Europees Landbouwfonds voor Plattelandsontwikkeling: Europa investeert in zijn platteland

# Nitrofungi Project

Applicatie van Mycorrhiza schimmels in combinatie met kunstmestkorrels

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# Content

# 1 Introduction, Nitrofungi

The focus in agricultural production has always been a maximization of crop yields, which has led to many technological developments to fulfill this demand. This demand for bigger yields is triggered by size of the human population and the subsequent bigger demand for nutrition to feed all mouths. Fears emerge that there could arise a shortage of food supplies worldwide. On the other hand there are signs that intensified land use can and sometimes will lead to loss of production capabilities and severe environmental damage. Due to these negative aspects, more and more emphasis is put into using natural processes instead of technological solutions for improving productivity, efficiency and soil quality.

The aforementioned recent developments as well as the decreasing availability of (partly) non-renewable resources, *e.g.* phosphate, shows that there is a need for innovation in order to secure the global food demands in the future. The best way to ensure this and to strengthen agriculture is by combining natural processes, which stimulate soil vitality and ongoing productivity, and technology with which yields can be planned.

The objective of the project is to combine the stimulating effects of natural soil fungi and artificial fertilizer coating technology. The best of both worlds can be used in agriculture and also they give a stimulus to a new sustainable and high-rate technology, the combination of coating technology and mycorrhizal bio-technology. The new product, a sustainable biofertilizer, will be called Nitrofungi.

Nitrofungi is considered to be innovative, as it brings the best of two worlds together, two worlds that were considered to be opposites and incompatible. To an extent the two are incompatible, but only in situations where the concentration of nutrients causes damage and misbalance to the soil ecosystem. For instance, when there is an abundance of phosphorous/phosphate in the soil (or in the fertilizer for that matter), it has been found that mycorrhizal fungi cease to exist and disappear completely. So the carrier for the mycorrhiza coating must be a fertilizer with a low phosphorous content. And so the incompatibility turns out to be only a technical challenge that can be overcome.

Neither the use of mycorrhizal fungi for improving soil conditions nor the technology to coat granulated fertilizers is groundbreaking new. Coatings are very divers and are applied in several fields of technology, such as coating of metals or paint. Therefore it is important to establish the right understanding of the type of coating we have in mind for this particular project. The coatings for fertilizers are a very thin protective layer over the fertilizer granule, with specific chemical characteristics. It is mostly applied as a release agent, so the fertilizer is released under the right condition, making it more accessible to plant uptake and less susceptible to run-off. Also it guarantees that fertilizer granulates do not stick together, forming a brick when kept in stock over a period of time.

Mycorrhizal coatings are often mentioned as commercial products. However, this relates to a powdery application that can attach to seeds for better and faster germination. This technology differs strongly from the technology to be studied in this project, as we aim on full integration of mycorrhiza species in a fertilizer coating, leading to more efficient agriculture for an attractive price.

Important part of the research with Nitrofungi is finding the right combination of fertilizer, mycorrhizal fungi and all the necessary additives to make a stable and effective end product. This has to do with the fact that Nitrofungi is also meant to make sure that less nutrients will be washed from the soil with far less pollution in ground- and surface water as a result. Nitrofungi not only provides nourishment for vegetation, it also builds a living subsoil matrix that is able to transport nutrients.

The project starts in Jan, 2012 and will have a stepwise approach. Novochem B.V is responsible to develop the coating, select the artificial fertilizer and bind mycorrhiza spores to carrier material for small scale testing. Three months after the project starts, Triple E received the first Nitrofungi prototype from Novochem B.V. As agreed, Triple E is going to test and evaluate this prototype in small scales.

Hence, the aim of this report is to describe and evaluate lab and greenhouse experiments on our first prototype.

# 2 State of the art

As mentioned in chapter 1, the objective of project Nitrofungi is to combine the stimulating effects of natural soil fungi and artificial fertilizer coating technology. Hence, the basic material of Nitrofungi consists at least two categories, which are:

- Biological coating materials

The mixture of pure Endomycorrhizal Glomus clarum, G.aggregatum, G.intraradices, G.mosseae, G.deserticola, G.etunicatum, Gagaspora margarita, G.brasilianum and G.monosporum spores have been chosen as the biological coating material. The total propagules in this spore mixture is 120,000 per gram.

- Chemical fertilizers (serves as coating/binding agent and core granule)

Artificial fertilizer urea is serves as the core granule. Three kinds of coating material which are widely used in artificial coating technology have been selected as binding and releasing agent.

