ERGO Meeting Report, 30 June 2009

ERGO is a research programme investigating the Ecology Regarding Genetically modified Organisms. The market approval track for new generations of modified crops might remain difficult, unless the authorities can make use of new relevant ecological information.

Professor Nico van Straalen highlighted in his opening address that the aims of this meeting include discovering opportunities for cooperation between the granted proposals. Another goal is to search for ‘gems beside the road’: interesting first findings that could trigger ‘topping up’ existing projects to deliver extra results on adjacent relevant fields. The realisation of ERGO’s secondary goals, ‘internationalisation’, ‘communication’, ‘embedding activities’, and ‘involving the private sector’, will be discussed. The exceptional nature of the ERGO programme allows for this greater flexibility. Finally, it was stressed yet again that the main reason why the ERGO Programme was set up, is a need for knowledge that can be used to address. In this respect the reaction of stake holders to the first results presented during this meeting were eagerly awaited. Each of the 11 ‘regular’ ‘Umbrella-projects’ introduced itself in a short presentation, followed by lively discussions, seeking for opportunities for common and/or additional activities. Below some general impressions (far from being scientifically correct or even complete) are written down.

Umbrella project 838.06.020, presented by Dr. Marcel van der Heijden.
“Effects of genetically modified crops on beneficial soil fungi”
Soil is often regarded a ‘black box’. Below our feet a fierce war rages on between an enormous diversity of, mostly unknown, creatures. Key components of soil biota, to a large extent responsible for key soil function are Arbuscular Mycorrhizal Fungi (AMF) and fungal decomposers. A gram of soil typically contains 10 to 200 meter of hyphae of these beneficial AMF, and AMF are associated with 80% of terrestrial plants.
The umbrella project encompasses projects addressing:

- Establishing a ‘fungal baseline’ over a wide range of agro-ecosystems;
- Developing Micro Arrays and other tools for ‘Rapid Assessment’ of the state of a soil’s fungal communities, for instance focusing on lignin decomposing organisms, using peroxidases, laccases and other enzymes, and/or their genes/mRNA’s, as markers;
- Investigating the impact of a gmo, e.g. Bt-maize, on beneficial soil fungi. It seems that this gmo maize (Mon810) shows a higher lignin content. It will be interesting to try to distinguish between any direct gmo-effect and indirect effects, like lignin content, on soil ecology.

First results include:

- Soils used for organic farming show a fungal biodiversity twice as high as in conventially farmed soil;
- Crop rotation is even more important for the level of fungal soil fungal biodiversity.

In both cases it has to be established whether these differences in taxonomical biodiversity reflect a difference in functional biodiversity.
Besides, it has to be established whether lignin decomposition experiments in glass houses reflect how lignin is broken down in natural soils. Root material from gmo’s grown abroad might be included in the experiments.

Umbrella project 838.06.030, presented by Dr. Klaas Vrieling.

"Quantifying introgression risks of transgenes with hazard rates, using carrot as a model species"

Carrot is used as a model species for Ecological Risk Assessment since wild carrot species (annual monocarpic perennial Daucus) grow in close proximity of (escaped) cultivated *Daucus carota*, which are biannual. Besides such morphological markers, metabolomical markers will be employed to trace introgression.

This allows for quantifying the probability of gmo introgression stochastically, address fitness effects and link long-term introgression probability with short-term fitness effects.

Introgression starts with hybridisation, followed by local establishment, followed by dispersal.

"Hazard Rate" (The probability something occurs, given it didn't occur previously) is a key element in analysing such processes quantitatively.

Cooperation with prof. Peter van Tienderen's ERGO-projects and with a Chinese project focussing on introgression between wild and cultivated rice, have already been established.

Daucus-populations in the Meijendel and Alkmaar, 2 regions where in the past flowering cultivated plants grew along wild relatives, are used to gather data.

The use of the results from this project will not be restricted to Daucus, hence the link with lettuce (van Tienderen) and rice (China). Efforts to include gmo carrot plants in the experiments have been unsuccessful so far.

Umbrella project 838.06.010, presented by Prof. Marcel Dicke.

