Wageningen University & Research Centre

Plant Sciences

Laboratory of Entomology

ENT-70436 Internship Entomology

Master of Plant Sciences

Research and Development

Koppert Biological Systems

Aguilas – Murcia, Spain

Carmen Isabel Castillo Carrillo

Supervisors:

Professor Dr Joop van Lenteren

Dr Jose Eduardo Belda

September - December 2007

Acknowledgements

I would like to thank my supervisors Professor Dr Joop van Lenteren and Dr Jose Eduardo Belda.

I also thank the head of R&D of Koppert Dr Karel Bolckmans for this opportunity.

I am very thankful to Javier Calvo, María José Lorente and Ana Gallego.

I also thank all the employees at Koppert - Aguilas.

Table of contents

Table of figures

Introduction

Kopper is an International Enterprise that operates in the production of biological control of pests and natural polinization around the world (Netherlands, England, France, Italy, Spain, United States, Canada, Mexico, Turkey, Kenya, Poland, Morocco, South Korea and New Zealand).

Koppert has a Research and Development Department oriented to find and study new parasitoids and predators to solve agricultural problems, also insects for polinization are produced. Under the supervision of this department, there is a very important branch dedicated to do the quality control of the production.

I have worked four months in R&D in Aguilas-Murcia (Spain) from September to December. Positive work experience has been obtained.

The pictures used in this report were obtained from the internet (mainly from Koppert and WUR web pages).

Objectives

- Learning and managing quality control techniques used for commercialized biocontrol agents.
- Knowing and recognizing the biocontrol agents.
- Develop experiments for biocontrol agents.
- Keeping the auxiliary insect rearing.
- Visiting greenhouses to observe biocontrol agents in the field.

Methodology

The quality control was made following the company's protocols. First learning how to recognize the biocontrol agents and their possible contaminants, and then getting acquaintance with the different steps defined in the protocol for each biocontrol agent. Quality control was done every day according to the market demand.

Experiments were developed to observe the establishment of *Nesidiocoris tenuis* in tomato plantlets in nursery and to observe the number of eggs of *Orius laevigatus* after certain storage time. *Ephestia kuehniella* was used as food in both experiments. The experiments were developed during three months with daily observations.

The rearing of *Nezara viridula* and *Trissolcus basalis* was maintained for future research. Twice per week both rearings were cleaned and fed.

One visit to greenhouses in Almeria was done. Some paprika greenhouses that have biological control pest management were visited.

Results

The aim of this internship was to be acquainted with the protocols for quality control of the products produced and received in Aguilas-Murcia and to recognize the biocontrol agents. I will describe the biocontrol agents in which I was doing the quality control.

Some experiments were developed to observe oviposition quantity of *Orius laevigatus* and the establishment of *Nesidiocoris tenuis*. Unfortunately, results could not be obtained due to death of insects and plants in spite of the fact that experiments were repeated. Also, the conditions inside the growth chamber were not stable.

Rearing of *Trissolcus basalis* and its host *Nezara viridula* were kept. I will describe the insects further in *Results*.

A field trip was done to visit some greenhouses around Almeria and biocontrol agents such as *Ambliseious swirskii*, *Eretmocerus* spp. and *Nesidiocoris tenuis* were observed in paprika crops.

Quality control of products

I will describe the products (biocontrol agents) in which I was doing quality control during my Internship and the main pests that are managed with them.

Products against acari

The pest

Tetranychus urticae (red mite) is the most spread and dangerous mite because of its poliphagous voracity and chemical resistance. The commercial history of biocontrol in horticultural greenhouses starts against this pest. In the 60´s, Koppert started to release *Pthytoseiulus permisilis* (Spidex) in pumpkins but nowadays there are other important products produced by the company based on predators as the mite *Amblyseius californicus* (Spical), the fly *Feltiella acarisuga* (Spidend) and the stinky bug *Macrolophus caliginosus*.

In favourable climatic and microclimatic conditions *T. urticae* population will grow extremely fast and with the use of chemicals the development of resistance could be also very fast. Another dangerous characteristic for having this mite as one of the most important pests is the high capacity of reproduction. One female can lay 130 eggs at 20-25^{o}C in roses. The regular sex-ratio is 1 male : 3 females. Only one fecundation is necessary to fertilize all the eggs.

Both, nymphs and adults cause damage in the host plant. It is common to find them in the back part of the leaves chewing cell tissues and drinking sap. The pant stars to show chlorotic symptoms as yellow regions in the leaves. The leaves dye and the whole plant can dye as well. Also, the production of nets by the mites can affect the final product (ornamentals).