The coatings for fertilizer are usually a very thin protective layer over the fertilizer granule, with specific chemical characteristics. It is mostly applied as a release agent, so the fertilizer is released under the right condition, making it more accessible to plant uptake. Mycorrhizal fungi forms a symbiosis relationship with plant root, enhances the plant nutrient uptake and disease resistance ability. In this particular project, mycorrhiza spores are mixed with a chemical binding agent homogenously. Then coated over the urea granule (Figure 1). Three chemical coating agents with different chemical and physical characteristics have been mixed with AM fungus spores and coated over urea under same circumstance.

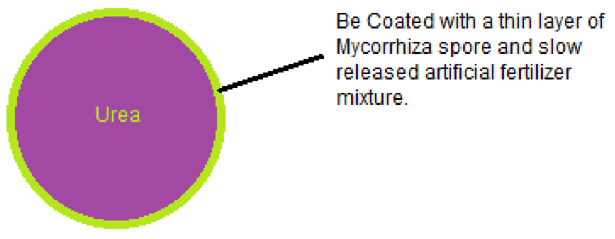


**Figure 1: The simple description of coating philosophy**

There is no clear indication that points out the potential influence of chemical and physical factors on AM fungus spores in fertilizer coating. The only parameter have been awarded and applied during the coating procedure is temperature, in order to avoid possible burnout.

We have been asked ourselves many questions, like:

- The behaviour of mycorrhiza in relation to fertilizer in mostly chemical nature.
- The influence of chemical and physical factors on AM fungus spore germination
- The interaction between AM spores, plant and chemical fertilizer
- The impact of external environments, such as temperature, pH, soil moisture
- The biological parameters, species preference or suitability

Either from macroscopically or microcosmic aspect, there are still many available we have not taken into consideration yet. Nevertheless, we are aiming to answer the most fundamental and urgent question, which is:

**How the AM fungus spores incorporating with artificial fertilizer?**

- The distribution of the mycorrhiza in the soil is random, not in close contact with the seeds, as you might wish. This could make it less effective in its colonization of plants in the Agricultural application. However, the application of fertilizer is limited to the technology on this field. Farmers will not use new machinery just for this product. Existing technology might be of interest however, such as fertilizer applied only in the seeding bed.
- Concentration of the mycorrhiza product and the amount that will be added, in relation to production by Triple E and the cost-effectiveness.
- If arbuscular mycorrhiza are added, only suitable for those plants (smaller green plants, except for cabbages). If for berries, trees or other, then different mycorrhiza needs to be added.

# 3 General materials and methods

This section describes the general materials and methods that were used in this experiment. Firstly I will describe which materials have been selected and then the method have been chosen for the experiment.

### *Mycorrhiza fungi species*

In this experiment, we did not use the spores we found in previous study. In order to provide the fine powder for coating process, we have chosen “White Widow” as the inoculation. White widow contains pure Endomycorrhizal Glomus clarum, G.aggregatum, G.intraradices, G.mosseae, G.deserticola, G.etunicatum, Gagaspora margarita, G.brasilianum and G.monosporum spores. It is very important to bear in mind those AMF species are only suitable for small green plants, not trees.

The total propagules is 120,000 per gram. That means, every kilo product contains more than 120 million mycorrhiza spores. We have made two brief calculations to indicate the spore distribution during if we apply nitrofungi in normal agriculture field. As you can see in table 2.1 and table 2.2, although the product we have been used is highly concentrated, however, if we transfer our data into normal field application the mycorrhiza spore distribution is still not high.

Novochem informed us that per ton fertilizer they will use about 1 kg coating material. We assumed that 5% and 1% of this coating material are AMF additives. As the result, there will be 6 million and 1.2 million spores in one ton fertilizer.

| Coating per ton fertilizer | gram |
|---|---|
| Total | 1,000 |
| Mycorrhiza additive | **5%** |
| Mycorrhiza additive (gram) | 50 |
| | |
| Million spores per kg mycorrhiza | 120 |
| Million spores per 50 gr | **6** |
| hectare per ton fertilizer | 3 |
| $m^2$ per ton fertilizer | 30,000 |
| spores per $m^2$ | 200 |