"Development of an ecological method to evaluate the effects of GM crucifer crops, altered in direct and indirect plant resistance traits, on non-target organisms in relation to baseline information"

Using non-GMO white cabbage (Brassica oleracea) and gmo and non-gmo version of Arabidopsis thaliana, at first an inventarisation of baseline data, effects of cultivars on non-target species is carried out. Cabbage field data are used to allow comparing results in the fields with those obtained in a greenhouse setting.

Questions like, "are there effects (and if, what effects) on predatory insects from the plant variety an aphid prey feeds on?.

"Gas Chromatography/Mass Spectroscopy demonstrate big differences in secondary metabolites, but most of them can be traced back to a large extend to differences in growing conditions. The gene responsible for a key-component (linaloon) will be introduced into Arabidopsis. Subsequently an effect of this introduced transgene will be investigated.

Umbrella project 838.06.050, presented by Prof. Dick (Jan Dirk) van Elsas.
"Baseline establishment and protocol development to assess the effects of genetically-modified crops on the structure and functioning of soil microbial communities"
This project aims at establishing the "Normal Operating Range" of a great many important soil parameters in two soils, which are typical for potato growing: Loamy Sand (Type B), and Sandy Peat (Type V).
Because 'nitrificiers' in soil seem to consist of a relatively limited group of organisms, relatively sensitive to disturbance, bacterial and archaeal *amoA* are investigated in soil under various conditions. Results from the past 6 years seem to indicate that archaea are responsible for roughly half the nitrification in soil. The Normal Operating Range seems to depend quite a lot on the growth stage of the (crop) plants. Young plants go along with a low diversity rhizosphere nitrifying community, whilst flowering plants have a very different low diversity community of nitrifiers present in their rhizosphere.
The *AsfA*-gene is followed as a marker for the desulfonator ß-proteobacteria.
PCR-DGGE is a widely used technique, but the coordinator would love to include pyrosequencing.
The second project within this umbrella project will try to find effects of gmo plants on soil community and soil functional processes. As an estimator for fungal biomass, the other project, ergosterol is used as a marker. Lacases are studied (lignine breakdown), cellulases and certain peroxidases to establish soil function activities.

The presentation gave rise to questions like:
Since the effects on any genotype (whether differing through conventional techniques or genetic modification) are expected to be relatively small, shouldn't they be established under controlled (phytotron or greenhouse) conditions?
The effect of gmo's could be mainly exerted via their root exudates. The root exudates of amylose-free potato seems less interesting (in a fungal community/fungal soil function kind of way) than would, for instance, be a phytophthora-resistant potato, isn't it?
"Should one expect effect of a starch-modified potato via decomposition of plant material?" was one of the suggestions that might be taken up in the experimental plan.

Umbrella project 838.06.040, by Dr. Clemens v.d. Wiel & Prof. Peter van Tienderen.

"Introgression of crop (trans-)genes into wild relatives: hybrid fitness, background selection and hitchhiking in Lactuca serriola; Tools for assessing the likelihood of the establishment of transgenes in wild relatives"

In the wild lettuce, both wildtype Lactuca serriola and cultivated Lactuca's, can encounter (combinations of) to a-biotic stresses, like drought, salt, and lack of nutrients.

If hybrids between these 2 Lactuca's occur (and subsequently back cross with the Wild type, introgression can occur. If the cultivated lettuce parent is a gmo, the gmo trait can become widespread in natural Lactuca populations (and these might expand as a result) if, either the introduced gene selects positively, the introduced gene is physically situated in a part of the DNA where many positively selected QTL's are situated, or by chance.

Much work can be done by using Recombinant Inbred Lines (RIL's).

This approach, which is based on a cooperation with Michelmore's lab, with 50% crop genes in various combinations is ideal for QTL-work, since there's an unlimited amount of seed available, and genotyping has already taken place. Besides Stress-related QTL's, QM's are involved for growth related characteristics.

Since its start, the methodology of the project has changed. By using the illumina-array analysis, developed at Michelmore's lab, 384 Single Nucleotide Polymorphisms (SNP's) can be addressed in a single run.

The second project in this cluster can be illustrated most simply by a 2-locus-model, e.g. a positively selected transgene in a chromosome background that is negatively selected.

Even if the initial occurence of outcrossing to the wild vegetation is very high, the frequencies dwindle, due to that part of the chromosome being selected against. Due to cross-over, the selection pressure around, and including, the transgene can change, and from this low initial incidence, e.g. due to the positively selected transgene, the frequency can, at this later stage, increase dramatically. The take home message being: "Initial frequency dynamics may differ enormously from ultimate dynamics". With regard to pollen dispersal, models need more realism, e.g. by including life histories, using QTL and fitness data. Furthermore, local and global modelling approaches will be linked.

