conducted under greenhouse conditions and were done in a similar way to keep the trials as similar as possible to each other.
To study the tritrophic interaction *B. afer* was reared on different host plants; sweet potatoes, chilli peppers, tomatoes, and beans. The host plants are chosen mostly for the reason that these plants are economically important cultivars where bio-insecticides are an interesting alternative to chemical. The second reason for choosing the plants is that a similar study was done using *B. tabaci* in 2006. In that study on *B. tabaci* it was shown that the mortality effect by insect pathogenic fungi might be different depending on host plant species (Åsman K. *et al*. 2007).

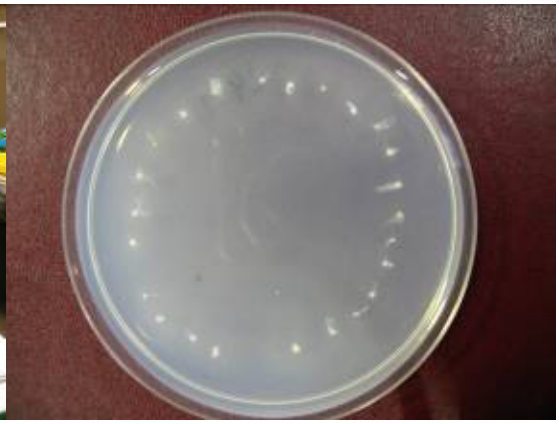
Tomato Bean Chilli pepper Sweet potato

Photos by Ethel Cavén

About 45 to 48 leaves were cut from each of the four host plant species, dipped in a root growing hormone powder, Rootone (Rooting Hormone with Fungicide, from Tech PAC, LLC Lexington KY), and placed four and four or five and five in small glass flasks with water. The flasks were placed in a cylindrical plastic container (12 cm high, 11 cm in diameter) with ventilation on top. On the day of exposure to test plants, about 80 adults from whitefly culture were aspirated into vials and then transferred to the plastic containers with leaves. The whiteflies were left with the leaves for 48-72 hours to lay eggs and then the adults were removed and the leaves were placed individually in glass bottles covered with the container with ventilation on top. The leaves were watered three times a week and kept under observation almost every day in a room with 16 hours of daylight and 8 hours dark and a temperature of 20 ºC.
Some days later, the leaves were checked under the microscope to see that eggs could be found on every leaf. 33 – 39 days after set up, fungi were prepared. Fungal spores were harvested by scraping conidia from 20 agar plates of each fungus and then adjusting to a concentration of 1 x 10^8 conidia/ml. Each fungus was then diluted into the three concentrations used in the tests: 10^6, 10^7 and 10^8.
After infestations were established, 34 – 40 days after start, the three different concentrations of all three fungi and a control with only water were sprayed to run-off on both sides of the leaves using a hand-held sprayer (200ml Nalgene plastic spray bottle, Thomas Scientific, Swedesboro, NJ) on 3rd - 4th instars whitefly nymphs.
The leaves were placed in containers without ventilation on top for two days under favourable conditions for the fungi to grow. 43 - 51 days after start the nymphs were taken to the laboratory and as many as possible or a maximum of 20 nymphs that had been marked out on each leaf in advance were placed on

agar plates. 52 – 61 days after start were the trials evaluated by looking at the agar plates for growing fungus on the nymphs.
Mortality confirmed by fungal growth on the nymphs was the response variable evaluated by ANOVA, which will be shown in the results.


Spraying of leaves


Fungi growing on agar plate

Photos by Ethel Cavén

Differences and difficulties between the three trials: Dates for different actions during the three trials are given in Table 2. **The first trial:** Some problems occurred during the trial e.g. in the beginning when four leaves were put in the same bottle, there was not enough water in the bottles and therefore some leaves wilted during the weekends and did not survive the trial.

The second trial: This time there were five leaves in every container because of lack of adult whiteflies. The trial was delayed and spraying the leaves was done later on in the whiteflies' life cycle. Therefore the nymphs were older and most likely in their fourth instars when the fungi were applied.

The third trial, even this time five leaves in every glass bottle and container were used because of lack of whiteflies.

Table 2 Data on trials, considering time and action.

Trial	Date	Action	Days from start
1	7 February	Start of trial	0 days
	13 March	Spray on leaves	34 days
	23 March	Nymphs put on agar	44 days
	4 April	Analyse	54 days
2	21-22 February	Start of trial	0 days
	4 April	Spray on leaves	39-40 days
	12-13 April	Nymphs put on agar	50 days
	23 April	Analyse	61 days
3	13 March	Start of trial	0 days
	18 April	Spray on leaves	36 days
	25 April	Nymphs put on agar	43 days
	4 May	Analyse	52 days

4.3 Material for the life cycle

The same four host plants as the trial above were used in the life cycle experiments. They were treated the same way as in the trials above. Five leaves, youngest fully developed, from each host plant were used.

4.4 Methods for the life cycle

Because the whiteflies were left only for 24 hours to lay eggs on the leaves it was easier to make sure that the life cycle studies had the same conditions for all four host plants. Eggs were counted on the day after the whiteflies were taken away. Eggs were found on both sides of the leaves and this specific behaviour is typical for this species of whitefly, *Bemisia afer*. In contrast, *B. tabaci* lays its eggs on the underside of the leaf.
The leaves were checked until the crawlers started to hatch from the eggs. As soon as the crawlers were settled down and transformed into second instars the nymphs were marked out with a pen. It was difficult to see if the crawlers actually settled down, they sometimes were found in another place the day after marking had been done. When all crawlers were marked out they were followed in their development every day or every other day (because the greenhouse was closed on weekends). The whiteflies were not followed individually and therefore there are no data from individuals, instead each leaf was checked as replicate out of five in total. The eggs that didn't hatch were counted as dead, and then every instar's stage was closely followed under stereoscope. The study followed the whiteflies until the leaf died or the nymphs had developed into adults or were found dead. As I didn't stay until the end of the study my assistant in Peru, Octavio Zegarra took over the study and followed the last sequence of the development. As the single leaves were hard to keep in a good condition during the long lifecycle were their some leaves that didn't make it to the end of the experiment and therefore there were some problems resulting analysing the results. Some leaves did, however, make it all the way to the end. Depending on host plant and their specific character some of them had quite a lot of roots, see picture above of sweet potato.
Differences between the host plants were seen both in survival of leaves and survival of whitefly on the leaves, this due to the condition and character of the host plants and leaves.

5 RESULTS

5.1 Results of the tritrophic interaction trial with entomopathogens.

Total mortality of *Bemisia afer* on bean, chilli pepper, tomato, and sweet potato after using entomopathogenic fungi species: *Paecilomyces fumosoroseus* strain CCB-LE818 (Senasa) and CIPWF24 (W24) and *Verticillium lecanii* strain S02 58-8 (now re-classified as *Lecanicillium* sp.) (Vlii), (Table 2) was determined. The mean mortality (± standard error) of nymphs in the controls was on bean 2 (± 2) %, on chilli pepper 0 (±0) %, on tomato 2 (±2) % and on sweet potato 1 (±1) % see table 3 and standard error se table 4.
All three fungal strains caused significantly more mortality to *B. afer* feeding on all four host plant species tested, when compared to the control mortality.

