Integrated Pest Management Component of the Ethio-Dutch Program for Horticulture Development

Contribution of Wageningen University and Research – Final Report

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Report WPR-711

WAGENINGEN
UNIVERSITY & RESEARCH

Referaat
De Ethiopian Horticulture Producer Exporters Association (EHPEA) is verantwoordelijk voor het Ethio-Dutch Program for Horticulture Development (EDPHD). Een van de componenten is het Integrated Pest Management (IPM) programma dat is uitgevoerd door Wageningen University and Research, Business Unit Glastuinbouw in samenwerking met Ethiopische onderzoeksorganisaties en EHPEA. Dit rapport van het WUR team geeft verslag van de volgende activiteiten: 1) Technisch advies op het gebied van formulering van de beste microbiële bca's die in het onderzoek zijn gevonden, 2) Zoeken, verzamelen en vercommercialiseren van inheemse natuurlijke vijanden voor het beheer van belangrijke plagen in de tuinbouw, 3) Ondersteuning en advies op het gebied van biologische studies van geïdentificeerde potentiële inheemse natuurlijke vijanden, 4) Ondersteuning en advies op het gebied van kleinschalige massaproductie voor evaluaties en methoden om persistente populaties in gewassen te vestigen, 5) Technisch advies op het gebied van efficacy (werkzaamheid) evaluaties in laboratorium- en veldomstandigheden voor de geïdentificeerde predatoren en parasitoïden, 6) Evaluatie van de IPM cursus gegeven aan de Jimma universiteit, 7) Het delen van informatie met tuinders en bedrijven, 8) Training van trainers, en 9) Alle verzamelmissies en andere missies.

Abstract
The Ethiopian Horticulture Producer Exporters Association (EHPEA) is responsible for the Ethio-Dutch Program for Horticulture Development (EDPHD). One of the components of this program is the Integrated Pest Management (IPM) which has been executed by Wageningen University and Research, Business Unit Greenhouse Horticulture in collaboration with Ethiopian research organizations and EHPEA. This final report of the WUR team reports on the following project activities: 1) Technical advice on formulation of the best microbial bca's obtained from the conducted research, 2) Survey, collection and commercialization of indigenous natural enemies for the management of major horticultural pests, 3) Support and advice on the study of the biology of identified potential indigenous natural enemies, 4) Support and advice on small-scale mass production for evaluation trials and methods to establish persistent populations in crops, 5) Technical advice on the efficacy trials under laboratory and field conditions on the identified predators and parasitoids, 6) Review of IPM course delivered at Jimma University, 7) Sharing information with growers and companies, 8) Train the trainer, and 9) All collection and other missions.

Photo backside cover (in the right circle):
Orius kokai, a new described species from Ethiopia and an important predator of thrips.

Reportinfo

Report WPR-711
Projectnumber: 3742214400
DOI number: https://doi.org/10.18174/428600

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Summary

The Ethiopian Horticulture Producer Exporters Association (EHPEA) is responsible for the Ethio-Dutch Program for Horticulture Development (EDPHD). One of the components of this program is the Integrated Pest Management (IPM) which has been executed by Wageningen University and Research (WUR), Business Unit Greenhouse Horticulture in collaboration with Ethiopian research organizations and EHPEA.

This is the final report of the team of Wageningen University and Research, Business Unit Greenhouse Horticulture, on its activities in this project. This document reports on the following project activities:

- Technical advice on formulation of the best microbial bca's obtained from the conducted research.
- Survey, collection and commercialization of indigenous natural enemies for the management of major horticultural pests.
- Support and advice on the study of the biology of identified potential indigenous natural enemies.
- Support and advice on small-scale mass production for evaluation trials and methods to establish persistent populations in crops.
- Technical advice on the efficacy trials under laboratory and field conditions on the identified predators and parasitoids.
- Review of IPM course delivered at Jimma University.
- Sharing information with growers and companies.
- Train the trainer.
- All collection and other missions.

1 Introduction

The Ethiopian Horticulture Producer Exporters Association (EHPEA) is responsible for a program by the name the Ethio-Dutch Program for Horticulture Development (EDPHD). One of the components of this program is the Integrated Pest Management (IPM) which is executed by Wageningen University and Research (WUR), Business Unit Greenhouse Horticulture in collaboration with Ethiopian research organizations and EHPEA.

The IPM component knows a number of foci:
1. DNA identification of antagonists.
2. Technical advice on formulation of bacterial control agents and small-scale field trials.
3. Collection, identification and research on indigenous predators.
4. University course review.
5. Sharing information with growers and companies.
6. Training of EHPEA trainers.

The project commenced with an inception mission in August 2015, resulting in a formal start early 2016. WUR has fulfilled its tasks by conducting a number of missions (see Annex 1), including their preparation and follow-up, and by identifying and researching indigenous predators from Ethiopia. Various external causes have resulted in undesired delays, however, at the time of the end-of-project workshop on February 28 and March 1, 2017, most activities have been fulfilled. A limited number of remaining activities will be reported as soon as they are finished in a final update of this intermediate report [DNA analysis, PCR training, identification of potential predators collected early March 2017, Technical advice on the small scale farm trial of the formulated entomopathogens and bacterial antagonists products].

We thank EHPEA, in particular its Director Tewodros Zewdie, its Coordinator IPM Dr. Adhanom Negasi, and its coordinator Training, Helina Getachew for their pleasant and fruitful collaboration. We also thank the Royal Netherlands Embassy and Dr. Eefje den Belder of Wageningen University & Research for their supportive roles.

Bleiswijk and Wageningen,
November 2017

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2 DNA molecular identification

This activity is numbered as follows in the project plan:

WP 1 Generation of IPM technology & on-farm implementation.

1.1 Development of sustainable fungal microbial control agents for major insect pests.

1.1.1 DNA molecular identification at the sub-species level of selected entomopathogenic fungi obtained from the trails conducted by Addis Ababa University.

1.2 Pilot scale formulation, production and application of biological control agents.

1.2.1 DNA and MALDI-TOFF molecular identification of the selected entomopathogenic bacteria obtained from the preliminary trials conducted by Addis Ababa University.

2.1 DNA molecular identification of selected entomopathogenic fungal antagonists

Fungal taxonomy has been revolutionized by the application of molecular methods based on DNA sequence-based classification and identification, namely, DNA barcoding. DNA barcoding is defined as the standardized analysis of an easily amplifiable PCR fragment for sequence-based identification of species.

In fungi, the internal transcribed spacer region (ITS) is the official DNA barcode. Sequence comparison of the ITS region is widely used in taxonomy and molecular phylogeny because it is easy to amplify even from small quantities of DNA and has a high degree of variation even between closely related species. Using the ITS marker alone for identification might not be sufficient in certain fungal clades, and it may be necessary to sequence one or more single-copy protein-coding genes for certain fungal genera and/or lineages to obtain a more precise identification at the species level. Due to the limitations of a single-marker barcoding system in fungi, it has been recommended to use the intron-rich 5' end of translation elongation factor 1-alpha gene (tef1) as the best potential to serve as a secondary DNA barcode, followed by partial nucleotide sequences of DNA-directed RNA polymerase II largest (RPB1) and second largest subunit (RPB2).

The present study aims to use this molecular tool for identification of *Beauveria bassiana* and *Metarhizium anisoplae* isolates from the University of Addis Ababa.

Materials and methods

DNA samples that had been obtained from preliminary trials were sent by Dr. Yonas Chekol Belay of the Addis Ababa University by courier to Wageningen University & Research Greenhouse Horticulture (Dr. Yaite Cuesta Arenas). The DNA from several isolates were re-suspended in 200µl Tris Elution buffer (Qiagen) since they were sent to WUR in filter paper. From these isolates, M1 was the only one which we could not be able to perform any PCR amplification due to the DNA quality.

Partial regions of translation elongation factor 1-alpha (EF-1α), internal transcribed spacer (ITS4) and RNA polymerase II second largest subunit (RPB1 and RPB2) genes were used for the identification of the isolates B1, B2,B5 B6 and M2.

PCR amplifications started with an initial denaturation at 94°C for 3 minutes, followed by 30 cycles of 30 s at 94°C, 45 seconds at 55°C and 1 minute at 72°C and a final elongation for 5 minutes at 72°C. The PCR reactions were performed with GoTaq® Colorless Master Mix (Promega) in a CFX96 Real Time PCR Detection system (Biorad). The amplified fragment sizes were confirmed on safe DNA electrophoresis gel at 80 V for 1 hour. Sequencing was performed in both direction by BaseClear BV (Leiden, the Netherlands) using the same primers for the PCR reactions. The resulted nucleotide sequences were aligned using BLAST software from NCBI Center and created as Fasta format with EMBOSS Seqret (EMBL-EBI).

Table 2.1

Primers used in the amplification of the genes (mentioned in the text above).

Pathogen	Gene	Forward 5′ ® 3′	Reverse 5′ ® 3′	Fragment length
Beauveria bassiana	EF-1α	CACGTCGATTCCGGCAAGTC	CGATCTTCTCGAGGAGCTC	1490
	ITS	GGAAGTAAAAGTCGTAACAAGG	TCCTCCGCTTATTGATATGC	550
	RPB1	CCATGAAGCTTTGGAGCTTGC	CCATCAAGTTGCCTCGCAGAC	752
	RPB2	GTTAAGCCTGGCACGCTTTCG	AATTGGCGCAGTTTTGCCAATG	1489
Metarhizium anisoplae	EF-1α	CACTTTTCGCCGTCTCGAG	AGGACACCAGTCTCGATACGGC	1145
	ITS	GGAAGTAAAAGTCGTAACAAGG	TCCTCCGCTTATTGATATGC	550
	RPB1	GAAGAACGGTCCCTTGATGGG	GCCTGTCTCTGCAGTCTTGACAG	957
	RPB2	TGTTGGTATCAAGCCTGGA	ACCCGAGTAGCAGGCAATG	1161

Results

These primers (markers) can be successfully used to identify *B. bassiana* and *M anisoplae* by PCR. Gene sequences generated for each isolate are given below. These can be used to generate shorter fragments for qPCR amplification. Furthermore they can be submitted to Genebank database.

The sequences are presented in Annex 9.

2.2 DNA molecular identification of the selected entomopathogenic bacterial antagonists

Unfortunately, DNA samples were sent but were not suitable for analysis.

3 Technical advice on formulation and on small-scale farm trials

This activity is numbered as follows in the project plan:

WP 1 Generation of IPM technology & on-farm implementation.

1.2 Pilot scale formulation, production and application of biological control agents.

1.2.2 Provide technical advice and a document on formulation of the best microbial bca's (*Metarhizium anisopliea*, *Beauveria bassiana*, *Bacillus subtilis* and *Pseudomonas flourescence*) obtained in the preliminary trials conducted by the Addis Ababa University.

1.2.3 Technical advice on the small scale farm trial of the formulated entomopathogens and bacterial antagonists products.

3.1 Technical advice on formulation of the best microbial bca's obtained from the conducted research

This item has been published before as: 'Upscaling and Formulation of BCA's in Ethiopia' by M. Streminska (2017).

3.1.1 Summary

This chapter contains information about mass production of bacterial isolates of *Bacillus* and *Pseudomonas*, as well as entomopathogenic fungi *Beauveria* and *Metarhizium*. These organisms have been isolated from soils in Ethiopia and are used as a biological control agents (BCA's) of *Fusarium* diseases (bacterial isolates) and insect pests (fungal isolates).

It describes briefly the methods and techniques currently available for upscaling of the production process of bacterial and fungal isolates. Additionally, possible product formulation options (to increase BCA's survival in the environment) are described in short.

Moreover, specific questions are answered such as: 1) question about the methods of preventing the bacterial isolates from losing their antifungal activity with time or 2) compatibility of isolates in prevention of disease when applied together.

3.1.2 Background information on BCA's obtained in Ethiopia

Bacillus subtilis and *Pseudomonas fluorescencens*, which had been collected by the team of prof. dr. Fasil of the Addis Ababa University, can be applied together (Prof. Dr. Fasil, personal communication). In general, the bacterial antagonists can be combined without negative effects on each other. Compatibility checks have been performed (isolates with chemical means to ensure good IPM). At present both of bacteria are mixed with local soil or alginates and freshly applied (with best effectivity). Question: Which carrier should be used and what type of formulation for upscaling and commercialization? Preference should be given to local biobased materials, such as rice grains/husks and coffee bran.

Both species (*Bacillus* and *Pseudomonas*) are isolated from black soil (clay) from carnation cultivation. They perform well in carnation, chickpea and hydroponics of peas. Owing to problems with Fusarium, freesia cultures have disappeared from that Ethiopian regions with black soil. So, these antagonists contain a promise for horticulture challenged with Fusarium pathogens. *Bacillus* and *Pseudomonas* isolates show PGPR activity, i.e., IAA production and siderophore production for enhanced Fe-uptake by plants. However isolates experience loss of virulence with aging cultures. Therefore, researchers from Ethipia think that re-isolation from soil will be needed in the future in order to ensure high quality isolates. Question: What are the options to minimise the loss of antifungal activity in isolated bacterial antagonists? Fast identification tools are necessary for bacteria. For *Bacillus*, the gyrA gene will be used for alignment and marker construction. For *Pseudomonas*, 16S rDNA will be used.

Beauvaria bassiana (2) and *Metarhizium sp.* (1) isolates were also obtained from nature in Ethiopia. They target whitefly, thrips, mealybugs (in roses). Advice on formulation is needed to ensure a long shelf-life and high virulence. Different carriers may be used, which one is the best within context of Ethiopia (costs and availability) and fitness of isolates, and large scale production (upscaling and commercialization).

3.1.3 Bacterial antagonists of Fusarium

3.1.3.1 Compatibility check between bacterial biocontrol agents

A check was performed by the team of Prof. Dr. Fasil to ascertain that biological isolates are compatible with chemical means of plant protection (pesticides). However, the question is whether checks were performed on compatibility of the two bacteria applied together. There are reports that co-inoculation of *Bacillus* and *Pseudomonas* species might not necessarily lead to higher biocontrol activity. This is possibly due to disruption of *Pseudomonas* biocontrol activity by *Bacillus* (quorum quenching in *Pseudomonas*).

Bacterial cells communicate with each other. This process is called quorum sensing. Many metabolic processes in bacteria, such as antibiotics production or virulence, are governed by quorum sensing. This basically means that bacteria will not start producing antibiotics until a certain threshold number of bacterial cells are present. Gram negative bacteria such as *Pseudomonas* use specific signal molecules for this cell-to-cell communication. Other bacteria, such as *Bacillus*, may produce enzymes which degrade the *Pseudomonas* signal molecules, while producing their own signal molecules. Generally quorum quenching (disruption of quorum sensing) has no effect on the numbers/growth of bacteria (in this case *Pseudomonas*) but its metabolism and biocontrol activity might be severely impaired. More information on quorum sensing can be found in the review article written by Grandclement *et al.* (2016).

3.1.3.2 Loss of biocontrol properties by aging cultures of *Pseudomonas* and *Bacillus*

It is advisable to not only maintain the isolates in a growth medium (on plates or in liquid medium). If they are maintained on nutrient rich medium (for example nutrient broth, Tryptic Soya Broth or Luria Bertani broth) they might lose their ability to produce siderophores and antibiotics over time. For each isolate, inoculum stocks should be prepared in sterile glycerol by mixing 0.5mL glycerol (80%) and 0.5mL of 24hrs liquid culture of specific isolate into a sterile Eppendorf tube (cryovials; 1.5mL). After thorough mixing stocks should be stored in -80°C freezer. Alternatively cultures of isolates should be freeze-dried. Inoculum samples prepared by freeze-drying can be stored for extended periods of time in the fridge (4°C) or at room temperature (22°C).

3.1.3.3 Production of bacterial biocontrol agents on the laboratory and semi-industrial scale

Bacillus

Bacillus sp. are aerobic endospore-forming bacteria. Their ability to produce endospores makes them by far the most researched group of bacteria in biocontrol. Endospores are produced when the environmental conditions are not favourable (for example lack of nutrients). Endospores are capable to withstand the stress caused by high temperature, high UV irradiation, desiccation, chemical damage and enzymatic destruction. Therefore using endospores as means of delivery of biocontrol to the soil has gained much attention.

Depletion of carbon, nitrogen, or phosphorous causes the process of sporulation to begin, however, the process needs to start before nutrients are exhausted of. Otherwise, the spore formation cannot be completed due to the fact that the nutrients are too low for the energy-requiring sporulation process.

Production of spores requires high cell density of bacterial cells and good sporulation efficiency. At laboratory scale, sporulation is normally induced by growth and nutrient depletion in media such as Difco Sporulation Medium (DSM) (Monteneiro *et al.* 2005; Monteneiro et. 2014). This medium should be prepared as follows (per litre): Bacto nutrient broth (Difco) 8 g, 10% (w/v) KCl 10 ml, 1.2% (w/v) $MgSO_4 \cdot 7H_2O$ 10 ml, 1 M NaOH ~1.5 ml (pH to 7.6). Adjust volume to 1 litre with ddH_20. pH to 7.6. Autoclave and allow to cool to 50°C. Just prior to use, add the following sterile solutions (and antibiotics if required): 1 M $Ca(NO_3)_2$ 1 ml, 0.01 M $MnCl_2$ 1 ml, 1 mM $FeSO_4$ 1 ml.

The process of sporulation usually begins after the exponential phase of bacterial growth, when nutrients become more depleted (however not completely). Under ideal conditions, the culture will initiate sporulation at a cell density of about 10^8 cells mL-1, and typical sporulation efficiencies will be in the range of 30-100%. During the growth phase of bacteria, samples should be taken to enumerate viable bacterial cells and endospores. For viable cell counts a standard method of dilution plating could be used. For counting endospores they should be first stained according to a protocol of differential staining technique (the Schaeffer-Fulton method). A primary stain (malachite green) is used to stain the endospores. Malachite green is forced into the endospores by heating. In this technique heating acts as a mordant.

There is also the possibility to produce bacterial inocula/spores on other substrates available locally. Examples of such substrates are: water extract of rice husks, molasses, organic wastes etc. (Abbasi *et al.* 2013; Korsten, 1996). In any case it is advisable to compare the production of viable cells/endospores on different substrates to the standard media (such as DSM or Luria Bertani).

Pseudomonas

Bacteria belonging to genus *Pseudomonas* are gram negative bacteria. In the laboratory they are usually grown on King B medium or standard non selective microbiological media such as nutrient broth, Luria Bertani broth etc. (Bisutti *et al.* 2015). However they also grow well on media based on agricultural waste products.

These bacteria, in contrast to *Bacillus* species, are not capable to produce endospores. Vegetative cells of *Pseudomonas* bacteria are far more susceptible to loss of viability during the process of drying, storage, and rehydration. Nonetheless gram negative bacteria have shown a high potential as a biocontrol agents. Usually they are cultivated in a liquid medium and added to the soil substrate as a liquid of freeze-dried powder. There are some carriers, for example talc, spent mushroom substrate, fly ash and lignite, which support survival of *Pseudomonas* species for more than 2 months when stored at the room temperature (Gade *et al.* 2014)

Few modifications to a production process were applied to increase the survivability of *Pseudomonas* inocula. During the cultivation process osmoprotectants (such as glucose, fructose, trehalose, raffinose and stachyose) are added to growth media (Cabrefiga *et al.* 2014; Bonaterra *et al.* 2006). Other osmoprotectants used successfully are for example lactose and skimmed milk (Cabrefiga *et al.* 2014).

3.1.3.4 Up-scaling of the production process (*Bacillus* and *Pseudomonas*)

The mass production of both *Bacillus* and *Pseudomonas* can be undertaken on a (semi-) industrial scale. Submerged fermentation (SmF) bioreactors are usually used for this purpose. Another option is using solid state fermentation (SSF), but it is less popular for bacterial inoculum production. In case of SmF substrate and organisms are present in bioreactor in a submerged form in large quantity of liquid medium. Advantages of SmF are: possibility to control the process parameters better (such as oxygen supply, management of pH of the medium). SmF can be set up as a batch or as semi-continuous process. For production of endospores of *Bacillus* batch or fed-batch process should be applied. Production of *Pseudomonas* could be optimised to be run continuously.

Mediums to use can be prepared from organic waste materials available locally (such as liquid manure, rice bran, cotton meal, molasses, and potato starch/dextrose, coffee husks) (Poopathi and Abidha, 2011; Abbasi *et al.* 2013). A medium should have a proper C:N ratio (optimal between 5-15). Preferable carbon source should be easily degradable sugars, such as glucose (around 20g/L medium). The choice of the medium for mass production of bacteria should be based on the results of comparison of bacterial performance (e.g. numbers and efficacy of endospore formation) in different media. The bacterial performance in a specific medium will depend on many factors and it is advisable to thoroughly check the bacterial growth parameters and production of active ingredients (such as siderophores or antibiotics) in the medium before starting a large scale production process.

The production process could be undertaken in laboratory based bioreactors with build-in environmental controls (pH, O_2, CO_2, mixing, aeration). Such bioreactors could be for example purchased from New Brunswick company. The disadvantage of this option is their relatively high price. Attempt could be made to design the bioreactor from materials which are cheaper and available locally. Important is to remember about incorporating the environmental controls into the design. Moreover a proper oxygen supply with a pump and means of mixing the growth medium are needed. Bioreactor should be made of a material which could be easily sterilised (for example by autoclaving it).

Formulation possibilities

Formulations are typically a mixture of "active" ingredient (in this case microorganisms, cells, spores in freeze-dried form or viable), carrier material and additives. Suitable carrier materials are fine clay, peat, vermiculite, alginate, and polyacrylamide beads, diatomaceous earth, talc, vermiculite, cellulose (carboxymethyl cellulose), biochar, organic waste materials (such as rice, wheat husks, biochar etc.). Additives such as gums, silica gel, methyl cellulose, and starch protect the micro-organisms from adverse environment conditions. Furthermore they influence the physico-chemical properties of formulations (Schisler *et al.* 2004). Extensive list and categories of different formulations available is given in the review paper by Malusa *et al.* (2012) and Mishra and Arora (2016).

To determine which formulation is the best under the Ethiopian conditions it would be advisable to choose a number the local agricultural waste products (rice husks, fly-ash, biochar etc.) and design the experiments to look at the survival of *Bacillus* and *Pseudomonas* in these carrier materials over an extended time period (e.g. 26 to 52 weeks) (Schisler *et al.* 2004; Hale *et al.* 2015). Bacteria could be added to the carrier as viable cells/ endospores (after removing the culture medium by for example centrifugation) or in a freeze-dried form.

Technical advice

Produce both bacterial cultures in a bioreactor with liquid medium (5-20L). Optimise *Bacillus* culture for a maximum production of endospores. *Pseudomonas* could be produced by continuous culture. Separate the bacteria/spores from the liquid medium by centrifugation at <5000rpm. Bacterial pellet can be re-suspended in buffered saline solution. Alternatively grow bacteria in the medium that would be suitable for freeze-drying the cultures.

3.1.4 Entomopathogenic fungi (*Beauveria* and *Metharhizium*)

3.1.4.1 Production of entomopathogenic fungi on the laboratory and semi-industrial scale

Beauveria and Metarhizium species are representatives of Ascomycete Hypocreales. In general, these fungi have three major propagule types that can be used. In nature, the aerial conidium is the primary infectious propagule. Conidia are the spores that are produced on the exterior of fungus-killed insects. Blastospores are the proliferative stages within the insect for these fungi and can also be produced in liquid fermentation. Under certain liquid fermentation conditions, mainly substitution of inorganic for organic nitrogen, Beauveria and Metarhizium can produce "microcycle" in which microconidia are produced. These conidia are not true conidia and are produced on the ends of hyphal strands. Conidia can be produced also under submerged culture or by a biphasic system. In the latter, fungus is first grown under submerged conditions (in liquid medium) to produce a large biomass of hyphae and then allowed to produce conidia in solid-state conditions. Conidia produced during solid state fermentation (SSF) are usually performing very well under field conditions, because the production process imitates the environmental conditions during the natural process of fungal multiplication.

Upscaling of the production process (*Beauveria* and *Metarhizium*)

There are two types of SSF used for production of entomopathogenic fungi on (semi-) industrial scale:

1. Cultivation on solid substrate acting as carrier and carbon source (mostly widely used).
2. Cultivation on inert carrier with addition of carbon source (rarely used), but could be advantageous. Inert carrier could be re-used after extraction of the spores and sterilisation.