As common output, the use of "hazard rates"

("what are the chances of something happening, knowing it hasn't happened yet")

will gain importance. Lettuce is to a very large extend self-fertilising. Do sativa and serriola crossbreed under natural circumstances? There's no lettuce seed production left in the Netherlands, so the oly source of gm-pollen would be bolting lettuce, outcrossing 1-5% with surrounding (serriola)-plants. In any way, the *Lactuca serriola*-outbreak of recent times seems not to be linked to outcrossing events of *Lactuca sativa* (the cultivated lettuce).

<u>Umbrella project 838.06.060, by Dr. Ron de Goede.</u>
"GM Crop Impact Assessment on Soil Ecosystems by DNA Barcode-based Monitoring of Nematode Communities (ERGONema)"
This cluster encompasses activities at the WUR and RIVM. The aim is to develop a DNA-based tool for qualitative nematode community analysis. Besides comparing, for instance, soil types and rotations, one could use such a tool to assess the impact (and the lasting effects) of growing gm-varieties. The tool to be developed should b:

- broadly applicable
- quick and affordable
- accurate and
- validated.

In this cluster, Plant-induced changes interact bi-directionally with the soil biota community(ies) which are in turn linked bi-directionally to Ecosystem services. More specific:
Brassica's with a range of glucosinolates-content (leading to toxic isothiocyanates) <--->
Nematode community <--->
Soil fertility and biodiversity.

In the relation Plant --> Rhizosphere/litter --> Ecosystem Services,
focus lies on N-mineralisation, the Carbon Cycle and CLPP (Community Level Physiological Profiling).

Due to its known toxigenic properties, differences in glucosinolate-content are expected to be reflected in one way or another in the nematode community (size/composition). Regarding to gm, this project should demonstrate its applicability. Will the effect of the gm plants on the soil biota community, more specific the nematodes, be high and significant enough to 'pick up' as a signal via this new tool?
This question can work both ways:

- 'ecologically insignificant' signals (and 'noise') could be picked up (false positives), leading to 'false alarms' or
- 'ecologically significant' signals could be missed, as a result of the tool lacking the necessary sensitivity (false negatives).

Umbrella project 838.06.080, by Prof. Dick van Elsas.
"The baseline of soil functioning across a representative range of Dutch soils"
This cluster aims at identifying chemical soil characteristics, as well as biological ones (C-cyle, Dead organic Carbon DOC, etc.) with microbial soil characteristics. Microbial soil characterisation focusses on Oligotrophs and Copiotrophs. Quantitative PCR on markergenes like *nifH* (marker for nitrogenase reductase) and *amoA* (ammonia monooxygenase marker for nitrification by nitrifying, amonium oxidizing, bacteria). Arcahaea and alfa-proteo-bacteria are included as well as elements to establish the Normal Operating Range for. To introduce standardisation, next to measurements directly form the field, soil samples will be put under standardised condition and re-measured by Sacha Semenov.

Drawing up models and developing instruments from molecular soil biota composition data have to overcome the fact that the vast majority of soil micro-organisms simply can't be cultured and/or may or may not be active under 'normal' soil conditions.

Michelle Pereira focuses more on Soil Respiration kinetics, DNA-SIP (DNA-Stable Isotope Probing) and the Carbon Cycle in soil. Especially for soil based systems it will be very difficult to get Stable Isotope Probing operational. The aim is to distinguish 'State variables' from 'Rate variables'.

Umbrella project 838.06.110, by Prof. Peter van Tienderen & Dr. Tom de Jong.
"Potential ecosystem effects of future GM crop introductions through establishment of crop/wild hybrids or feral populations"
This project from ERGO's 3rd call addresses the question 'How do you recognize a new genetic trait, introduced in a GMO, that has an extraordinary risk aspect. Such an extraordinary risk might be: 'the potentially irreversible and damaging effect to the recipient ecosystem'. A transgene escape may:
- Have/show no effect whatsoever;
- Lead to a higher density of this crop species in its 'own' habitat;
- The transgenic crop could, as a result of this extra trait, expand beyond its 'normal' range;
- The transgenic crop could, because of this trait, invade other habitats.

