Proposal for Biological Pest Control

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OUTLINE FOR BIOLOGICAL CROP PROTECTION

Introduction

On Wednesday 16th of December 2015, I have visited the horticulture farm in ■■■■.

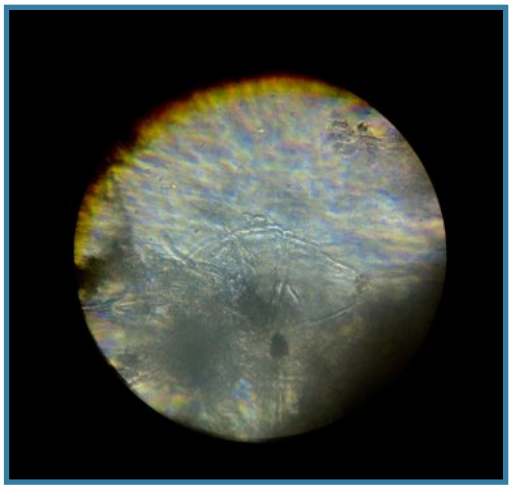
The reason of my visit was to make first observations and exchange ideas on introducing on Biological Pest Control on the farm to bring part of the production proces to a more 'ecologically responsible' level, specifically, by the use of *Trichoderma* sp. In this document I will describe the road map to large-scale production of locally obtained *Trichoderma*.

Definition of the Problem

Crops that are grown at the farm near ■■■ are: (mainly?) *Hypericum* and *Veronica*. The production of ornamental thistles was discontinued because of its high vulnerability to *Fusarium*.

The ***Hypericum*** plantation is based on well developed root systems which are 5 to 7 years old. It shows little vulnerability to *Fusarium*. However, nematode infestations of the root systems can cause signification damage to the plantation.

The other crop, ***Veronica***, shows more disease symptoms. Plants show leaf deformations, (probably?) caused by *Rhodococcus fascians*. Furthermore, the plants show infections at the cutting location, which is spreading through the base of plant. Sample taking and analysis was not performed appropriately due to the lack of the right equipment. However, a hight amount of mycelium was observed under the microscope, implying it to be a fungal infection. Unfortunately, the department for Phytopathology of the Addis Ababa University refused to study the sample (and the sample was therefore discarded).



Trichoderma is used as biological pest control for the protection of crops against pathogenic fungus species. It is an ecological alternative for the management of plant diseases that are important in agriculture and horticulture.

Commercial *Trichoderma* can be used to inoculate the soil in the farm. The main disadvantages with this procedure:

1) The product is expensive.
2) The commercial *Trichoderma* formula is likely to be less-effective since it contains a species that is probably not optimally adapted to the local climate, such as soil structure, pH, nutrients, temperature, humidity, etc.

Proposed solution

Veronica cuttings.

It is likely that the infection is spread by contaminated pruning scissors. Scissors are decontaminated using formaldehyde. However, it is a highly carcinogenic and allergenic substance. Alternatives for the use of formaldehyde are soil-infusion with hydrogen-peroxide and/or fungicides (if the infective agent is a fungus). Scissors can be decontaminated with 1000 ppm of active chlorine (0.1%) with at least 30 second exposure time. Chlorine is corrosive and scissors need to be rinsed thoroughly with sterile water and/or 70% ethanol and air-dried. Scissors can also be decontaminated by high-intense UV light. Proper eye and skin protection would need to be put in place. Scissors need to be turned after 30 minutes. Still, the UV light is not able to penetrate the small slits.