In general there was no significant differences in mortality when comparing host plant species, except of the *Paecilomyces fumosoroseus* strain CCB-LE818, with concentration 10^7 that had a significant difference between sweet potato and tomato. There were large variations between the replicate results. This can be compared with the other study done by Karolina Åsman on *B. tabaci* where *Paecilomyces fumosoroseus* strain CIPWF24 showed significant more mortality between host plant species. The trial was analyzed by generalized linear models (Proc Glm SAS).
ANOVA, Analysis of Variance with F value, Df = Degrees of freedom, and P = significance < 0,05.

Table 3 Mean value of the mortality of nymphs on host plant and the effects on activity of fungi against whiteflies

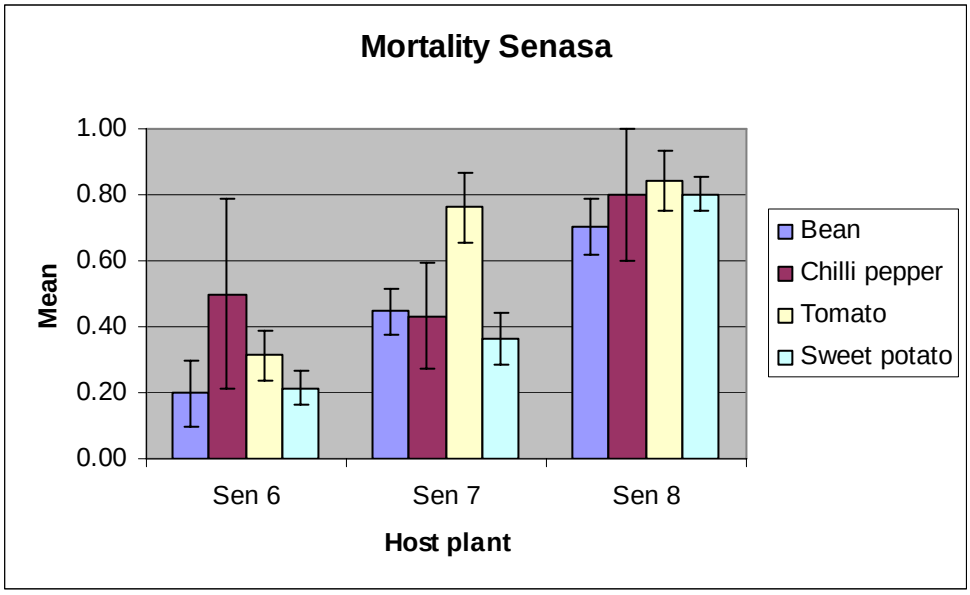
Mean	Bean	Chilli pepper	Tomato	Sweet potato	F	Df	P
Control	0.02	0.00	0.02	0.01	0.33	3.24	0.80
Senasa 10^6	0.20	0.50	0.31	0.21	1.18	3.25	0.34
Senasa 10^7	0.45 ab	0.43 ab	0.76 a	0.36 b	3.79	3.32	0.02
Senasa 10^8	0.70	0.80	0.84	0.80	0.43	3.29	0.74
W24 10^6	0.17	0.07	0.22	0.17	0.42	3.31	0.74
W24 10^7	0.35	0.38	0.39	0.47	0.33	3.27	0.80
W24 10^8	0.57	0.69	0.54	0.76	1.20	3.31	0.33
Vlii 10^6	0.13	0.29	0.37	0.19	1.13	3.19	0.36
Vlii 10^7	0.40	0.25	0.44	0.35	1.36	3.26	0.28
Vlii 10^8	0.60	0.27	0.72	0.69	1.15	3.26	0.35

Significant difference between tomato and sweet potato with Senasa 10^7, marked with a, b and ab.

Table 4 Standard error

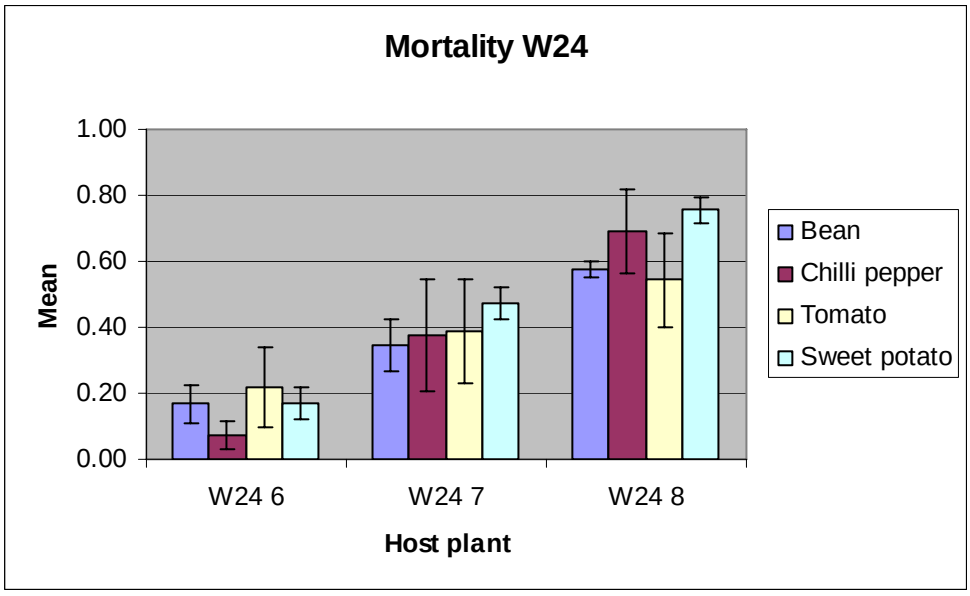
Std Error	Bean	Chilli pepper	Tomato	Sweet potato
Control	0.02	0.00	0.02	0.01
Senasa 10^6	0.10	0.29	0.08	0.05
Senasa 10^7	0.07	0.16	0.11	0.08
Senasa 10^8	0.09	0.20	0.09	0.05
W24 10^6	0.06	0.04	0.12	0.05
W24 10^7	0.08	0.17	0.16	0.05
W24 10^8	0.03	0.13	0.14	0.04
Vlii 10^6	0.05	0.21	0.02	0.05
Vlii 10^7	0.05	0.06	0.22	0.05
Vlii 10^8	0.26	0.16	0.10	0.05

Diagram 1 Mean Mortality by Senasa on four host plants. Standard error bars are shown for each host plant.



Mean mortality of nymphs 7 to 10 days after inoculation ranged between 20 %±10 for nymphs reared on beans and 50 % ±29 for nymphs reared on chilli pepper with Senasa 10^6.
With Senasa 10^7 the mean mortality was between 36% ±8 for nymphs reared on sweet potato and 76% ±11 for nymphs reared on tomato. With Senasa 10^8 the mean mortality ranged between 70 % ±9 for nymphs reared on bean and 84 %±9 for nymphs reared on tomato
The only significantly differences comparing mean mortality of nymphs between the host plants were on tomato and sweet potato on the concentration of 10^7 of Senasa but not between the others host plants or doses of concentration.