The most common natural substrate for the production of entomopathogenic fungi is rice grains. They are widely available and have good characteristics as a dispersal carrier. The other option is using barley grains. However it would be beneficial to find an alternative substrates, due to the fact that both grains are also a staple food. Therefore, more research is needed into the use of agroindustrial waste such as wheat bran and rice straw, residual potatoes, sugarcane bagasse, coffee husk (Dalla Santa *et al.* 2005). Some of the substrates used for this purpose so far include: bagasse ± 2% dextrose, barley, beetroot, broken rice, broken rice + $CaCl_2$, carrot tubers, cassava chips, chickpea, coconut cake, cottonseed cake, finger millet, groundnut cake, maize, maize bran ± 2% dextrose; neem cake, potato tubers. Also waste products of animal husbandry could be used. In United States a process was developed for production of spores from whey (a lactose rich waste material from cheese production) (Kassa *et al.* 2008). There are also a few inorganic substrates in use: calcined diatomaceous earth (diatomite), clay granules (e.g. Seramis®). For the low cost production polyethylene bags filled with agro-industrial wastes could be used. Otherwise it is advisable to determine if building for example a packed bed reactor is a viable option. Conidial yields can vary among strains of each fungus species. For example, Arcas *et al.* (1999) determined that one strain of *B. bassiana* produced three times as many spores as a second under identical fermentation conditions. Therefore it is important to determine the conidial yields for each strain used. Jaronski *et al.* (2012) noted that conidial production of 15 *B. bassiana* isolates ranged from 1.11×10^{11} to 2.25×10^{13} conidia per gram of initial dry substrate when grown under identical solid substrate fermentation conditions.

Formulation possibilities

If the fungal conidia are not to be used directly the moisture content in the product of SSF should be lowered. Refrigerating of the whole sporulated solid substrate (not dried) will also result in a prolonged shelf life. It will however not be more than a few weeks.

If conidia are to be stored for a longer period of time they must be dried down to a moisture content <9% w/w or aw ≤ 0.3 (Jaronski, 2013). This low moisture is necessary for optimal shelf life regardless of whether conidia are formulated or not.

Drying methods include: 1) opening of plastic fermentation bags; 2) transfer of sporulated substrate to open trays or table tops; 3) transfer to Kraft paper sacks (Jaronski and Jackson, 2012) and 4) use of air-lift devices. In general, Beauveria conidia can be dried relatively quickly (within 2–3 days) without loss in viability, whereas Metarhizium conidia require slower drying (5–9 days) (Jaronski and Jackson, 2012). The most popular method to separate conidia from substrate is sieving (mechanical separation).

Conidia of entomopathogenic fungi could be formulated into the oil-based (eg. emulsifiable adjuvant oils, vegetable oils or mineral oils), water or solid formulations. There are contradictory reports in scientific literature about the possible influence on any type of formulation on survival of conidia. It seems like test are needed to evaluate the survival of conidia each time when starting to use a new formulation.

Technical advice
Consider producing the conidia of both entomopathogenic fungi in biphasic process (start with liquid inoculum; then continue with solid substrate fermentation) on rice as a solid substrate. The detailed protocol for the procedure can be found in an article by Seema *et al.* (2013). This procedure is relatively easily to adapt for other solid substrates (such as agro-industrial wastes). For the process 500 g polyethylene re-sealable bags could be used.

3.2 Technical advice on the small scale farm trial of the formulated entomopathogens and bacterial antagonists products

The degree of disease control obtained depends on the density of the biocontrol agent, the density of the pathogen, the efficiency of the biocontrol agent in suppressing the pathogen, and the proportion of the pathogen population that is potentially affected by the agent (Montesinos and Bonaterra, 1996; Smith *et al.* 1997). Differences in the mechanism of action of the biocontrol agents also affect the dose-response relationship of the isolates (Larkin and Fravel, 1999). Biological control agents must be at adequate population levels and be capable of effectively interacting with the pathogens or host to provide acceptable disease control. Knowledge on the relationships between biocontrol agent and pathogen inoculum concentration can determine the population levels of the biocontrol agent that are required to achieve adequate disease control, as well as the pathogen population levels at which the control agent will or will not be effective.

Here we provide an example how to evaluate the inoculum concentration relationship between the biocontrol agent and pathogen inoculum on biocontrol of Fusarium wilt with *Pseudomonas* fluorescens and *Bacillus subtilis* isolates.

Fusarium oxysporum
F. oxysporum strains are grown at 25°C on potato dextrose agar (PDA) plates by inoculation with conidia from frozen (-80°C) stock solutions. Flasks containing 250 ml of sterile 2% malt extract broth are inoculated with agar plugs (0.5 cm) overgrown with *F. oxysporum* from 10 days old PDA culture plates. Liquid cultures are grown for 10 days on a rotary shaker at 135 rpm at 25°C. Subsequently cultures are filtered through glass wool to remove mycelial mats. Conidia in the filtrate are pelleted by centrifugation (10 min, 5000 x g) and washed twice with 0.01M $MgSO_4$. The propagule suspension is adjusted to 1 x 10^7 conidia/ml.

Bacillus
The isolates are cultured for 2 days in tryptic soy broth at 28°C on a rotary shaker at 150 rpm. The culture is centrifuged at 3500 × g for 5 min at room temperature, the supernatant is discarded, and the bacterial pellet is washed three times with tap sterilized water. This pellet is re-suspended in tap sterilized water. The bacterial density is determined spectrophotometrically OD_{600} and adjusted to 2x10^9 CFU/ml.

Pseudomonas
P. fluorescens is first grown (from the stock) on King's B agar plates for two days at 27°C. Then, one colony is picked up and suspended in 10 ml King's B broth overnight at 27°C under constant agitation (150 rpm). The bacteria are centrifuged at 3500 × g for 5 min and the pellet is washed once and re-suspended in 0.01M $MgSO_4$ The bacterial density is determined spectrophotometrically OD_{600} and adjusted to obtain a suspension of 10^8 CFU/ml.

Soil preparation
For fungal inoculation: Potting soil is mixed with quartz sand in 1:2 ratio (vol/vol). This mixture is sieved (5 mm mesh) and autoclaved (120°C, 20 min). Conidial suspension is introduce into the soil mixture at concentrations ranging from 10^4 to 10^5 conidia/ g soil. Then, the soil samples are incubated for 5 days in the dark at 24°C for 16h, followed by 8h at 20°C.

For bacterial inoculation: Serial dilutions ranging from 10^6 to 10^9 are introduced (20ml/600 g soil) into autoclaved potting soil mixed with quartz sand 1:2 ratio (vol/vol). then the soil is incubated for 12 h at 25°C. In case of having a formulated product, this should be prepared in different percentages and mixed with soil. In case of not having sand, only soil can be used.

Bioassay
Fungal inoculated, bacterial inoculated and soil-quartz sand are thoroughly mixed in 1:2:4 ratio (w:w:w). Subsequently, pots are filled with 750 g soil on top of a bottom layer (3 cm) of hydrogranules. (It has to be adjusted to the size of the pots).

Fusarium disease assessment is arranged in a completely randomized design experiment (see PP 1/152, you can find it as appendix) with the following treatments: *Bacillus* or *Pseudomonas* isolates (1×10^9, 1×10^8, 1×10^7 and 1×10^6 CFU/ml). Pathogen soil treatments (inoculation with Fusarium. at 1×10^4, 1×10^5 conidia/ g soil). Controls included seeds treated only with bacterial isolates, seeds non-treated planted in soil inoculated with or without the pathogen. Five replicates are used for each treatment. Each replicate consisted of 6 plants. Seeds are surface disinfected by immersion in a solution of commercial bleach of 1% NaClO and 0.5% Tween 20 for 5 min and rinsed five times in sterile deionized water under agitation. Bacterial isolates inoculation is made by placing surface disinfected seeds in beakers containing each biomass inoculum, shaking continuously for 24 h at 28°C followed by air-drying of the seeds. Non-inoculated seeds are shaken under the same conditions but soaked in sterile tap water. Seeds are sown in the soil mixture at a depth of 1 cm. The plants are grown at 24°C and 70% relative humidity. Plants are watered twice per week - once with tap water and once with a nutrient solution-.

Disease severity is monitored for 30 days after planting and assayed as the total percentage of seedlings showing any symptoms of Fusarium disease (yellowing, dropping of leaves or vascular discolouration).

Disease incidence is confirmed by plating slices cut from lower stem and roots from diseased seedlings, surface-disinfected in 1% sodium hypochlorite, on Komadas Fusarium-selective medium. The experiment is repeated twice.

Data analysis: All data are analysed using SAS (SAS Institute Inc., 1990). Statistical significance was determined at $p<0,05$.

Entomopathogens
For technical advice on entomopathogens we refer to paragraph 4.6.

4 Indigenous natural enemies

This activity is numbered as follows in the project plan:
WP 1 Generation of IPM technology & on-farm implementation.
1.4 Survey, collection and commercialization of indigenous natural enemies for the management of major horticultural pests by on-location MSc program students.
1.4.1 Support on taxonomic identification of indigenous natural enemies (Chapters 4.1 and 4.2).
1.4.2 Support and advice on the study of the biology of the 4 identified potential indigenous natural enemies (life cycle, population dynamics, host preference, ecology, feeding behaviour, etc.) (Chapter 4.3).
1.4.3 Support and advice on small scale mass production for evaluation trails and methods to establish persistent populations in crops (Chapter 4.4).
1.4.4 Technical advice on the efficacy trials under laboratory and field conditions on the identified predators and parasitoids (Chapters 4.5 and 4.6).

This project element contains the following activities:
a. Collection.
b. Morphological identification.
c. Taxonomic identification.
d. PCR training.
e. Develop long-term activity plan.

An intense collaboration with JKUAT was envisaged, however, due to various reasons, collaboration remained restricted to joint collection of organisms. Morphological and taxonomic identification has taken place at WUR. PCR training proved not possible due to the absence of enzymes. The development of a long-term activity plan is part of the planning of the next IPM project.

4.1 Methods for collecting natural enemies and pests

This paragraph will be published as a chapter in the project report 'Indigenous natural enemies for biological pest control in Ethiopian horticulture' (Messelink *et al.* 2017).

4.1.1 Focus

The main focus was to find and collect natural enemies of thrips and mealybugs as these two pests are considered as the most important in rose crops near lake Ziway and other ornamental crops in horticultural areas. For thrips it was also not clear which species are dominant and causing damage. So in addition, we also collected several thrips species for identification. Besides natural enemies for thrips and mealybugs, we also collected species that can potentially be used for biological control of spider mites, Tuta absoluta (tomato), whiteflies, leaf miners and aphids.

4.1.2 Collection of natural enemies

Natural enemies were collected during 4 missions:
1. March 2016: in the area of lake Koka, near Ziway and on the road to Jimma (road number 5 , see map Figure 4.1).
2. June 2016: in the area of Adama.
3. September 2016: in the area of Jimma and Adama.
4. March 2017: in the area of Debre Zeit (at the moment of writing this intermediate report, this mission still has to be executed).

Details of these missions are given in Annexes. Survey sites were selected mostly along the main roads on wild vegetation or in unsprayed vegetable gardens. Plants were inspected by using hand held magnifiers or by naked eye. Predatory insects were collected with an aspirator and stored in alcohol (70%) for further identification. Small predatory mites were kept on leaves and in the laboratory slide mounted for microscopic identification. Small cultures of some predators were maintained for taxonomic descriptions and some basic laboratory trials. Also leaves from plants with prey (whiteflies, aphids, scale insects, spider mites) were collected in plastic bags and further inspected for the presence of natural enemies under a binocular microscope. Each time a species was collected, we registered the host plant, associated pest, location, position (coordinates) and altitude and we made a picture of the host plant where the species was found.

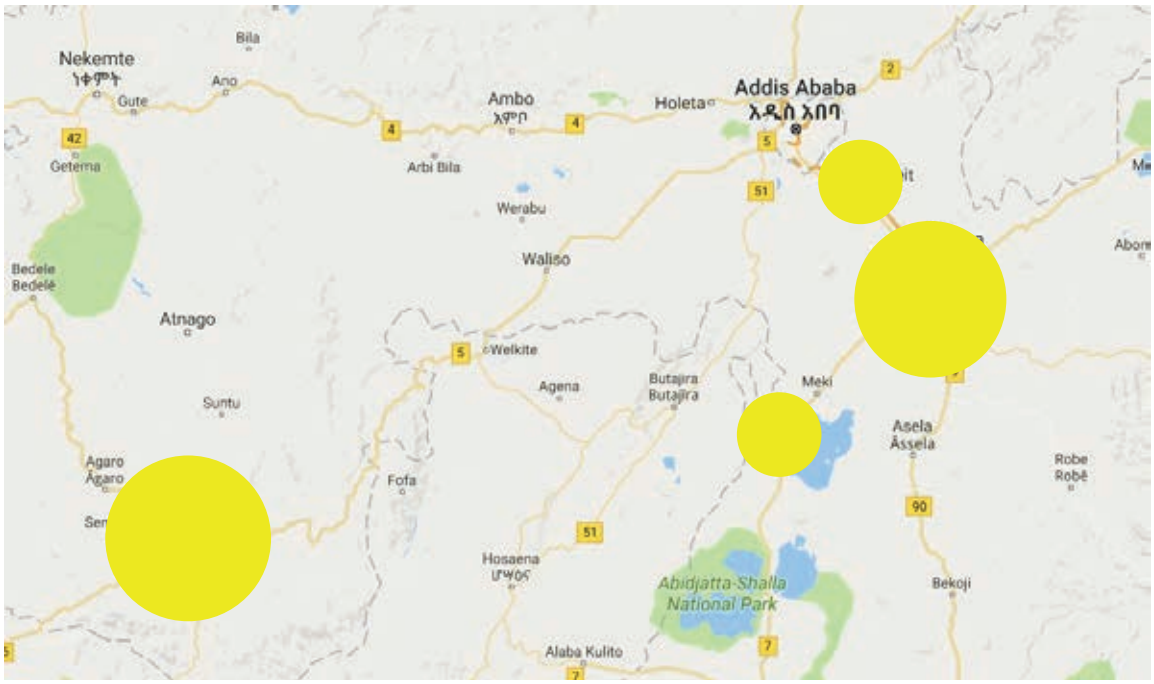


Figure 4.1 *Yellow highlighted areas indicate survey sites where most natural enemies were collected.*

4.1.3 Bait technique for collecting natural enemies of mealybugs

Predatory midges are interesting predators of mealybugs. The larvae are yellow, orange to red and prey upon mealybugs (Figure 4.2). The adults are no predators but they lay eggs in colonies of mealybugs (Hayon *et al.* 2016). The predatory midges can be collected with a bait trap, which consists of sprouted potatoes with mealybugs (Figure 2.2). The bait has to be protected from ants by putting glue on the wire and from other predators like birds.

Figure 4.2. *Larvae of a predatory midge of mealybugs (in orange, left) and an example of a trap to catch these midges (right) (source: Netta Dorchin, Tel Aviv University, Israel).*

The following procedure was developed to collect predatory midges in the area of Lake Ziway.

1. Take some (old) potatoes and let them sprout in the dark.

2. Infest the potato sprouts with citrus mealybugs, collected from roses that are free of natural enemies and strong chemical sprayings and let them grow on it in the dark at 20 - 25°C, until there is a colony of 300-400 mealybugs/potato. (this can be done in the big plastic box that has two small ventilation holes in the side wall).

3. Make the traps (4 in total): put the infested potato in a transparent plastic box and but that inside small baskets. Cover the basket with transparent plastic to protect it against rain.

Hang the traps in randomly chosen plants in the greenhouse area up to 1.5 m above ground in order to attract predatory gall-midge females for oviposition. To prevent ants from attending the mealybug, be sure that the traps do not touch the plants. Cover the strips used for hanging the baskets with grease to prevent ants to enter the traps. Keep the traps for 14 days outside and then collect the potato with mealybugs. When predatory midge larvae are present, then keep the sample for further identification.

4.1.4 Collections of thrips

Several thrips species were collected during the four missions in the area near greenhouses from outdoor vegetable crops and wild vegetation. In addition, thrips was weekly collected from the company AQ Roses in Ziway. The purpose was to identify which thrips species occur and to analyse the seasonal abundance of those thrips species. Employees of this company collected each week 100-200 thrips by the following procedure:

1. Let some rose flowers grow a bit longer in the greenhouse till they are open and accessible for thrips. Collect those flowers.

2. Hit the flowers on a white sheet of paper or in a white plastic box so that the thrips that drops out of the flower can be seen easily.

3. Collect the thrips one by one with an aspirator (manually sucking) with small pipet point at the end with a hole that is big enough for thrips to enter. (the smaller the hole, the easier the thrips is sucked with the aspirator, but this can be adapted when necessarily by making the hole of the pipet pointy a big larger by cutting it with a knife). Try to collects mainly adult thrips (winged) of different sizes (males and females).

4. Put the collected thrips in a glass with 70% ethanol (alcohol) to kill the thrips.

5. Use the plastic funnel to get the thrips in the small glass vials, or put them one by one with a small brush directly in the glass vial (try what is easier). Try to have 50-100 thrips per vial and close them well so that the ethanol cannot evaporate.

6. Label each vial with a pencil and write the date of collection and rose cultivar. Do not use a pen, because ink can be wiped out when getting in contact with ethanol.

7. Do this whole procedure every week and store the vials in a dark place.

This procedure started in week 29 and is still ongoing till the thrips is collected year round. Samples are so far analysed for the first 21 weeks (week 29-49).

4.2 Collected and identified species

This paragraph will be published as a chapter in the project report 'Indigenous natural enemies for biological pest control in Ethiopian horticulture' (Messelink *et al.* 2017).

4.2.1 Predatory mites

In collaboration with mite taxonomist Professor Dr. Eddie Ueckermann (South Africa), we identified 10 species of predatory mites, which are listed in Table 3.1. Nine of them belong to the family of phytoseiidae, which are worldwide the most used natural enemies for pest control. All species are new for Ethiopia, as so far, no predatory mite surveys for Ethiopia are published.

Table 4.1

Identified predatory mites and their associated host plant and food sources.

Identified species	Family	Host plant	Associated food/pest	Location/area
Phytoseiulus persimilis Athias-Henriot, 1957	Pytoseiidae	unknown	Spider mites	Adama
Phytoseius amba Pritchard & Baker 1962	Pytoseiidae	Regi tree and on Croton macrostachus	Spider mites or no prey at all	Jimma
Neoseiulus teke (Pritchard & Baker 1962)	Pytoseiidae	cabbage	Spider mites	Jimma
Typhlodromips near culmulus (Van der Merwe, 1968)	Pytoseiidae	Pepper and amaranthus	Pollen and nectar	Jimma
Amblyseius herbicolus (Chant, 1959)	Pytoseiidae	avocado	Scale insects/honey dew	Jimma
Euseius sp.	Pytoseiidae			
Euseius near vandenbergae Ueckermann & Loots 1988	Pytoseiidae	Croton tree	Spider mites and thrips	Jimma
Amblyseius sp.	Pytoseiidae	Phaseolus sp.	unknown	Adama
Iphiseius degenerans (Berlese, 1889)	Pytoseiidae	Castor bean	Pollen and nectar	Jimma
Proctolaelaps sp.	Melicharidae	Lantana camara	unknown	Lake Koka

A taxonomic descriptions, their world distribution and potential target pests of the 5 collected predatory mites that were identified without doubt are given below.

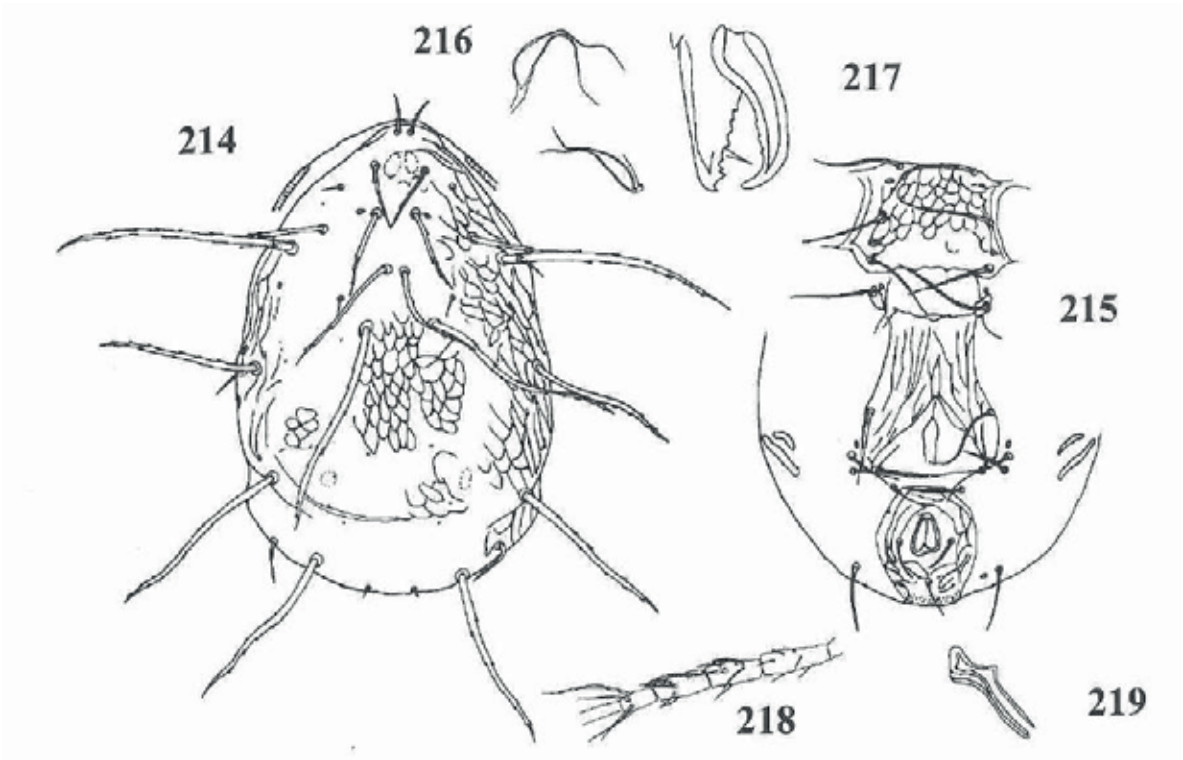


Figure 4.3 *Phytoseiulus persimilis Athias-Henriot. Dorsal shield (214), female ventral surface (2015), spermatheca (2016), chelicera (2017), leg IV (2018) and male spermatophoral process (2010). Source: Chant and McMurtry (2007).*

World distribution: probably all over the world due to wide application in biological control programmes.

Potential target pest: This predator is highly specific on spider mite of the genus Tetranychus, like *Tetranychus urticae* (McMurtry *et al.* 2013). It is one of the first species used in augmentative biological control and on the market since 1968 (van Lenteren 2012). The application is worldwide in both greenhouse and field vegetable and ornamental crops and mass produced by almost all large biocontrol companies.

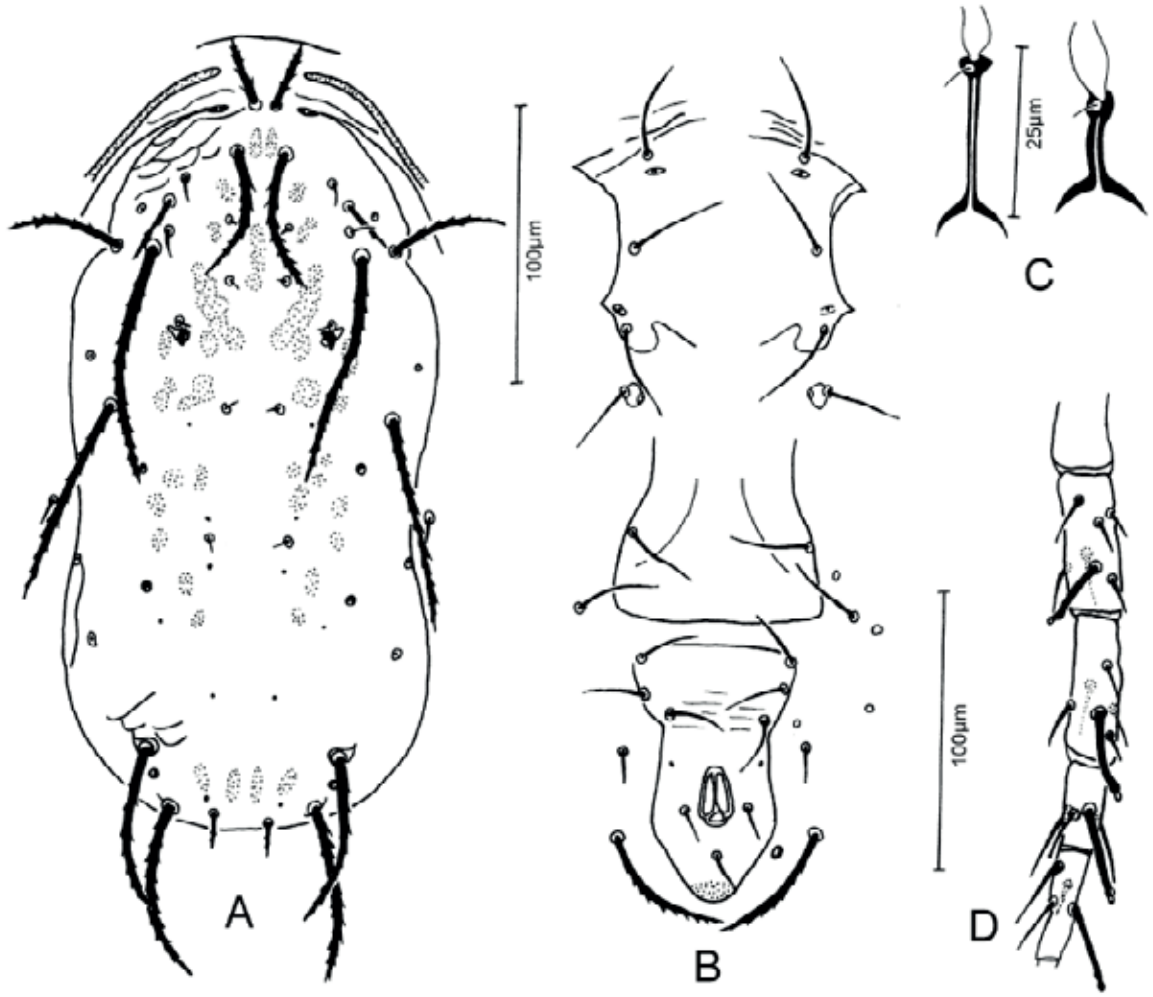


Figure 4.4 *Phytoseius amba Pritchard & Baker. Female dorsal shield (A); Female ventral shield (B), Spermatheca (C) and Leg IV(D). Source: Ueckermann et al. (2007).*

World distribution: Benin, Burundi, Cameroon, Cape Verde, Democratic Republic of Congo, Ghana, Kenya, Madagascar, Malawi, Mozambique, Nigeria, Reunion Island, Rwanda, South Africa, Tanzania, Zambia and Zimbabwe

Potential target pests: Mites are probably one of the potential prey of this predatory mite. This species is so far only described and little to nothing is known about the biology and ecology. In one study it is mentioned to occur in Malawi on cassava at the end of the rain season in association with the cassava green mite *Mononychellus tanajoa* (Bondar) (Zannou *et al.* 2005).

