Technology Updates — Crops, Environment and Land Use

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Assessing and Monitoring Impacts on Agro-ecosystems of Genetically Modified Potatoes with Late Blight Resistance

Key external stakeholders:

Tillage farmers, agri-food sector, policymakers, general public

Practical implications for stakeholders:

While previous Teagasc research has indicated the potential role a GM potato engineered with late blight resistance could have in reducing fungicide applications, assessing and monitoring the impact of cultivating such a crop could have on the agri-environment is of paramount importance. Specifically, what would be the impact of cultivating a GM (cisgenically engineered) late blight resistant potato variety on soil health, which is the most important ecosystem service associated with sustainable land use. In addition, how durable would the resistance of such a crop be against the late blight organism *Phytophthora infestans* and could this crop be included in an IPM tailored management regime? The output from this multi-disciplinary study has addressed these issues while in parallel an extensive programme of knowledge transfer was delivered to ensure the subsequent public debate was guided by impartial, science-driven knowledge.

The outcome/technology or information/recommendation is that, based on this field study:

- Engineering potatoes (via cisgenics) presents a viable option to combat late blight disease
- Cultivation of the cisgenic potato had no significant impact on soil microorganisms, which are essential for soil fertility
- Proactive knowledge transfer that occurs in parallel to an active research programme can impact positively on public attitudes towards technologies of societal concern

Main results:

- The cultivation of a GM potato variety with late blight resistance exerted no significant impact on soil biodiversity compared to a non-GM potato variety
- The GM potato displayed durable resistance to late blight disease over the 3 years of the field study and when incorporated into an IPM strategy can reduce the fungicide usage by up to 75%

Opportunity / Benefit:

Output from this project has generated large datasets that describe the comparative impact of cultivating GM v. non-GM potatoes on soil microbes including, bacteria, fungi and nematodes. In addition, the agronomic performance of a late blight resistant GM potato grown in a field setting has been recorded. Combined with the public outreach programme that occurred in parallel, the AMIGA project has addressed many of the preconceptions around GM crops from an Irish perspective and informed stakeholders of the opportunities that genetic engineering can provide in light of the challenges faced in the tillage sector.

Collaborating Institutions:

Wageningen University (The Netherlands), Thünen Institute of Biodiversity (Germany), Slovak Agricultural University (Slovakia)

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1. Project background:

Sustainable land use has been identified as a primary environmental protection goal to be considered before approving the cultivation of new GM crops. The most important ecosystem service associated with sustainable land use is creating and maintaining soil health, which is largely influenced by soil inhabiting organisms, which are differentiated into chemical engineers (bacteria, fungi), biological regulators (nematodes) and ecosystem engineers (earthworms). To preserve soil function and enhance natural soil fertility, all three groups must interact and thus specific attention must be paid to minimizing the impact of specific crop cultivation and related management practices on their community diversity and structure.

Most soil inhabiting organisms are highly sensitive to crop species, agricultural management practices, landscape factors, and weather conditions. This causes a high level of variability that may mask effects triggered by GM-plants. A certain response of soil organisms to a GM crop can only be interpreted against this background of natural variability, as it occurs with other crops, tillage practices or environmental triggers. Furthermore, changes must consider temporal and spatial scales in order to allow conclusions to be formed on the potential adverse effects on soil health. Certain GM crops offer potential advances in our ability to effectively manage agricultural pests (e.g. late blight disease of potato), if management is tailored to regionally variable agro-ecosystems. The importance of Integrated Pest Management (IPM) for GM crops has been well established as a mechanism to ensure the durability of the novel trait as the pressure from the target pest increases.

Potato remains an important food crop in Ireland and across the world but potato late blight disease (caused by *Phytopthora infestans*) continues to threaten the sustainability of the crop such that farmers in Ireland typically apply ~15 fungicide applications per crop. Generating genetic resistance in commercial varieties is the most cost effective and environmentally friendly way to control late blight disease. However, the issue of breeding resistance is complex with the development of a novel variety taking ~13 years. From 2006 – 2015 the Wageningen University and Research Centre's DuRPh project aimed to develop a proof-of-concept that existing potato varieties could be made durably resistant to late blight when provided with stacked R genes through cisgenic modification and when combined with adequate IPM strategies. As part of this programme, they developed a cisgenically modified potato line of cv. Desiree, containing the *Rpi-vnt1.1* late blight resistance gene which was derived from the wild potato *Solanum venturii*. Based on field studies previously completed in the Netherlands, this GM Desiree line has displayed strong resistance to late blight disease.