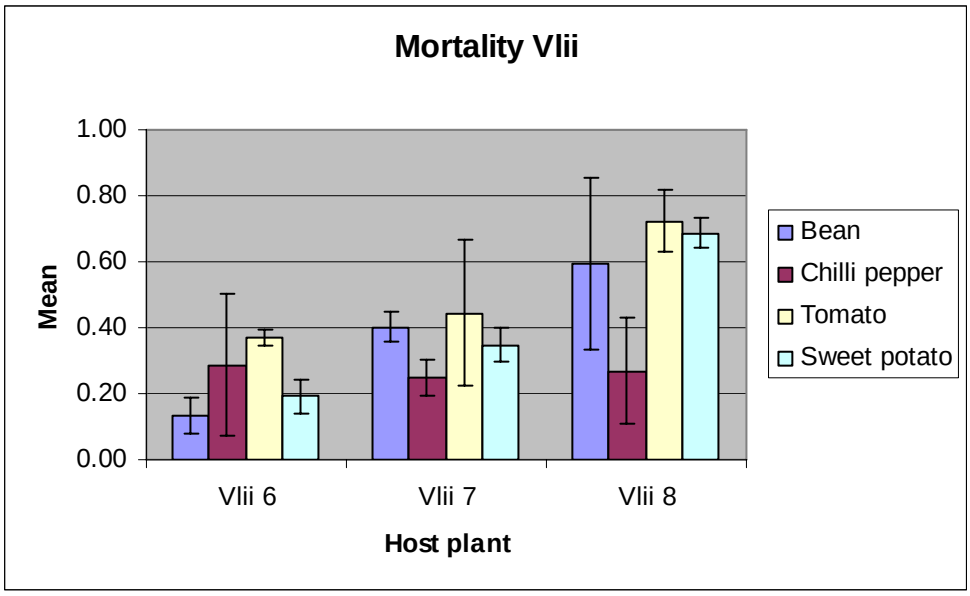
Diagram 2 Mean Mortality by W24 on four host plants. Standard error bars are shown for each host plant.



Mean mortality of nymphs 7 to 10 days after inoculation ranged between 7 % ±4 for nymphs reared on chilli pepper and 22 % ±12 for nymphs reared on tomato with W24 in a concentration of 10^6.
With W24 10^7 the mean mortality ranged between 35 % ±8 for nymphs reared on bean and 47 % ±5 for nymphs reared on sweet potato. With W24 10^8 the mean mortality ranged between 54 % ±14 for nymphs reared on tomato and 76 % ±4 for nymphs reared on sweet potato

The W24 showed no significantly differences between the four host plants and the three different concentrations. The only effect was that the higher the concentration the higher the mortality of the *B. afer* nymphs.

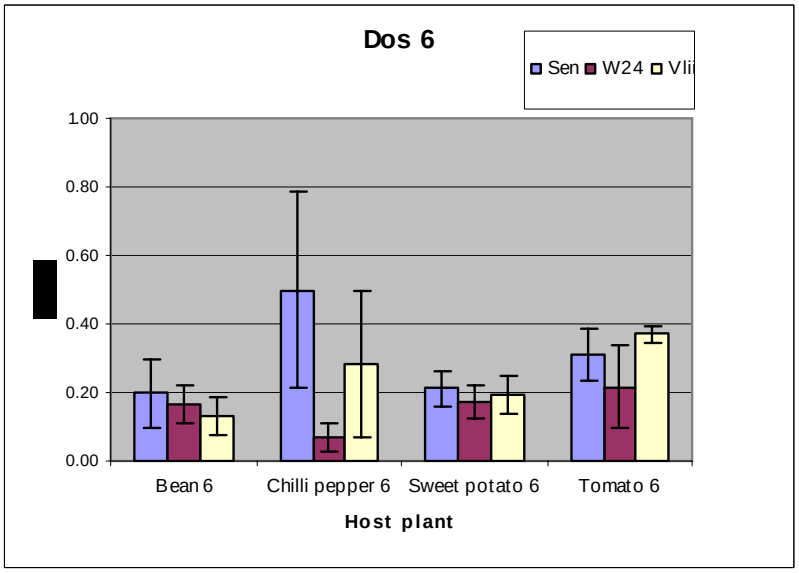
Diagram 3 Mean Mortality by *Verticillium lecanii* on four host plants. Standard error bars are shown for each host plant.



Mean mortality of nymphs 7 to 10 days after inoculation ranged between 13 % ±5 for nymphs reared on bean and 37 % ±2 for nymphs reared on tomato with Vlii in a concentration of 10^6.
With Vlii 10^7 the mean mortality ranged between 25 % ±6 for nymphs reared on chilli pepper and 44 % ±22 for nymphs reared on tomato. With Vlii 10^8 the mean mortality ranged between 27 % ±16 for nymphs reared on chilli pepper and 72 % ±10 for nymphs reared on tomato. There were no significant differences between host plant species and this fungi and the variation among the replicates were quite large.

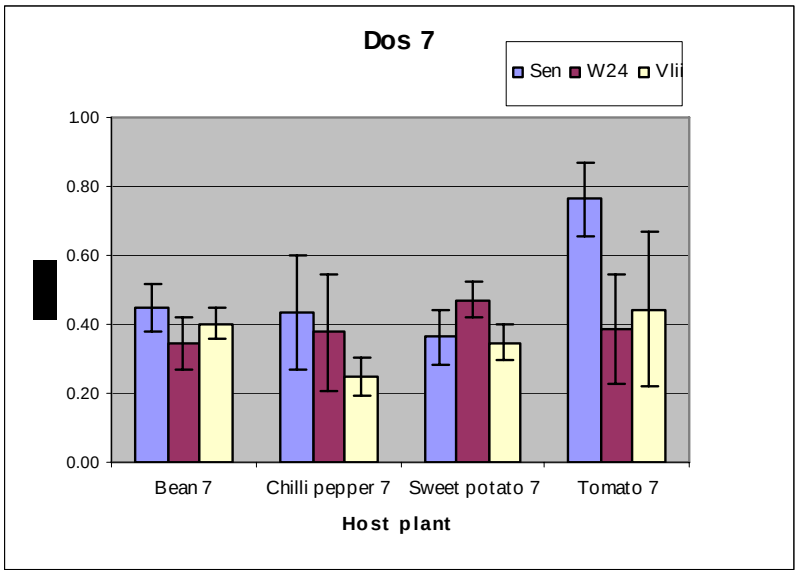
Comparing doses and host plant species gives us another perspective on the results. This would show us if there were any differences between host plants, but not even here we could see any significant differences. Even here the variations between the replicates vary a lot and the results are hard to evaluate.

Diagram 4 Nymphs infested by fungi dos 10^6 on different host plants Standard error bars are shown for each host plant.



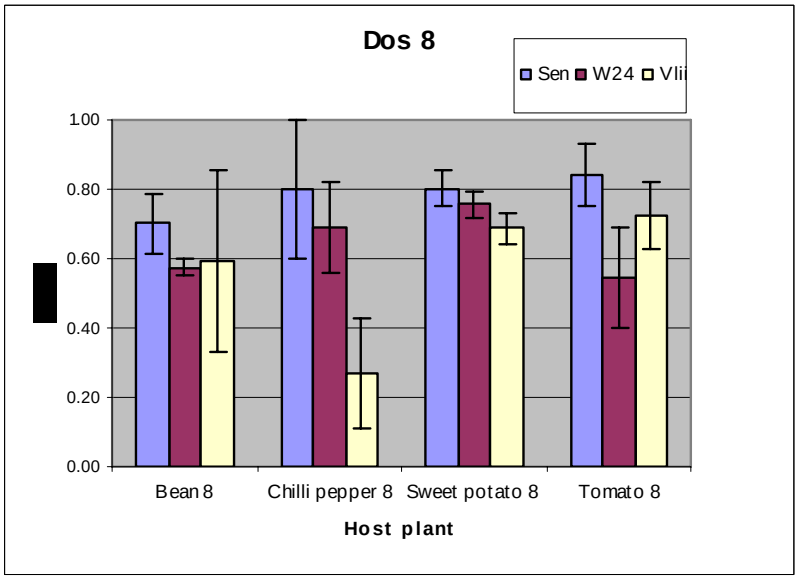
There were fewer replicates done on chilli pepper with this dose and in general fewer nymphs on the leaves than the others host plant species, the variation was therefore where quite large on this dosage.