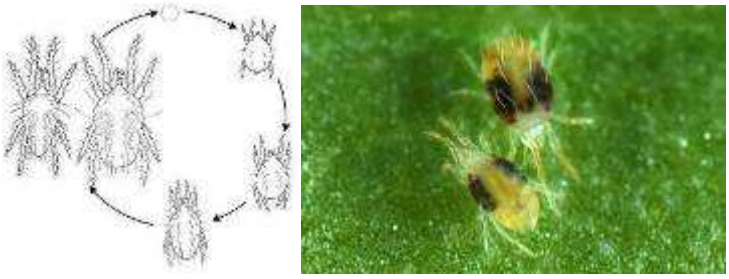

Figure 1 ***Tetranychus urticae*****. Life cycle and adults.**

Spidex

The predator

Phytoseiulus persimilis is a predator mite that belongs to Phytoseiidae family. Its origin is South America and it was accidentally imported to Europe in 1958. Once it was found by scientists, it was distributed around the world.

P. persimilis is a specific and a very efficient predator of *T. urticae* and it is better when the temperatures are around 20 ^{o}C. Its sex-ratio is 1 male : 4 females, superior to mite pests. Another advantage is the shorter life cycle of this predator. Therefore, the increment of the predator population will be faster than the one from the mite pest.

Nymphs and adults of *P. persimilis* feed on every stage of the red mites. The best temperature for this predator is between 15 and 25 ^{o}C. Changes in temperature and humidity of the environment can limit the efficiency of the predator due to the different behaviour and distribution of the predator and its prey in low humidity and high temperature.

Figure 2 ***Phytoseiulus persimilis*****. Scheme and adult feeding on a mite pest.**

Products against whitefly

The pest

Thialeurodes vaporariorum and *Bemisia tabaci* belongs to Aleyrodidae family. These pests are important in many crops around the world. Its wide range of plant hosts, direct feeding, capability of virus transmission, high level of reproduction and chemical resistance make this pest as a real problem.

Nymphs and adults suck sap mainly from the back part of the leaves of their host plants. Also it is important to mention that nymphs secrete sugar components in which *Cladosporium* spp. grow abundantly and diminish the quality of the products and reduces photosynthesis.

Since pesticides start to be overused, these pests acquired resistance and the damage became devastating and uncontrollable. The biological control of the whitefly complex is taking place as the most clean and efficient way to manage these pests. *Eretmocerus mundus*, *Eretmocerus eremicus*, *Encarsia formosa* as parasitoids, *Macrolophus caliginosus, Amblyseius swirskii* as predators and *Verticillium lecanii* as entomopathogen are important natural enemies of whiteflies.

Figure 3 ***Bemisia tabaci*****. Life cycle, adult emergency and adults.**

Bemipar

The parasitoid

Eretmocerus mundus is a parasitoid wasp belongs to Hymenoptera: Aphelinidae. Aphelinidae are minute parasitic wasps (body length from 0.58 to 0.8 mm) that primarily attack whiteflies, armored scales, aphids and other Aphelinidae. *E. mundus* parasitize *Bemisia tabaci* and females prefer to lay eggs underneath the third instar of its host. E. mundus is naturally distributed in the Mediterranean countries and spontaneously appears where *B. tabaci* is present. Its sex-ratio is a 40% of males and a 60% of females. Females lay a rate of 171 eggs on pepper during its life period (around 10 days).

Figure 4 ***Eretmocerus mundus*****. Adult is laying an egg underneath a whitefly nymph.**

Ercal

The parasitoid

Eretmocerus eremicus belongs to Aphelinidae family. This species is from the South West of the United States. *E. eremicus* parasites Aleyrodes sp., *B. tabaci*, T. vaporariorum and *T. abutilonea*. The advantage of the parasitoids is they develop faster than their hosts and have less sensibility to pesticides than *E. Formosa*. It is important as well to mention *E. eremicus* maintains a normal activity in high temperatures: 30-40 ^{o}C. One female can

oviposit from 50 to 200 eggs. Female adults need to feed on nymphs of *T. vaporariorum* for the egg production thus one young female can kill 30 nymphs per day.

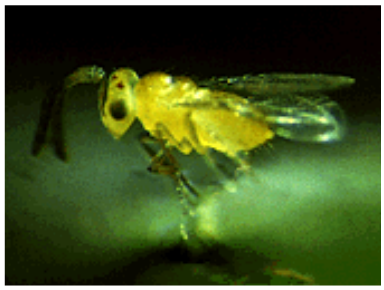

Figure 5 *Eretmocerus eremicus*. Adult.