Taking this into account, the primary goal of this project was to assess and monitor the agri-environmental impact of cultivating a GM late blight resistant potato at a field site at the Oak Park research centre. To achieve this, baselines for microbial diversity (bacteria, fungi and nematodes) in the soil rhizosphere of the GM Desiree potato line, a non-GM Desiree comparator and an additional potato variety, Sarpo Mira were calculated through current DNA-sequencing methods. In parallel the agronomic performance (disease resistance and yield response) of the GM Desiree line (A15-031) was recorded in the presence of various IPM strategies. Finally, the practical research was combined with a pro-active programme of knowledge transfer via on-line, print, radio, summer schools and TV to answer the many questions that arose around the subject of GM field studies and the principles of environmental risk assessment.

2. Questions addressed by the project:

- What is the environmental impact on soil health of cultivating a GM late blight resistant potato?
- Can IPM strategies be implemented in tandem to cultivating such a crop?
- Can a practical, hands-on approach to knowledge transfer make a constructive contribution to addressing the many issues that center on the issue of GM crops?

3. The experimental studies:

Prior to initiating the field studies in 2013, 2014 and 2015, a submission was made to the EPA seeking a field license for the controlled release of a GM potato engineered with late blight resistance. EPA consent was

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granted under Notification No. B/IE/12/01 and field studies were completed at Oak Park, in Carlow. Two potato cultivars and one potato clone were used in the field trials: the susceptible cultivar Desiree, the highly resistant cultivar Sarpo mira and the engineered, highly resistant GM Desiree line (A15-031). For planting, seed tubers (Desiree and GM Desiree) were generated under glasshouse conditions to offset potato virus transmission. Sarpo Mira seed potatoes were obtained from commercial sources. Each field study was laid out as a randomized block experiment including the 3 potato genotypes plus three late blight control strategies (unsprayed control, weekly spray schedule and an IPM2.0 control strategy) with 6 replicates per site and 2 sites in total. Plots measured 3m x 3m and were separated by 3m of grass on all sides. The IPM2.0 control strategy for potato late blight can be summarized as "we do not spray unless":

- virulence to the R-gene(s) in the potato genotypes grown is locally present and
- an infection event is predicted in the near future or detected in the recent past and
- the remaining fungicide protection is/was insufficient to cover this infection period.

To sample the rhizosphere for microbial community diversity, one flowering plant per plot was selected and loosely adhering soil removed by shaking. Microbial cells (bacteria, fungal mycelia and spores) adhering to the roots were detached by suspending root material (~8 g) in 30ml of saline water for 30 min at 4 °C. Microbial cells were collected by centrifugation and the cell pellets stored at -80°C. DNA was extracted from the frozen cell pellets using the FastDNA SPIN kit and then split into equal volumes with one processed at the Thünen Institute for bacterial sequencing while the other by the Teagasc Sequencing Centre, Moorepark, for fungal diversity. For nematode sampling 7 plots per treatment (1 plant / plot) were randomly sampled and after soil removal, nematodes extracted by processing 100g of the homogenized soil/plot (7 samples/treatment) via an Oostenbrink elutriator, followed by passage through a series of sieves (45 - 180µm mesh size) and then a cotton wool filter. After a 48 hr incubation, a volume of 50 ml was recovered, from which nematodes were collected into 10mL. For DNA sequencing purposes, DNA was extracted using the Purelink Genomic DNA kit. For target amplification, the 5' end of the 18 small subunit rDNA gene (~1000bp) was amplified using a set of universal primers. All reactions were completed in a 50ul volume containing 50 ng DNA, 5 ul buffer, 1 ul each primer (10mM) and 200 uM dNTP, with conditions; 95^{o}C – 5min, 30 x (95^{o}C – 30sec, 60^{o}C – 60sec, 72^{o}C – 5min), 72^{o}C – 10min. Five reactions were completed/sample, with these replicates pooled post-PCR before being verified via gel electrophoresis for appropriate amplicon size. Amplicons were subsequently cloned and target sites sequenced by an external provider. Bacterial, fungal and nematode community structure and diversity levels were assessed by calculating established ecological indices.

4. Main results:

Across the three years of the study, the GM Desiree line maintained a high level of resistance during the blight periods (July – Sept.) of each season (Fig. 1). Compared to the standard commercial practice of spraying on a weekly basis, integrating the GM potato line into an IPM strategy (IPM 2.0) eliminated the need for spraying in the IPM2.0 strategy. This compared against the non-GM Desiree plots which required ~11 sprays per season as per the 'weekly schedule' to protect the plants against late blight disease. Across the three years, the unsprayed control plots containing the susceptible non-GM Desiree recorded ~60% plant death by the end of the growing season. For the corresponding unsprayed GM Desiree plots, no disease was recorded on the engineered plants. For Sarpo Mira cultivated plots, resistance levels were high as expected with *P. infestans* lesions recorded on foliage towards the end of the growing season as the plants began to senesce. In regards to yield there was no significant difference between the yield of the unsprayed GM Desiree plots versus the non-GM Desiree plots that received a weekly fungicide application.

