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Genetically Modified Potato with Increased Resistance to *P. infestans*- Selecting Test Species for Environmental Impact Assessment on Non-Target Organisms

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Summary

Infestations of potatoes with *Phytophthora infestans*, also known as "potato late blight" is the most devastating potato disease worldwide. Its occurrence often results in huge economic losses for potato producers. Current control measures – involving extensive use of fungicides – come with environmental costs. Efforts have been made to develop commercial potato varieties with increased resistance to *P. infestans* (the causal agent of late blight) using a variety of approaches. Due to the remarkable ability of *P. infestans* to overcome resistance, potato breeders have not yet succeeded in developing commercial potato varieties with resistance that is lasting. One approach, where genetic engineering is used to 'stack' (i.e. insert in tandem) genes with broad-spectrum resistance to *P. infestans* in commercial potato varieties, has recently been employed as a means to create genetically modified (GM) potato varieties with more durable resistance. Several European companies and research institutes are involved in this research. Field trials with this type of GM potato have taken place in several localities in Europe since 2006. It is likely that Norwegian authorities, in the future, will receive applications for regulatory approval of GM potato with increased resistance to *P. infestans*.

The mandate for the project reported here was to conduct a trial run of a procedure developed to select the ecologically most relevant test species for assessing potential impacts on non-target organisms of GM potato plants with increased resistance to *P. infestans*. The species selection procedure is part of the Problem Formulation and Options Assessment (PFOA) framework and was performed by conducting an expert workshop. The project took place between January and December 2011. The workshop was organized in August 2011. The report concludes with the following recommendations for follow up research and analysis:

- Increase funding for baseline studies to generate background knowledge about the current level of biodiversity of fauna and flora in potato agro-ecosystems in Norway, particularly with regard to the presence of species that are not known to be pests or beneficial organisms from an agronomic point of view.
- Conduct follow up workshops involving experts from all Nordic countries to identify the ecologically most relevant test species for assessing impacts on non-target organisms from GM potato with increased resistance to *P. infestans* and to evaluate the risk for virulence development in the Nordic *P. infestans* populations.
- Facilitate a full Problem Formulation and Options Assessment (PFOA) of GM potato with increased resistance to *P. infestans* to explore whether this approach is a viable solution to the problems of the late blight disease in Norway.

1. Introduction

The deliberate release of genetically modified organisms (GMO) in Norway is regulated under the Gene Technology Act (1993)¹. This act dictates that deliberate release of a GMO can only take place if there is no risk of adverse effects on human health or the environment, and if it fulfils social utility and sustainability criteria. Regulations relating to the impact assessment pursuant to the Act (2005) describe the risk assessment criteria, including criteria for environmental risk assessment (ERA), but give no suggestion of specific methodologies or standardized testing procedures to evaluate these criteria.

This report focuses on identifying potential impacts of GM potato plants on non-target organisms, i.e. species that are directly and/or indirectly exposed to the GM potato plants, but which are not targets of the expressed transgene products in these plants. When a GM plant is released into the environment, it will interact with other species in this environment at different levels and, possibly, affect a wide range of non-target organisms and ecological functions. When testing for potential impacts on non-target organisms, it is of course not possible to include all species that can be exposed to the GM plant as test species. Hence, the basis for selecting test species is essential in determining which non-target impacts that are investigated.

Our approach is to contribute to the development of a methodology for selecting ecologically relevant test species, i.e species that represent important ecological functions and may be likely exposed or vulnerable to effects related to the GM potato in question. The purpose is to uncover which potential non-target impacts should be investigated in the conduct of a risk assessment or in the development of a monitoring program. We report from an expert workshop where we applied the initial steps of a proposed procedure for selecting non-target test species for an ERA or monitoring of GM plants (Hilbeck et al. 2008; 2011). By adopting a functional approach to biodiversity, this procedure aims to identify the ecologically most relevant test species. The rational is that significant adverse impacts on these non-target species could impact the conservation or sustainable use of biological diversity (Cartagena Protocol, 2003), or on the overall productivity (including ecosystem services) of the entire potato agro-ecosystem. The selection procedure was developed by an international group of public sector scientists who worked together in an international project on GMO ERA methodologies (for further details see www.gmoera.umn.edu). It has already been tested on various GM plants around the world (Hilbeck and Andow, 2004; Hilbeck et al., 2006; Andow et al., 2008; Hilbeck and Römbke, 2009), and was recently, at least in part, included in the revised guidance document on ERA of GMOs in EU (EFSA, 2010).