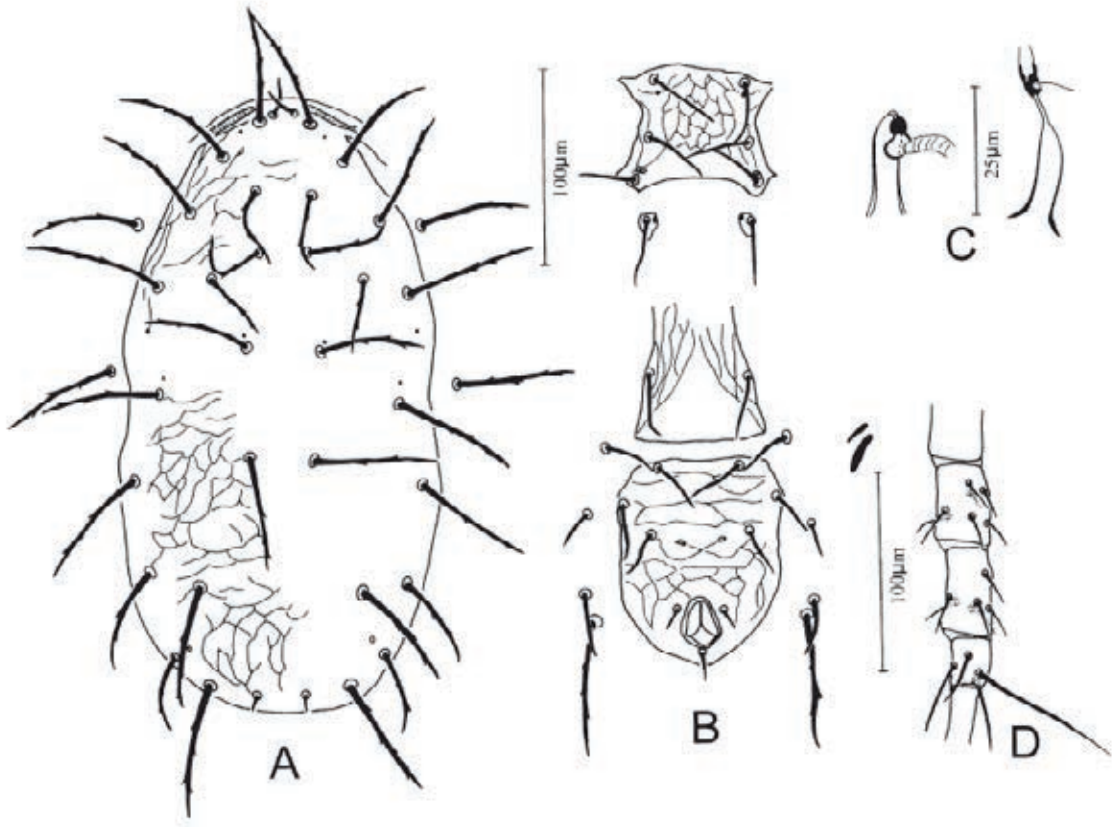


Figure 4.5 *Neoseiulus teke (Pritchard & Baker). Female dorsal shield (A); Female ventral shield (B), Spermatheca (C) and Leg IV(D). Source: Zannou et al. (2006).*

World distribution: Burundi, China, Democratic Republic of Congo, Ghana, Germany, Kenya, Malawi, Mozambique, Reunion Island, Rwanda, Sierra-Leone, South Africa, Tanzania, and Zimbabwe.

Potential target pests: Mites are probably one of the potential prey of this predatory mite. The species is known as a good predator of the cassava green mite (Nwilene and Nachman 1996). Whether other pests species are also a potential prey is not known.

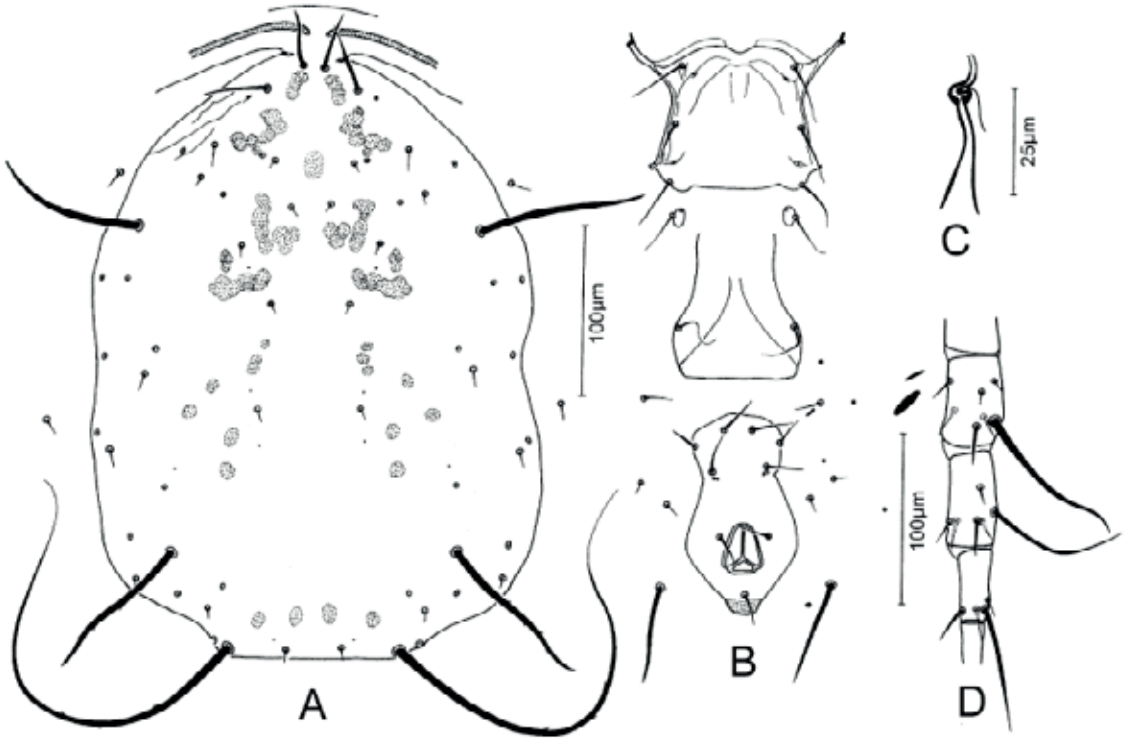


Figure 4.6 *Amblyseius herbicolus (Chant). Female dorsal shield (A); Female ventral shield (B), Spermatheca (C) and Leg IV(D). Source: Zannou et al. (2007).*
World distribution: cosmopolitan.

Potential target pests: This species seems to be a generalist predatory mites. It is reported to be a excellent predator of broad mites, Polyphagotarsonemus latus and the tobacco whitefly Bemisia tabaci, and it also reproduces op spider mites and pollen (Rodriguez-Cruz *et al.* 2013, Cavalcante *et al.* 2015, Duarte *et al.* 2015).

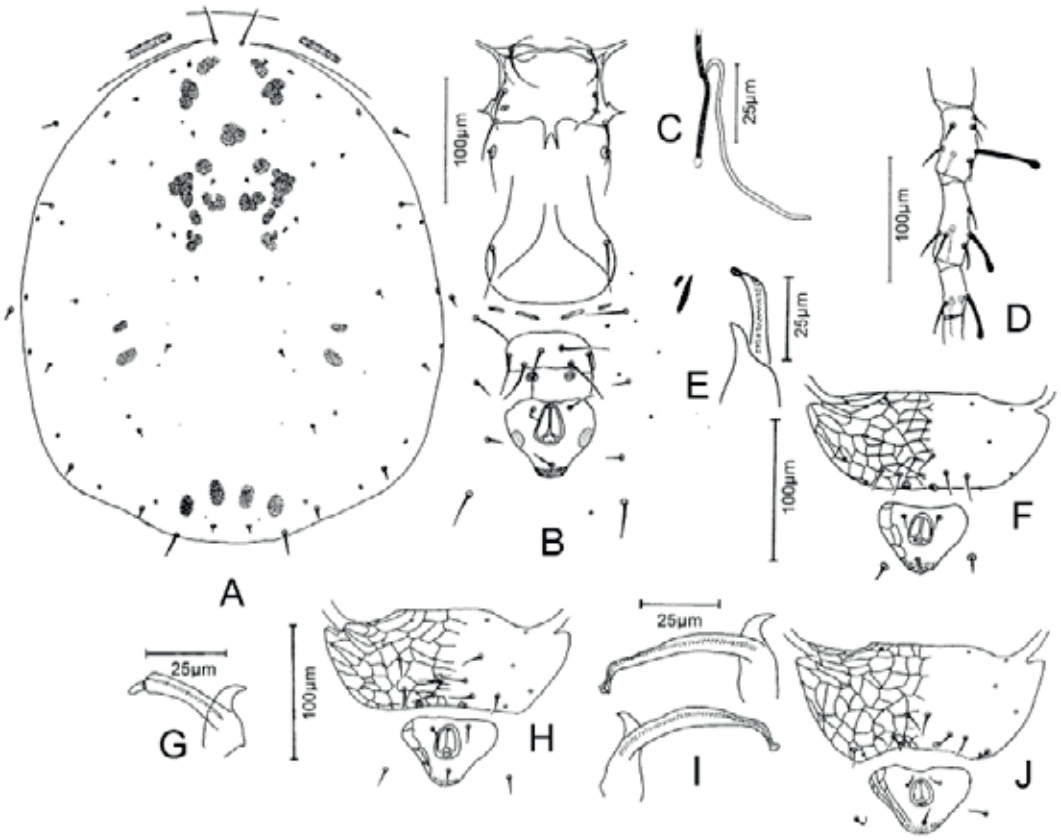


Figure 4.7 *Iphiseius degenerans (Berlese): A. Female dorsal shield; B. Female ventral surface; C. Spermatheca; D.Female leg IV; E. Short type spermatodactyl of a male (from Kericho, Kenya) with 3 pairs of preanal setae; F. Ventrianal shield a male (from Kericho, Kenya) with short type spermatodactyl; G. Short type spermatodactyl of a male (from Kericho, Kenya) with 4 pairs of preanal setae; H. Ventrianal shield of a male (from Kericho, Kenya) with short type spermathodactyl; I. Long type spermatodactyl of a male (from ca. 10 km E Kisii, Kenya) with 4 pairs of preanal setae; J. Ventrianal shield of a male (from ca. 10 km E Kisii, Kenya) with long type spermatodactyl. Source: De Moraes et al. (2007).*

World distribution: Algeria, Benin, Burundi, Cameroon, Canary Islands, Cape Verde, Democratic Republic of Congo, Egypt, Ghana, Georgia, Greece, Israel, Italy, Kenya, Lebanon, Madeira Island, Madagascar, Malawi, Morocco, Nigeria, Portugal, Rwanda, Sierra Leone, South Africa, Tanzania, Turkey, Uganda, Yemen and Zimbabwe.

Potential target pests: This species is well known for biological pest control in greenhouse crops. It is mainly used to control thrips in sweet pepper (Houten *et al.* 2005), but also in ornamental crops like roses they can give a good control of western flower thrips (Messelink *et al.* unpublished). The species is a so-called type IV predatory mites and reproduces very well on pollen (van Rijn and Tanigoshi 1999), which is a benefit for supporting populations in crops. This predator also develops on several types of factitious prey and on spider mites (Vantornhout *et al.* 2004).

4.2.2 Predatory bugs

Three species of predatory bugs were identified as potential natural enemies for horticultural pests in Ethiopia (Table 3.2). Two of these species were new and need to be described by taxonomist. The first one is named *Orius kokai* by taxonomist Dr. B. Aukema, named after the place where this species was found, near lake Koka. The paper that describes this species will probably appear in 2017. The morphological characteristics of this species are shown in Figure 3.6.

Table 4.2
Identified predatory bugs and their associated host plant and food sources.

Identified species	Family	Host plant	Associated food/pest	Location/area
Orius kokai Aukema, 2017 (new species)	Anthocoridae	maize	aphids	Awash river bedding near lake Koka N08° 24'24.2, E039° 01'14.3 Altitide: 1598 m
Orius naivashae (Poppius, 1920)	Anthocoridae	Meskel flowers, Datura stramonium cotton	Thrips and spider mites	Adana outskirts N08°33'07.550, E039°18'43.778 Altitude: 1544 m
Nesidiocoris new species	Miridae	cucumber	whiteflies	Jimma, campus of the agricultural faculty of Jimma University N07°41'12.657, E036°49'47.842 Altitude: 1722 m

Figure 4.8 *Female (left) and male (right) of Orius kokai. Photo credit T. Heijerman.*

The second identified anthocorid predatory bug was *Orius naivashae*, which was abundantly present in meskel flowers feeding on pollen and thrips, but it was also found in spider mite colonies. The morphological characteristics of this species are shown in Figure 3.7. This species was for a long time only known from field collections in Kenia in cotton, where it was observed to feed on the caterpillar *Helicoverpa armigera* (Hernandez and Stonedahl 1999). In 2009, this species was also found in South Africa on sugarcane (Bonte *et al.* 2012) and laboratory studies showed this species is an excellent predator of thrips, aphids and spider mites (Bonte *et al.* 2015). Our study is the first showing this species also occurs in Ethiopia. Several other species of Orius are worldwide used for biological control of pests, particularly for the control of western flower thrips in pepper crops with species like *Orius laevigatus* (Europe) and *Orius insidiosus* (Northern America) (Van den Meiracker and Ramakers 1991).

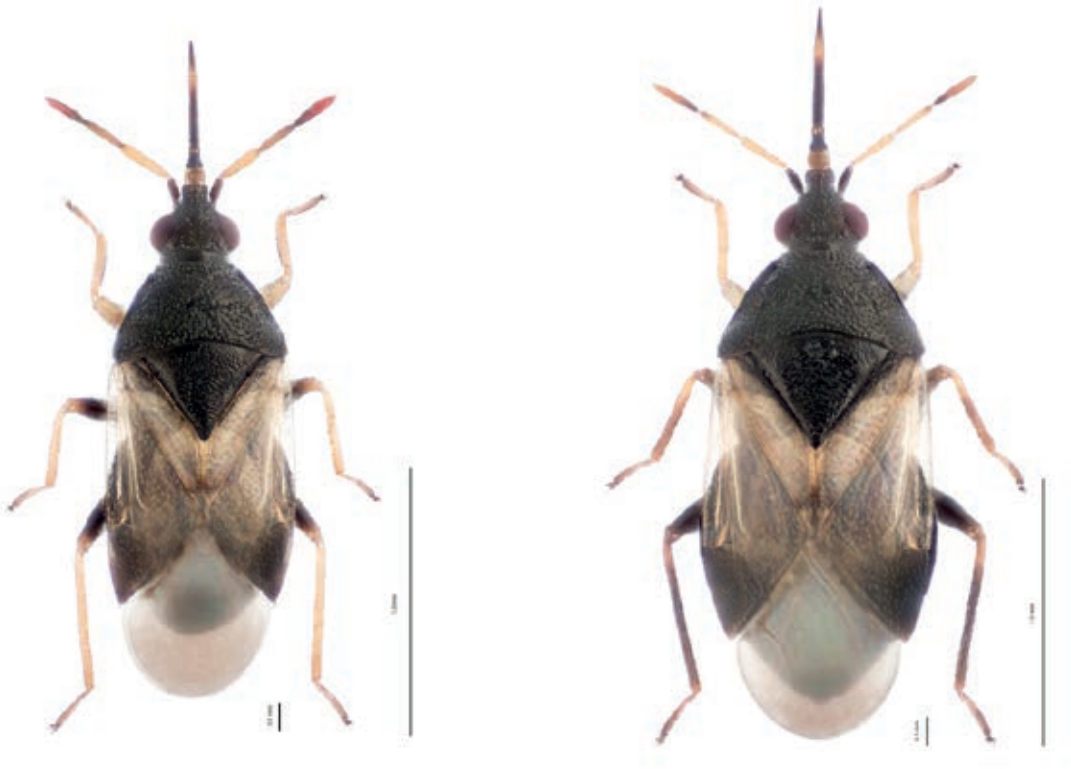


Figure 4.9 *Female (left) and male (right) of Orius naivashae. Photo credit: T. Heijerman.*

The third identified predatory bug with potential for pest control was again a new species, this time belonging to the family of the Miridae. Predators of this family are zoophytopghagous, which means they feed both on plants and prey (Coll and Guershon 2002). Well known European species that are used in biological pest control are *Macrolophus pygmaeus*, *Nesidiocoris tenuis* and *Dicyphus errans*, all feeding on important pests like *Tuta absoluta*, whiteflies, spider mites, leafminers and aphids (Urbaneja *et al.* 2009, Perdikis *et al.* 2011, Ingegno *et al.* 2013, Messelink *et al.* 2015). The species found on cucumber plants in Ethiopia is a new species belong to the genus Nesidiocoris and needs a taxonomic description (Figure 3.7).

Figure 4.10 *Female of a new species of Nesidiocoris. Photo credit: W. van Egmond.*

4.2.3 Predatory beetles

Four species of predatory beetles from different families were identified as potential predators of pests. The most abundant was a short-winged predatory beetle identified as *Holobus fageli*. This genus was formerly included in the subgenus Oligota. There is not so much know about this specific species, but the related species *Oligota kashmirica benefice* is a well-studied specialist spider mite predator in Japan (Shimoda *et al.* 1997). The species we found was always present in spider mite colonies, so it is likely to be also a specialist spider mite predator. The larvae showed the same typical predation behaviour as the Japanese species (Figure 3.8)

The 3 other species were identified as specialist predators of scale insects (Table 3.3, Figure 3.9 and 3.10). Although scales were originally not a target pest in this project, it is still interesting to mention these predator species as they might potentially be useful to control the rose scale Aulacaspis rosae. This pest is currently one of the biggest problems in the Dutch rose greenhouse industry. So far this pest has not been observed on roses in Ethiopia (as far as we know), but it might be a potential threat for the future.

Table 4.3

Identified predatory beetles and their associated host plant and food sources.

Identified species	Family	Host plant	Associated food/pest	Location/area
Holobus fageli (Wiliams)	Staphylinidae	Cabbage, croton, bean	Spider mites	Several places in Jimma and Adama
Cybocephalus sp.	Cybocephalinae	Unknown tree	Scale insects and whiteflies	near lake Koka N08° 24'24.2, E039° 01'14.3 Altitude: 1598 m
Lotis sp. (near neglecta (Mulsant))	Coccinellidae	Avocado	Scale insects	Bore, near Jimma N07°34'17.571, E036°51'38.831 Altitude: 1948
Scotoscymnus sp.	Coccinellidae	Avocado	Scale insects	Bore, near Jimma N07°34'17.571, E036°51'38.831 Altitude: 1948

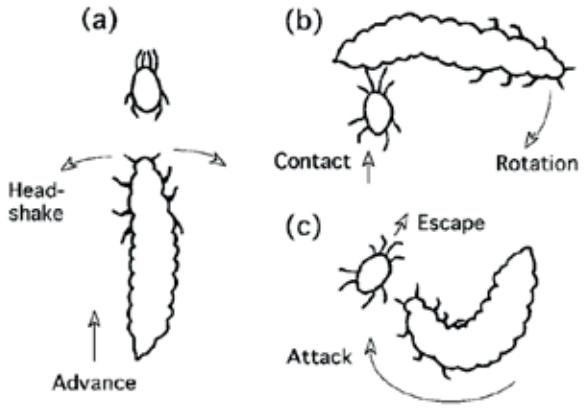


Figure 4.11 *Schematic representation of (a) active searching behaviour and (b) and (c) ambush-like behaviour of third instar larvae of Oligota kashmirica benefica (Shimoda et al. 1997).*

Figure 4.12 *Picture of Cybocephalus sp. Photo credit S.E. Thorpe.*

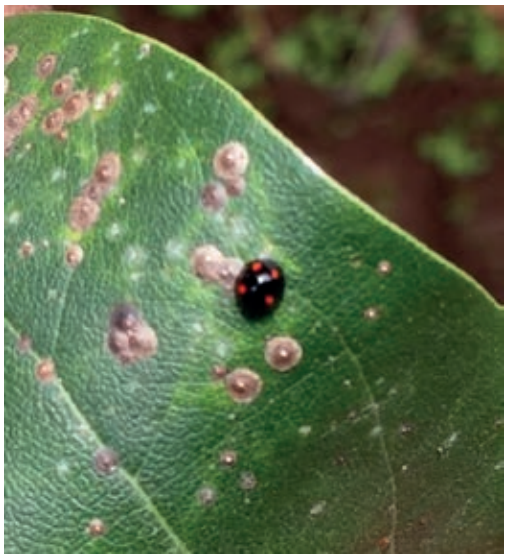


Figure 4.13 *Coccinellid beetle (Lotis sp.) feeding on scale insects on avocado. Photo credit G.J. Messelink.*

4.2.4 Parasitoids

Collecting parasitoids did not receive much attention in this project and requires much more research. However, we were still able to identify one important parasitoid of whiteflies, which was identified as *Encarsia Sophia* (Girault & Dodd) (Hymenoptera: Aphelinidae). This parasitoid was collected from whiteflies on a unknown weed in the outskirts of Adama (Nazreth), Coördinates: N08°33’07.550, E039°18’43.778, altitude 1544 m.

The species is cosmopolitan and as far as we know, for the first time described to be present in Ethiopia. This tiny parasitoid is well studied and know to be an excellent parasitoid of several whiteflies species, including the major pests *Bemisia tabaci* (tobacco whitefly) and *Trialeurode vaporariorum* (greenhouse whitefly) (Luo and Liu 2011). Because mass production systems of Encarsia systems are well-known, this species could easily be implemented in biocontrol programmes. Another option is to use a non-crop banker plant system that employs *Encarsia sophia* with whitefly, *Trialeurodes variabilis* (Quaintance) (Hemiptera: Aleyrodidae), as an alternative host for rearing and dispersal of the parasitoid to the target pest (Xiao *et al.* 2011).

Figure 4.14 *Female of Encarsia Sophia parasitizing tobacco whitefly. Photo credit: Dan Gerling.*

4.2.5 Thrips

The weekly collections of thrips at the rose farm AQ roses in Ziway shows that at least 6 species of thrips can occur in one rose crop, but the most abundant two species were western flower thrips, *Frankliniella occidentalis*, worldwide the most plant damaging thrips species and the common blossom thrips *Frankliniella schultzei*, which is known as an important pest in tomato and other vegetables. The weekly observations show a clear pattern of increasing numbers of *F. schultze*i in the dry season (Figure 3.12). *Haplothrips ganglbaueri* is a flower thrips and not known as plant damaging. Anaphothrips sudanensis is known as a tropical thrips species from grasses and not known as a pest species. Thrips cacuminis has been observed earlier on roses samples imported from Ethiopia (Vierbergen 2014), but this is the first time this species has been found in Ethiopia itself.