Rhodococcus fascians

Veronica is in particular susceptible to infection and disease from *Rhodococcus fascians*. Maybe the bacterial disease has been brought in on propagation material. The bacteria can be further transmitted by clonal propagation and / or via infected infected pruning tools and via water splash and in flood irrigation systems. *R. fascians* is a very persistent soil bacteria and there are no specific products that can be used to prevent the disease. Controlling measures that can be taken are:

* Discard infected plants before they shed bacteria onto surrounding plants
* Discard any plants that had contact with the infected plant.
* Decontaminate the surfaces on which the plant had been growing
* Decontaminate surfaces which had contact with the plant (pruners, water irrigation hoses)

Nematodes

Nematocides are used for the control of root-nematodes. Several nematocides have been withdrawn from the market because of environmental en health concerns. A number of commercial ‘Biological' products based on nematophagous fungi and bacteria have been developed, but so far they appear to have limited success. One fungus that has most potential is *Verticillium chlamydosporium* (not to confuse with *Verticillium lecanii*). The fungus can be easily produced. Still, the efficacy of the fungus is dependent on nematode species, density and plant-host. Despite its limitation, it may be a useful management tool when integrated with control measures, including chemicals.

Trichoderma

The application of *Trichoderma* at the *Hypericum* plantation will not be economic beneficial. Instead, the fungus may be used at the nursing station where the fungus may have beneficial properties on root

development and pest protection. A small scale production facility and testing can be considered to be setup at ■■■■ flower company.

Project Outline

The following steps do not necessarily need to be executed in exact order.

1. SETUP INITIAL *TRICHODERMA* ISOLATION LAB

Equip the 'clean-lab': a detailed list for the purchase of consumables equipment and the facilities is available and can be discussed in detail with the management and the purchase office. The aim is to find items as much as possible on the local market.

Table 1 shows a rough estimation of the involved cost for the setup of the initial 'research lab'. Costs of molecular characterization of *Trichoderma* are not included (probably around $ 350,-)

TABLE 1		
Facility	$ 1.490,00	not including building of lab.
Equipment	$ 3.350,00	not including Biosafety Cabinet Class II
Disinfection	$ 70,00	
Consumables	$ 604,92	
Lab technician 50%	$ 130,00	Bases on salary of ETB 2800,-/month
Lab specialist	$■■■■	Based on fee of € ■■,00/hour, rough estimate
Total	**$ ■■■■**	

There are several health and security regulations involved when the laboratory is setup. The main requirements for the Clean-lab:

- At least 25m^2 surface
- One entrance/exit, to minimize air-draft.
- Sink and electricity.
- Lab well separated from other production facilities.
- Minimize dust forming. Room should be easily cleaned

A detailed description on the lay-out of the lab and proper lab procedures can be provided on request.

Appoint a position for a laboratory technician. The lab technician will eventually have a full-time position on the farm. In case of absence, a second person needs to be able to continue the routine lab procedures. The persons needs to have basic knowledge on good laboratory procedures. A local employee can be appointed and trained on the job. Additional intensive training can be provided by the lab-specialists.

2. ISOLATE LOCAL TRICHODERMA STRAIN.

Collect soil samples. The soil sample needs to be taken from location with endemic vegetation, at close range from the farm. The sample should be taken from a territory where the soil has not been disturbed by digging or pesticides. Preferable, a soil sample containing some rhizoderma from an old endemic healthy tree.

Grow *Trichoderma* on plates according to prepared protocol. *Trichoderma* can grow on phyton-yeast-agar, Cornmeal Dextrose Agar (CDA) and/or Potato-Dextrose-Agar (PDA) (1st choice).

Isolate several clean *Trichoderma* strains by transferring spores from a single colony to a new plate. 'Clean' strains can be kept in refrigerator or (for long time storage) mixed in glycerine and put in -80°C (or -20°C).

Write Protocols and Standard Operation Procedures for *Trichoderma* culture using the lay-out that is used at Abyssinia Flowers.

3. CHARACTERIZE OBTAINED TRICHODERMA STRAINS

Morphological identification of cultures (limited to Genus-level). An experienced person might be able to distinguish some *Trichoderma* species.

Send samples to a researchers in Europe experienced with *Trichoderma*. The research department is able to sequence the fungus and perform a molecular characterization of the strain.