GM potato with increased resistance to *Phytophthora infestans* was used as the case example for the workshop. P. infestans is the causal agent of potato late blight which is described as the most devastating potato disease, and results in large economic and ecological costs in potato production worldwide (Fry, 2008). In Norway, late blight causes losses of about 55 to 65 million NOK annually (Sæthre et al., 2006) and populations of P. infestans have shown increased aggressiveness in the last two decades (Brurberg et al., 2011; Cooke et al., 2011). Breeding for increased resistance to P. infestans in commercial cultivars has been one of the main goals in traditional potato breeding programs, but due to the remarkable ability of P. infestans to quickly overcome resistance, there has been no success yet in developing commercial potato varieties with durable resistance (Champouret et al., 2009; Haltermann et al., 2008; Vleeshouwers et al., 2011). Using GM approaches in conjunction with conventional potato breeding is suggested as one possible strategy to develop varieties with more durable resistance (Vleeshouwers et al., 2011). Several research institutes and companies are involved in this development. Field trials with GM potatoes with increased resistance to *P. infestans* have taken place at several locations in Europe since 2006 (European Commission, 2011), with 'Fortuna' – a variety developed by BASF Plant Science GmbH – probably closest to commercialisation (BASF, 2010; Storck et al., 2011).

¹ The act regulates all GMOs excepts for human biotechnology and non-viable processed GM products.

The mandate of the project reported here was to conduct a trial run of the proposed procedure (Hilbeck et al. 2008; 2011) for selecting test species to assess impacts on non-target organisms, using GM potato with increased resistance to *P. infestans* as a case. The report starts with a brief introduction to potato production in Norway and prevalence of the potato late blight disease. Then we describe different control strategies that are currently practised to combat late blight in Norway. We focus on presenting research efforts and challenges related to breeding commercial potato varieties with increased resistance to *P. infestans*, including the most recent developments using GM approaches. We then give a brief summary of the test species selection procedure, before we describe the expert workshop (where the initial steps of the procedure for selecting test species were applied) and its findings. The report concludes with recommendations and suggestions for follow up research and analysis.

2. Background

2.1 Potato production in Norway

Potato (*Solanum tuberosum L*.) is a member of *Solanaceae* – an economically important family that includes tomato, pepper, aubergine (eggplant), petunia and tobacco. It is the world's number one food crop in terms of productivity relating to yield and consequently one of the three most consumed crops globally (along with wheat and rice). The global production of potatoes approached 330 Megatons in 2009, with Asia and Europe representing the regions of the globe with the largest areas of potato production (FAOSTAT, 2011).

Potato is an important food crop in Norway. In total, 321 100 tons potato were produced in Norway in 2010, on an area of 13212,4 ha cultivated land (Statistics Norway, 2010). This includes 167,5 ha which is approved for organic potato production (Debio, 2010) and 888,8 ha which is used to produce certified seed potatoes (Nowegian Food Safety Authority, 2011). About one third of the potato produced in Norway is directly consumed while two thirds are further processed (e.g. to produce flour, chips, spirits, feed, etc.) (Møllerhagen, 2011). Potato is grown all over the country, under widely varying climatic conditions from the marginal sub-arctic climate with 24-h day lengths in the north (70°N), to a temperate climate in the south (58°N) (Johansen et al., 2007). Most of the potato production is concentrated in the south- central parts of the country, with the areas around Lake Mjøsa, the areas around the Oslo Fjord, Nord-Trøndelag, Rogaland and Troms representing the five most important production areas. In fact, almost half of all the potatoes produced in Norway are cultivated in the areas around Lake Mjøsa (Statistics Norway, 2010). The trend is that the number of potato producers in Norway is decreasing while the area per potato producing unit is increasing. The average size of a potato farm was 4,78 ha in 2010 which is an increase of 0,34 ha compared to the previous year. There are however big regional differences in terms of farm size; in Hedmark the average size of a potato farm was 14,4 ha in 2010, while it was only 1,4 ha in Troms (Møllerhagen, 2011).

Planting of seed tubers in Norway usually takes place in May and the potato tubers are harvested from July – September, depending on the region and type of potato cultivated. Due to cold temperatures during winter, potato tubers that are left in the field after harvest are usually killed by frost. However, in the southern regions of Norway, tubers may survive and develop into potato plants in the following season. This may in some instances also be the case in the northern parts of the country, where thick snow cover may prevent freezing of the soil. Hence, volunteer plants may occur all over the country (Cooke et al., 2010).