In regards to microbial diversity; examining the fungal communities in the soil rhizosphere of potato varieties the Ascomycota were the most dominant phylum accounting for 63.0 % of all sequences. Zygomycota was the second most dominant phylum with potato (19.7 %) followed by the Basidiomycota (10.8 %). All other fungal phyla were poorly presented or assigned to "unclassified" phyla, representing for potato 6 %. Surprisingly, the phylum Glomeromycota, which includes all known endomycorrhizal taxa, was practically fully absent in potato rhizospheres, underlining that this assumingly plant growth promoting symbiosis was completely irrelevant under the respective cultivation conditions. Based on the analysis completed, the cultivation of the GM did not induce a significant impact on fungal diversity compared to the non-GM Desiree cultivar. For bacterial diversity, the library coverage was ~98%, indicating that practically all of the soil-derived bacterial community was assessed. While a total of 9693 species were detected, a single potato sample contained ~9151 species with the 10 most dominant representing 17% of the total diversity captured. No differences in the overall bacterial community structure were detected by the respective genetic modifications of potato. In contrast, a clear distinction was possible for the years of cultivation and cultivars grown.

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An analysis of nematode ecological indices revealed the rhizospheric nematode community did not register any significant affect of the cultivation of the cisgenic Desiree line compared to its comparator, cv. Desiree in the presence or absence of fungicide management. Neither was there a significant difference between crop cultivars (cv. Desiree and cv. Sarpo Mira) or crop management. A qualitative analysis of the maturity indices did indicate differences between the potato genetic background and their interaction with the disease management strategies (the no spray control v. weekly chemical applications). Also the year of study exerted a significant effect, which was due to the drought conditions experienced in July 2013, which would have supported those nematodes less sensitive to environmental disturbance. In contrast, the weather conditions of 2014 and 2015 were more supportive of an enrichment condition, which would have provided equal opportunities for a broader range of nematode families.

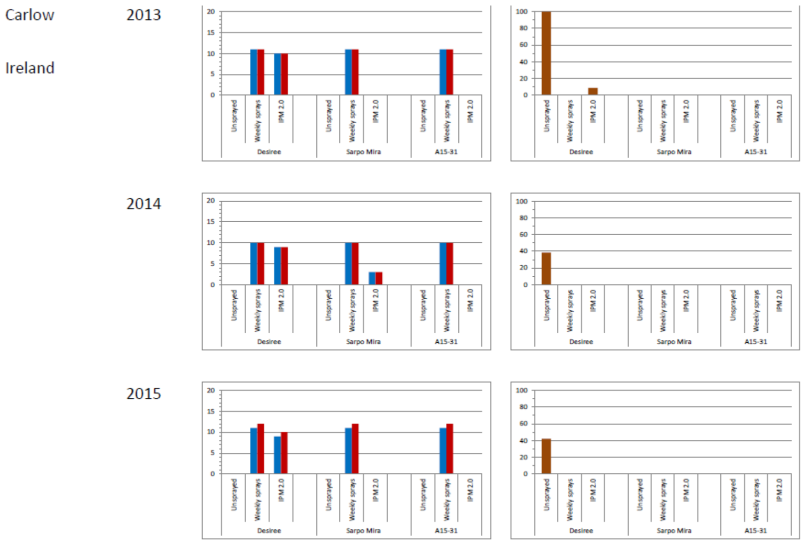

Fig. 1. Fungicide input (left graphs) and end of season severity (right graphs) for each year of AMIGA Irish study. Fungicide input represents the number of spray applications (blue column) and the number of 'full dose sprays' applied (red column) to GM Desiree (A15-031), Desiree and Sarpo Mira.

5. Opportunity/Benefit:

This project provided an opportunity to initiate a large-scale knowledge transfer exercise to address the many issues and perceptions that exist around GM technology. The benefit of completing this project is that a baseline of data detailing the microbial community structure and diversity has now been compiled. This acts as a benchmark from which to gauge the impact of cultivating a GM potato. Based on this, the GM potato line studied induced no greater environmental impact than its non-GM comparator. In addition, the GM potato line maintained its resistance to late blight disease for the three years of the study, which implied there was no requirement for fungicide applications in contrast to the ~11 sprays required to control late blight in the control non-GM Desiree plots. Overall, the introduction of genetic resistance using cisgenesis presents a sustainable strategy for the control of late blight disease with no detectable negative environmental consequences, based on the study adopted here.

6. Dissemination:

OrtizCortés, V., Phelan, S. and Mullins, E. (2016). A temporal assessment of nematode community structure and diversity in the rhizosphere of cisgenic *Phytophthora infestans*-resistant potatoes. BMC Ecology.

In addition, over 85 KT events were completed during the project through TV, radio, print, on-line media as well as public debates, workshops, national and international scientific presentations and 2 summer schools for early –stage researchers.

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