Diagram 5 Nymphs infested by fungi dos 10^7 on different host plants. Standard error bars are shown for each host plant.



There were no significantly big differences between doses and mortality of nymphs. In general there was more mortality on tomato on Senasa compared with the other host plants.

Diagram 6 Nymphs infested by fungi 10^8 on different host plant Standard error bars are shown for each host plants.



Out of this diagram 6 we can read; higher mortality in general on this higher dose of each fungus, but no significant differences between the fungi. There is a tendency to a higher mortality with the Senasa fungi compared with the W24 and V.lii. This could be compared with the K. Åsman study were the W24 had a higher mortality compared with the others.

5.2 Results of life cycle of Bemisia afer.

There is little information on the life cycle of *B afer* on tomato, bean, sweet potato and chilli pepper. In my exam thesis therefore life cycle study was conducted on the whitefly species *B afer* on four different host plants. My stay was short in Peru and the life cycle of *B. afer* was long and there was not time to do more than one complete life cycle study. First I'll show the longest duration of each development stage for different host plants.

Diagram 7 Duration of *B. afer's* lifecycle by stage on four different host plants.

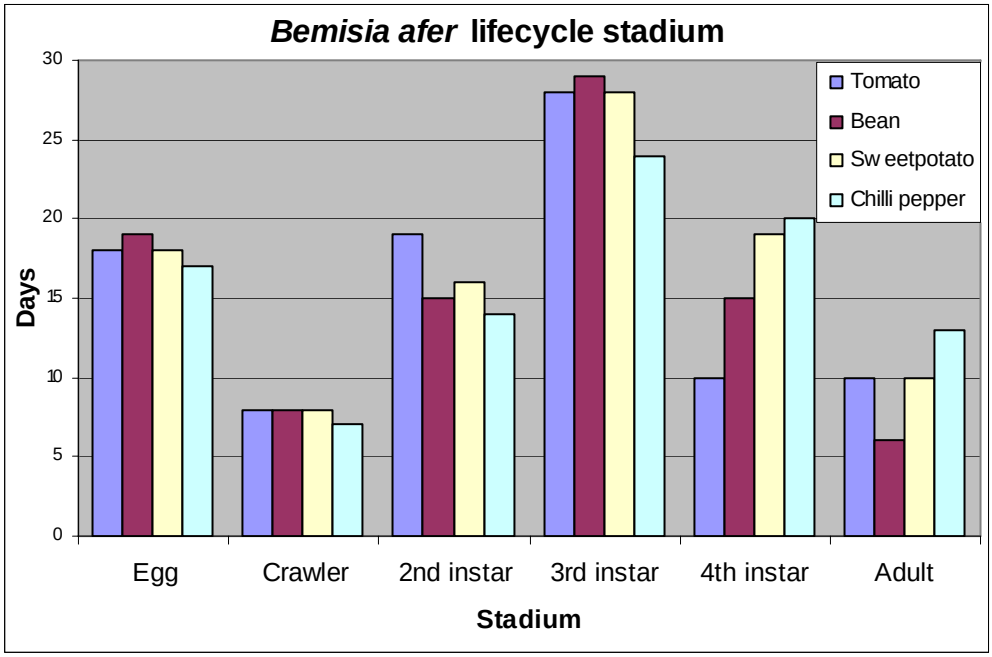
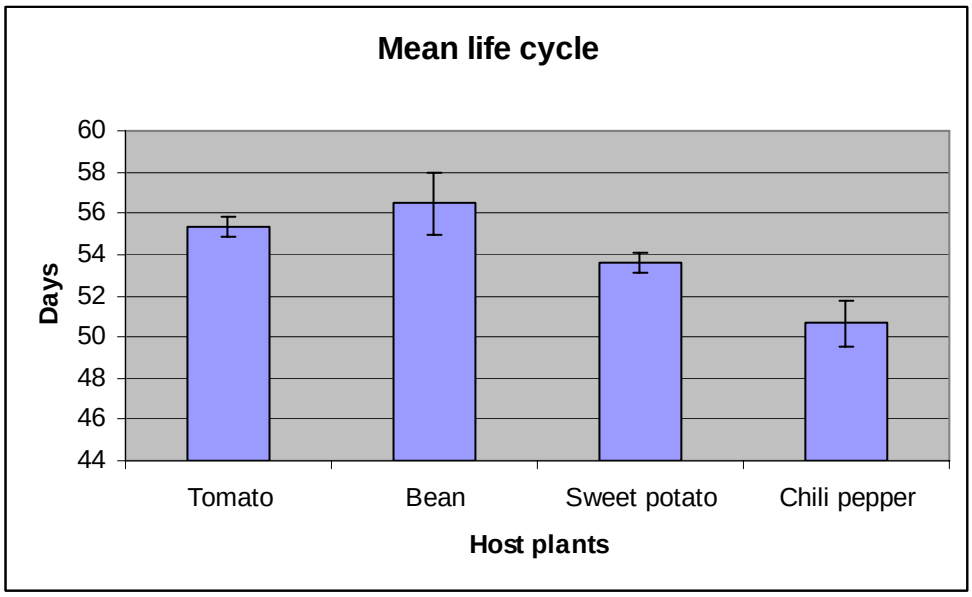


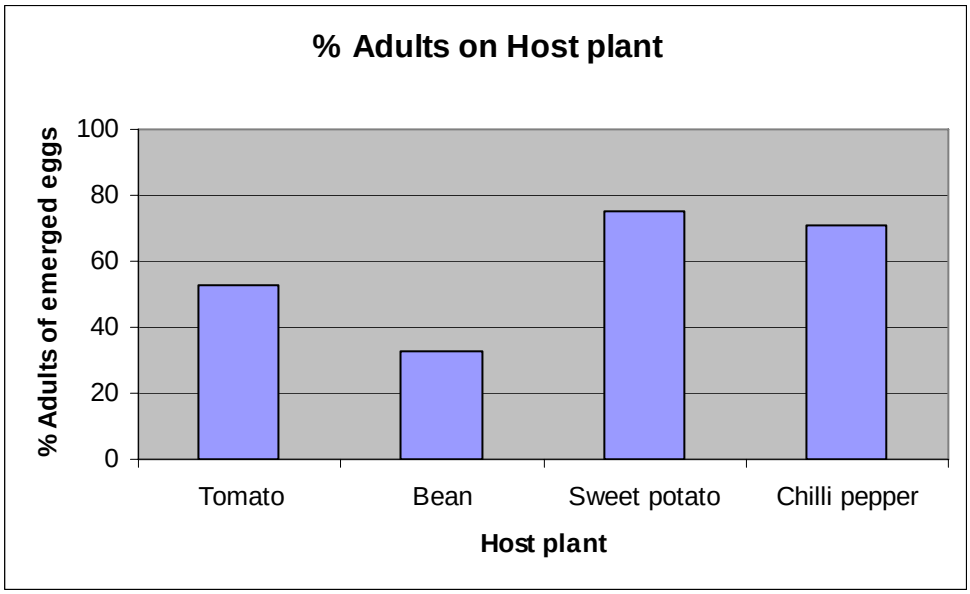
Diagram 7 gives a picture of how long time the nymphs stayed in each stage of the life cycle. The largest differences could be seen in the 2nd, 3rd and 4th instar and the period to adult.
In total chilli pepper gave the shortest life cycle compared with tomato and beans that had much longer life cycles. This can be seen in diagram 8 where chilli pepper had a significant shorter life cycle than the others.