4. SENSITIVITY TEST OF STRAINS

If more than one species / strain is isolated, a sensitivity test can be performed to identify the best performing strain. The *Trichoderma* is put on plate together with *Fusarium*. The antagonistic properties are measured from the strains that are found. As a reference, the *Trichoderma* strain from the commercial company can be used.

5. BULK PRODUCTION OF A DESIGNATED STRAIN.

Trichoderma bulk production similar to the set-up at ■■■■ ■■■■ plc.

Field test. Compare performance of plants growing on *Trichoderma* inoculated soil and non-inoculated soil (conventional growth conditions) by measuring standard quality parameters. During the process the field test set-up will be discussed and agreed upon with the management of ■■■■ ■■■■.

When a local *Trichoderma* strain is isolated, the next phase can be started by the setting up the production lab. A rough estimation of additional involved costs are listed in table 2.

TABLE 2		
Facility	$ 1.290,00	not including building of lab.
Equipment	$ 6.781,00	including Biosafety Cabinet Class II
Disinfection	$ 70,00	
Consumables	$ 1062,36	
Lab technician 100%	$ 260,00	Bases on salary of ETB 2800,-/month

TABLE 2		
Lab specialists	$■■■■	Based on fee of € ■■,00/hour
Total	**$ ■■■■**	

6. SETUP DIAGNOSTIC SYSTEM FOR MONITORING AND QUALITY ASSURANCE.

Routine *Trichoderma* detection by culture and spore-counting. Bulk production need to be analyzed for uniformity and purity. Inoculated soil samples can be analyzed over time for the presence of the mould.

FUTURE

The initial investments are high. However, if the lab is sufficiently equipped, it can perform other general lab analyses, such as soil analysis, nematode counts, production of other microbiological agents for pest control and diagnostics of plant diseases.

An other option is to setup a central laboratory in collaboration with other farms interested in the production of local *Trichoderma* strains. One other farm interested in *Trichoderma* production is ■■■■■■■■. A survey can be performed at other farms for their interest.

OBJECTIVES

Main objectives, strain isolation:

- Identify the optimal performing *Trichoderma* species.

Specifics:

- Provide a detailed list of lab-equipment and consumables.
- Purchasing and equip the lab
- Isolate Trichoderma strains from environment.
- Submit *Trichoderma* obtained strains to Europe for DNA sequencing.
- Sequences and strain descriptions; literature search.
- Write protocols and Standard Operation Procedures on: Good Laboratory Practice, *Trichoderma* culture, bulk production, quality assurance, routine detection procedures…
- Train lab-tech on good laboratory practices.

Main objectives, bulk production:

- Testing the selected *Trichoderma* with a field test and assess its effectiveness.

Specifics:

- Support lab-tech on bulk-production
- Analyse and report obtained data.
- Conduct monthly evaluation and reporting on the progress of the project.
- General trouble shooting.

Expected results

- optimal *Trichoderma* identified and selected
- selected *Trichoderma* tested in vivo and in vitro before introduction on the farm
- selected *Trichoderma* multiplied and when proven successful during tests introduction on the whole farm
- at the end a more ecologically dynamic farm

TIME FRAME

2016	Planning
January	Elaborate on *Trichoderma* project Discuss list of equipment and consumables for lab. Purchase of equipment and consumables
February	Employ lab-tech. Order laboratory equipment and products Write lab procedures
March	Equip laboratory. Write SOPs Collect soil-samples Train lab-tech
April	Grow separate strains and submit for genetic analyses
May	Wait for results
June	Analyse sequences and identify local strains Sensitivity testing of designated strain.
July	Bulk production of designated strain
August	Application of *Trichoderma* product. Field testing of *Trichoderma* formula
September	Collect and analyse field-data
October	Evaluation
Future / Optional	Design molecular detection system Setup other biological pest control methods