En-Strip

The parasitoid

Encarsia formosa is a parasitoid wasp of *T. vaporariorum* that belongs to Hymenoptera: Aphelinidae. *E. formosa* parasites *B. tabaci* as well but prefers Trialeurodes. This parasitoid develops faster than its host. In temperatures lower than 12 ^{o}C and higher than 38 ^{o}C the wasp does not survive. *E. formosa* has a population conform only by females due to the presence of *Wolbachia* bacteria inside its organism. These bacteria induce changes in the reproduction then generations of females are produced by parthenogenesis. In optimum conditions a female can lay eggs in a rate from 5 to 15 per day in a total of approximately 150 eggs.

Figure 6 *Encarsia formosa*. Adult.

Nesibug

The predator

Nesidiocoris tenuis belongs to Miridae family and it is a zoophytophagous insect with biological control actibity. *N. tenuis* can control pests as whiteflies, leafminers, butterflies and spider mites. However, since it is a zoophytofagous insect it can cause fruit blemishes and flower abortion in plants as tomato. Nevertheless, *N. tenuis* cannot complete its development on a strictly phytophagous diet. With supplentary food such as *Ephestia kuehniella* eggs it is possible to allow colonization of this predator early in the crop before damage is likely to occur and before pests become established.

Figure 7 *Nesidiocoris tenuis*. Adult.

Swirski-mite

The predator

Amblyseius swirskii is a predator mite that belongs to Phytoseiidae family. Its origin is the west part of the Mediterranean region. This generalist predator feeds on thrips larvae, eggs, larvae and adults of *B. tabaci*, *T. vaporariorum* and mites. Also, *A. swirskii* can feed pollen meaning it can be established in the field before the pests arrive to the host plant. Since it comes from desert areas it can bear extreme conditions inside the greenhouses. At 26 ^{o}C the life cycle can be completed in 5-6 days.

Figure 8 *Amblyseius swirskii*. Predator adult feeding on whitefly and predator adults feeding on a thrips nymph.

Products against aphids

The pest

Aphids are important pests that ingest sap from the host plant resulting in yield reduction inclusive a complete lost of production is common. Aphids secret honeydew and *Cladosporium* spp. grows fast causing quality reduction in fruit and ornamentals. Aphids are considerate important pest in many crops due to their capacity of virus transmission and their high reproduction rate. Aphids are viviparous and their reproduction is by parthenogenesis. In 6-7 days nymphs become adults and one female can have a progeny of 40–100 individuals. In few days, some individuals can increase the population very fast and causing complete damage in the host plant.
Species like *Myzus persicae*, *Aphis gossypii*, *Macrosiphum euphorbiae*, *Aulacorthum solani* are polyphagous on a wide host range. Aphid pests such as *Sitobion avenae* and *Rhopalosiphum padi* are used as host for parasitoids commercial production. Biological control took place first in Netherlands in 1988. Aphids can be controlled with natural enemies such as *Aphidius colemani* (Aphipar), *A. ervi* (Ervipar) and *Aphelinus abdominalis* (Hymmenoptera) (Aphilin), *Chrysoperla carnea* (Neuroptera) (Chrysopa, commercial

name), *Episyrpus balteatus* (Syrphidend) and *Aphidoletes aphidimyza* (Diptera) (Aphidend), *Adalia bipunctata* (Coleoptera) (Adalia, commercial name), *Orius* spp. (Hemiptera), and *Verticilium lecanii* (Fungus).

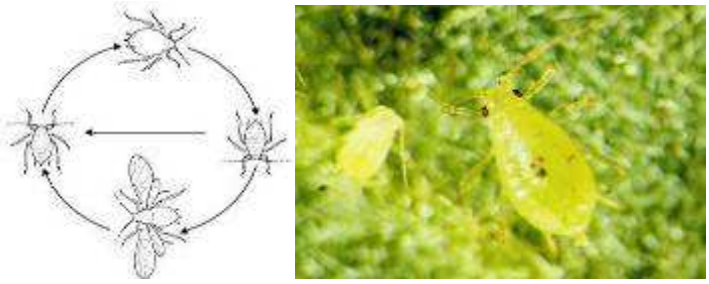

Figure 9 *Myzus persicae*. General life cycle and nymphs.