More thrips species were collected from wild vegetation close to the Maranque farm. The following species were identified:

- *Ceratothripoides cameroni* (Priesner) on Lantana camara.
- *Haplothrips gowdeyi* (Franklin) on Lantana camara.
- *Thrips gowdeyi* (Bagnall) on Lantana camara.
- *Thrips* new species on Calotropis procera.
- *Haplothrips articulosus* Bagnall in unknown flowers.

In the area of Jimma, we found Hydatothrips adolfifriderici Karny on a climbing plant.

Ceratothripoides clarisetis (Shumsher) is a serious polyphagous pest and a vector of a virus of tomato. It is possibly a synonym of *C. cameroni*, indicating *C. cameroni* may have a serious pest potential. Both species found on desert apple are very likely new to science. Hydatothrips species are leaf feeders, which can give damage to leaves, which are in a young stage. H. adolfifriderici is especially a pest species on Fabaceae. *Thrips gowdeyi*, *Haplothrips articulosus* and *H. gowdeyi* are commonly found in sub-Saharan Africa in all kinds of flowers. They are not known as pest species.

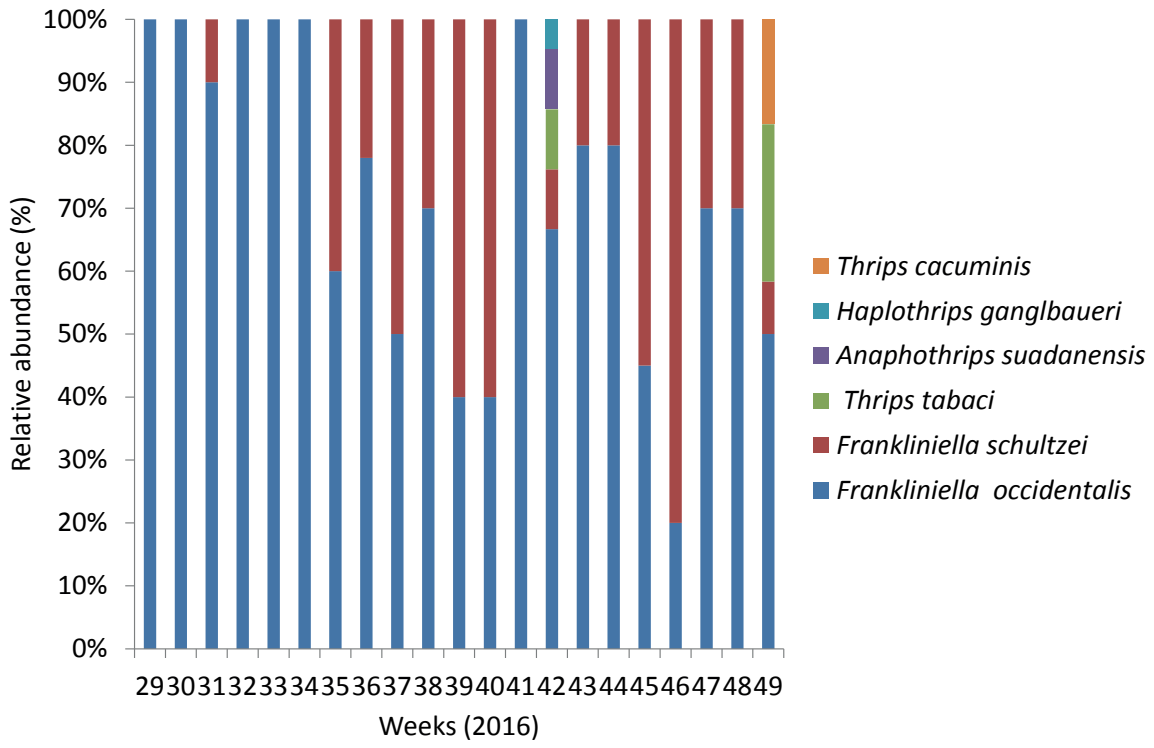


Figure 4.15 *Seasonal abundance of thrips species in a rose crop at lake Ziway.*

4.3 Host range tests of collected predatory bugs

Studies on the biology of natural enemies for horticultural pests can be complex. However, they are essential to understand the interactions between pest, predator, crop, and food sources, and to identify options for pest management. It is not effective to jump from a recently collected predator to on-farm implementation; intermediate research steps that gradually identify the most promising pest management options are required. Also here, an intensive collaboration with JKUAT was envisaged. In the absence of this, we here report on the activities of WUR.

This paragraph below will be published as a chapter in the project report 'Indigenous natural enemies for biological pest control in Ethiopian horticulture' (Messelink *et al.* 2017).

4.3.1 Introduction

In this project we collected 2 new species of predatory bugs that need to be described. So nothing is known about their potential for pest control, but since they are related to species that are well known for their capacity to control pests, it is expected that also these species can be important predators for pest management in Ethiopia. In order to know a bit more about the potential host range of these predators we did some small scale lab trials. First for the species *Orius kokai* we tested predation rates on western flower thrips and peach aphids. Secondly, we tested predation rates of the new Nesidiocoris sp. On greenhouse whiteflies and the tomato moth Tuta absoluta.

4.3.2 Material and Methods

Predation rates of the predatory bugs were tested in the laboratory and conducted in a climate room under 16 h of artificial illumination per day, at 25°C and 70% RH. Predation and oviposition rates of O. kokai were measured with one-week-old mated females, which were starved for one day on bean pods to ensure they were motivated to feed. We used plastic boxes (5 cm high, diameter 6 cm) with a sweet pepper leaf disc (diameter 6 cm) that was embedded upside-down in water agar (1% agar), making the abaxial side of the discs available to the prey species and predators. Either 40 second instar thrips larvae or 40 third instar aphid nymphs were added to the discs, so ample prey was present in all treatments. Subsequently, one starved female *O. kokai* was added to each box. The boxes were placed upside down on a tray covered with gauze in order to have the abaxial side of the discs facing downwards as on plants. Ventilation was possible through a hole in the lid covered with insect gauze (mesh size 80 µm). The bugs were transported to a new box with the same prey densities after 24, 48 and 72 hours. Predation and oviposition rates were measured after the predators had been transferred. Eggs were mainly deposited in the leaf veins and could easily be counted under a binocular microscope (40x). Each treatment was replicated 10 times.

A similar trial was done for the new Nesidiocoris species, but this time with Tuta absoluta eggs and greenhouse whitefly pupae, Trialeurodes vaporariorum, as prey.

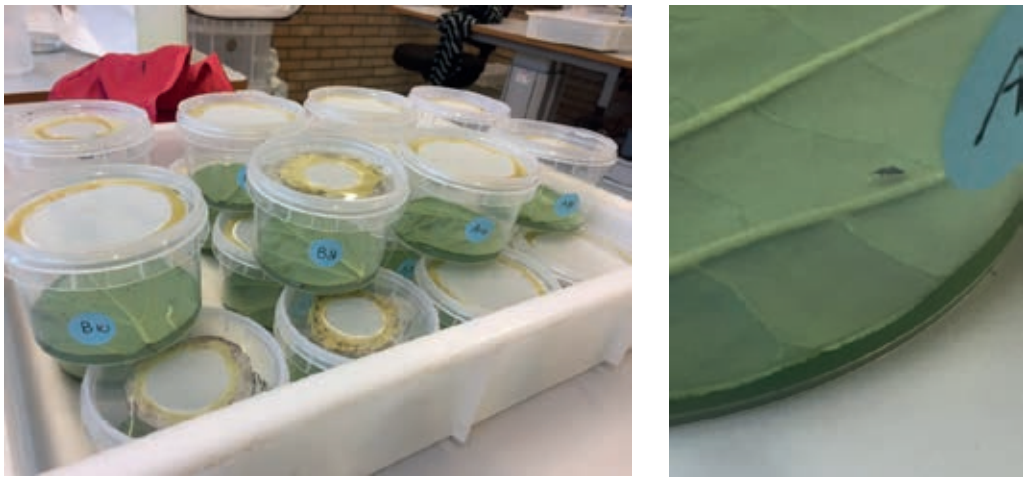


Figure 4.16 *Small units used for laboratory trials and Orius kokai feeding on thrips.*

4.3.3 Results and Conclusions

Orius kokai appeared to be an excellent predator of western flower thrips and peach aphids with daily predation rates of 15 thrips larvae per day and 3-4 aphids per day (Figure 4.2). Both prey were also suitable for oviposition, but the average egg production was much higher with thrips than aphids as prey (Figure 4.3). The predation rates are very similar to the European species Orius majusculus (Messelink *et al.* 2013).

The new *Nesidiocoris* species also showed promising results. Both whiteflies and eggs of Tuta absoluta were consumed. On average 18.3 whitefly pupae per day and more than 25 T. absoluta eggs per days. These trials need more replications with higher numbers of prey to assess the real predation potential. Nevertheless, for so far it shows that this predator is at least promising for control of two major pests on vegetables cucumbers and tomato.

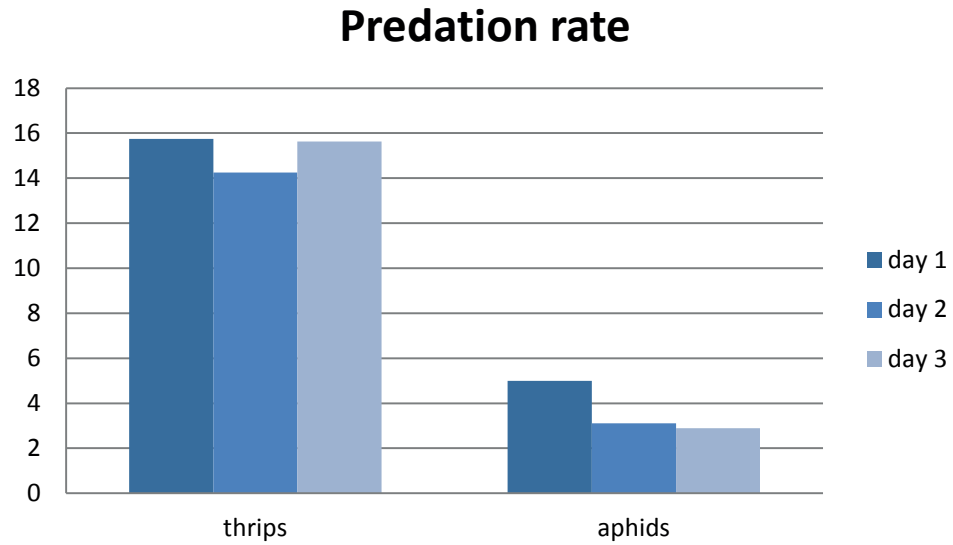


Figure 4.17 *Daily predation rate of western flower thrips and peach aphids by Orius kokai females.*

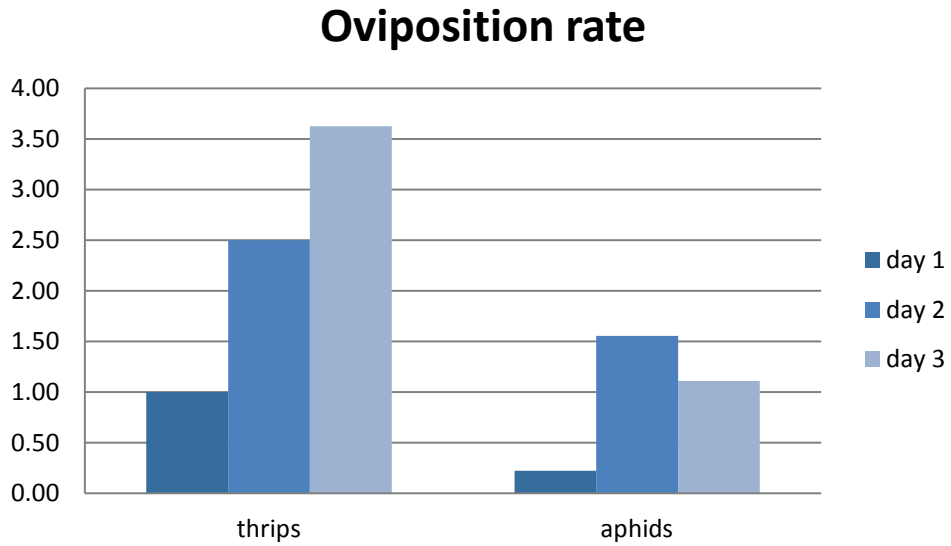


Figure 4.18 *Daily oviposition rate of Orius kokai with western flower thrips of peach aphids as prey.*

4.4 Possible systems for mass production of natural enemies

4.4.1 Introduction

Mass production of arthropod natural enemies has been developed the last decades by professional industries together with universities and research stations. An important organization for exchanging knowledge is the International Organization for Biological Control (IOBC). One of the working groups is specifically devoted to sharing knowledge about mass rearing quality assurance (MRQA) and meets every 3 years (www.amrqc.org). One of the products the IOBC developed is the book about mass rearing and quality control (Figure 5.1). A new book about mass rearing of beneficial organisms appeared in 20014 and can also be consulted for specific techniques (Figure 5.1)

Figure 5.1. *Two important books with procedures for mass production of natural enemies.*

For mass production of arthropod natural enemies we can in general separate 4 types of rearing systems:

1. The classical tri-trophic system of a plant, a pest species and the natural enemy of that pest species.
2. A system with natural enemies and pests reared on an artificial rearing substrate that replaces the plant.
3. A system with natural enemies fed with factitious prey without plants.
4. A system with natural enemies directly reared on an artificial substrate.

More examples of these 4 type of rearing systems are given below.

4.4.2 Tri-trophic systems with plants, pests and natural enemies

The most well-known examples of a classical tri-trophic earing systems is that of the spider mite predator *Phytoseiulus persimilis*, which is usually reared on the spider mite *Tetranychus urticae*, which is reared on bean plants. These reared systems require good greenhouse facilities with separate units for the production of pesticide-free plants, spider mites on plants and eventually predatory mites on spider-mite-infested bean plants. Similar principles can be used for other species of natural enemies. For example, the parasitoid *Aphidius colemani* can be reared on cucumber plants infested with the cotton aphid *Aphis gossypii*. Again here separate units are extremely important. Also greenhouses should be closed with insect gauze to prevent invasions of unwanted other pests of natural enemies that can disrupt the rearing systems. For example, western flower thrips can destroy the rearing of spider mites and hyperparasitoids can totally take over the rearing of parasitoids.

4.4.3 Natural enemies and pests reared on artificial substrates

A huge advantage of replacing plants by an artificial rearing substrate for the pest is the ability to move the production to closed climate chambers where the climate can be optimized and kept stable. Also the risk of contamination by unwanted organisms is much lower in climate chambers than in greenhouse compartments. An interesting technique is the use of artificial substrates for aphids. Through this techniques, aphid size is much more stable than on plants and thereby also the size of aphid parasitoids can be stabilized. However, the technique for doing this is patented and not freely available. The company Viridaxis is using this high tech technique. Another more basic example is the
use of potato sprouts to rear mealybugs. This can be done in the dark in closed boxes. The mealybugs can be used for rearing specialised mealybug predators of parasitoids.

4.4.4 Natural enemies reared on factitious prey

More advanced rearing systems have been developed by looking for factitious prey that do not need plants or specific substrates. A major development was for example the discovery that prey mites reared on bran can be used to mass produce some species or predatory mites (Ramakers and van Lieburg 1982). This principle has been further developed by using different species of prey mites, and by the development or rearing sachets. Another huge development has been the use of sterilized eggs of the flour moth *Ephestia khueniella*. This moth is nowadays mass produced to produce hundreds of kilograms of *Ephestia eggs* per day. The method for rearing Ephestia has been described by Cerutti *et al.* (1992)
The eggs are used for mass production of several predatory bugs which are together with Ephestia eggs provided with bean pods as an oviposition substrate. But also several coccinellid species can be completely produced on Ephestia eggs.

4.4.5 Natural enemies reared of artificial substrates

Some species of natural enemies that are generalists can also be reared on artificial substrates, such as lacewing larvae coccinellid predators and generalist predatory mites. However, in practice these systems are hardly applied, because they are often too expensive for mass production. Moreover, in many cases it has been shown that artificial diets can have a negative impact on the long term quality.

4.5 Recommendations for developing IPM systems

This paragraph below will be published as a chapter in the project report 'Indigenous natural enemies for biological pest control in Ethiopian horticulture' (Messelink *et al.* 2017).

We identified 19 interesting species of natural enemies, but the field surveys need to be continued.

Collections need to be continued. For example, the important predatory mite Amblyseius swirskii has been found in the neighbouring countries Egypt and Kenia. Hence, it is very likely this species also occurs in Ethiopia. If the presence is confirmed by field surveys, it would be easier to recommend this species for biological control programmes. In the present 4 field surveys, this species was not yet found and more efforts are needed. Also we did not yet succeed in finding mealybug predatory midges. This also needs to be continued as these predators have great potential to improve biocontrol of this pest in greenhouse rose crops.

Next step: test on field level en develop mass rearing systems.

4.6 Recommendations for efficacy testing of natural enemies

This paragraph below will be published as a chapter in the project report 'Indigenous natural enemies for biological pest control in Ethiopian horticulture' (Messelink *et al.* 2017).

Efficacy trials are required to evaluate whether laboratory findings uphold under near-commercial or commercial conditions. Ethiopia does have some small-scale research facilities, and a large number of commercial farms, but lacks near-commercial research facilities. Establishment of the latter is be very welcome as efficacy trials in a production greenhouse easily interferes with the production goal of the farm. For example, if the efficacy trial on a certain pest is accidently infected with a disease, this can harm the entire farm.

4.6.1 Criteria for selecting natural enemies

The selection of natural enemies for biological control was traditionally focused on specialist enemies that were released to obtain rapid control of the pests (van Lenteren and Woets 1988). Well-known examples are the spider mite predator *Phytoseiulus persimilis*, the whitefly parasitoid *Encarsia formosa*, and aphid parasitoids of the genus *Aphidius*, which are still successfully used in many crops and countries (van Lenteren 2012). These natural enemies are released as soon as the pests are observed, the socalled augmentative biological control. Criteria for selecting the most effective natural enemies for augmentative biological control according to van Lenteren and Woets (1988) were:

- Internal synchronization with the pest (able to develop to the adult stage).
- Adaptation to the greenhouse climate.
- No negative effects on other natural enemies.
- Mass production is possible.
- High pest kill rate.
- Good searching ability.

Although specialists are well adapted to their host and can be very effective, they often disappear when prey densities have been reduced and need to be released whenever pests resurge. As they are used mainly to obtain rapid control of specific pests, their efficacy requires high quantities and quality and intensive monitoring to assure accurate timing of the intervention. Currently, biological control of pests is increasingly based on generalist predators combined with methods to conserve and augment them (Messelink *et al.* 2014). For this approach, different criteria for evaluation are
needed. These could be:

- Ability to establish on alternative or plant-provided food sources.
- Behaviour in food webs with multiple prey and food sources.
- Adaptation to the greenhouse climate.

Other criteria such as the development on the target pest are less important as generalists can complement their diets with supplemental food sources. However, negative effects on other insects can often not be excluded, as most generalist predators not only feed on pests, but also on other natural enemies. This is not necessarily bad for biological control (Messelink *et al.* 2012). Mass production is still important, but released densities of generalist predators can be rather low when crops are "inoculated" with natural enemies, thus generalists predators can even be interesting with a suboptimal mass production system. The high pest kill rate and good searching ability are also less relevant, as these criteria can be compensated by a good establishment and high predator densities. For evaluation experiments with natural enemies, these different criteria need to be considered. A common mistake is that natural enemies are mostly judged on their efficacy when applied curatively or on their predation capacities per individual, thereby underestimating the effects on a population level. For example, generalist predatory mirid bugs were not effective in controlling aphids when released curatively, but preventive releases was very effective (Messelink *et al.* 2015).

4.6.2 Quality control of natural enemies before trials begin

Natural enemies obtained from commercial producers should always be checked for their quality. Quality control criteria include, for instance, the number of enemies delivered, their emergence rate, fitness, fecundity, sex ratio etc. Some practical guidelines are provided by the International Organization for Biological Control (IOBC) (Van Lenteren 2003), which can be used in research. The natural enemies supplied by producers are not always of good quality and this may become worse during transport. Moreover, the age and storage conditions of natural enemy products are often unknown and this can significantly affect their performance. For these reasons it is highly recommended to rear natural enemies for at least one generation for trial purposes in order to know the exact age, and to get the right stage and "fit" natural enemies. The same may count for the pest species that are released to test natural enemies. It is finally important to realize that some pest stages are invulnerable to their natural enemies. Thereby, multiple releases of the pests and predators may be required to obtain a stable stage distribution.

4.6.3 Setting up a greenhouse trial to test the efficacy of a natural enemy

Greenhouse trials for evaluating natural enemies can be complex, as many factors may influence the outcome of the trial. Firstly, the plants and the soil or growing media used should be free from pesticide residues. This is less of an issue in a dedicated organic research facility but organically acceptable chemicals will have effects on beneficial organisms. Even when pesticides are not applied, they could still unintentionally be added to plants or soil when low levels are present in, for example, the watering system. This should be checked carefully before starting an experiment. Secondly, vapours of earlier applied pesticides in greenhouse compartments can affect pests and natural enemies afterwards. Thirdly, pesticide applications used to control other organisms should be avoided as much as possible as these can strongly affects the results of the experiments. For example, sulphur used against powdery mildew will also affects predatory mites and bugs.

The setup of a greenhouse trial needs to take into account deviations in temperature, humidity, watering and light. Randomized block designs are often required to correct differences in greenhouse climate and sunlight within greenhouse compartments. Also the watering and nutrient supply should be homogenized. Fluctuations in the watering systems which may strongly affect the behaviour of certain pests such as aphids should be prevented. Experimental units need to be separated, particularly when natural enemies and pests are able to fly or migrate. This can be achieved with separated greenhouse units. As budgets and facilities are often a limiting factor, scientists may choose cages for the experiments. Then the mesh size of the insect gauze needs to be suitable to prevent insects from escaping. However, this also needs to be in balance with the ventilation capacity and light transmission. Finally, the number of plants per experimental unit should be high enough to handle a population increase (i.e. the carrying capacity). More plants are often also needed to get higher humidity levels. Plants should also be old enough to represent a real crop situation. For example, sweet pepper plants that have started flowering may give totally different results than non-flowering plants, as the pollen and nectar can be alternative food sources for both the pests and natural enemies.

4.6.4 Methods for observations

Populations of pests and natural enemies can be followed by direct observations on the plants, but often samples need to be taken to do observations under a binocular microscope. Leaves or other plant parts can be cut from the plants and transported in separate bags to the laboratory. At low densities more samples should be taken; it is important to have enough plant material in each experimental unit to be able to do this. Some samples can be stored at about 10°C when it is not possible to do all observations on one day. This works well for predatory mites, thrips, and whiteflies, but less so for aphids that start to walk around. The observed natural enemies and pest should always be identified to species level. This is particularly important for small species that are difficult to distinguish, such as predatory mites or thrips. It often happens that species of natural enemies are found in experiments other than those that were released through invasions of naturally occurring species - even in cages that you would expect to keep out unwanted organisms. Such organisms always need to be mounted in microscopic slides and identified under a microscope.

4.6.5 Summary of tips and tricks

Selection criteria of natural enemies depend on the species and intended application:

- Always check the quality of products with natural enemies, but preferably produce your own natural enemies and pests for experiment.
- Check the planting material, soil and facilities for pesticide residues.
- Homogenize your greenhouse set-up for effects of light, temperature, humidity, and water and correct with block designs.
- Use the right insect cages to separate units.
- Adapt the sampling to the insect/mite densities.
- Always check the identity of the observed species.

5 University course review

This activity is numbered as follows in the project proposal:

WP 1 Generation of IPM technology & on-farm implementation.

1.4 Survey, collection and commercialization of indigenous natural enemies for the management of major horticultural pests.

1.4.5 IPM course review for industry-oriented horticulture. Review and evaluate an IPM course delivered at Jimma University, identify gaps and thereby suggest inputs and establish the practicality of the course.

5.1 Review of IPM course delivered at Jimma University

The following material has been received:

1. Nationally Harmonized Curriculum for Horticulture Programme. Ministry of Education, 2103.
2. Jimma University, College of Agriculture and Veterinary Medicine, Department of Horticulture and Plant Sciences. Competence-Based Curriculum for MSc in Horticulture. November 2015.
3. IPM Course Descriptions.
4. Jimma team. Shortcomings identified in the existing Integrated Pest Management course at (the) Department of Horticulture and Plant Science, Jimma University.

On the basis of this material, we came to the recommendations below. By Anne Elings and Gerben Messelink.