Diagram 8 Mean life cycle of *B. afer* in days with standard error**.**



The variation of days to emerging adults on tomatoes was between 53 to 61 days, on beans between 53 to 59 days, on sweet potato 49 to 61 days and on chilli pepper between 46 to 61 days. Because the nymphs were not followed individually the exact data of when they entered one stage to another can't be described but though I have the data of when they emerged to adult this last step can be tested statistically.
Mean lifecycle, see diagram 8, for the nymphs of *B. afer* on tomatoes was 55.3 (with standard error ± 0.5) days, on beans 56.5(± 1.5) days, on sweet potatoes 53.6(± 0.47) days and on chilli peppers 50.7(± 1.12) days. The mean life cycle had a P value of 0.0005, and shows that chilli pepper was significantly different from tomato, bean and sweet potato in this test.

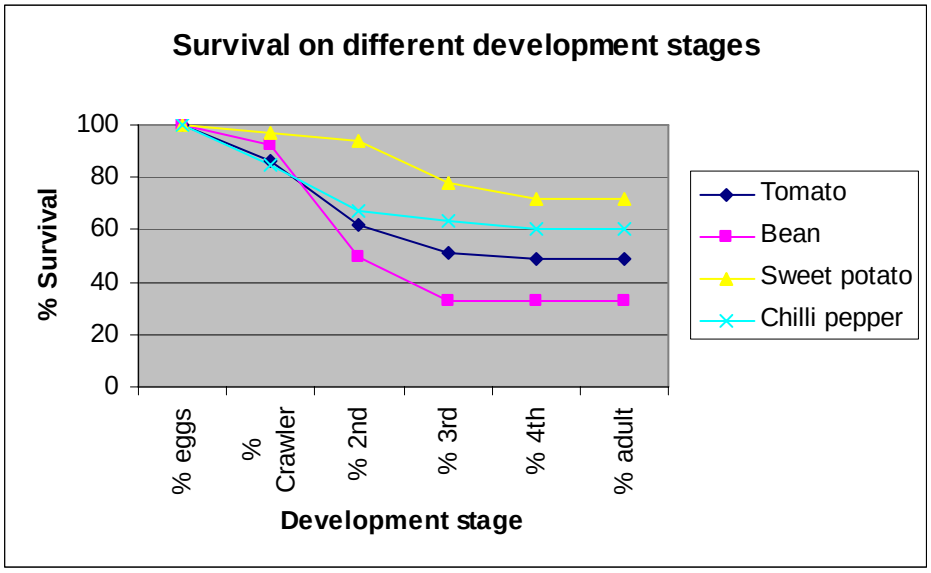
Diagram 9 Percent adults that emerged out of different host plants.



Results of the life cycle study on the four host plants see diagram 9. The survivals of hatched eggs to adults were on average: tomato 53 %, bean 33 %, sweet potato 75 % and on chilli pepper 71 %. This shows that tomatoes and beans may not be the best host plants for *B. afer* and that if the whitefly eggs hatch on chilli pepper they have a good chance to survive to adults. It does not show though that chilli pepper might not be

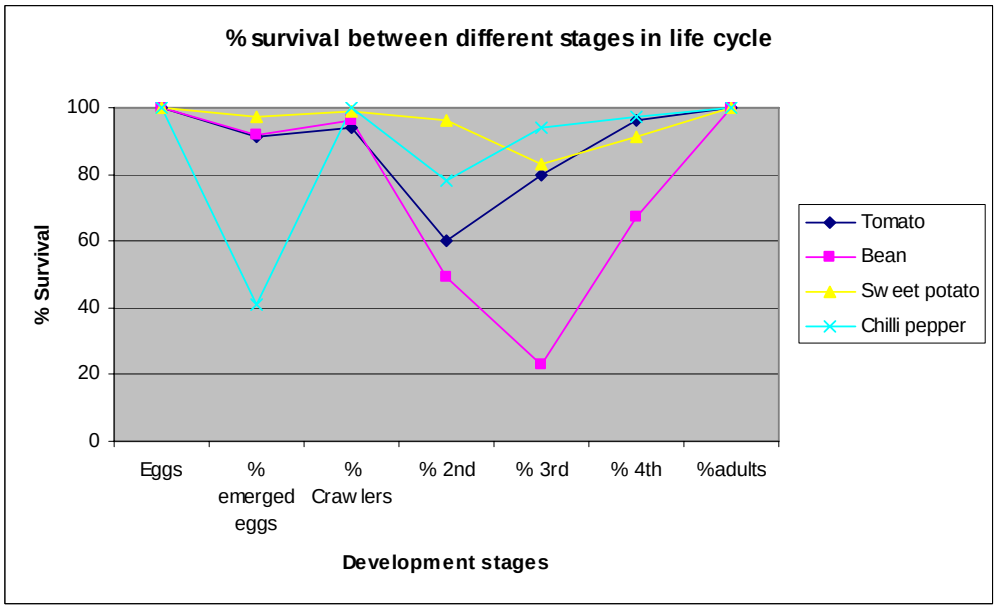
the most popular host plant to lay eggs on. Sweet potato shows good results of surviving all the way to adult.

Diagram 10 Survival on different development stages on host plants.



The diagram 9 shows the survival in percent on different host plants from emerged eggs. The most critical stadium was from crawler to 3rd instar for almost all host plants, except chilli pepper where it seems to be from egg to crawler. Nymphs on beans died mostly between crawler and 3rd instar and nymphs on chilli pepper had it hardest to survive from hatched eggs to 2nd instar. The survival on beans was as low as 33 %, while sweet potato had 72 % survival adults.
Differences in the lifecycle were significant between chilli pepper and tomato and bean, but not between chilli pepper and sweet potato. This shows again that sweet potato is a highly suitable crop for *B. afer* reproduction and population growth according to data obtained in this study, whereas chilli pepper, tomato and bean are clearly a poor hosts for this insect.

Diagram 11 Percent of survival between different stages in lifecycle on four host plants.



This diagram 11 shows where the critical stage is for each host plant during the whitefly development cycle.

6 DISCUSSION

6.1 Results and effects of the tritrophic interaction trial with entomopathogens

Difficulties to find the best tritrophic interaction seem to be harder than we think. Only to find good methods to study what happens in the plant, the insect and the fungi, while we are doing experiments on them in the same system seems to be hard enough. Many factors will have to be considered and a lot of studies done to understand the complexity of what happens in a tritrophic interaction. As my study was a part of a larger study with tritrophic interaction between host plant, whitefly, and fungi the methods were already decided.
Through my study I have found that the fungi that I used had certain mortality on the specie *B. afer* but from the specific point of view that the interaction with host plant and fungi would have some effect to alter the mortality of the insect there were no significant proof. Neither were there any significant differences between different doses and fungi.
Greenhouse experiments may be hard to compare with real circumstances or "real life", and therefore hard to apply straight away. To avoid unexpected problems and to get a "as fair judgment" the circumstances have to be special and strictly under control, this to avoid other influences and other unexpected factors in the trial.
This study was held under greenhouse conditions and was not at all implemented in the field. The concentrations of fungi that were used in this trial and the best possible circumstances for the fungi that were in the laboratory gave us the results that have been shown earlier.