Tabel 3.1

| Coating per ton fertilizer | gram |
|---|---|
| Total | 1,000 |
| Mycorrhiza additive | **1%** |
| Mycorrhiza additive (gram) | 10 |
| | |
| Million spores per kg mycorrhiza | 120 |
| Million spores per 50 gr | **1.2** |
| hectare per ton fertilizer | 3 |
| $m^2$ per ton fertilizer | 30,000 |
| spores per $m^2$ | 40 |

Table 3.2

***Prototypes:***

In total Triple E have received 10 samples from Novochem B.V. The experiment samples consisted of one urea granule which they have been uses as core material, three coated chemical fertilizers (Urea and coating agent), six integrated fertilizers / Nitrofungi prototype 1 (the combination of different coating material and AM fungus spore) and one biological fertilizer (pure AM fungus spore which have been used as mother material) as follows:

S1: Urea

S2: Urea + NF5040

S3: Urea + NF165

S4: Urea + NF4020

S5: Urea + NF5040 +1% Mycorrhiza

S6: Urea + NF165 + 1% Mycorrhiza

S7: Urea + NF4020 +1% Mycorrhiza

S8: Urea + NF5040 +5% Mycorrhiza

S9: Urea + NF165 +5% Mycorrhiza

S10: Urea + NF4020 +5% Mycorrhiza

S11: Pure Mycorrhiza inoculum

***Sample preparation and location:***

The lab experiment will be executed in the seed trays at the Watermuseum, in Arnhem. Samples from Novochem are used as inoculation and finely mixed with sterilized sand. These mixture can create an infection of seedlings in the seed trays. Seed trays will be placed in the third floor of the water museum, where most people will not pay a visit. The seed trays on which the sample pots are to be placed will be covered with plastic covers, to decreases the chances of infection with external spores. The material that is to be used is all cleaned with 60% alcohol, to remove any micro-organisms present.

***Field Application Dosage***

Four field application dosage have been chosen in order to provide a brief idea of the interaction between urea, AMF and Alfalfa plant, which are: 200 kg/ ha, 400 kg/ha, 800 kg/ha, 1600 kg/ha. To be noticed that, 200 and 400 groups are based on the normal filed application rate (FAR). 800 and 1600 groups are much more than normal FAR, from that we might get some information about the toxicity index.

The reason we did not chose other FAR, like 100kg/ha or 300 kg/ha is the minimal weight of our scale is 0.1 gram. According to the pot size, 0.1 gram equals to 200 kg/ ha FAR.

# 4 Experiment design

**Phase one, prime selection coating/AMF/fertiliser**

An Important part of the research with Nitrofungi is finding the right combination of fertilizer, mycorrhizal fungi and all the necessary additives to make a stable and effective end product.

| | |
|---|---|
| The biological coating materials | AMF spores |
| | Nature mineral / clay / algae |
| | Biological Binding agents |
| Chemical fertilizers | Urea / or other core granules |
| | Chemical Binding agents |

For the use of mycorrhiza in the coating, a number of things needed to be investigated, among which will be the selection of mycorrhizal fungi species. The research was done and coordinated by Triple E, supported by our partners in the related field. A list of questions was answered in the preparatory research.

- Which naturally occurring mycorrhizal species prevail in the European soil?
- Which of the mycorrhizal species would be most beneficial for the agricultural crops of interest and suitable for incorporation in coating?
- Is the combination of mycorrhizal spores with the selected type of artificial fertilizer harmful for mycorrhizal spore activity?
- Is the process of coating the fertilizer granulates of influence on spore survival?
- What is the interaction between AM spores, plant species and chemical fertilizer?
- What is the behavior of mycorrhizal fungi in relation to fertilizer in mostly chemical nature?

In order to develop the coating, the following research questions were stressed:

- Which artificial fertilizer could be selected for the specific purpose?
- Which binding agent could be used in the coating?
- Which environmental parameters should be taken into consideration during the coating process?

After answering all those preparatory questions, we entered the first phase of this project, **the phase of direct coating.**

In the phase, artificial fertilizer UREA serves as the core granule. Three kinds of coating material which are widely used in artificial coating technology have been selected as binding and releasing agent. The coatings for fertilizer are usually a very thin protective layer over the fertilizer granule, with specific chemical characteristics. It is mostly applied as a release agent, so the fertilizer is released under the right condition, making it more accessible to plant uptake. In this phase, mycorrhiza spores were mixed with a low concentration chemical binding agents homogenously. Then coated over the urea granule (Figure 2). Three kinds of chemical binding agents were mixed with AM fungus spores under different dosage settings and tested under lab conditions.