Aphipar

The parasitoid

Aphidius colemani belongs to the Braconidae family. It parasites 40 aphid species but is specific for *Aphis* and *Myzus*. The development of a new population of this wasp can be very fast. One female oviposit more than 300 eggs. Almost all the eggs are laid in the three next days after adult emergency. The sex-ratio is 2 females per one male. *A. colemani* is very precise and fast finding its host insect even if the aphids are in low densities.

Figure 10 *Aphidius colemani*. Typical position of an Aphidiidae during oviposition and mummies.

Products against thrips

The pest

Detrimental thrips belong to Thripidae family (Thysaoptera). Thips are important pests world wide because they transmit virus and cause severe damage in ornamentals and vegetables. Species such as *Frankliniella occidentalis*, *Thrips tabaci*, *T. fuscipennis* and *Echinothrips americanus* are most common present in greenhouses. Thrips scrape superficial plant tissue and suck sap. The surrounded tissue becomes silver-grey and together with their black excrement are symptoms and signals for their presence in the crop. Its reproduction can be sexual or asexual (parthenogenesis). Biological products such as Thripex (*Amblyseius cucumeris*), Thripor (*Orius* spp.), Thripans (*Amblyseius degenerans*), Swirskii-mite (*Amblyseius swirskii*), Mycotal (*Verticillium lecanii*) and Entomite (*Hypoaspis aculeifer*/*miles*) are used agains this pest.

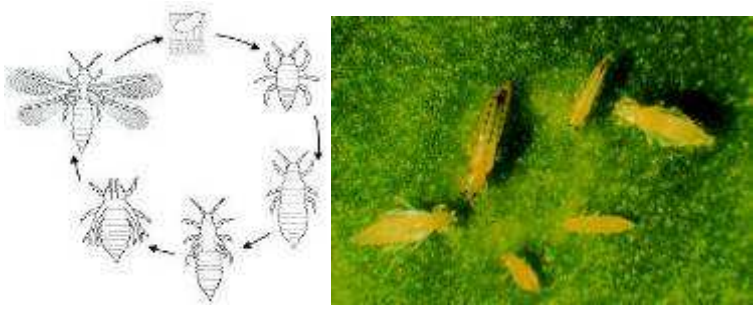

Figure 11 *Frankliniella occidentalis*. Life cycle and adults / nymphs.

Thripor

The predator

Orius laevigatus are good flyers and fast hunters so they excellent biocontrol agents against thrips and whiteflies. Nymphs and adults eat 12 thrips per day. *O. laevigatus* is well distributed in the Mediterranean region and the north of Africa. Females lay around 165 eggs during its life. Lower temperatures than 11 ^{o}C stop the normal development, meanwhile between 25 and 30 ^{o}C the development of eggs and nymphs are 12-18 days.

Figure 12 *Orius laevigatus*. Adult and its prey (whitefly nymph).

Rearing

Nezara viridula

The pest

Nezara viridula belongs to Pentatomidae family. *N. viridula* is an important pest around the world in tropical and subtropical areas. This stink bug causes damage in tomato, pepper and eggplant. Females lay eggs in groups of 30 – 130 on the back side of the leaves. The development takes place during 3-10 weeks depending on the environmental conditions. The best temperature for its development is 30 ^{o}C.

Figure 13 *Nezara viridula*. Adult laying eggs.

Trissolcus basalis

The parasitoid

Trissolcus basalis belongs to Pentatomidae family. It is a solitary egg parasitoid of *Nezara viridula*. This biocontrol agent is important and widespread, having already been used in biological control programs in many countries around the world.

Figure 14 *Trissolcus basalis*. Adult over *Nezara* ovipositions and *Nezara* ovipositions parasitated by *Trissolcus*.

Field trip

Figure 15 Greenhouses visited in Almeria.

References

Calvo J & A Urbaneja, 2004. *Nesidiocoris tenuis*, un aliado para el control biológico de mosca blanca. Horticultura Internacional, 44: 20-25.

Correa-Ferreira BS & E Moscardi, 1996. Biological control of soybean stink bugs by inoculative releases of *Trissolcus basalis*. *Entomologia Experimentalis et Applicata* 79: 1-7.

Malais MH & WJ Ravensberg, 1992. Conocer y reconocer las plagas de los cultivos protegidos y sus enemigos naturales. Koppert B.V. Netherlands.

Urbaneja A, G Tapia & P Stansly, 2005. Influence of host plant and prey availability on developmental time and suviorship of *Nesidiocoris tenius* (Het.: Miridae). Biocontrol Science and Technology, 15: 513-518.

Appendix

Figure 16 Some staff members and guests (Koppert Almeria and Aguilas).