The host plants characters are very different and this may be important to have in mind when considering the results, this not only in substances or secondary metabolites, but even in structure and surface characteristics. Some leaves show different structures, i.e. bean very hairy or pubescence while chilli pepper has a glossy and harder cuticle. This might play a roll both for the whitefly when choosing a host plant or surviving on a host plant, but also for how the fungi react with the host plant species in their interaction with the insect. The pubescence makes a special microclimate on leaf and might have some influence on the insect or the fungi.

In this study there were no significant differences among the three fungi and host plants species, with the exception of Senasa at the concentration of 10^7 on tomato. Comparing with other studies conducted on *Bemisia tabaci,* it seems like the secondary metabolites (caffeic acid, capsaicin and tomatine) in the plants may have a larger influence on the nymphs and their mortality. The secondary metabolites can also inhibit fungal growth and germination, but this doesn't seem to be the case in this study. Earlier studies with the same fungi but on *B. tabaci* were not done on the host plant of beans, so a comparison of the two studies on that host plant species cannot be done. The spore germination of insect pathogenic fungi strains from three different genera (two *Paecilomyces fumosoroseus* strains, and one *Verticillium lecanii* strain) was in the other study affected by caffeic acid, capsaicin and tomatine but to different extents depending on fungal strain (Åsman, K *et al* 2007). In that case the strain CIPWF24 caused the highest infection rate of *B. tabaci* nymphs regardless of host plant species, and seemed to tolerate low dosages of capsaicin, and spore germination was not negatively effected by caffeic acid at all.

Another factor is the necessity of water during germination of fungi; it has led to the general belief that moist conditions are essential to the use of fungi in microbial control against insects.

Without these circumstances with moist conditions and high amount of conidia or fungi the effect will be much lower and it might not even be an economically sound investment for the farmer.

6.2 Discussion of life table on different host plants

Whiteflies are multivoltine and have several generations yearly. Most species are recorded from tropical or subtropical regions, and such species may develop and breed continually so long as temperature conditions admit (Poprawski, T. J. 2000). Development times for multivoltine whiteflies vary usually with the season, but most species reported develop from egg to adult in from 25 to 50 days under field conditions (Byrnes D.N. & Bellows, T.S. 1992). This compares well with results of this study, the variation between days of emerging adults on tomatoes was between 53 to 61 days, on beans between 53 to 59 days, on sweet potato 49 to 61 days and on chilli pepper between 46 to 61 days under greenhouse conditions and with a constant temperature of 20 degrees C. The constant temperature may affect the life cycle as a slow down effect.

Compared to other studies done on other species of whitefly the egg stage lasts 8 days, crawler 5 days, 2nd instars 2 days, 3rd instars 5 days, 4th and the longest 9 days instars, this all together more or less a 40 day life cycle on *Trialeurodes vaporariorum* from egg to adult in an observation condition with 20°C, on tomato (Malais, M., Ravensberg, W.J. 1991).
This study had a much longer life cycle and depending on host plant the *B. afer* egg hatches between 13 to 19 days. Crawlers varied between 3 to 4 days during a 7 to 8 day period. 2nd instar stage was harder to see but varied between 3 to 10 or 16 days during a 14 to 19 day period depending on host plant. 3rd instar was the longest stage for all host plants. The nymphs were in this stage between 14 and 20 days in a 24 to 29 day long period. This is a total of between 46 to 61 days, depending on host plant species and a mean life cycle, see diagram 8, for the nymphs of *B. afer* on tomatoes 55.3 days, on beans 56.5 days, on sweet potatoes 53.6 days and on chilli peppers to 50.7 days.

Another study has been done on *B. afer* on Cassava (*Manihot esculenta* (Crantz)) in Africa under field conditions during the cold months at 20 degrees C, where the results of the longest mean developmental was a period of 59,5 days (Munthali DC, 1992).

Other observations during the trial were that if the nymphs were able to make it all the way to adult, on for example beans and tomatoes the whiteflies were weak and did not survive long even if they emerged. Some had problems with their wings or other not fully developed functions.
Other observations were that on chilli pepper leaves the nymphs and adults seemed smaller than average, this only by sight because no measuring was done.
As discussed earlier the surface of the leaves vary in pubescence, thickness, stiffness, substance etc., between the host plant species and maybe that is one reason why the nymphs develop differently.

Crawler mortality has been attributed to several plant characteristics, including the thickness of the cuticle, pubescence and nutritional factors; this might be one reason why the whitefly didn't survive on chilli pepper as well as on the others or on beans as they are much more pubescence than the others.
It seems that while the nymphs were growing the harder it got for the nymphs to develop in-between the hairs and often they did not get in good contact with the leaf surface. This may be a reason why some host plants were not as good as others in the development of the whitefly *B. afer*.
In Munthali's study in Africa it was found that pubescence of cassava variety was only weakly associated with resistance to *B. afer* (r=0,48) suggesting that other factors are more responsible for resistance to this pest than hairiness(Munthali DC, 1992).

It appears as though the third instar is the longest stage in time in all four host plants. Some problems with identifying exact stage for a first time checker occurred as well. The result of not being able to look at the nymphs every day as well makes the data a little unreliable.

Another observation done at the same time was that four different host plant leaves were put in the same container with 80 *B. afer*, to see on which one of the host plant leaves the whitefly preferred to oviposit. The results of the few tests that were done gave this response: most eggs; beans >> sweet potato > tomato > chilli pepper; with least eggs.
This can be compared with another study made by Pedro Morales and Mario Cermeli in Venezuela on *Bemisia tabaci* and on sesame (*Sesamum indicum* L.), beans (*Phaseolus vulgaris* L.), cucumber (*Cucumis pepo* L.), cantaloupe (*Cucumis melo* L.) and tomato (*Lycopersicon esculentum* Mill.). The order of preference for oviposition and nymphal development was tomato > melon = sesame > cucumber = beans (Morales, P. and Cermeli, M. 2001).
There might of course be differences between the two species of whitefly and their host plant of preference.

7 THANKS

I wish to thank everyone that has supported me during this thesis, who with their knowledge as much as their kindness helped me under the visit at CIP in Peru and during the writing in Sweden.

I wish to thank Karolina Åsman who inspired me, and helped me with the application of the MFS, which in the end resulted in this thesis and study at CIP, Peru.
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Thanks to SLU and the people with a great knowledge for a better world, don't forget to get it into practice!

To all that reads this thesis; Thanks for showing interest!

Mostly thankfully!

Yours Ethel Cavén
Soon to be an Agronomist.

8 REFERENCES

8.1 References from newsletters, books etc.

Anderson, P. K. Martin, J.H. Hernandez, P. & Lagnaoui, A. 2001. *Bemisia afer* sens. Lat. (Homoptera: Aleyrodidae) outbreak in the Americas. Florida Entomologist 84(2):316-317.

Byrnes D.N. & Bellows, T.S. 1992. Whitefly Biology. Annual review of Entomology 36, 431-457.

Böttger, J. A. A. 2006. Tamizado de patogenicidad de 20 aislamientos de *Paecilomyces fumosoroseus* (Wize) Brown & Smith para el control de *Bemisia tabaci* Biotipo B (Gennadious) Bellows & Perring en camote *Ipomoea batatas* (L.) Lam. Thesis. Universidad Nacional Agraria La Molina, Peru.

Cisneros, F. & Mujica, N. 1999. Biological and Selective Control of the Sweetpotato Whitefly, *Bemisia tabaci*. Impact of a Changing World. Program report 1997-98. International Potato Center Lima, Peru.

Faria, M. and Wraight, S.P. 2001. Biological control of *Bemisia tabaci* with fungi. Crop Protection 20, 767-778.