5.1.1 Academic education at the BSc level

Although formally not part of the assignment, we nevertheless gave some comments on the BSc curriculum, as described by the 'National Harmonized Curriculum'. The document says, in summary, the following:

- There is a shortage of professionals in the horticultural labour market.
- The curriculum is developed to contribute to the economic development of the country.
- The needs of the labour market demand a modular approach: task oriented, student centred and competency based.
- Objectives of the 3-year BSc programme:
  - Provide students with basic and applied knowledge.
  - Enable the students to understand and realize problems in horticultural crop production and seek solutions through exposure to research, extension and management.
  - Provide students with the knowledge of handling plants and their products.
  - Integrate teaching-research activities with extension activities.
- The training comprises:
  - Teaching and learning facilities such as laboratories and computer trainings.
  - Courses, laboratory exercises and field visits.
  - Seminars prepared and presented by students.
  - Practical field attachment.
  - Senior research projects.

The following courses are relevant:

Module 9. Greenhouse operation, in production and management of horticultural crops. This module covers a broad set of issues in 16 lecture hours, 49 laboratory/field work hours and 17 hour of home based activities. Greenhouse pest management in greenhouses is part of this module (2 hrs lecture, 3 hrs practicals). It appears an introductory course. The objectives of this module are to have detailed knowledge and practical (skills) on greenhouse management, and the ability to operate greenhouse systems and facilities.
Comments:

- The objectives can only be met after a much more intensive course. After this course, a student can almost certainly not operate a greenhouse. It is recommended to revise the objectives towards something like 'Students possess introductory knowledge on major greenhouse issues, such that communication with the sector is enabled'.

Module 10. Horticulture Pathology & Horticultural Entomology. These module might be called 'Introduction to Horticulture Pathology / Entomology', given their broad character and objectives.
Comments:
- It is not clear why Agricultural Microbiology is a prerequisite for Horticulture Pathology.
- References appear a little out-dated.

Module 11. Vegetable Crops Production and Management. This course gives an introduction to vegetable production and contains elements of types of growing conditions (e.g., greenhouses), quality seed preparation, seedling management, crop protection.
Comments:
- While crop protection is mentioned, the course appears not to approach crop protection in an integrated manner with the other issues. This would require a systems approach in which the elements of the production system are inter-related.

Module 14. Floriculture production and management. This course is a general but comprehensive one, and from the perspective of large-scale commercial flower production an important one. It knows 32 lecture hours, 48 laboratory/field work hours and 44 home based hours. Pest and disease management knows 5 lecture hours (integrated pest management, monitoring, major pests and diseases, control strategies, sanitation, cultural, physical, biological and chemical control, and safe pesticide use).
Comments:
- It is difficult to bring the students to a level at which they can be directly fully operational at a large-scale commercial farm. That would require a fair amount of practical exposure.
- If the practical exposure is required anyway, a good theoretical basis would be a valuable contribution by the University.
- References: Texts specific for the Ethiopian situation are required.

Module 17. Postharvest Physiology and Handling of Horticultural Products. The course knows 5 lecture hours on postharvest diseases.
Comments:
- Plant Physiology is a prerequisite for this course. The consequence is that students specializing in crop protection who skip plant physiology, lack teaching in postharvest crop protection.

5.1.2 Academic education at the MSc level

- The document 'Competence-Based Curriculum for MSc in Horticulture' says, in summary, the following:
- The fruit and vegetable sub-sector in Ethiopia has a high potential.
- Curriculum has been developed after consultation with stakeholders.
- Graduate programme since 2006.
- Principles of competence-based education:
  1. Competence profiles for the foundation of the educational program.
  2. Core occupational problems and key elements of the program.
  3. Assessment of competence development.
  4. Stimulating learning in practice.
  5. Integration of knowledge, skills and attitudes.
  6. The development of self-directed learning.
  7. The teacher as coach.
  8. The support of the attitude towards lifelong learning.
- Competence-based education ensures a close link between what is being taught and the actual situation at the labour market.
- Collaboration with Ehpea (an many more institutions and universities).
- Objectives:
  - Produce highly qualified, competent and skilled human resource in Horticulture.
  - Engage in intensive horticulture research and generate applicable technologies that will be used by horticulture industry and farmers for sustainable Agriculture.
  - Involve in horticultural extension and advisory services.

- Graduate profile:
  - As a researcher, they can engage in research institutes, universities, and other higher education institutes.
  - As a teacher or trainer, they will have a broad range of opportunities, varying from teaching on a university, development worker for an NGO or a governmental body, as a consultant for private entrepreneurs and companies, as an extension worker for farmers and regional communities and so forth.
  - But MSc graduates might also opt to take up their own business as a private investor or entrepreneur or start being a manager within a commercial farm or business and of course there are still different job sectors where they might be engaged in.

The major courses are (see further below):
HORT 552 Integrated Pest Management of Horticultural Crops
PLPA 512 IPM, Agrn 532 IPM, Ento 612 IPM, IPM Course Descriptions for MSc in Plant Pathology, Agronomy and Agricultural Entomology

HORT 552 Integrated Pest Management of Horticultural Crops

This course, with a total of 26 contact hours, 6 hrs field practical and 4 days (24 hrs) excursion, is obviously an important one. It prepares the students for:
- Manipulating plant growth and environments to keep pests below damaging levels.
- Pest scouting, monitoring and forecasting.
- Implementing and monitoring different pest management practices.
- Developing and implementing safety guidelines.

Course components are:
- Introduction.
- Principles of pest management.
- IPM components.
- Emerging trends in pest management.
- Pesticides and their application.
- Development and implementation of IPM.

PLPA 512 IPM
Agrn 532 IPM
Ento 612 IPM
IPM Course Descriptions for MSc in Plant Pathology, Agronomy and Agricultural Entomology
The course descriptions are relatively compact. However, a number of distinct elements are worth mentioning:
- Estimation/determination of pest damage intensity and yield loss, and economic injury levels.
- Quarantine and legislation.
- Integration of different methods of pest and disease management.
- Modelling and systems analysis.
- Socio-economic aspects.

Jimma's own assessment is:
1. The course focuses on insect pests with little consideration of diseases, weeds and other plant protection issues. Thus the course focuses on insect pests with classical IPM approaches instead of principles and practices of holistic system strategies.
2. The social interface and values are not properly captured.
3. There is lack of uniformity in course content, mode of delivery and credit hours across the programs.
4. Above all, the laboratory and case studies are not well-designed. Besides, the crouse is deficient in field visits and practical activities that help the student to contextualize the IPM notion to the realm of the growers in a given agro-ecosystem.

Observations Wageningen University & Research:

1. No systems approach.
2. Little integration with crop management.
3. Pesticides and their application: Is there enough attention for the bad side of pesticides? Risks for human health, environmental pollution (silent spring), negative effects on natural enemies (pesticide-induced pests) and pesticide resistance development in pests?
4. Is there sufficient attention for biological control, how to identify potential predators, research pest-predator-plant interactions, etc.
5. Are the different approaches in biological control well explained ”classical, augmentative and conservation biological control?
6. Is there sufficient human and infrastructural capacity at Jimma to research pest-predator-plant interactions.
7. Does Jimma have sufficient taxonomic capacity?
8. Does Jimma have sufficient long-term stability in terms of human capital?
9. Are the crop protection scientists in Ethiopia sufficiently collaborating? Is there a strategy to distribute tasks and interact?
10. Where is the microbiological / plant strengthening component?
11. Would it be an option to integrate the three courses on IPM in Plant Pathology, Agronomy and Entomology, as they seem to overlap? Specialization, if necessary at all, can be in the examples or e.g., the thesis.
12. Are the teachers up-to-date with practical reality? Jimma is pretty far away from the commercial flower farms, a natural forum where farmers and academics meet seem to be almost absent. Commercial research projects are absent (?). A near-practice greenhouse is not available at Jimma.

Recommendations Wageningen University & Research:

1. Introduce one IPM course, with sufficient possibilities for elaboration of details.
2. Ensure that the staff is more up-to-date with practical reality.
3. Ensure a more structured approach to research, such that long-term large projects that really contribute to knowledge development form the backbone of Jimma’s academic agenda.
    a. A Dr. from the University leads the project.
    b. One of more PhD’s are fully involved.
    c. MSc are involved in the short-term.
    d. Wherever necessary, scientists from other disciplines are involved.
4. Invest in young scientists and have a national placement programme. Established scientists that already have a job can be offered specialized refreshment courses in which they interact with farms.
5. Carefully analyze the knowledge and competences available at Jimma. If not present and if difficult to develop (e.g., specialized taxonomic knowledge) establish links with scientists elsewhere. This can be part of projects. Lacking appears for example:
    a. Quantitative systems knowledge.
    b. Taxonomic knowledge.
6. Perform a reality check on the balance between problems in practice and the research investment required to solve these problems. Realize that problems at a farm can rarely be solved by an MSc student research. Usually, a long-term comprehensive research programme is needed. An MSc student can do a quick-scan, collect basic data, etc.
7. With regards to deficiency in field visits: it is not clear to us as to why this is not simply organized. Should not be too difficult.
8. Instead of focusing on emerging technologies that are not applied in Ethiopia (sterile insect technique, genetic engineering) rather focus more deeply on biological control and particularly on conservation biological control. Try to clarify which non-crop plants near crop plants are important for conserving natural enemies. Try to identify which species are key natural enemies and what their needs are for survival and development. This can be nicely combined with field trips to pesticide-free small holder farms with vegetable crops and by describing the natural enemy communities in the surroundings. This should be combined with ongoing research to describe the functionality of biodiversity for crop protection (ecosystem services).

Other relevant courses are:

HORT 521 Advanced Post Harvest Handling of Horticultural Crops

Comment: Post-harvest pests and diseases are lacking.

HORT 502 Greenhouse Horticulture Production and Management
The course knows 16 hours of lectures, 4 hrs of self study, 4 hrs of individual paper/project work, 6 hrs of intensive practical class and 3 days of excursion. In total, this is 30 hrs + 3 days. Pest and disease management (spraying, biological control, sanitation) is one of the core elements of this course.

HORT 532 Current Topics in Horticulture.
The course provides basic knowledge on general horticulture. Crop protection appears to be a minor subject.

HORT 542 Vegetable Crop Production and Management
The course covers detailed production and management aspects of individual vegetable crops. Post-harvest pests and diseases are lacking.

HORT 602 Development Team Training Program
This community-based course can be given an IPM focus.

6 Sharing information with growers and companies

This activity is numbered as follows in the project proposal:

WP 1 Generation of IPM technology & on-farm implementation.

1.5 Sharing experiences with growers groups and related companies.

1.5.1 Take role in the establishment of 1, and later more farm groups. Summarize latest findings from academic and on-farm research. Facilitate discussions during growers group meetings, and share knowledge.

1.5.2 Take a role in facilitating, through a seminar, the dissemination of the work, together with the growers and others from the IPM project.

Real groups of growers that were meeting and sharing information on a regular basis had not been established. WUR has participated in a number of meetings with farmers and gatherings with a wider group of stakeholders (see list below, and Annex for a more detailed report).

During the meetings with growers, latest developments in IPM were presented and discussed.

Meetings with growers:

August 2015	Meeting at AQ Roses with its staff at Ziway; Gerben Messelink and Anne Elings.
	Meeting at Florensis, Koka; Gerben Messelink and Anne Elings.
March 2016	Meeting at Ziway with a diverse group of growers; Gerben Messelink.
May 2016	Meeting at ET Highland; Andre van der Wurff and Anne Elings.
August 2016	Meeting at Maranque Farm; Marjolein Kruidhof and Ada Leman.

Meeting with wider group of stakeholders:

August 2015	Stakeholder workshop to present the WUR assignments in the IPM2 programme, provide explanation, and obtain from the stakeholders additional suggestions and comments for further improvement of the assignments; Gerben Messelink and Anne Elings.
February/March 2016	Final project workshop; Gerben Messelink and Anne Elings.

7 Train the trainer

This activity is numbered as follows in the project plan:
WP 2 Research and knowledge capacities.
2.3 Training of EHPEA trainers.
2.3.1 Preparation of training.
Deliver in-service training on recent and relevant IPM technologies to EHPEA trainers such as an IPM decision support system, seasonal pest distribution, population dynamics, and biology of economically important pests and diseases on horticulture crops and pest resistance management.

7.1 Deliver IPM training to EHPEA trainers

The preparation of the training consisted of:
- Intense communication by Helina Getachew and WUR on many details of the course.
- Preparatory discussions during the March and May missions.

The training itself was provided by Marjolein Kruidhof and Ada Leman on August 16 and 17, 2016.

7.1.1 Training report

7.1.1.1 Preparations

Marjolein Kruidhof and Ada Leman arrived to Addis Ababa on Monday morning August 15. In the afternoon they had a meeting with Dr. Adhanom Negasi and Helina Getachew to discuss the layout and content of the workshop and the organization of the trip to Maranque farm.

7.1.1.2 First training day

All participants (Helina Getachew, Yordanos, Yeneneh, Seble, Yodit, Selam, Bayana, Fikre, Wondemagegn, dr. Adhanom Negasi) and trainers left Addis Ababa on August 16 and went to ET Highland farm for the workshop. Upon arrival, we first hung several sticky traps on different places on the farm (yellow and blue) in order to evaluate them the next day. The workshop started at 9.30.

Morning session

IPM Workshop EPHEA day 1:
- Workshop approach and expectations.
- Introduction to Integrated Pest Management.
- Components of an IPM systems approach.
- Thresholds.
- Knowledge and recognition of pests (practical part).
- Knowledge and recognition of beneficial organisms (practical part).
- Monitoring & scouting.
  - Basic concepts (practical exercise).
  - Advanced scouting tools (theory).

We started with an introduction round. After this, Marjolein gave an introduction to Integrated Pest Management. We discussed different approaches to pest management and distinguished calendar spraying, 'tactical IPM' (use of pest thresholds to determine the need for pesticide application) and 'ecologically-based' IPM (use of preventative biological measures to keep pest number under threshold levels, use of selective pesticides only as a corrective measure). We then discussed about the current approaches to pest management in roses in Ethiopia. We decided to take roses as an example to keep sufficient focus during the course.

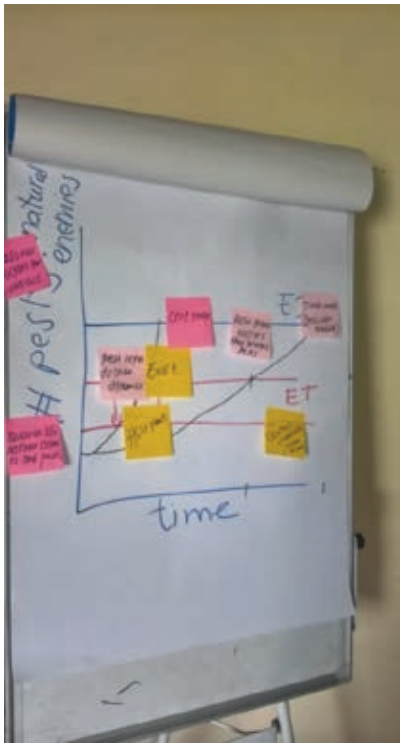
Present situation in Ethiopian roses: 70% calendar spraying, 20% tactical IPM (based on scouting results), 10% ecologically-based IPM. Growers change to IPM to be more cost effective, calendar spraying is very expensive. Growers don't follow the climate inside of their greenhouses. They estimate temperature relying on outside temperature. Relative humidity(RH) is not measured at all.

>> When using ecologically based IPM, it is very important to know the relative humidity (RH) in the crop. This can be easily measured with small data loggers.

We subsequently discussed the different components of an IPM approach. Both a 'traditional' IPM pyramid was presented as well as an 'updated' IPM triangle for an IPM systems approach, with the three main 'pillars' a) host plant (crop), b) environment and c) natural enemies. In an IPM systems approach, not only the direct effects of the different IPM components are important, but also the interactions between the different IPM components.

Examples. For example, an optimal environment for crop growth is crucial to create strong crops that can resist and/or tolerate pests (interaction crop – environment). Moreover, one of the most important considerations for the choice of natural enemies is their response to the climatic conditions in the greenhouse (interaction environment – natural enemies). Also, a crop can have certain traits that benefit natural enemies (such as provision of food (pollen, nectar), shelter or the production of volatiles that attract natural enemies) (interaction crop-natural enemies). On the other hand, some crops have defense mechanisms (toxic compounds, (glandular) trichomes) that are not only effective against certain pests, but also against natural enemies. If the crop itself does not have the right traits to support natural enemies, the environment can be modified to preserve the natural enemies. For example through the provision of complementary food (interaction environment – natural enemies).
We subsequently discussed the difference between preventative and curative control measures. The farmer should always start with taking so-called 'preventative measures', which are implemented before the pest becomes a problem. When the grower works according to an IPM SYSTEMS approach, he/she will implement measures of all three main 'pillars' of the IPM triangle and seek how to best combine them. However, when – in spite of the preventative measures taken - the pest numbers increase too much, the grower will need to resort to so-called 'curative measures'. These 'curative' measures can immediately reduce the numbers of already established pest populations below economic thresholds. These may include both chemical (selective !) pesticides, as well as biological (e.g. entomopathogenic fungi, insectparasitic nematodes, inundative release of natural enemies) or physical/mechanical measures (e.g. mass trapping). It is important that these 'curative' measures interfere as little as possible with the already established preventative control measures.

To be able to determine when to implement different types of IPM measures, it is important to correctly interpret the concept of 'thresholds'. First, Marjolein presented the 'classical' concepts of thresholds in IPM and gave the definitions of Economic Threshold (or Action Threshold) (ET/AT) and Economic Injury Threshold/Level) (EIL). We subsequently discussed about the factors that influence the Economic Threshold and the factors that influence the Economic Injury Level.



Picture 7.1 *Outcome of the discussion about the factors that influence the Economic Injury Level (EIL) and the Economic Threshold (ET).*

Factors that were brought up by the participants and were discussed include:

In relation to ET:
- Balance between natural enemies and pests.
- Control measure (type, time).
- Pest reproduction dynamics.
  - Environment.
  - Host plant.

In relation to EIL:
- Time/ money (decision making).
  - Crop stage.
  - Host plant.

The most important outcomes of the discussion were:

- The ET should ideally be based on the balance between natural enemies and pests, and not on pest numbers alone.
- The ET depends on the type of control measure taken, and the time it takes before the control measure exerts an effect on the pest population. For control measures that take time to exert an effect, the ET should be lower (i.e. preventative measures, inoculative introduction of natural enemies). For control measures that exert a more immediate effect (curative measures) the ET must be higher.
- The ET depends on the pest reproduction dynamics. The faster the population growth of the pest, the lower the ET. The pest reproduction dynamics in turn depend on the environment and the host plant (crop species/ cultivar). And – as mentioned earlier - the number of natural enemies.
- The EIL is mainly determined by the farm manager. The cheaper the control measure, the lower the EIL can become (benefits of the control measure will outweigh the costs at lower pest numbers). Costs of a control measure should not only be measured in the purchase costs of the control agent, but also in 'ecological costs' (i.e. negative side effects on natural enemies).
- EIL can also depend on the crop stage. For example (e.g. higher numbers of pests can often be tolerated without immediate economic consequences when the crop is not in the reproductive stage) or the crop species/ cultivar (e.g. on white roses the pest damage will be more visible than on red roses, hence IEL on white roses will be lower).

Subsequently, the principles of 'tactical' IPM were compared with the principles of 'ecologically-based' IPM, basically wrapping up the several issues that were discussed earlier during the morning.

Afternoon session

After lunch we continued with the practical parts of 'knowledge and recognition of pests and natural enemies' and 'monitoring and scouting'. Ada introduced a tool based on photographs of crop damage, pests and natural enemies that can be used for training scouts on the recognition of pests and natural enemies. Subsequently, the practical scouting exercise was continued in the rose greenhouse of ET Highlands. During this practical part, the differences between Dutch and Ethiopian conditions and the different ways of scouting were discussed.

Mostly, scouts in Ethiopia have received limited education and scouting is considered 'a less job' and is not taken sufficiently seriously. This is a problem at the moment the growers start to work with ecological IPM, because the whole system is based on scouting.

>> It is very important to make growers and farm managers aware of the importance of scouting and required education / training.

After the practical part Marjolein continued in the classroom with advanced scouting tools. At this moment the most important advances scouting tools are offered by the companies Scarab and Koppert. These systems are based on data collection on PDA devices with GPS, with which high-resolution maps of the prevalence of pests and diseases, as well as natural enemies in the crop are generated. This allows for early and targeted pest and disease interventions. See the additional materials for a more detailed description of the Scarab Precision advanced scouting system. The drawback of these systems is that there is the need for at least one WIFI spot on the farm. We learned that most farms in Ethiopia do not have a WIFI connection. Hence, the implementation of this type of advanced scouting systems is currently limited.

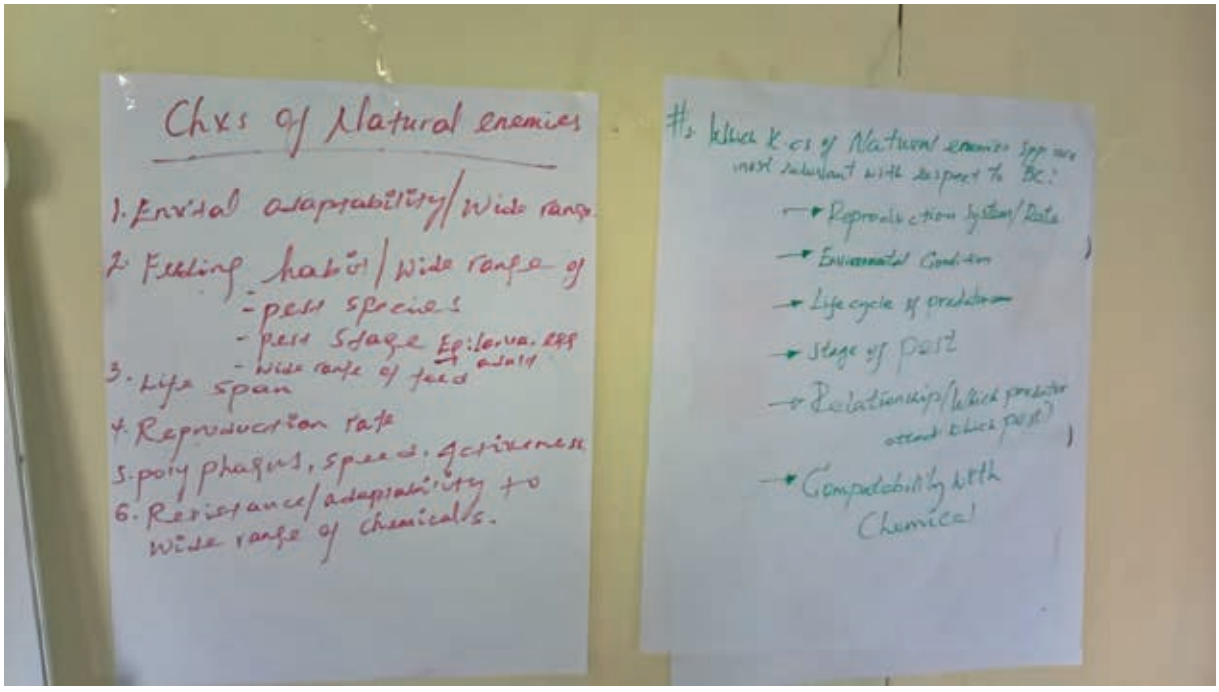
7.1.1.3 Second training day

Morning session

IPM Workshop EPHEA day 2:
- Knowledge and recognition of beneficial organisms (theoretical part).
- Combining different IPM measures.
  - Beneficial organisms + pesticides (side-effects).
  - Different beneficial organisms (intraguild interactions).
  - Different pesticides (prevention of pesticide resistance).
- Exercise: choosing a control measure.
- Applying beneficial organisms.
- Discussion: IPM 'state of the art' and future directions.
- Course evaluation.

The morning session was started with a summary of the first day of the workshop. We continued the morning session with a brainstorm about the important traits of natural enemies. The participants discussed in two groups the question: 'which characteristics of a natural enemies species are most relevant with respect to biological control'? This was followed by a presentation and discussion of these characteristics with the whole group together (see Picture 2) and a powerpoint presentation by Marjolein.

>> An important point when training scouts on the recognition of pests and natural enemies (and which was not included in the course material of this workshop) is to give a clear indication of the size of the pest/natural enemies. If people picture a large insect from studying a photograph, whereas in reality it is much smaller, they will not be able to recognize or find it.