Consequences of the speed at which this occurs:
- There could be just a higher presence of the crop compared to the previous situation;
- Other habitats are invaded (comparable to the introduction of exotic species in nature, or escaping from fields or gardens).

The systems investigated are *Brassica rapa* (fodder rape, koolraap) versus *Brassica napu*s (oil seed rape, koolzaad) and *Lactuca serriola* (prickly lettuce, kompassla) versus *Lactuca sativa* (lettuce, sla). Wild prickly lettuce invades/invaded stands of cultivated lettuce. But why? This observation gave rise to this theoretical ERGO-study in Amsterdam.

In Leiden the work focusses on *Brassica napus* and *Brassica rapa*. What had long been suspected was confirmed by comparing chromosome numbers and morphological characteristics: 'Yes, the morphological characteristics of the two species are different :-) , but, alas, they overlap :-(.
During the initiative "Fietsen voor Koolzaad", some 100 volunteers inventarised the yellow-flowering Brassica's, the results are at waarneming.nl. Real oilseed rape populations being rare, one of the notable exeptions being next to the railroad track at (or in the vicinity of) Gouda railway station.

Umbrella project 838.06.090 (Dr. T.F.M. Roelofs), by Elaine van Ommen Kloeke.
"Design of a decision matrix to assess GM crop impact on the detrital food web"
Glucosinolates, secundary metabolites in Cabbages-crops like broccoli (*Brassica oleracea* var. *Italica*) (also) have beneficial effects on consumers. Myb28 and Myb51 attract much attention in this respect. The effect of Sulforaphane from broccoli on the occurence of cancer is documented best.
While 'High Glucosinolate' broccoli are being developed the question arises what effect this elevated glucosinolate content will have on soil evertebrates.
The difference between Genetically modified crops and non-GMO-crops could turn out to be that more plant biomass remains on and in the soil and accumulates there, while non-GMO-crops is broken down in soil faster.
There also exist traditionally bred 'Double Zero' Brassica crops, very low in Glucosinolates and Erucic acid (Erucazuur). These show no harm to soil and soil evertebrates, but lack the beneficial effect upon human consumption of specific glucosinolates.

Umbrella project 838.06.100 (Dr. J.M. Raaijmakers), by Rodrigo Mendes.
"Whole-cell biosensors to monitor and assess the effects of transgenic crops on soil health"
The project aims at developing bioindicators for soil health. Like Bacterial species strains that would behave as whole cell biosensors responsive to soil perturbations, for instance those which might occur by the effects of GM crops on soil. This project might also add to our understanding of natural disease/pest suppressiveness of soils. In some soils were sugar beet is grown, 'damping off', wilting of seedlings, caused by Rhizoctonia solani' does occur much less than is the case with other soils. Another example form soils that are able to suppress the occurence of 'Take-all' in Wheat, (caused by a fungus called: *Gaeumannomycis graminis* var. *tritici*). There are some indications a Pseudomonas-bacterium, producing a fungicide (cocktail) is a key player among the culturable microbiota isolated from such soils.
The strategy is to combine microbial indicators (e.g. in Psuedomonas/Bacillus) with Biosensory functions.

Umbrella project 838.06.070 (Dr. P.G.L. Klinkhamer), by Paul Wakefield.
"Developing baselines and protocols for evaluating the direct and indirect effects of GMP's on the above-ground insect community using GM potatoes as a case study"
This study uses Genetically Modified potatoes with modified starch composition. The effects on ladybirds predating on aphids sucking up phloem juice from GM potatoes will be used.
The project is a combination of Metabolomics, field monitoring en Bio-assays in the field/greenhouse as well as in the lab. The starch potato will only have effect after the flowering stage, when colons and tubers (potatoes) grow out, but. since gmo plants are involved, one needs a permit. Such a permit can result in the plants not being allowed to flower, and hence, in such cases the effect of pollen, or on pollen-eating insects, are difficult to identify.
The project uses 2 locations.
One location has many insects, including Colorado beetle, a well-known, and voraceous insect pest. The other location shows hardly any insects.

General Presentation on Ecological Risk Assessment, by Dr. Hans Bergmans (some additions, explanations, not necessarily reflecting Dr. Bergmans' views were added afterwards by Theo Saat).