Hall, R.A.1993.The use of pathogens to control whiteflies in Europe and the Tropics: Possibilities for Integrated Control. Memoria II Taller Latinoamericano Y del Caribe Sobre Moscas Blancas y Geminivirus. Managua, Nicaragua

Inglis, G. D. M.S. Goettel, T.M. Butt, & H. Strasser 2001. Use of Hyphomycetous Fungi for Managing Insect Pests. In Fungi as Biocontrol Agents: progress, problems and potential. Ed. T.M Butt, C.W Jackson & N. Magan. CABI publishing.

Lagnaoui, A. Mujica, N. Zegarra, O. & Anderson P. K. 2001. The whitefly complex affecting sweetpotato in the Cañete Valley of Peru. Sweetpotato: Food and health for the future. International Society for Horticultural Science, CIP and Universidad Nacional Agraria La Molina, Lima, Peru.

Martin, J. 1987 Tropical Pest Management 33(4): 298-322

Malais, M., Ravensberg, W.J. 1991. Knowing and recognizing. The biology of glasshouse pests and their natural enemies. First edition 1992. Koppert B.V. Berkel en Rodenrijs, the Netherlands

Morales, P., Cermeli, M. 2001. Evaluación de la preferencia de la mosca Blanca *Hemisia tabaco* (Gennadius) (Hemiptera: Aleyrodidae) en cinco cultivos agrícolas. Entomotropica Vol. 16(2): 73-78.

Munthali DC. 2001. Effect of cassava variety on the biology of *Bemisia afer* (Priesner and Hosny) (Hemiptera, Aleyrodidae). Insect science and its application 13(3):459-465 Jun 1992

Naranjo, S.E. 2001.Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci.* Crop Protection 20, 835-852.

Nava-Camberos, U., Riley D. G., Harris, M. K. 2001. Temperatura and Host Plant Effects on development, Survival, and Fecundity of Bemisia argentifolii (Homoptera: Aleyrodidae) Environ. Entomol. 30(1): 55-63.

Oliveria, M.R.V., Henneberry, T.J., and Andersson, P. 2001. History, current status, and collaborative research projects for Bemisia tabaci. Crop Protection 20, 709-723.

Poprawski, T. J. Greenberg, S.M., Ciomperlik, M.A. 2000. Effect of Host Plant on *Beauveria bassiana*- and *Paecilomyces fumosoroseus*- Induced Mortality of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) Environ. Entomol. 29(5): 1048-1053 (2000)

Poprawski, T. J. & Jones, W. J. 2000. Host plant effects on activity of the mitosporic fungi *B. bassiana* and *P. fumosoroseus* against two populations of *Bemisia* whiteflies. Mycopathologia 151, 11-20.

Zare R and Gams W. 2001. A revision of Verticillium section Prostrata. IV. The genera Lecanicillium and simplicillium gen.nov. Nova Hedwigia 73:1-50.

Åsman, K., Zegarra O., Kroschel, J., Ekbom, B. 2007. Tritrophic interactions between whiteflies, insect pathogenic fungi, and host plant.

8.2 Photos and References from Internet

Ethel Cavén, CIP, Lima, Peru 2007.

Kathryn Sparks, AQIS, Victoria, www.padil.gov.au/img.aspx?id=1967&s=s

9 APPENDIX

9.1 TABLES AND DIAGRAMMES

Complementary diagrams and tables for the thesis.

Diagram 12 Mean mortality of B. afer with three different fungi and three doses on beans.

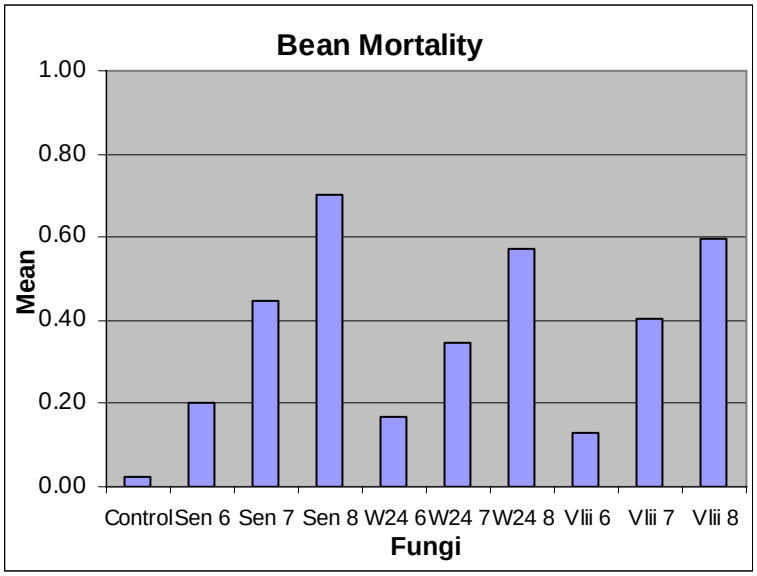

Diagram 13 Mean mortality of B. afer with three different fungi and three doses on chilli peppers.

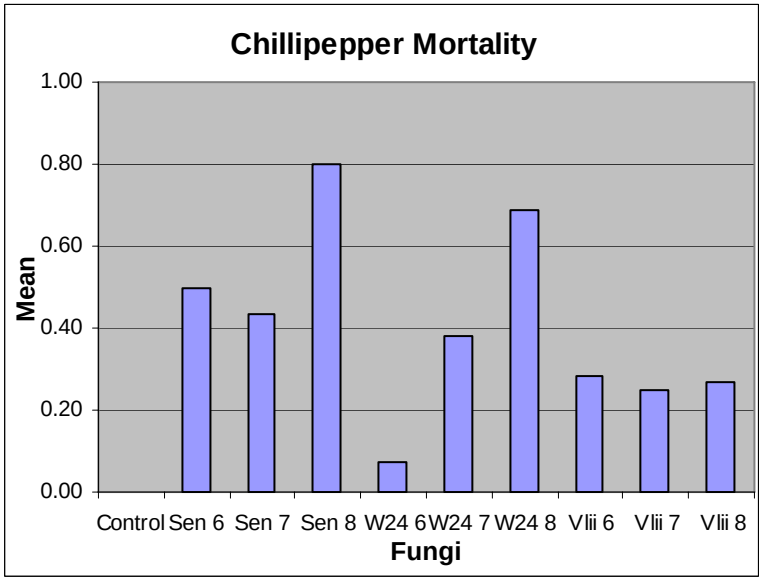

Diagram 14 Mean mortality of B. afer with three different fungi and three doses on tomatoes.

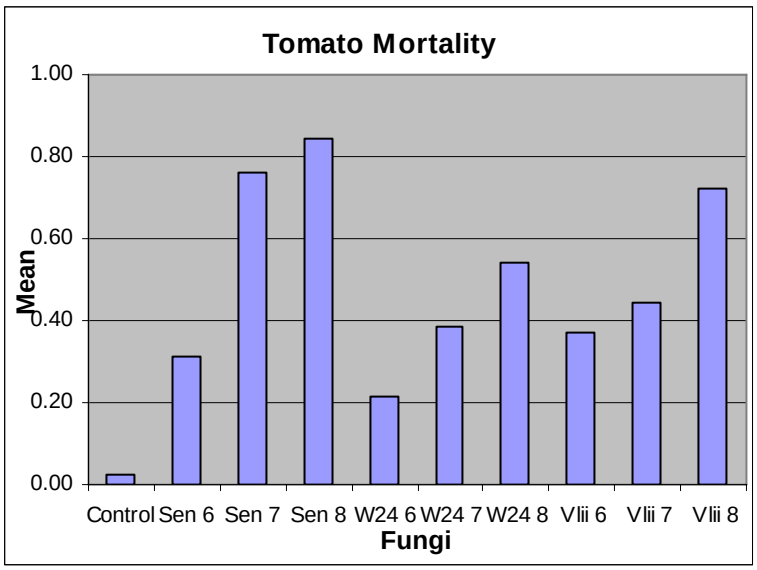

Diagram 15 Mean mortality of B. afer with three different fungi and three doses on sweet potatoes.