Picture 7.2 *The result of the brainstorm session on the characteristics of natural enemies that are most relevant with respect to biological control.*

Afternoon session

Combining chemicals and natural enemies (side-effects)
During the afternoon we discussed the importance to use pesticides and fungicides with minimal side-effects on natural enemies if implementing ecologically-based IPM. We provided the participants with a list of side-effects of the most important chemicals used in ornamentals in Ethiopia. It is important to consider both the severity of the direct effects of chemicals to different types of natural enemies, as well as the persistence of these chemicals on the crop. When available, information is provided on both these aspects. The list of side-effects was made by combining the information provided by Koppert and Biobest, both companies that produce natural enemies.

>> Because new insights on side-effects of chemicals continue to develop, it is important to up-date the information on the side-effects of chemicals regularly by consulting the following websites:
- https://www.koppert.com/side-effects/explanation-of-the-side-effects-database/
- http://www.biobestgroup.com/en/side-effect-manual

Subsequently a practical exercise was performed, for which Ada sketched different situations (combinations and severities of pests and diseases) in a rose crop, and the participants had to come up with solutions.

Combining different species of natural enemies (intraguild predation)
We then very briefly talked about combining different natural enemies. In most cases more than one species of natural enemies needs to be used to manage pests. Some combinations of natural enemies may strengthen each other. However, especially when using generalist natural enemies one has to also be aware of the negative interactions that can occur between natural enemies, such as intraguild predation (i.e. one natural enemy feeding on the other natural enemy). There is a continuous flow of studies that deals with intraguild predation, and it is wise to consult this literature when designing IPM programs. However, during this workshop there is no time to go into details about this topic.

Combining different pesticides (prevention of pesticide resistance)
Because it is difficult to explain the concept of pesticide resistance to growers and scouts, several people have made an effort to develop tools to create awareness of this problem and to make the concept of pesticide resistance, and the measures that can be taken to prevent this, understandable. On the websites of IRAC (Insecticide Resistance Action Committee) and FRAC (Fungicide Resistance Action Committee) you can find important information on this topic.
- http://www.irac-online.org/about/resistance/management/
- http://www.frac.info/

On the IRAC website you can also find a video that explains in layman's terms how resistance to pesticides develops and how it can be prevented. During the training we played a role play that is described in the book "Vegetable IPM Exercise Manual" compiled by Dr. J. Vos of CABI Bioscience. The whole book is available online. A file with the full description of the role play has been provided.

Picture 7.3 *Role play to explain the development of pesticide resistance to growers*

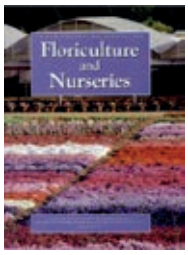
To prevent pesticide resistance it is advised to alternate the use of chemicals with a different mode of action (so not just chemicals with a different active substance, but preferably chemicals which have a distinct mode of action). The IRAC has classified all different pesticides based on the mode of action. A report with this information has been provided as reference material. It is also available online and can be found on their website.
We continued with an explanation by Ada about different ways to apply beneficial organisms in the greenhouse .

>> Very logically, but often overlooked: growers need to always check whether the beneficial organisms are alive before they introduce them to the crop.






Picture 7.4 *Overall impression of the IPM workshop EPHEA.*



Supplementary materials of the workshop:

- Book 'Knowing and recognizing' (Koppert).
- Book 'Integrated Pest management for Floriculture and Nurseries' (University of California).
- Educational videos on the life cycles of important horticultural pests and their natural enemies (made by Koppert).
- Background material on pesticide resistance (IRAC classification of mode of action of pesticides; description of the role play on pesticide resistance development).
- Overview of side-effects of pesticides and fungicides allowed for use in ornamentals and vegetables in Ethiopia.
- Background information on Scarab advanced scouting system.
- Pictures of pests and natural enemies.
- Workshop presentations.
- Course evaluation questionnaires.

Annex 1 Reports

The following reports have been produced by the team of Wageningen University & Research Greenhouse Horticulture:

Elings, A. and G. Messelink, 2015.
Inception Mission IPM2ETH August 2015.

Elings, A., G. Messelink, M. Kruidhof, A. Leman, Y. Cuesta Arenas and A. van der Wurff, 2017.
Integrated Pest Management Component of the Ethio-Dutch Program for Horticulture Development. Involvement of Wageningen University and Research – Intermediate Report.

Kruidhof, M. and A. Leman, 2016.
Report of Training Mission to Ethiopia.

Messelink, G. and A. Leman, 2016.
Report first mission of the Ethiopia IPM Project (March 2016).

Messelink, G., Leman, A., Vijverberg, R., Kruidhof, M. and A. Elings, 2017.
Indigenous natural enemies for biological pest control in Ethiopian horticulture. The results of 4 field trips for collecting and identifying natural enemies in Ethiopia 2016-2017. Wageningen University and Research, Business Unit Greenhouse Horticulture, Report GTB-xxx (in preparation).

Messelink, G., 2016.
Report of September Mission to Jimma and Nazareth (Adama).

Streminska, M., 2016.
Upscaling and Formulation of BCA's in Ethiopia. Technical Advice. Wageningen University and Research, Business Unit Greenhouse Horticulture, Report GTB-1428. 10.18174/405707 (doi.library@wur.nl).

Wurff, A. van der, and A. Elings, 2016.
Trip Report May 2016.

Annex 2 Missions

August 2015:	Inception mission by Anne Elings and Gerben Messelink. Also farmer's meeting at Ziway and meeting with a wide range of stakeholders.
March 2016:	Collection mission by Gerben Messelink and Ada Leman to Jimma and Koka. Also farmer's meeting at Ziway.
May 2016:	Planning meeting by Anne Elings and Andre van der Wurff to universities of Jimma and Addis Ababa. Also review of Jimma curriculum and farm visit.
August 2016:	Train the Trainer mission by Marjolein Kruidhof and Ada Leman. Also collection and farm visit.
September 2016:	Collection mission by Gerben Messeling to Jimma and Adama. Also review of Jimma curriculum.
February + March 2017:	Final workshop mission by Anne Elings and Gerben Messelink. Also final collections.

Annex 3 Mission August 2015

The outcome of the inception mission (by Anne Elings and Gerben Messelink) was the project plan.

The reports of meetings with growers and with a wider group of stakeholders during the mission are placed in other Annexes.

Annex 4 Collection mission March 2016 to Jimma and Koka

Period: March 13-19 2016

Purpose of the mission:

1. Support the survey, collection and commercialization of indigenous natural enemies for the management of major horticultural pests by on-location M.Sc. program students (WP1.4).
2. Participate in a farmers group and present latest results of Dutch pest management research(WP1.5).
3. Prepare the training of Ehpea trainers: discuss with EHPEA trainers to obtain clarification on their training needs and training materials.

Partners involved:

EHPEA: Dr. Adhanom Negasi + EHPEA IPM trainers
Wageningen University & Research: Gerben Messelink and Ada Leman
Jimma University: Entomologists of Jimma University, Dr. Adugna Debele Duguma, MSc. students involved in MSc programme.
Growers in farmers group meeting.

Schedule of the mission

Sunday March 13	travel to Ethiopia
Monday March 14	arrival at 6:20, straight from the airport to Nazareth vegetation fields for collecting natural enemies.
Tuesday March 15	full day travel to Jimma University and collecting natural enemies while traveling
Wednesday March 16	Full day at Jimma University: give a lecture about new developments in biological control. Discuss strategy for project, meet students and researchers
Thursday March 17	Travel back to Addis Ababa and collect while traveling
Friday March 18	visit roses growers near Lake Ziway in the morning, a short stop near lake Koka for further collection of natural enemies in the afternoon meeting at EPHEA for discussion with EPHEA IPM trainers Flight back: 23:59

Vegetables near Lake Koka.

Pepper plants where flower thrips were collected.

Vegetation near the river bedding of the Awash river.

Maize crop in Teji.

Area near Gibber Shet river.

Ricinus communis and wanza tree in Jimma.

The "students" that did the collections of insects , from left to right Beyene Hailu (spider mites), Dr. Afdhanom Negasi (project leader EPHEA), Girma Kebede (mealybugs), Buid Daba (aphids), Fentale Boru Roba (whiteflies), Musa Mohammed (Tuta), Gerben Messelink and Ada Leman.

The species collected and kept alive for further rearing:

1. Orius sp. on bean pods and *Ephestia kuehniella* eggs.
2. Predatory mite Iphiseius degenerans on *Typha latifolia* pollen.
3. Spider mite predatory mites on green beans leaf with *Tetranychus urticae*.
4. Coccinellid predatory beetle of whitefly (Delphastus sp.?) on Mandevilla leaves with *B. tabaci* and *E. kuehniella* eggs.
5. Predatory beetle of spider mites (Oligota sp.) on green bean plant with *T. urticae*.
6. Mealybug species on potato sprouts.

Alcohol samples for future identification:

1. Parasitoid from mealy bug on Solanaceae sp., Koka, 14- 03.
2. Parasitoids (2) collected from Ricinus plant infested by mealybugs, Koka, 14-03.
3. Unknown bugs (2), Koka, 14-03.
4. Predatory beetles of whitefly, Koka, 14-03.
5. Flower Thrips, Solanaceae (pepper), Koka, 14-03.
6. Mealybugs, Lantana camara, Koka, 14-03.
7. Beetles (3x big), Koka, 14-03.
8. Mealybug, Xanthium sp., Koka, 14-03.

Discussion with Jimma university and further actions

Contact persons for future collaboration.

person	email	role
Esayas Mendesil	emendesil@yahoo.com	entomologist (did PhD in Sweden)
Wakuma Bayissa	wakumabh@yahoo.com	entomologist, did recently PhD in ICEPE Nairobi
Zeleke Mekonnen	zeleke.mekonnen@ju.edu.et	head molecular lab en responsible for molecular identifications of collected insects
Adugna Eneyew Bekele	adugna.eneyew@ju.edu.et adugna_e@yahoo.com	dean
Gezahegn Berecha Yadessa (PhD)	gezahegn.berecha@ju.edu.et gberecha@yahoo.com	Director International Institute of Coffee Research (did introduction and also contact person for next mission)
Fikre Lemessa (Prof.)	fikre.lemessa@ju.edu.et	president Jimma University

- The collected insects by the MSC students were all used for DNA extraction, so we could not bring the material to support in morphological identifications. Jimma University will themselves try to identify them to species levels, but this depends on the availability of the molecular markers.
- We agreed to do more collections of natural enemies in the Jimma area, as this seems to be very rich in species. The next visit will be used to start small cultures of collected natural enemies in Jimma. The plan is that Gerben will go there in September. He will come up with a plan to prepare this trip and prepare materials for rearing natural enemies in Jimma.
- We agreed there is a the need to extent the project in order to achieve what we want: the identification of endemic natural enemies that can be mass produced and used biological pest control in the Ethiopian horticulture.
- We discussed some future possibilities for further research:
  - Start collaborative research projects with Ethiopian PhD students that work on biological pest control.
  - Do applied research in greenhouses near the rose growers of Lake Ziway under controlled conditions, because the step between lab and practice is now too big.

Annex 5 Mission May 2016

Andre van der Wurff & Anne Elings

1. Addis Ababa University

AAU: Prof. Dr. Fassil Asefa, PhD student Yonas Chekol
EHPEA: Dr. Adhanom Negasi
Wageningen University & Research: Dr. A. van der Wurff, Dr. A. Elings

Activity 1.2.1.: DNA and MALDI-TOFF molecular identification of the selected entomopathogenic bacteria obtained from the preliminary trials conducted by Addis Ababa University.

Fast identification tools for the biocontrol species *Metarhizium*, *Beauvaria* (against insects), *Bacillus*, *Pseudomonas* (against *Fusarium* pathogen). Two studies were send in advance, to show progress of the group of Prof. Dr. Fassil, i.e. describing trials with the entomopathogenic species (whitefly, thrips, mealybug) and the antagonists against Fusarium. All show very good prospects with high percentage of kill-off. They sampled the population of pest species to determine the effect: eggs and adults seem most sensitive to the entomopathogenic strains.

a. *Bacillus subtilis* and *Pseudomonas fluorescence* can be applied together (Prof. Dr. Fassil). In general the bacterial antagonists can be combined without negative effects on each other. Compatibility checks have been performed (isolates with chemical means to ensure good IPM). However, now there are mixed with local soil or alginates and freshly applied (best effectivity); but what carrier should be used and what type of formulation for upscaling and commercialization. Focus could be on local biobased materials, such as rice and coffee bran. The species are isolated from black soil (clay) Carnation culture. They perform well in Carnation, Chickpea and hydroponics of peas (all 3 species). Owing to problems with Fusarium, Freesia cultures have disappeared from that region of black soil. So, these antagonists contain a promise for horticulture challenged with Fusarium pathogens. Isolates show PGPR activity, i.e., IAA production and siderophore production for enhanced Fe-uptake by plants. The group experiences loss of virulence with aging cultures. Therefore, there will be needs in future to re-isolate from nature in order to ensure high quality isolates. Thus fast identification tools are necessary. For *Bacillus*, the gyrA gene will be used for alignment and marker construction. For *Pseudomonas*, 16S rDNA will be used.
b. *Beauvaria bassiana* (2) and *Metarhizium sp.* (1) isolates (PhD Yonas). They target whitefly, thrips, mealybugs (in roses). Advice on formulation is needed to ensure long shelf-life and high virulence. Different carriers may be used, which one is the best within context of Ethiopia (costs and availability) and fitness of isolates, and large scale production (upscaling and commercialization). Together with botanical extracts, they enhance IPM to 100% reduction of pests. Focus genes are ITS, TEF, RPB1, RPB2. (3 genes will be used to construct alignments and marker construction).

MALDI-TOFF identification is a time-consuming activity, and in order to finish this task before the project deadline of December 31, 2016, Wageningen University & Research had already prepared for (DNA and) MALDI-TOFF molecular identification. It requested for early sending of isolates to The Netherlands. However, AAU argued that immediate export of living organisms from Ethiopia is difficult (indeed, a Material Transfer Agreement is needed as mentioned in the action plan), and requested more explanation of the differences between DNA and MALDI-TOFF identification before taking a decision.

AAU needs kits for DNA extraction. This can be arranged. Wageningen University & Research can purchase the kits with the regular supplier of AAU and ask this supplier to send the kits through their handling agent.

The following was decided:
- AAU: does PCR analysis.
- Wageningen University & Research: does DNA sequencing.
- Dr. Andre van der Wurff (Wageningen University & Research): Within two weeks A4 is send to Addis Ababa university presenting the added value of the MALDI-TOF procedure in addition to sequencing.
- Prof. Dr. Fassil Asefa (AAU): decides as soon as possible upon arrival of the above-mentioned A4 document whether AAU wants to send isolates for MALDI-TOFF or extend the sequencing activity with more antagonists.
- Prof. Dr. Fassil Asefa (AAU): checks with Ethiopian Biodiversity Institute a Material Transfer Agreement. This will be required in case isolates are sent to Wageningen University & Research.
- Dr. Adhanom Negasi (EHPEA): contacts Yonas this week for contact details of custom handler and producer of a) Taq polymerase and b) DNA extraction kit. Details are made available to Jimma University.
- Wageningen University & Research: does potential MALDI-TOFF identification.

Activity 1.2.2.: Needs assessment with regards to formulation of entomopathogens and bacterial antagonists at laboratory level. Discussions with the AAU staff with regards to their precise needs, to be followed later on by some home-work by us and the documented advice.

&

Activity 1.2.3.: Needs assessment and preliminary discussion on the small scale farm trial of the formulated entomopathogens and bacterial antagonists products. Discussions, to be followed by a documented advice later on.

Training/ recommendation for formulation and means for upscaling of Metarhizium, Beauvaria (fungi against insects) and *Bacillus*, *Pseudomonas* (against Fusarium) in context of possibilities of Ethiopia (High versus Low-tech: costs and availability of means). Examples of High Tech are Solid State Cultures (SSC) with liquid medium and (semi) submerged cultures with either airconditioning and Relative Humidity control (Ye *et al.*), agitation or trays (but lower heat removal). Commercial examples are BotaniGard, Mycotrol, Ostrinil. Low Tech example are PUR (Polyurethane foam=carrier) systems which can be recycled and are quick in production (Banik *et al.*) with 1% rice and 0.5% gram powder impregnation.
What tests/ trials checks have to be done in order to safeguard bioethics and environmental ethics, i.e. what checks to do to ensure human safety, and wat checks to ensure there is no threat for the environment. This is needed for accreditation and further commercialization. There is an universal checklist available. The knowledge on these checks is needed to (future) admittance to Ethiopia. Strict regulation on microbials and macrobials are there for Ethiopia. Registration is easier.
The following was decided:
- Dr. Andre van der Wurff (Wageningen University & Research): Writes recommendation on formulation and upscaling and checklist for safe use (human, environment). Date of delivery to be determined.

OVERALL PRIORITY
1. Import of kits (and polymerase for Jimma).
2. Decision on MALDI-TOFF.
3. Training/ recommendation for formulation and means for upscaling.
4. What tests/ trials checks have to be done in order to safeguard bioethics and environmental ethics.
5. Fast identification tools for the biocontrol species.

2. Jimma University, College of Agriculture and Veterinary Medicine

JUCAVM: Dr. Adugna Eneyew (Dean), Dr. Duguma Adugna, Dr. Gezahegn Berecha (chair of meetings and contact person)
EHPEA: Dr. Adhanom Negasi
Wageningen University & Research: Dr. A. van der Wurff, Dr. A. Elings

We were warmly welcomed by the Dean of JUCAVM and various IPM scientists (see attendance list at the end of this report). Major subjects of discussion were:
- Linkage of the university with the private sector.
- Integration of IPM in the curriculum.
- Needs assessment for the PCR training.
- Options for future collaboration.

Activity 1.4.1.: Support on taxonomic identification of indigenous natural enemies. During this mission, we will identify the PCR training needs and training possibilities. The training itself will be provided later. Project has to be re-scheduled for this.

The following was agreed upon:
- A 3-day PCR training course will be given by Dr. van der Wurff and Dr. Yaite Cuesta Arenas by the end of June 2016.
- The presence of all equipment and materials is ultimately the responsibility of JUCAVM. However, WUR will try to assist where needed and possible.
- 10 persons, well-skilled.
- Training takes form of a Training-of-Trainers.
- Dr. van der Wurff will try to donate a PCR machine that is not used any longer. Dr. Berecha will send a template of a donation letter for custom clearance. Jimma has a clearance office in Addis.

Activity 1.4.5.: IPM course review for industry-oriented horticulture. Materials have been received. Materials will be discussed. To be continued and completed later.

The following was agreed upon:
- JUCAVM summarizes shortcomings by the end of May.
- WUR provides feedback and recommendations by the 3rd week of June.

Future collaboration:

- Parties agreed that strengthening their collaboration is desirable.
- IPM can serve as an entry point to develop project proposals to address the problems of the horticultural sector, creating opportunities for capacity building at the university.
- Jimma produces a concept note on future collaboration by the end of June.
- WUR will discuss during the mission at the Netherlands Embassy.
- Strengthened links with the private sector are required.

In addition, a presentation by Dr. Andre van der Wurff on latest scientific developments in crop protection through strengthening of crop resilience was given.

3. Growers

See Annex 9.

4.EHPEA

Preparation of August Training.

5. Royal Netherlands Embassy

Embassy: Martin Koper, Deputy Head of Mission; Betselot Mesfin Admassu, Agriculture Assistant
EHPEA: Dr. Adhanom Negasi
Wageningen University & Research: Andre van der Wurff, Anne Elings

Mr. Hans van den Heuvel was not available and was represented by Mr. Martin Koper.

A status update was provided, and a possible follow-up project was discussed.

Annex 6 Collection mission August 2016 to Maranque Farm

Period: August 18 2016

Partners involved:
EHPEA: Dr. Adhanom Negasi
Wageningen University & Research: Marjolien Kruidhof and Ada Leman
Maranque Farm: Robert ten Hove

After providing the train-the-trainer IPM course to EHPEA staff, the group left Addis Ababa on August 18, 2016, to drive to Maranque farm at 6 AM. On the way, collected of insects and mites were collected and preserved:

- Mites predating on spiders mite on bean plants.
- Thrips from flowers of desert apple.
- predatory mite *I. degenerans* on castor bean.
- predatory bugs on maize (Anthocoridae).
- and single beetles and other insects.

Picture 5 *Collecting beneficial insects and mites and the way to, and around Maranque farm.*

The group arrived to Maranque around noon. Maranque farm was founded in 2004 by Marc Driessen and mainly produces cuttings of chrysanthemum, and besides produce some other ornamentals as well (e.g. Poinsettia, Impatiens, Dahlia, Celosia) for the Beekenkamp Group of the Netherlands. The farm is situated south-east from Addis Ababa (see Figure 1) at an altitude of approximately 1223 m.

Maranque (Coordinates 8.53776 39.64217)

Figure 1 *Location of Maranque farm.*

Upon our arrival there was a meeting with Bart Ridder and Biruk Hailemariam on the situation at the farm and possible ways to control thrips. Thrips and leaf miners are the main pests. Spider mites are not a problem, which is attributed to the effect of overhead irrigation. Several greenhouses with cuttings of chrysanthemum in different growth stages were visited. Thrips outbreaks are prevented, amongst others, through strict hygiene protocols that prevent virus contamination (mainly problems with TSWV) and steaming of the soil and fogging of the greenhouse space with chemicals when preparing their greenhouses for a new cropping cycle. Thrips are scouted not only inside the greenhouse, but also in the surrounding area.
Later in the afternoon more beneficial agents were collected in the field and preserved:

- Predatory bugs on Cucurbita (Miridae).
- Eggs of lacewings.
- Thrips species from flowers of *Lantana camara* and Solanaceae.

After the collection, the collected insects were distributed over several sample containers, making use of the Maranque laboratory. Back in the Netherlands the collected insects will be identified and if possible a rearing will be started.






Picture 6 *Overall impression of our visit to Maranque farm.*

Annex 7 Collection mission September 2016 to Jimma and Adama (Nazareth)

Period: September 10-17 2016

Purpose of the mission:

1. Support the survey, collection and commercialization of indigenous natural enemies for the management of major horticultural pests by on-location M.Sc. program students (WP1.4).
2. Review of the IPM course of Jimma University.

Partners involved:

EHPEA: Dr. Adhanom Negasi (only for field collections near Nazareth)
Jimma University: entomologists Dr. Esayas Mendesil, Dean Adugna Eneyew Bekele and director of IICR, research assistants Daniel (field work) Abebe (lab), MsC student Inge of Leuven University (field work).
For Nazareth collections: MSc student involved in the IPM project.
Wageningen University & Research: Dr. Gerben Messelink

Deliverables of the mission:

- Collections of potential natural enemies in the surroundings of Jimma en the cotton fields near Nazareth with special focus on predatory mites and mealybug predatory midges.
- Advice for changes in the Jimma IPM course.

Schedule for the mission:

date	activity
Saturday Sept 10	19:40 departure from Amsterdam
Sunday Sept 11	Arrival 5:55 in Addis, flight at 11:10 am to Jimma, arrival at 12.05, take a rest
Monday Sept. 12	full day field collections in Jimma area
Tuesday Sept. 13	morning field collections and afternoon short lecture about small scale rearing of natural enemies
Wednesday Sept. 14	Morning field collections, afternoon prepare collected natural enemies for transport at Jimma lab + discuss the Jimma IPM course
Thursday Sept 15	10:25 flight back to Addis, 12:40 straight from the airport to Nazareth cotton fields for collecting natural enemies. Stay 1 night in Nazarath area
Friday Sept 16	morning: field collections in Nazareth area cotton fields, afternoon: travel to Addis. Flight back: 23:59

Field collections in Jimma

Collections on Monday 12 September 2016 with Esayas Mendesil (lecturer entomology) and Daniel (research assistant of Gezahegn Berecha, collected also coffee pollinators)
Collections at the Highland Vegetable Research Center near Jimma, the "Eladale research farm"
Altitude: 1818 m
Coördinates: N07°41'00.010, E036°49'46.908
Spider mite beetle *Oligota* sp. on spider mite infested leaves of a climbing plant (produces sticky white stuff when breaking the leaves (see picture). Collected adults both on leaves and on alcohol.

Climbing plant with spider mites and a spider mite beetle.

Also at the vegetable research center
Altitude: 1782 m
Coördinates: N07°42’04.104, E036°48’40.743
Mirid predatory bug from sticky plant tufo also called “Mich” in Ethiopia (see picture). Scientifica name is *Guizotia abyssinica*. We found many individuals that probably feed on entrapped insect carrion. The plant was very sticky.

Sticky plant “tufo” on which we found many individuals of a mirid predatory bug.

At the campus if the agricultural faculty of Jimma University
Altitude: 1722 m
Coördinates: N07°41’12.657, E036°49’47.842

- Spider mites on roses in the greenhouse campus. No predatory mites were found, but leaves were collected to check this.
- Predatory mites from castor bean *Ricinus communis* (brown mites, probably Iphiseius sp.).
- Predatory mites from Croton tree (the leaves have extra floral nectar en some mites can be found between the veins at the base of the leaf).
- A mirid predatory bug from cucumber (Korean variety, was left after a trial). Some whiteflies were present, but most leaves were very clean. Both nymphs and adults of the mirid predator were present, indicating reproduction on the plants. Many individuals were collected with an aspirator.