"What did I hea today in relation to ERGO, and how does this relate to what I heard yesterday in Germany during BioSafeNet."

Hans pointed out that "there is low impact of biosafety research on GMO decision making". We must therefore ask ourselves: Does (our) research answer the questions/challenges of:

- decion makers
- opinion makers
- general public.

Specific attention went to 4 issues:

1) Protection goals

It is not easy to translate something like 'concern' in 'scientific terms'.
The law often speaks of 'protection goals', but addressing 'concerns' with sound scientific research by biologists, chemists, statisticians and other experts within the 'natural sciences' is not an easy road. Therefore, Biosafety research has a platform to exchange experiences, ideas, approaches.... called BioSafeNet.

2) Problem formulation

Working with GMO, or rather GMO-related issues, requires a very well-wrought methodology. If only to be able to add/combine results obtained from different sources. Just 'doing things like we've always done' might make it more difficult to use results and data in the most optimal way possible. Design and validation of methodology & tools is of great importance. Data management is a problem for all sciences generating large amounts of data. But with GMO-related research, the results and conclusions have to be understandable for experts and general public alike. Furthermore we have to be aware that data and results from objective and good scientific research may be used, and made to fit, a specific conclusion, whether justified or not. Accidental inappropriate use can be prevented to some extent by defining the problem to be solved very carefully.

3) Reference

In order to draw valid conclusions on effects and how important a certain effect is, one needs a proper set of references. If one has no information on how a certain biological or abiotic factor tends to change over time, soil type, crop rotation scheme, climate and micro-climate, it will remain difficult to distinguish a 'signal' signifying an effect, from natural background variation and other 'noise'. Ecology is notoriously difficult in this respect, and the a-biotic part of the hypervariable three-phase substrate we tend to simply call 'soil' isn't making it any easier either.
An theoretical example mayIf a GMO-crop would diminish the occurrence of a certain species of soil biota by, say 30%. This might sound alarming, but perhaps only until one would learn that the population were to collapse by 99% during next year's crop rotation ányhow. On the other hand, knowing that a soil biota species will drop by 99% the next year, would not make ány change in ány species, as an effect of growing gmo's, a priori acceptable. Soil resilience (veerkracht) might become impaired, or vital soil functions, normally performed by several soil dwelling species, might no longer take place.

4) Application

It is not the intention of the ERGO programme, just to knów more. If this programme would enable us to ask better questions, it still would be falling short of delivering the results expected.
The ERGO programme is primarily about application: the generated knowledge and insights must be implemented in the decision taking process regarding ecological risk assessment. It must become one of the exceptions and examples of biosafety research on NWO that dóes how an impact on decision making. The results, besides being used by other scientists, stakeholders and/or the general public, múst be ready to use for decision makers who have to deal with gmo-related ecological risk assessment.
This sometimes results in dilemma's.
For scientific reasons, to more, and in depth, on the soil ecosystem, it may be favourable to use sophisticated and expensive, even 'fancy' techniques. But to deliver to our 'applicability target', we must make sure that we do not overburden the applicants! If a simple blot or PCR will do, we must be alert not to turn pyrosequencing into a de facto standard!

Dr. Bergmans illustrated this by a project by Clemens van de Wiel on 'Hitch-hiking and drag'.
He can, of course, use 'safe places' to insert genes. But his choice might influence whether applicants will be advised to use safe places for insertions, or whether it becomes mandatory. Being 'extra careful' holds the risk of the extra careful turning into the standard, and the approach called 'careful' before, all of a sudden be regarded as 'risky'.

Although but an example, dr. Bergmans warned that, scientifically speaking, all projects looked solid and useful to him. From the perspective of application, still "some is to be desired".

"If we all switch to applicability-mode it will be good and useful for all", therefore was his message to the ERGO community.

Finally, as befitted a discussion on biosagfety and gmo's, dr. Bergmans turned to semantics.
We aim at the "identification of hazard".
But what ís 'hazard'?
Hazards need not be 'convenient'.
Aren't we focussing on quasi-hazards, just because they are easy to detect, or easy to quantify?
Again, hazards need not be convenient:
they should fit the general idea of what has to be protected.
To get some idea, and 'feeling' about what to look form, Hans Bergmans,
in his last remark, adviced researchers to look in European Regulations
for a general definition of 'adverse effects'