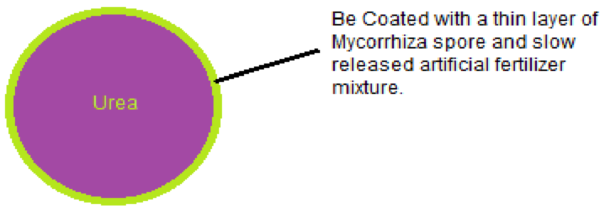


*Figure 2: The simple description of coating philosophy*

Seeds of interesting agriculture crops were selected not only base on economic interests but also because of its high mycorrhizal potential. Sterilized soil, seeds and equipment were used in the experiment in order to avoid undesirable soil contamination and microorganisms.

The first lab test of our very first prototype was not successful. There was no colonization found in any of the tested groups. In order to figure out the problem, questions listed below were asked:

- Where does the pressure come from? From binding agents or urea granules?
- Test which binding agent could be used, coating fertilizer with living material inside (the mycorrhizal spores).

In order to answer above mentioned questions, the different components were tested separately:

- Part 1: The interaction between Urea and Mycorrhizal fungi
- Part 2: The interaction between Mycorrhizal fungi and binding agents
- Part 3: the time control of release

In order to answer all addressed questions, following experiment were conduct:


*Figure 3: The overview of experiments conduct in phase 1*

At the end of the phase one, we conclude that only a few kinds of natural slow-released binding agents can be used together with AM fungi in the coating. Urea will bring negative effect in mycorrhizal colonization in the beginning stage, but the problem can be solved by adding an extra slow release layer during the coating.

### Phase two, coating with a protection layer

The design of this prototype is based on the result of previous experiments. The thick protection layer is added in between Urea core and AM fungi to protect the fungi from the high concentrations of urea for a period of 3 to 5 weeks.

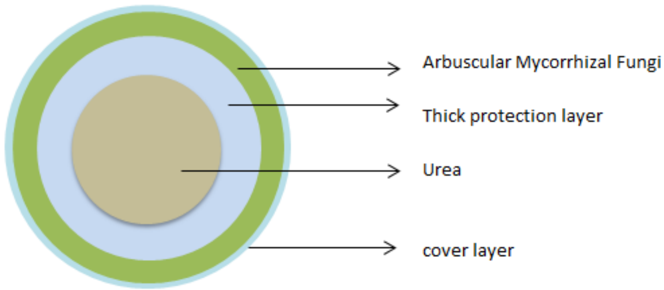


*Figure 4: The simple description of coating philosophy of phase 2.*

Unlike phase one, in phase two the interaction between biological and chemical materials were tested separately in order to be able to indicate all potential stresses. Experiments listed in figure 5 were conducted to validate our hypothesis step by step:

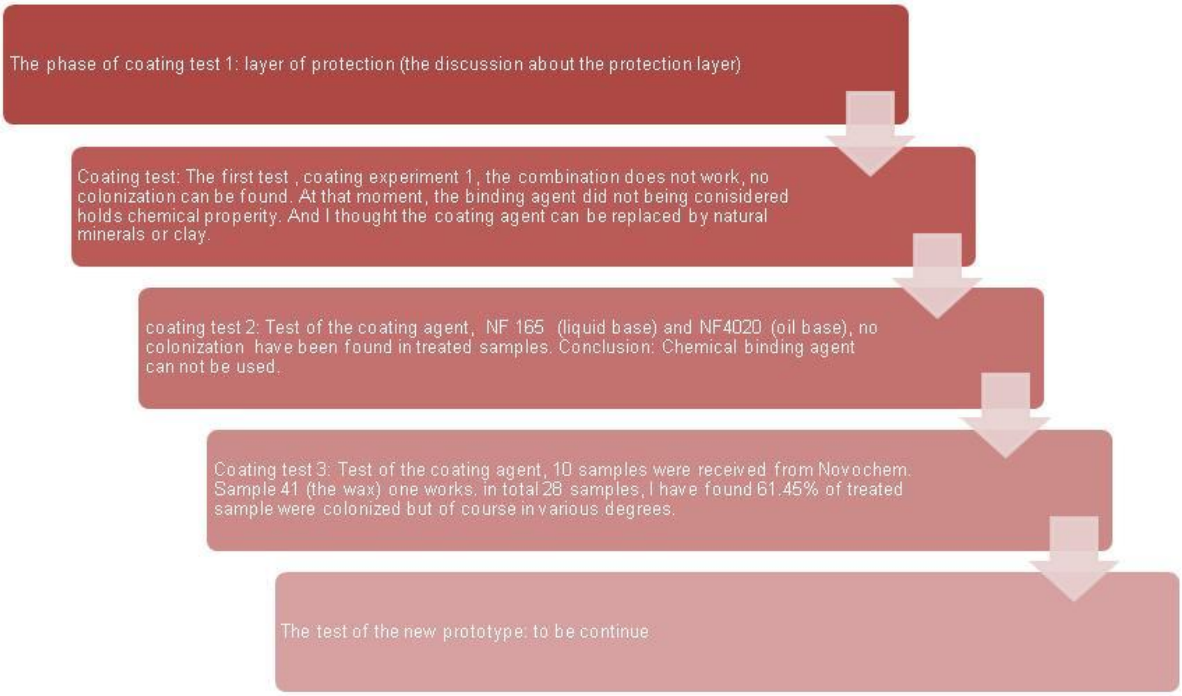


*Figure 5: The overview of experiments conduct in phase 2*

In coating test 3, we found out that Arbuscular Mycorrhizal Fungi just works fine in combination with nature wax. Hence, 10 new prototype were coated by Novochem and now testing under lab condition.

At the end of the experiment, the related data like the root colonization rate, the plant shoot mass and the dry weight of different treatment group will be collected and anal sized. And the R&D process will be continued based on the result of nearest experiment.

**The possible technical difficulties**

- The distribution of the mycorrhiza in the soil is random, not in close contact with the seeds, as you might wish. This could make it less effective in its colonization of plants in the Agricultural application.

- The nature characteristic of AMF brings some limitation in the coating process, the temperature, the moisture concentration and some other parameters have to be carefully controlled.

- The life cycle of Arbuscular Mycorrhizal Fungi is highly linked with plant root. It takes at least 26 days for AMF to be colonized with plant root. But it is also highly depends on the growth condition of the plant, hence, the experiment duration is longer than other fertilizer application experiments.

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## Eind fase van het Nitrofungi project

Vanwege het faillissement van de onderzoekspartner, Kenniscentrum Triple-E, is er geen eind rapportage beschikbaar van deze partij. Het was ook niet mogelijk om tussentijdse rapportages te verkrijgen. Hieronder is een gemankeerde rapportage van de kolonisatie testen bij planten uitgevoerd in 2014.

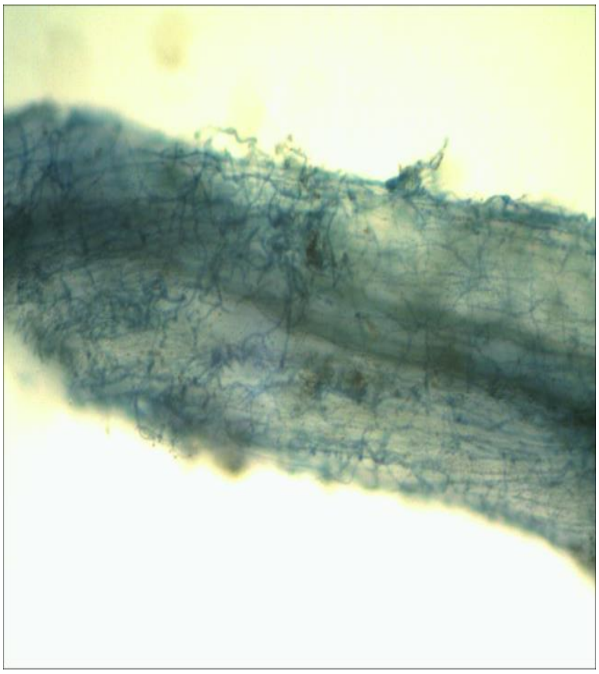
In 2014 zijn 2 experimenten gepland en uiteindelijk slechts 1 uitgevoerd. De tweede was ingezet maar uitslag onbekend vanwege faillissement.