Greenhouse with roses at the campus. Spider mites were found on some plants.

Left: The castor bean Ricinus communis. The is a very common plant in the Jimma area and the bigger plants that have been flowering are always colonized by an Iphiseius sp. predatory mite.
Right: Small croton tree with some predatory mites on the leaves with extra floral nectar.

Cumcumber plants growing in between a grass variety. On these cucumber plants we collected many individuals of a specific mirid predatory bug.

Collections day 2 on September 13, 2016

Visit to small holder farms in the village "Dalle" in the "outskirts" of Jimma.
Altitude: 2019 m
Coördinates: N07°43'43.111, E036°46'43.381
3 collections:

- Predatory mites from Croton tree leaves which were also infested by thrips (only larvae, so identification not possible). 4 mites were put in alcohol They were relatively big and a bit red coloured.
- Tufo (*Guizotia abyssinica*): again mirid predaory bugs, just like collected on 12/9.
- Sunflower: some larvae and 1 adult ladybird beetle in association with mildew. The larvae were also feeding on mildew, so probably a mildew specialised ladybird beetle.

Also lacewing eggs were observed, but not collected.
We checked flowering sunflowers that still produced pollen, but here no natural enemies like Orius sp. were found. Only pollen feeding beetles. WE also checked maize with aphids for Orius predators, but they were not found.

A bit further on the road, village called "Gube i Muleto"
Altitude: 2038 m
Coördinates: N07°43'57.002, E036°45'56.868
Collections were made from *Ricinus communis*, which were colonized by black colored predatory mites, probably again Iphiseius sp.

Again at the vegetable research center we now checked the cassava plants
Altitude: 1782 m
Coördinates: N07°42'04.104, E036°48'40.743
We found some spider mites, but no predatory mites or other natural enemies. In general the plants were very clean.

Sunflowers on which we found a mildew specialized ladybird beetle and many pollen beetles, but no predators like Orius sp.

Croton tree where we found predatory mites and some thrips on the leaves.

The sticky plant "Tufo"(Guizotia abyssinica) with mirid predatory bugs.

Ricinus communis plant which was colonized by many Iphiseius sp. predatory mites.

These cassava plants were inspected, but only some spider mites were found.

Collections day 3 on September 14, 2016

Visit to small holder farm in the village "Bore" near Jimma. These small holder farms have several crops mixed with coffee, banana, avocado, papaya and none of these crops are treated with pesticides.
Altitude: 1920 m
Coördinates: N07°37'30.046, E036°49'50.561
2 collections:

- Predatory mites from hot pepper of the local and common red variety "Marako fana". Pale coloured mites were abundant and collected for further identificatins (both put in slides and smal culture with Typha pollen on leaves). Leaves were very clean in general. Plants were flowering, so the mites probably survive on the pollen as food source.
- A few predatory mites of Amaranthus between a maize field. Probably Amarathus viridis (needs to be checked). These were pale coloured phytoseiid predatory mites. Also here no prey was detected, but plants were also flowering. I t could also be that maize pollen has been a food source, but these were not flowering anymore.

Also maize, cucumber and bean crops were inspected for pests and natural enemies, but nothing was found.

Along the road: Altitude: 1943 m (I noted 1743m, that is probably wrong)
Coördinates: N07°35'09.380, E036°51'27.144
1 collection:

- Predatory mites on leaves of the "Regi" tree, scientific name: Vernonia amygdanila, a tree also used in the biofuel industry. The leaves have a high trichome density. Leaves were rather clean, some smal thrips larvae were present, still on each leaf a few predatory mites were present. Also one red coloured long-legged mites was present. Both mites were mounted in slides for further identification and some leaves were used for starting a small culture by providing some Typha pollen.

Visit to small holder farm in the village "Dedo" near Jimma. These small holder farms have several crops mixed with coffee, banana, avocado, papaya and none of these crops are treated with pesticides.

Altitude: 1948 m
Coördinates: N07°34'17.571, E036°51'38.831
3 collections:

- On avocado trees with scale insects: a small black coloured Coccinellid predatory beetle. Both adults and larvae were put in 70% ethanol for further identification. Most scale were predated (checked in the laboratory). We also found one other coccinellid predator, black coloured with 4 spots and a bit bigger, predating on the scales.
- On the same avocado tree: a pale coloured predatory mites with typical two long dorsal hairs (checked in the lab). Mites were slide-mounted for further identicifation and some individuals were kept on leaves with Typha pollen. The leaves seem suitable for predaory mites with good hiding places and soem trichomes near the veins. Mites were always present near the veins.
- On the chat tree: Some dark coloured and fast running predatory mites, probably Iphiseius sp. Mites were slide-mounted for further identicifation. The plant seems not very suitable for predatory mites: it has smooth and tough leaves. Some spider mites were present, which might have bee a food source for the predatory mites. Als one red coloured mite with long legs was found, which we stored in 70% ethanol.

2 places along the road with *Ricinus communis* trees:

Boni Hotel:
Altitude: 1718 m
Coördinates: N07°40'16.874, E036°50'20.279

And in Jimma centre:
Altitude: 1846 m
Coördinates: N07°39'29.621, E036°50'36.552

Plants were inpected for the presence of Iphiseius sp. In both cases they were abundantly present, so this mite seems to be strongly associated with this tree. No mites were collected for further identification as is is very likly to be the same species as found on the other Ricinus trees.

In the front pepper plants that were colonized by predatory mites.

Pepper plants were flowering and the pollen might have been a food source for the predatory mites, although no mites were found in the flowers. They were all on the leaves.

Amaranthus plant between maize plants. A few predory mites were found on the leaves on this plant.

Regi tree which had many predatory mites on the leaves.

Huge avocado tree with fruit.

Abaxial side of avocado leaves, where preadtory mites were present near the veins.

Scales on avocado leaves, where many small black coccinellid predators were found.

The four-spotted coccinellid predator feeding on avocado scales.

Chat leaves that were colonized by an Iphiseius sp. predatory mite.

Ricinus tree in the JImma center.

Collections day 4 on September 15, 2016

Travelled to Adama (Nazareth) and along the road just outside the city, we collected some natural enemies from flowering plants. The landscape is after the raining time very green and many plants are now flowering. The location of the Adana outskirts:
Coördinates: N08°33’07.550, E039°18’43.778
Altitude: 1544 m

4 collections:

- Orius predatory bugs from yellow flowering plants that produced a lot of pollen. The plant is called the Meskel flower or "Aday Abeba". The flowers contained also many black coloured thrips. The predatory bugs, ca. 10 individuals are kept on the flowers together with the thrips for starting a small culture for identification.Th thrips is also stored in 70% ethanol for identification
- Small beetles, looking like the big-eyed bug, colected from teh same flowers as above. Two individuals were stored in 70% ethanol.
- Predatory mites from an unknown flowering weed with hairy leaves and a low infestation by whiteflies. Only a few individuals wer detected. Leaves were randomly picked and put in a back for isolation of mites later on.
- On Datura stramonium we found some leaves infested by spider mites. We found 3 possible natural enemies on these leaves: a red coloured predatory mite, an Orius adult and one black coloured coccinellid beetle. The mites and Orius are kept alive on leaves in a plastic tube covered with coton. The beetle is stored in 70% ethanol for further identification.

The meskel flowers that were colonized by a thrips sp, an Orius sp and a predatory beetle.

An unknown weed that was infested by whiteflies and some predatory mites.

Datura stramonium with spider mites, an Orius sp and a black coloured coccinellid beetle.

Collections day 5 on September 16, 2016

We continued our collection in the surroundings of Adama. Tow student of Jimma joined us for the collections: Beyene Hailu (spider mites) and Buid Daba (aphids). Collections were done along the road on various plants. The first stop was in the village "Adulala"near Adama
Coördinates: N08°28'15.280, E039°17'57.251
Altitude: 1656 m

2 collection:

- Again Orius predatory bugs from yellow Meskel flower or "Aday Abeba". The flowers contained this time alos a yellow thrips species. Flowers with thrips and Orius were put in tubes for further identification.
- From an unknown plant that looked liked a type of bean. Several red mites that were very fast running were collected. The Looked like a Balaustium sp. A few individuals were stored on 70% ethanol.

Coffee stop at Hotel Alamaayyoo
This is the place where Ada collected a spidermite predatory mite.
Coördinates: N08°24’32.577, E039°19’58.196
Altitude: 1538 m
One collection (by Buid Daba)
Mummies of parasitozed aphids were collected from a tree and kept dry in a tube for emergence of the parasitoid.

The third stop was near a huge sugercane field in the area “Sodare”near Adama. The crop was 3-4 meter high and only sprayed against weed. Spider mites were expected but not found. However, the plant near the sugar cane field contained often spidermites: on Datura and an unknown bean plant and an unknpown solanaceaous plant.
Coördinates: N08°24’22.881, E039°22’03.235
Altitude: 1457 m
A few collections:

- Spider mites and probably a phytoseiid predatory mite from Datura. Extremely high densities of spider mites were observed.
- In a spider mite colony one individual of an Oligota sp. Spider mite beetle was found and collected.
- One red coloured predatory mite was found running fast on bean leaves with spider mites. The mite was stored on 70% ethanol.
- Larvae of a spider mite predatory midge were found in a spider mite colony. Only 2 larvae were found, so not collected. Adults are needed for a proper identification.

Fourth stop:
Coördinates: N08°24’12.341, E039°22’22.159
Altitude: 1440 m

Ricinus communis trees were checked for the presence of Iphiseius predatory mites. They weer abundantly present. No other natural enemies were found. Many huge pollen feeding beetles were present, but we did not collect them.

Bean like plant with many red fast running Balaustium predatory mites.

Cotton type plant. Orius was also found in spider mite colonies.

Huge sugarcane field.

Solanacaeous crop on which the spider mite beetle Oligota sp. was found in a spider mite colony.

Vegetation around the sugarcane fields.

Datura stramonium infected by spider mites and cpolonized by a phytoseiid predatory mite.

Ricinus communis plants with Iphiseius sp. predatory mites.

The "good wish smoke"of the coffee ceremony. Let's wish all collections lead to the identification of several species that have a great potential for biocontrol in Ethiopia.

Annex 8 Mission February / March 2017; Collection mission to Debre Zeit and Holeta

Gerben Messelink & Anne Elings
Wageningen University & Research Greenhouse Horticulture

Objectives of mission
End-of-project workshop
Administrative arrangements
Collection of predators

1. End-of-project workshop

The end-of-workshop was organized to present all contributions of Wageningen University & Research to the project. These results were presented on the first day of the workshop by Dr. Gerben Messelink and Dr. Anne Elings, as shown below. The presentations were made available to Dr. Adhanom Negasi of Ehpea. The first day was chaired by Dr. Duguma Adugna, representing Jimma University, and reported by Dr. Fikre Markos, representing the Ministry of Agriculture. The few pending activities of WUR were not yet presented. The workshop was attended by more than 40 representatives from farms, universities, governmental bodies, the Embassy of the Kingdom of The Netherlands, and Ehpea.

The second day of the workshop focused on detailed discussions on future IPM activities. The second day of the workshop was attended by a smaller group of persons, still a wide representation of the sector. The second day was chaired by Dr. Adhanom Negasi of EHPEA and reported by Dr. Fikre Markos, representing the Ministry of Agriculture.

WORKSHOP ON 'PAST ACHEVEMENTS REVIEW AND SUMMARY OF RECENT PROJECT RESULTS'

CAPITAL HOTEL, ADDIS ABABA – FEBRUARY 28 - March 1, 2017

no	Title of presentation	Presenter	Time
Tuesday, February 28: Morning Session Chairperson: Dr. Duguma Adugna; Rapporteur: Dr. Fikre Markos			
1	Registration of participants		8:30-9:00
2	Well-coming address	Mr. Frank Amerlaan	9:00-9:10
3	Introduction to the IPM project	Dr. Adhanom Negasi	9:10-9:25
4	Overview of the Wageningen supported IPM activities in Ethiopia	Dr. Anne Elings	9:25-9:40
5	Biological control opportunities for tomato pests: whiteflies and *Tuta absoluta* Current strategies in Europe New identified natural enemies in Ethiopia Opportunities for biocontrol in Ethiopia	Dr. Gerben Messelink	10:00-10:45
6	Coffee break		10:45-11:30
7	Biological control opportunities for thrips and spider mite control in ornamental and vegetable crops Identified thrips species in Europe and Ethiopia Current strategies in Europe and Ethiopia New identified natural enemies in Ethiopia Opportunities for biocontrol in Ethiopia	Dr. Gerben Messelink	11:30-12:15
8	Biological control opportunities for mealybugs in roses and other ornamental crops Current strategies in Europe New identified natural enemies in Ethiopia Opportunities for biocontrol in Ethiopia	Dr. Gerben Messelink	12:15-12:45
9	Lunch		12:45-14:00
	Afternoon session Chairperson: Dr. Duguma Adugna; Rapporteur: Dr. Fikre Markos		
10	IPM training, interaction with growers	Dr. Anne Elings	14:00-14:30
11	Up-scaling of fungal and bacterial antagonists	Dr. Anne Elings	14:30-14:45
12	Need assessment Round table general discussion with expected output SWOT analysis Priorities	All the group	14:45-16:00
Wednesday, March 1 Chairperson: Dr. Adhanom Negasi; Rapporteur: Dr. Fikre Markos			
1	Opening of the day	Dr. Adhanom Negasi	9:00
2	Development of shared perspectives Expected output: Long-term goals Short-term goals Intervention (linked to SWOT analysis)	Selected group	9:00-12:00
3	Lunch		12:00-13:30

Dr. Fikre Markos reported the following on the workshop:

Introduction

The workshop was scheduled to commence registration of participants at 8:30 a.m. and conclude the registration by 9:00 a. m. This was done accordingly and was followed by the opening processes including the welcome, introduction of the workshop and highlighting the major milestones of the project by Dr. Adhanom Negasi. Mr. Frank Ammerlaan delivered the official welcoming address of the workshop.

Presentations

The session was chaired by Dr. Duguma Adugna from Selale University (former Djimma University staff) supported by a rapporteur from the Ministry of Agriculture and Natural Resources. Dr. Anne Elings and Dr. Gerben Messelink from Wageningen University presented according to the schedule set out in the program of the workshop (Refer workshop program annexed). Presentations were tuned both to the national and international practices in integrated pest management of horticultural crops with specific reference to the application of biological control agents. In the presentations overview of the Wageningen supported IPM activities in Ethiopia, biological control opportunities for tomato pests (white flies and Tuta absoluta), thrips and spider mites control in ornamentals and vegetables, mealybugs in roses and other ornamentals were covered. IPM training interactions with relevant stakeholders and growers and up scaling of fungal and bacterial antagonists were also covered in the presentations. At every presentation, questions suggestions and comments were provided by participants and the presenters.

Among the comments underscored by the participants, the finding of new records of beneficial biological agents in the country were considered as a possible gap filling opportunity of the long standing constraint of the lack of technology availability for integrated pest management. These findings were conceived as potential for commercialization parallel with the biological agents already in use in the country.

In the general discussion session issues related to the following subjects were raised and discussed:

- The need for putting in place the required legal framework for the importation and release of the biological control agents.
- Ensuring the availability of demonstrable technologies that are compatible with other alternative IPM components.
- Demonstrating the economics of IPM for the users.
- Encouraging the producers to adopt the technology.
- The possibility of combining biological control agents and other alternatives like cultural practices.
- Improving the quality of graduates in horticulture through competence based curriculum development.
- The need to initiate mass rearing of the indigenous biological control agents.
- The need to transfer technology through partnership with the technology developers.
- The challenges faced with the use of pesticide and pesticide resistance.
- Incentivizing those who wish to engage in commercializing the technologies giving due attention to small companies or enterprises.
- Improving the linkage among the research institutions, higher learning institutions, regulators and the industry.
- The need to give equal access to the technology providers to enable the market to operate in a competitive manner and other related issues were discussed.

Finally participants suggested prioritized interventions giving responsibilities to the relevant institutions but recommended a smaller group to finalize the prioritization based on the directions provided by the participants of the plenary session.

Among the priority areas the following were at the top list of the participants:

- The legal framework should receive due attention and get finalized by the Ministry of Agriculture and Natural Resources. Expertise and funding can be thought from relevant bodies including the research, the higher learning institutions and the industry.
- Incentivize those who engage in the commercialization of the technologies. This can be worked out based on experiences from elsewhere and may need to be stated in a policy document for the sector.
- Design a mechanism where the research institutes, the regulators and the industry work in close partnership to alleviate the prevailing challenges.
- Follow up the implementation of the recommendations of the previous project out puts.

Day Two

This session is convened to discuss the issues raised in the workshop in a more detailed manner and to prioritize the activities to be implemented during the project life of the forthcoming project which is under process. Dr. Adhanom starting by welcoming the participants introduced the overall task of the group and highlighted what is expected from the group and invited Dr. Anne to explain the detail task.

Dr Anne explained the outline prepared for funding and would like to identify the activities to be implemented by the project which will last for three years. He noted the timing is not yet clear as to when the project proposal will be approved. In any case there is a need to identify the activities in a prioritized but with no budget ear markings as this would be done at a letter stage after the approval of the project proposal. Consequently the following areas were identified as the main activities to be implemented by the project.

1. Continue in the identification and testing of new endemic biological control agents. This task requires:
   - Agent identification.
   - Analysis of the behavior of the agent.
   - Testing the efficacy of the agent in the field (the issue of where this can be done is yet to be determined as it requires medium or semi commercial greenhouse establishment).
2. Evaluation of the test result Adoption and registration for commercialization (Rules and procedures need to be set out as a legal frame work).
3. Training and capacity building.
4. Creating a platform for technical consultations.
5. Establishing and sustaining funding for the training center in Horticulture.

In reaching consensus, pests and crop combinations were taken as the bases for prioritizing the activities. In the course of the discussion Tuta absoluta on tomato, Thrips on roses, Mealybug on roses, Aphids, White flies, Leaf miners, Bemisia Tabacci, Spider mites, were believed to be the main pests in the green house raised crops. On the other hand Strawberries, Herbs, Peas roses, tomatoes and green beans were the main crops affected by the pests mentioned above.

The usage of pesticides by the smallholder's pest management practices is affecting the interventions in the integrated pest management in the commercial farms. The small holder farmer is not using biological agents but rather use pesticides. The pesticides through drift effect damage the biological agents used to manage pests by the commercial farms.

Based on the identified of biological control agents, the following crop pest combinations were discussed:

Table

Focus species of natural enemies, pests and cropping systems.

		roses				carnation		strawberry		herbs	cuttings		tomato	
species of natural enemy	category	thrips	mealy-bugs	aphids	spider mites	thrips	spider mites	thrips	spider mites	thrips	thrips	white-flies	Tuta	white-flies
Nesiodocoris sp	a			x								x	x	x
Orius naivashae	a	x		x		x		x		x	x			
Orius kokai	a	x		x		x		x		x	x			
Amblyseius herbicolus	b	x				x		x		x	x	x		
Amblyseius swirskii	c	x				x		x		x	x	x		
Hypoaspis/Macrocheles	c	x	x			x		x		x	x			
mealybug predatory midge	c		x											
mealybug parasitoid	c		x											

category

a	identified and reared
b	identified but needs to be re-collected and reared
c	needs to be collected, identified and reared

Finally the session was closed by Dr. Adhanom Negasi thanking the participants, presenters, and all those who contributed to the workshop.

2. Administrative arrangements

a. Project termination

An intermediate report had been prepared and was submitted to EHPEA to make available the second payment, according to the contract.

The third payment has to be before March 31, which is the final deadline set by the Embassy of the Kingdom of The Netherlands. Although most activities will have been finished by then, some activities may be pending:

- 1.1.1 DNA molecular identification of selected entomopathogenic fungal antagonists (work by WUR is in progress)
- 1.2.1 DNA molecular identification of the selected entomopathogenic bacterial antagonists (WUR is still waiting for DNA)
- 1.4.1 Identification of predators collection on March 2 and 3.

A gentlemen's agreement: With Mr. Tewodros Zewdie, EHPEA Director, it was cordially agreed that the final payment from EHPEA to WUR will be made before the mentioned deadline, and that WUR will fulfil its project obligations at the earliest possible moment.

It was agreed that the PCR course is cancelled.

b. Future project

The proposal for IPM phase 3 has been submitted and is currently in The Hague for assessment. Approval is expected shortly, after which a new workshop will be organized to develop a detailed work plan.

WUR was requested by EHPEA to participate in IPM phase 3.

3. Collection of predators

Two field days were devoted to the collection on endemic natural enemies and thrips. This was the fourth time species were collected and we decided to focus this time on the collection of predatory mites. Besides the predators, we also collected thrips species to increase our knowledge about the species that can occur in the areas surrounding greenhouses. The collection area was different from earlier missions. The first day, species were collected in the surroundings of Debre Zeit. This area was rather dry, but plenty of green vegetation was present around the lakes and resorts. The second day we went to the area around Holeta. This has a higher altitude than Debre Zeit and therefore also more green vegetation. Collections were made along the road, in a wild park and at the Holeta Bee Research Center. In total, 16 samples with natural enemies and thrips species were collected and put on alcohol for further identification. Annex 3 gives an overview of the collected samples. The final identification of all collected species will take several months because of the need of specific taxonomic expertise.