2.2 Prevalence of potato late blight disease in Norway

Potato late blight is the most devastating potato disease resulting in high yield loss, and consequently economic losses to the potato producers worldwide. In 2006 it was reported that the total annual cost caused by potato late blight in Norway is about 55 to 65 million NOK depending on the year (this includes expenses related to fungicide use to protect the potato from the disease) (Sæthre et al. 2006). It is particularly prevalent in Rogaland and the areas around the Oslo Fjord. It also causes problems, but to a lesser extent, in the areas around Lake Mjøsa and in Nord-Trøndelag, while it is only a minor problem in northern parts of Norway (A. Hermansen personal communication, 2011). 5

2.2.1 Epidemiology and population characteristics

Potato late blight is caused *Phytophthora infestans* (Mont.) de Bary which belongs to the oomycetes (a diverse group of eukaryotic microorganisms, including pathogens of plants and animals). Late blight epidemics are most often caused by asexual clones of *P. infestans* that spread and amplify through aerial dispersal of sporangia. If the sporangia are spread to potato leaves or stems and conditions are cool and wet they will release zoospores which germinate and infect the potato plants. Symptoms of infection (i.e. brown lesions on the leaves) become visible after a short latent period (at optimal conditions as short as 3 days). New sporangia may form in the border between healthy and injured tissue and may spread and infect new leaves. As a result, several asexual generations of spores can be produced, which may ruin the entire potato crop within a few weeks. During the growing season, sporangia may also enter the soil and produce zoospores that may be transported via soil water and infect the potato tubers. Tuber infection may also occur during harvest if infected soil and haulm come in contact with tubers and these are wet for some time afterwards (Kamoun and Smart, 2005; Sæthre et al., 2006).





Symtoms of P. infestans infections on potato leaves and potato tuber. Photos: Ethan Hack / Agricultural Research Service

There are two mating types of *P. infestans* (A1 and A2). When both of these are present in the same field the pathogen can reproduce sexually and form oospores which can survive outside the host. Originally, the two mating types were only known to exist in Mexico, which is the centre of origin of *P. infestans*. In the mid-19th century, the pathogen migrated and became well established in potato production throughout the world. Initially, the global spread of *P. infestans* probably only consisted of mating type A1, whereby only clonal asexual lineages of the pathogen occurred outside Mexico. *P. infestans* has been present in Norway at least since 1841 and has been recorded in all counties except for Finnmark (however, there are indications that the pathogen is also present there, see Hermansen, unpublished data reported in Sæthre et al., 2006).

Late 20th century, mating type A2 also migrated from Mexico to Europe, probably in a shipment of potato tubers in the summer of 1976, and since then this mating type has spread throughout Europe (Fry et al., 2008; 2009). Hence, both mating types are now present in Europe. Current monitoring in Europe shows prevalence for mixed mating types and increasing genetic diversity, with distinct regional differences. In the Nordic countries, the prevalence of the A2 mating type was at the level of 36-49 % in 2003 (Fry et al., 2009). A recent study by Brurberg and co-workers (2011) shows a highly diverse population of *P. infestans* in the Nordic countries (approx. 40 % A2), with a large number of genotypes (169 multilocus genotypes based on 7 loci from 191 isolates). This indicates that sexual reproduction is common among *P. infestans* populations in the Nordic countries and this potential is further strengthened by the fact that both matingtypes were present in 40 % of the fields sampled (Brurberg et al., 2011). Sexually reproducing oospores may survive in the field and infect potato crops over multiple seasons. Hence, in addition to infection from seed tubers, late blight is

also a soil borne disease. In fact, cold winters with frozen soil help to conserve oospores between growing seasons and there are indications that they may survive at least five winters (Nordskog et al., unpublished reported in Cooke et al., 2011).

Box 1: The Norwegian P. infestans population

- Key characteristics & issues:
- High genetic diversity
- Both mating types (A1 and A2) are present in the same fields
- Increased virulence
- Sexual reproduction is probably common
- Formation of oospores makes late blight a soil borne disease
- Cold winters with frozen soils may increase the longevity of the oospores

Taken together, these factors indicate that the Norwegian *P. infestans* population has a strong adaptive potential. This may influence the durability of the resistance to *P. infestans* in GM potato, if cultivated here

The *P. infestans* population in the Nordic countries have similar prevalence of the same genotypes. Therefore, it is likely that the Nordic *P. infestans* lineages belong to the same population (Brurberg et al., 2011). Genetic analyses of *P. infestans* populations reveal that the genetic diversity of the pathogen is particularly high in the Nordic countries and some areas of northern Europe, when compared to the rest of the world (Brurberg et al., 201; Cooke et al., 2011; Sujkowski et al., 1994; Drenth et al., 1994). In fact, the frequency of the presence of both mating types and the level of genetic diversity described in the Nordic countries, are only matched by the *P. infestans* populations in the centres of origin in central Mexico (Goodwin et al., 1992) and the southern Andes (Gomez-Alpizar et al., 2007).