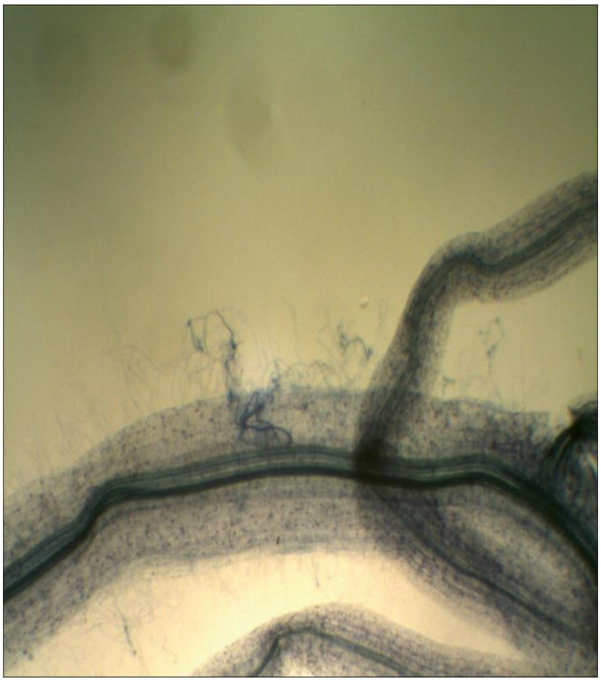
Identiek aan 2013 zijn er kolonisatie testen uitgevoerd in het laboratorium van Triple-E. Aanvullende testen waren nodig vanwege de matige resultaten tot nu toe; nauwelijks kolonisatie van de Mycorrhiza-fungi bij de initiële wortelgroei. De combinatie Mycorrhiza-sporen en kunstmest zoals afgebeeld in figuur 3 was op diverse punten aangepast om vooral de interactie tussen kunstmest (Ureum en Calcium Ammonium Nitraat) te verhinderen evenals de (te) vroege release van de kunstmest. Aanpassingen bestonden uit modificeren van de laagdiktes, type Mycorrhiza-sporen en type coating.

Het belangrijkste resultaat van de eerste experimentele test ronde in 2014 zijn weergegeven in de bijgevoegde foto's. Hierop is te zien dat prototype nummer 5 enige kolonisatie veroorzaakte tijdens de initiële wortelgroei. Dit was tot nu toe nog niet geconstateerd. Het was nog gering maar gaf aan dat de twee negatieve effecten; interactie kunstmest/Mycorrhiza en kunstmest/initiale ontwikkeling plant, overwonnen kunnen worden. Prototype 5 is opgebouwd uit CAN kunstmest met 3 coating lagen;

1. 20.000 ppm slow release coating laag

2. 1.000 ppm coating laag met daarin 5% Mycorrhiza sporen.
3. 1.000 ppm beschermlaag





Een tweede experiment werd ingezet met een aangepaste versie van prototype nummer 5. Deze keer slechts veranderingen in de hoeveelheid Mycorrhiza-sporen en laagdikte van vooral de slow-release laag. Dit experiment is wel ingezet maar uitkomst onbekend.

# 5 Conclusie en discussie

Het is mogelijk om een plant te laten groeien met kunstmestkorrel/Mycorrhiza sporen combinatie. Of dit een duidelijk verbetering van de plantengroei geeft, is niet bewezen gedurende dit project. Net zo min of het principe werkt onder meer praktische omstandigheden, bijv. tijdens een veldproef. Laat staan of het principe een voordeel geeft bij plantengroei onder ongunstige omstandigheden zoals droge of schrale gronden.

Tijdens het project kwamen de volgende problemen naar voren die verhinderde dat er uiteindelijke een werkend prototype geselecteerd kon worden voor een veldtest, ondanks een jaar extra onderzoek;

1. De kunstmest (stikstof op basis van ureum of nitraat) taste de Mycorrhiza-sporen aan.
2. De kunstmest kwam te snel vrij zodat de initiale plantengroei werd gedood vanwege overbemesting.
3. De gevoeligheid van de Mycorrhiza-sporen voor vocht en hitte was een sterke beperkende factor voor type coating en productie van het prototype.
4. Onbalans in de vraag (van de plant) en het aanbod (de combinatie kunstmest/mycorrhiza-sporen). De mycorrhiza moet de initiële wortelgroei koloniseren terwijl de kunstmest in een later fase nodig is.

Factor 1,2 en 3 zijn nagenoeg opgelost maar de laatste factor vereist nog de nodige onderzoeken op laboratoriumschaal en in het veld.

Conclusie

Op basis van de project resultaten moeten wij constateren dat we (nog) geen functionerend “kunstmest/Mycorrhiza-sporen” prototype hebben gevonden die de plantengroei verbeterd. Vervolg onderzoek moet daar uitsluitsel over gaan geven.