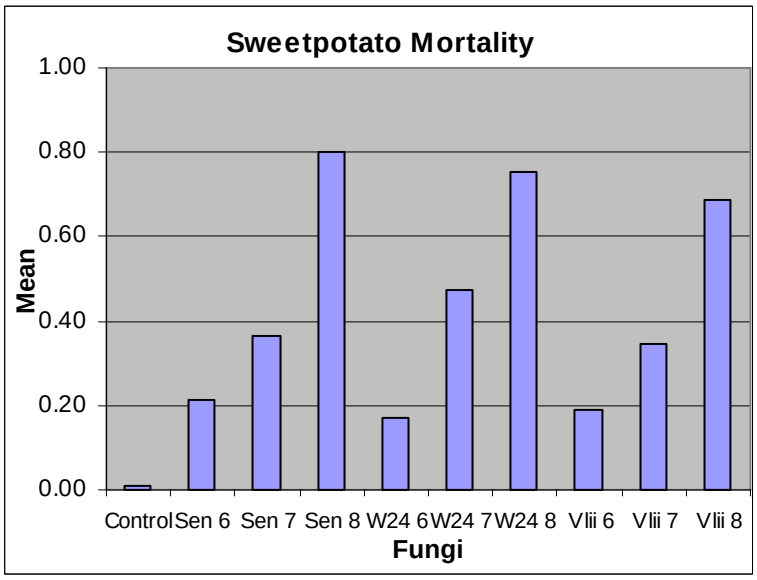

Table 5 Mean value of the mortality of nymphs on host plants and the effects on activity of fungi against whiteflies

	Sen	W24	V. lii	F	Df	P
Bean 10^6	0.20	0.17	0.13	0.16	2.21	0.85
Bean 10^7	0.45	0.34	0.40	0.62	2.21	0.55
Bean 10^8	0.70	0.57	0.60	0.26	2.22	0.77
Chill pepper 10^6	0.50	0.07	0.29	1.13	2.80	0.37
Chill pepper 10^7	0.43	0.38	0.25	0.58	2.18	0.57
Chill pepper 10^8	0.80	0.69	0.27	2.03	2.12	0.17
Sweet potato 10^6	0.21	0.17	0.19	0.15	2.30	0.86
Sweet potato 10^7	0.36	0.47	0.35	1.19	2.31	0.32
Sweet potato 10^8	0.80	0.76	0.69	1.53	2.31	0.23
Tomato 10^6	0.31	0.22	0.37	0.47	2.16	0.63
Tomato 10^7	0.76	0.39	0.44	2.31	2.15	0.13
Tomato 10^8	0.84	0.54	0.72	1.65	2.21	0.22

Table 6 Date in life table, in between which days in the study the development stages occurred.

	Egg	Crawler	2nd instar	3rd instar	4th instar	Adult
Tomato	9 March-27 March	22 March – 30 March	26 March - 14 April	29 March – 26 April	24 April – 6 May	26 April - 8 May
Bean	9 March – 28 March	22 March – 30 March	26 March - 10 April	28 March – 26 April	17 April – 2 May	30 April - 6 May
Sweet potato	9 March – 27 March	22 March – 30 March	26 March - 11 April	29 March – 26 April	17 April – 6 May	26 April - 8 May
Chilli pepper	9 March – 26 March	22 March – 29 March	26 March – 9 April	30 March – 23 April	13 April – 2 May	23 April - 8 May

Table 2 shows by date when the study were done and in between which days the different development stages occurred.

Table 7 Lifecycle stages at there longest stay in each development stage, in days.

	Egg	Crawler	2nd instar	3rd instar	4th instar	Adult
Tomato	18	8	19	28	10	10
Bean	19	8	15	29	15	6
Sweet potato	18	8	16	28	19	10
Chilli pepper	17	7	14	24	20	13

Table 7 shows the longest period of each development stage and the differences between the host plants.

Table 8 Lifecycle stages and time variations between different host plants and development stage, in days.

	Egg	Crawler	2nd instar	3rd instar	4th instar	Adult
Tomato	16±3	6±2	11±8	27±1	7±5	10±5
Bean	16±3,5	6±2	9,5±6,5	24,5±4,5	14±1	6±4
Sweet potato	16±3	6±2	9±6	23±5	14±5	5±5
Chilli pepper	16±3,5	5,5±1,5	14±5	24±5	16±4	9±4

Table 8 shows differences between *B. afer* lifecycle stages in time between different host plant species. Egg hatches between 13 to 19 days as longest. Crawlers varied between 3 to 4 days during a 7 till 8 days period. Second instars stage was harder to see but varied between 3 till 10 or 16 days during a 14 to 19 days period depending on host plant. Third instar was the longest stage for all host plants. Between 14 and 20 days in a 24 to 29 days long period lasted the nymphs in this stage. The fourth instar varied between 3 to 5 days in a 10 till 20 days long period.

Table 9 Survival stages on *B. afer* on four different host plants.

	Total eggs	Crawler	2nd	3rd	4th	adults	% eggs	% Crawler	% 2nd	% 3rd	% 4th	% adult
Tomato 1	9	9	6	4	4	4	100	100	67	44	44	44
Tomato 2	4	4	4	4	4	4	100	100	100	100	100	100
Tomato 4	20	19	14	11	10	10	100	95	70	55	50	50
Tomato 5	14	8	5	5	5	5	100	57	36	36	36	36
Total Tomato	**47**	**40**	**29**	**24**	**23**	**23**	**100**	**86**	**62**	**51**	**49**	**49**
Bean 2	2	2	1	1	1	1	100	100	50	50	50	50
Bean 5	11	10	5	3	3	3	100	91	45	27	27	27
Total Bean	**13**	**12**	**6**	**4**	**4**	**4**	**100**	**92**	**50**	**33**	**33**	**33**
Sweet potato 1	17	15	15	8	8	8	100	88	88	47	47	47
Sweet potato 2	37	37	35	29	28	28	100	100	95	78	76	76
Sweet potato 3	29	28	27	27	26	26	100	97	93	93	90	90
Sweet potato 4	11	11	11	9	6	6	100	100	100	82	55	55
Total Sweet potato	**94**	**91**	**88**	**73**	**68**	**68**	**100**	**97**	**94**	**78**	**72**	**72**
Chilli pepper 1	6	6	3	3	3	3	100	100	50	50	50	50
Chilli pepper 2	20	20	16	16	15	15	100	100	80	80	75	75
Chilli pepper 3	13	8	8	6	6	6	100	62	62	46	46	46
Chilli pepper 4	9	7	5	5	5	5	100	78	56	56	56	56
Total Chilli pepper	**48**	**41**	**32**	**30**	**29**	**29**	**100**	**85**	**67**	**63**	**60**	**60**

The table 9 shows both in quantity and percent how much survival on each leaf and total of nymphs on different development stages. The data shows also how many adults survived all the life cycle.