Collections Ethiopia on March 2-3 2017 near Debre Zeit and Holeta

#	date	place	coördinates	altitude (m)	host plant	collected species	remark
1	2-mrt	along the road in Debre Zeit	N8°45'30.114, E38’58'21.735	1910	Lantana camara	staphyllinid predator/thrips	
2	2-mrt	Adulalaresort & spa, Lake Babogayo	N8°47'31.745, E38°59'45.932	1985	cultivated red gerranium	phytoseiid predatory mites	3 tubes from 2 locations in the resort
3	2-mrt	Adulalaresort & spa, Lake Babogayo	N8°47'31.745, E38°59'45.932	1985	pink flowering succulent plant	thrips	next to the gerranium, might have provided pollen for the predatory mites
4	2-mrt	Adulalaresort & spa, Lake Babogayo	N8°47'31.745, E38°59'45.932	1985	bourgainvillea tree with pink fowers	phytoseiid predatory mites and one balausteum feeding on an whitefly adult	leaves looked clean, occasionally some whiteflies and aphids
5	2-mrt	onion field in Debre Zeit, close to greenhouses	N8°46'06.405, E38°00'18.785	1898	onion	thrips in flowers	these thrips may migrate from the field to the greenhouses
6	2-mrt	parc in Debre Zeit	N8°45'25.869, E38°56'58.625	1894	unknown climbing plant (looks like bean)	spider mites and thrips in spider mite colonies with coloured wings	predatory thrips?
7	3-mrt	Wild life centre Ensessakotteh (way to Holeta)	N9°03'53.362, E38°32'53.359	2408	unknown tree with hairy leaves	phytoseidd predatory mites	no prey observed
8	3-mrt	Wild life centre Ensessakotteh (way to Holeta)	N9°03'53.362, E38°32'53.359	2408	unknown tree with hairy leaves	thrips and spider mites + 1 Ologota + on one sample degerenans?	
9	3-mrt	Holeta Bee Research Center	N9°03'28.997, E38°30'22.934	2390	Veronia amygdalina (flowering tree)	2 species of predatory mites, including Iphiseius degenerans	
10	3-mrt	Holeta Bee Research Center	N9°03'28.997, E38°30'22.934	2390	Wanza tree, Cordia africana	many black big mites	predators?
11	3-mrt	Holeta Bee Research Center	N9°03'28.997, E38°30'22.934	2390	Koso tree, Hagenia abyssinica	very tiny predatory mites and some thrips	
12	3-mrt	Holeta Bee Research Center	N9°03'28.997, E38°30'22.934	2390	yellow flowers of a composite	thrips (a lot)	
13	3-mrt	Yilma restaurant Holeta	N9°04'18.990, E38°29'56.228	2396	Lonicera sp?	parasitized whiteflies (black pupae)	put in tubes to let them emerge
14	3-mrt	road from Holeta to Addis	N9°03'19.667, E38°33'34.641	2519	Veronia amygdalina (flowering tree)	thrips in purple flowers	

#	date	place	coördinates	altitude (m)	host plant	collected species	remark
15	3-mrt	road from Holeta to Addis	N9°03'19.667, E38°33'34.641	2519	Croton tree, Croton macrostachyus	predatory mites + thrips	mites and thrips probably feed on the extra floral nectar
16	2-mrt	farm maranque			composite flower	thrips	sample received from Marc Driessen

Annex 9 Results DNA molecular identification

Beauveria bassiana

B1-EF

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B1-ITS4

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 421 GGCCCTGAAA TGGAGTGGCG GCCCGTCCGC GGCGACCTCT GCGTAGTAAT ACAGCTCGCA
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B1-RPB1

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B1-RPB2

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B2-EF

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B2-RPB2

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B5-EF

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B5-ITS4

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B5-RPB1

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B5-RPB2

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B6-EF

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 781 GGAAATCATC AAGGAGACTT CCAGCTTCAT CAAGAAGGTT GGCTACAACC CCAAGGCTGT
 841 TGCTTTCGTC CCCATCTCCG GTTTCAACGG CGACAACATG CTTGAGCCCT CCACCAACTG
 901 CCCCTGGTAC AAGGGCTGGG AGAAGGAGAC CAAGGCTGGC AAGTCTACTG GCAAGACCCT
 961 TCTCGAGGCC ATCGACGCCA TTGAGCCCCC AAGCGTCCTA CCGACAAGCC TCTCCGTCTT
1021 CCCCTCCAGG ATGTTTACAA GATCGGTGGT ATCGGAACGG TGCCCGTCGG TCGTGTTGAG
1081 ACTGGTATTA TCA
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B6-ITS4

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   1 AAACGTAACA AGGTCTCCGT TGGTGAACCA GCGGAGGGAT CATTACCGAG TTTTCAACTC
  61 CCTAACCCTT CTGTGAACCT ACCTATCGTT GCTTCGGCGG ACTCGCCCCA GCCCGGACGC
 121 GGACTGGGCC GGCGGCCCGC CGGGGACCTC AAACTCTTGT ATTCCAGCAT CTTCTGAATA
 181 CGCCGCAAGG CAAAACAAAT GAATCAAAAC TTTCAACAAC GGATCTCTTG GCTCTGGCAT
 241 CGATGAAGAA CGCAGCGAAA CGCGATAAGT AATGTGAATT GCAGAATCCA GTGAATCATC
 301 GAATCTTTGA ACGCACATTG CGCCCGCCAG CATTCTGGCG GGCATGCCTG TTCGAGCGTC
 361 ATTTCAACCC TCGACCTCCC CTTGGGGAGG TCGGCGTTGG GGACCGGCAG CACACCGCCG
 421 GCCCTGAAAT GGAGTGGCGG CCCGTCCGCG GCGACCTCTG CGCAGTAATA CAGCTCGCAC
 481 CGGGACCCCG ACGCGGCCAC GCCGTAAAAC ACCCAACTTC TGAACGTGAC CTCGAATCAG
 541 G
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B6-RPB1

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   1 AGCGATCCGA TTCGTCACAG CTATTCATAC TCGCGATCCG AAACTCCGAT TCAAGCGCGT
  61 TTGGGCCGTA TGCAAGAAGA AGCGCAAATG CGAGAATGAG GAGCGGCAAG ACAAGAATAA
 121 AGACGAAGAG TTCGCTCCAG GTGTCAAGAA CGTCGTTCTC GAAGGACATG GCGGATGTGG
 181 CAATATGCAG CCGCAGGTGA GACAGGCTGC GCTGCAACTC AAAGCTGCCT TCGAGGTTAC
 241 TTCGGAAGAG GGTCCCAAGA GGAAAGAGAC GGTTAATATC AGCGCCGAGA TGGCGCATGG
 301 TATCCTTCGC CGCATCTCTG AGCGCGATCT GCACAATATT GGTCTTAACT CAGACTATGC
 361 TCGTCCCGAG TGGATGATCA TCACTGTCCT GCCTGTACCC CCTCCTCCCG TGCGTCCTAG
 421 TATTTCCATG GATGGTACTG GTACTGGCAC GAGAAACGAG GATGATCTGA CTTACAAGCT
 481 TGGTGACATT ATCCGCGCCA ACGGTAATGT CAAGCAGGCC ATTCGTGAAG GATCACCGCA
 541 ACACATCGCG CGTGATTTTG AGGAGCTGCT GCAGTACCAT GTTGCGACCT ACATGGATAA
 601 CGATATTGCG GGTCAGCCGC GGGCGCTCCA AAAGAGCGGT CGTCCTGTCA AGGCGATTCG
 661 CGCCCGTCTC AAGGGCAAGG AGGGTCGTCT GCGAGGCAAC CTTGATGGA
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B6-RPB2

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   1 CTGGTATTGG GGTGACCAGA AAAAGCTATG AGCTCAACTG CTGGTGTGTC CCAGGTTTTG
  61 AACCGATATA CTTTTGCCTC AACACTATCC CACCTTCGAC GTACCAATAC ACCCATTGGC
 121 AGAGATGGCA AACTGGCAAA ACCTCGTCAG CTTCATAATA CTCATTGGGG TCTTGTCTGC
 181 CCTGCTGAGA CACCTGAAGG CCAGGCTTGC GGTCTTGTCA AGAATCTGTC TCTCATGTGC
 241 TACGTCAGCG TCGGCTCCCC AGCCGAGCCG CTCATTGACT TCATGATCAA CAGGGGTATG
 301 GAGGTCATTG AGGAGTACGA ACCACTCAGA TACCCACACG CTACCAAGAT TTTTGTCAAC
 361 GGGACCTGGG TTGGAGTTCA CCAGGACCCC AAGCACCTTG CTGACCAGGT ATTCGACACC
 421 CGCCGCAAGT CCTACCTGCA GTATGAGGTG TCTCTTGTCA GAGAAATCCG TGACCAGGAA
 481 TTCAAGATCT TCTCTGATGC TGGCCGAGTC ATGCGGCCTG TTTTTACTGT GCAAAGTAAA
 541 AATGACCCGG AGACTGGCCT TGAAAAGGGA CAGCTCGGTC TCACCAAGGA TTTAGTCAAC
 601 AGACTGGCGC AAGAGCAAGC CGACCCGCCA GATGATCCAG AAATGAAGAC GGGTTGGGAG
 661 GGCTTGATCA AAGCTGGCGC TGTCGAGTAT CTAGACGCTG AAGAAGAGGA GACGTCAATG
 721 ATTTGCATGA CCCCGGAGGA TCTGGAGCTT TATAGACTTC AGAAAGCGGG TGTAGCTGTC
 781 GACGACGACC ATGGCGATGA TCTGAACAAG CGCCTGAAGA CTAAAACCAA CCCAACTACA
 841 CACATGTACA CTCACTGTGA AATTCACCCG AGTATGATCT TGGGTATCTG CGCAAGCATT
 901 ATTCCTTTCC CAGACCACAA TCAAGTAGCT TCTCATCACA TATACGCCGG CAATTCAGTT
 961 ACTGACCAGA GTTGTAGTCG CCTCGTAACA CCTACCAATC CGCAATGGGT AACAAGCCAT
1021 GGGCTTCTTC CTGACAATTA TTCTCGTCGT ATGGACACTA TGGCAAATAT CCTGTACTAC
1081 C
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Metarhizium anisoplae

M2-EF

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   1 ACTGGTCACT TGATCTACCA GTGCGGTGGT ATCGACAAGC GTACCATTGA GAAGTTCGAG
  61 AAGGTAAGCC AAACCACTCC GATTAATGAT CTGCTATTGT TTGGCGATGA ACATTATTGG
 121 GTTTCCCGCT GCCTGTCGGC CATTACCCCT CACTGTGGCA CGAAAATTTT CGCGGGGCCT
 181 TATCTTGGAC TTTGGTGGGG CATCATACCC CGCCAGCTGT CGAGGGTGTC TCTGTGTGTC
 241 TCTGGCTGTT GAAACCACAA TATTGTCGTT GCTTTCAGAG GGAAAAAACA TGAAACTAAT
 301 TTGGATCGCT GTATAGGAAG CCGCTGAACT CGGCAAGGGT TCCTTCAAGT
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M2-ITS4

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   1 CGTTGGAAGA ATAAACGTAA CAAGGTCTCC GTTGGTGAAC CAGCGGAGGG ATCATTACCG
  61 AGTTATCCAA CTCCCAACCC CTGTGAATTA TACCTTTAAT TGTTGCTTCG GCGGGACTTC
 121 GCGCCCGCCG GGGACCCAAA CCTTCTGAAT TTTTTAATAA GTATCTTCTG AGTGGTTAAA
 181 AAAAAATGAA TCAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA TGAAGAACGC
 241 AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG
 301 CACATTGCGC CCGTCAGTAT TCTGGCGGGC ATGCCTGTTC GAGCGTCATT ACGCCCCTCA
 361 AGTCCCCTGT GGGACTTGGT GTTGGGGATC GGCGAGGCTG GTTTTCCAGC ACAGCCGTCC
 421 CTTAAATTAA TTGGCGGTCT CGCCGTGGCC CTCCTCTGCG CAGTAGTAAA GCACTCGCAA
 481 CAGGAGCCCG GCGCGGTCCA CTGCCGTAAA ACCCCCCAAC TTTTTATAGT GACCTCGAAT
 541 CAG
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M2-RPB2

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   1 GCAGCAGCTC ACGGAACTTG AGAACTCCAT GGATCGAGTG GTGGCCAGAG GTTTCTGGGG
  61 ATAATAGAGA ATATTTGCCA TGGTGTCCAT ACGGCGAGAG TAATTGGTGA GGAAGAAACC
 121 CATGGCTTGT TTACCCATGG CGGATTGATA GGTGTTACGA GGTGACTAAA CAAGACTGTC
 181 AGTAGAGCAT GAGACAATTC TTGACAAAGG CTGCTTACCT GATTGTGATC GGGGAACGGA
 241 ATAATACTAG CGCAAATACC AAGAATCATA CTGGGGTGAA TTTCACAATG CGTATACATG
 301 TGCGTTGTGG GGTTGGTCTT GGTCTTGAGA CGCTTATTCA GGTCATCTCC AATATCGTCA
 361 TCAAGAGCAA CACCGGCTTT CTGCAGACGA TACAGCTCAA GATCTTCTGG CGTCATGCAG
 421 ATCATTGATG TCTCTTCTTC TTCGGCATCG AGGTACTCGA CGGCGCCGGC ACGAATCAGT
 481 CCTTCCCAGC CAATTTTCTC GCTTGGGTCT TCGGGTGGTT CAGCTTGTTC TTTAGCAAGC
 541 TTGTTAACCA ACTCTTTGGT CAAAACGAGA TGGCCTTTTT CAATGCCAGT CTCGGGATCA
 601 TCTTCTTGCT GCACAGTAAA CACTGGTCTC ATAACTCGGC CAGCGTCGGA GAAAATCTTG
 661 AACTCTTGAT CCCTGATTTC TCGGACGAGA GACACCTCGT ACTGCAGATA CGACTTTCGT
 721 CTAGTATCCA AGACTTGACT GACCAGGTGC TTAGGATCTT GGTGTACACC AACCCAAACA
 781 CCATTGACAA AGATCTTGGT GGCATGGGGA TATCTCAGCG GCTCGTACTC CTCTACCACT
 841 TCCATGCCAC GGTTGATCAT GAATTCAATC AATGGCTCGS CCGGTGAACC CACACTGACG
 901 TAACACATCA ATGACAGGTT CTTGACCAGG CCGCAAGCCT GACCTTCTGG CGTCTCGGCA
 961 GGACAGACCA AGCCCCAGTG TGTGTTGTGC AGCTGACGCG GTTTAGCGAG CTTACCATCT
1021 CTACCAATTG GTGTGTTGGT TCGTCSCAGT GAGAGAGTGT CGAAGCAAAA GTATACCTAT
1081 TCAAC
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M2-RPB1

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   1 TGGCGTGAAG AGAATTCAGA GGGTGTGAGA CCACGAAGGT AAGAGTTCTC GACAAAACCA
  61 CGTGCTTCAG GAGAATAGTC ATCCTTTGTG AAATGGGGCA GTGTTCGATA CTTGAAACCG
 121 AACGGAATAC GCTTTCCTTC CACAATTTGC TGGCCGACGA GGGCTGTCAT TTGCGAGATG
 181 TTAATTGAGG ATCCCTTGGA ACCAGATTCA GACATGGTTA CGGCATTGTT CGAGTCCTTC
 241 AAACTTTTCT GTGTGGTGGT ACCAGCCTGG TCACGGGCGG AGTTGAGAGC CATGGAGACT
 301 TTGTTCTCGA ATGTAGCTCG GACATTCATA CCTGGCAGGG CTTCGAGCTC GTTTGCCGTG
 361 GCCTGGGCCG TGAGCTTGGC GACTTCGGCC TTCTGTGTGT CAATGTGAAC CTGAATCTTT
 421 TCAATGGTCT GCTTGTCTGG AACAGTATCT CCAATACCGA TACTGTGACC GGTGTTGAGA
 481 AGCCAATAAG TGACTACTTG TTGAACACCA TTCAGAAAAG CCATGGCACC ATCGGGGCCA
 541 AGCTCATTGT AGCACAGATG AATAATACCA CCAGCCGCTG CACCCACATT TTTCTTCTTC
 601 AGAAGACCAT ACAGAAGTTC GCCGCTCTGG ATAAGGAGCC CGGTATCCTT GAGGGGGATA
 661 TCAGAGTCAC CTTCAGGGGC ATGCAAACTA ATCTCCTGAG GAATGACCAT GCTGATGAGC
 721 TGTTTTCCGG TCCAGCGAGG CCGCGGTTTG AGAATGGCTG GTTGGGGAAT AACACCGTCC
 781 CAATTAGGCA CCCAGAGCAT CATGTTCATG ACCATTTCCT TGTCGATAAR GTGTCTCGTC
 841 TGCAGAGTTT GTAAACACCA GCAAGGGAGT CCTGCACAAT ACCCATCAAG GGA
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Annex 10 Meetings with growers

Tuesday August 18, 2015

Visit to AQ Roses, Ziway by Gerben Messelink and Anne Elings

We met with Mr. Wim Ammerlaan, Mr. Ron van Hoorn, and Mr. Mohammed Ayalew, +251 916 580196. Transport was kindly provided by EHPEA.
AQ Roses in Ziway is a large rose producing company (http://www.rosaplaza.nl/AQ-Roses).
Pests:

- Western flower thrips and mealybugs are the 2 most import pests that became problematic after they started with biocontrol of spider mites. Thrips are moving in from outdoor crops when these are harvested.
- The spider mite control strategy is: preventive release of Koppert rearing sachets with *N. californicus* and scouting and hot spot treatments with *persimilis*.
- *Amblyseius* swirskii was used before, but not anymore, not effective enough. They now use biopesticides, such as neem-based products.
- They did trials with rearing their own swirskii on Ricinus banker plants, but they were soon outcompeted by the naturally occurring Iphiseius degenerans. Release of degenerans in their roses from Ricinus leaves was not successful (obviously because of a lack of food).
- They have been using *cryptolaemus* larvae against mealy bugs, but the stopped as it was not effective enough and in their opinion too expensive.
- Dudutech and RealIPM are conducting trials at other farms.
- Options for thrips control we discussed:
  - *Hypoaspis* or other endemic soil-dwelling predatory mites.
  - Natural occuring *Euseius* spp. in combination with a natural pollen source (could be degenerans + ricinus pollen, also maize is growing everywhere). Besides degenerans, also other predatory mites were walking around on the Ricinus plants besides the greenhouse, they should be identified).
  - Increase mass trapping with odours, they observed to catch 10 times more thrips with sticky plates just above the soil compared to sticky plates above the crop.
  - Search for an endemic *Orius* and introduce them on banker plants like the ornamental sweet pepper
  - Entomopathogens (Dudutech and real-IPM claim to have thrips specific strains).
- Options for mealybugs we discussed:
  - Endemic parasitoids such as *Leptomastix*, which can be introduced on an open rearing system such as kalanchoe with *Planococcus citri*, or another endemic mealybug species.
  - New entomopathogen, such as the *Beauveria bassiana* strain of the Addis Ababa university (PhD-student Yonas did a trial on the farm to test efficacy and look for synergistic effects with the neem product). This strain seemed to infect mealybugs, but the trial was disrupted by sprayings against powdery mildew after one week.

Diseases:

- Fusarium, powdery mildew, downy mildew, botrytis. Chemical measures are used, applied seasonally (on a need basis only), combined with cultural practices (maintain an open crop).
- Sulphur is used for powdery mildew control, which seems to be not an issue for californicus and *persimilis* (there is a danger of affecting predatory mites).
- Downy mildwew occurs only in the rainy season, they try to solve that with crop management (keep it open) and they spray.

Nematodes:

- *Meloidogyne* can be an issue, but is solved by using rootstocks, additionally they use a Sesam oil (seems to help) and Trichoderma (does it help?)

On-farm trials should be safe for the company. In a recent example, that was part of the MSc programme, the experiment had to be ended because of risky disease development. Actually, semi-commercial greenhouses that represent the circumstances in commercial greenhouses and in which control measures can be evaluated, are lacking.
On-farm trials should be solution-oriented.


Figure 1 *Left: group discussion at AQ Roses; right: Gerben inspecting indigenous predators at a ricinus plant.*

Visit to Florensis, Koka by Gerben Messelink and Anne Elings

We met with Jan-Willem van der Meijden, Senior Agronomy Manager. Florensis was an active participant in the first phase of the project, when efficacy trials to determine the effect of *Amblyseius swirskii* and *Eretmocerus* spp. on white fly management in herbs were conducted.
Florensis currently mainly produces potted and border plants. The company is still very active in introducing IPM. It is involved with conducting a number of efficacy trials, some of which receive financial support from Ehpea from the IPM programme.

March 2016

Visit to Ziway by Gerben Messelink

The growers that attended the meeting were Frank Ammerlaan (AQ roses), 3 people of Barnhoorn (Sher) and 2 more growers (Arie Braam and Ziway Roses??) + the MSC student of Jimma University and Dr. Duguma.
Things discussed:

- Thrips and mealybugs are the most important pests, but the growers disagree which of thoses is the most important (HG roses- thrips, Sher- mealybugs).
- The project should also focus on finding endemic soil-dwelling predatory mites that may also contribute to the control of thrips. This can be included in the next missions.
- It is unclear when which species if thrips occur. In some periods, large numbers of thrips fly in from field with Teff or other crops. We decided that the people of Jimma will organize to monitoring the seasonal abundance of thrips in roses, by weekly catches of adult thrips and store them on alcohol. Duguma will organis this, WUR will support he thrips identifications.
- The presentation about thrips and mealybugs will be send to HQ roses.
- The results of biological control of mealybugs with cryptolaemus larvae were discussed. They are only successful in "hot-spots" , but very sensible for pesticide treatments against other pests and diseases. Thus additional biological control agents are required.

May 2016

Visit to ET Highland by Andre van der Wurff and Anne Elings

We met with Ms. Emebet Tsegaye and Mr. Wondwossen Legesse, Farm Manager and Crop Protection Manager, respectively, of ET Highland at Sebeta. It was a warm welcome after some 6-7 years of not having met. We were shown around the farm, that is expanding from 12 ha with another 7.5 ha. The new greenhouses are approximately 1 ha each and know a span with of approximately 12 m.

Latest IPM developments were discussed, both in Ethiopia and in The Netherlands.

Some relevant IPM points:
- 50% biological.
- Problems:
  - Thrips (nation-wide), possibly because the soft chemicals that are required by biological control are not sufficiently effective. Black thrips is a new species occurring.
  - Mealy bug.
  - Agrobacterium (crown gall).
- Spider mite is controlled by 'Silvet Gold' (Bayer), an organic oil, which is effective and much cheaper than predators.

August 2016

Visit to Maranque Farm by Marjolein Kruidhof and Ada Leman

We left Maranque farm during the morning. On our way back to Addis Ababa we visited the rose farm ZK Flowers in Debre Zeit. This farm is owned by Zelalem Messele, chairman of EPHEA. This rose farm is situated approximately at 50 km distance from Addis Ababa and lays on an altitude of approximately 1800 m above sea level. Temperature range in this area is between 17-25 degrees with an average rainfall of 1100 mm per year (source http://www.jittuhorticulture.com/?q=farms). They have almost 11 ha of greenhouses, with 8 varieties of mainly long-stem roses. They export 60-70% of their roses to the Netherlands.

We spoke with Beniam Getachew about their problems with thrips. At the moment of our visit they did not experience problems with thrips. The major problems start in December, following the harvest of teff. They observe flower damage, but not leaf damage. They also find the pupal stage in the beds. They try to control the trips with Diazinon; they drench the soil and spray at the same time. They also use mesurol against thrips. Against spider mites they use a product that kills the mites through suffocation. They only scout for thrips inside the greenhouse, but not outside. We discussed that it would be good if they also scout for thrips outside the greenhouse, to detect the thrips before it enters the greenhouse.

Moreover, we discussed with Dr. Adhanom Negasi about making a small questionnaire for rose farms in different rose growing areas of Ethiopia with questions about thrips problems. This to find out how thrips densities fluctuate over the year in the different rose growing areas, and which chemicals are used for the control of thrips and other pests.

Annex 11 Meetings with stakeholders

Thursday August 20. 2016

9:00-13:00: Stakeholder workshop.

The purpose of the workshop was to present the WUR assignments in the IPM2 programme, provide explanation, and obtain from the stakeholders additional suggestions and comments for further improvement of the assignments.

Programme:

Ethiopian Horticulture Producers and Exporters Association (EHPEA)
Integrated Pest Management (IPM) Component

Workshop agendas for WUR involvement in EDPHD IPM program

Time: 9:00-1:00 AM

Venue: EHPEA meeting hall

Attendees: Refer the list

Time	Agendas	Presenters
9:00-9:15 AM	Welcoming and brief introduction of participants	
9:15-9:35 AM	Progress of the IPM component of EDPHD	Adhanom Negassi (PhD)
9:35-9:50 AM	WUR and areas of excellence in crop protection	Gerben Messelink (PhD)
9:50-10:30 AM	Open discussion on activities to be handled by WUR in the IPM component program of EDHDP (Refer the compiled document)	
10:30-10:45 AM	Coffee break	
10:45-11:15 AM	Policy and registration bottlenecks for the use of biological products in Ethiopia.	
11:15-11:45 AM	Issues related to permit, verification trials, possibility of registration and/or permit for commercial applications of biological products in Ethiopia	
11:45-12:30 AM	Final Question and Answer	
12:30-1:00 PM	Lunch and wrap up	

Highlights:

- Major stakeholders were present; only individual farmers were absent (but of course represented by EHPEA).
- Recommendation: develop an IPM website under the EHPEA website.
- Recommendation: Should there be established a Monitoring and Evaluation Committee to review experiments?
- Koppert and RealIPM would be most happy to consider in a sort of business deal. Koppert has tried to establish a local production facility, but has given up because of bureaucracy. Koppert has much interest in using biological products in outdoor crops such as vegetables, barley and oil seeds.
- There is 30% tax on (the import of) bca's, which are considered regular life animals. This leads to high prices. There is a role for the Ministry of Agriculture.
- There is no proper regulatory framework for the import and registration of biological control agents.
  - Registration is not possible. Efficacy trials and permits per product, per farm.
  - Import per farm, not in bulk, so very expensive.
  - Import organization that imports for the entire country not possible.
  - Does MoA need assistance in designing a protocol?
  - EIAR is very busy has barely has time to execute efficacy trials. MoA should have specific group of scientists for this job.
- Recommendation: The Association should put mass rearing strongly on the agenda.

Figure 3 *Workshop participation.*

Monday May 9, 2016

presentation by Dr. Andre van der Wurff on latest scientific developments in crop protection through strengthening of crop resilience was given at JKUAT.

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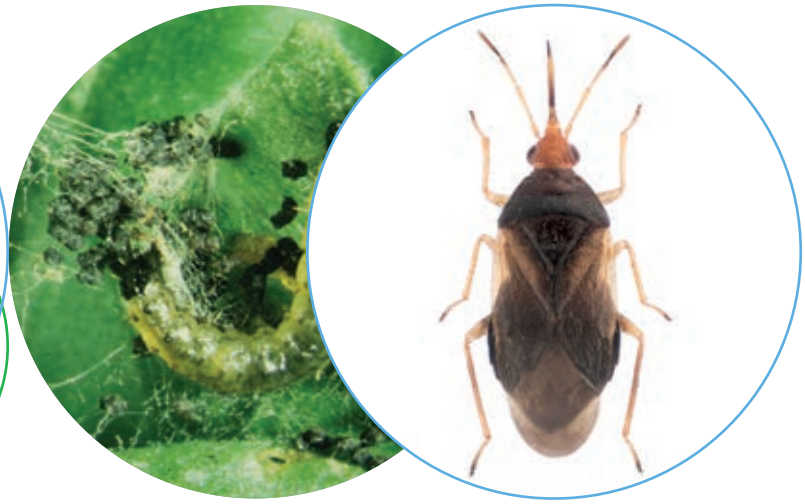

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