2.2.2. Control strategies

The application of synthetic fungicides (particularly products containing cyazofamid, the active ingredient e.g. of the fungicide Ranman, mandipropamid (e.g. of Revus), as well as mancozeb-based products) during the growing season is currently the most widely practised strategy to fight *P. infestans*. The number of fungicide applications needed for adequate control varies considerably between seasons, climatologically diverse regions and production types, with an average number of 5 to 6 sprays per growing season (Cooke et al., 2011; Sæthre et al. 2006). No fungicides for seed tuber treatment are approved for use in Norway, but all marketed seed tubers are certified and this includes controlling for *P. infestans* infections. This control is however difficult, as mother plants that have been treated regularly against late blight during the growing season may nevertheless have infected tubers. Therefore, infected tubers are frequently released on the market and if farmers suspect that the seed lot is infected they are advised to use fungicides early in the production season to delay the first infection (Cooke et al., 2011).

However, such control strategies may begin to lose their efficacy with increased fungicide use. Excessive use of fungicides under limited rotation schemes or application of different control strategies imposes pressure on the pathogen for developing fungicide resistance (Fry, 2008). This potential has also been strengthened by the presence of both mating types in Norway (Hermansen et al, 2000). No synthetic fungicides or copper solutions are approved for use to control late blight in Norwegian organic potato production (Tamm et al., 2004). A forecasting and decision support service is established in Norway where information on late blight control (i.e. related to the environment, the host and the pathogen) is disseminated via the Internet (www.vips-landbruk.no) to help farmers make decisions on fungicide use.

Killing of the haulm prior to harvest is a normal procedure to reduce the risk of tuber blight (Sæthre et al., 2006). Sound crop rotation is an important and effective way to reduce the risk of soil borne infections of *P. infestans*. Cooke et al., (2011) suggest three years between each potato crop 7

production, but indicate that in some situations longer periods are needed to prevent infection from surviving oospores.

Finally, as will be described in more detail in the next section, breeding for commercial varieties with increased resistant to *P. infestans* is an important strategy to fight the pathogen.

2.3 Breeding for increased resistance to *P. infestans*

Resistance to *P. infestans* is one of the most important targets in potato breeding (Park et al., 2009). Plants may achieve specific resistance to pathogens primarily through two different mechanisms.

In 'single gene' disease resistance (also known as 'gene- for-gene', 'race specific' or 'qualitative' resistance) defence responses are invoked through interactions between specific avirulence (Avr) gene products (effector proteins) produced by the pathogen and single resistance (R) gene products produced by the plant. Disease resistance starts with a recognition of the pathogen Avr factors by plant R proteins, followed by signal transduction leading to a hypersensitive response (HR) and death of the infected cell. If a plant lacks the correct R gene to match at least one of the Avr genes possessed by an invading pathogen, that plant will be unable to use its R genes to detect and stop the pathogen(Kamoun and Smart, 2005; Tuzun, 2001). In 'multigenic' resistance, (also known as 'horizontal', 'quantitative' or 'partial' resistance), a plant's defence mechanisms are generated via interactions between the products of multiple plant genes. Hence, the plant and the pathogen do not require matching R and Avr genes for a timely plant defence response to occur (Kamoun and Smart, 2005; Tuzun, 2001). However, research has shown that it is difficult to transfer 'multigenic' resistance to commercial potato varieties. Additionally, this type of resistance is day-length dependent and strongly correlated with late maturity under long-day conditions – characteristics which are not suitable for commercial potato production in some environments (van der Vossen et al., 2003). Therefore, most breeding programs focus on 'single gene' resistance (Park et al., 2009, van der Vossen et al., 2003), through identifying and introgressing R genes from wild Solanum species into commercial potato varieties.

Wild *Solanum* species that have coevolved with *P. infestans* in its centre of origin in central Mexico constitute the primary source for *R* genes in potato breeding. To date, 21 *R* genes that confer differential resistance specificities to *P. infestans* isolates have been cloned from various *Solanum* species (Vleeshouwers et al., 2011). In the earliest attempts to breed potato with *P. infestans* resistance, starting in the first half of the twentieth century, breeders particularly worked with genes from *Solanum demissum*. As of today, eleven *S. demissum R* genes have been identified and introgressed into commercial potato varieties through traditional breeding methods (Vleeshouwers et al., 2011).

A major challenge in resistance breeding is however that *P. infestans* has a remarkable ability to rapidly adapt to and overcome *R* genes in the potato plants. Sequencing the *P. infestans* genome has shown that most *Avr* genes occupy highly plastic and dynamic areas (gene spares, repeat rich areas) in the genome. This provides one explanation for *P. infestans*' extraordinary ability to evolve (Vleeshouwers et al., 2011). Moreover, as the pathogen is also able to reproduce sexually it can form larger numbers of recombinants than if it only reproduced through asexual clones – which contributes to increased genetic diversity and consequently improved evolutionary potential. For instance, as both mating types of *P. infestans* are now present in Europe, the pathogen has shown increased aggressiveness in this region in the last two decades (Cooke et al., 2011; Vleeshouwers et al., 2011). This constant 'evolutionary arms race' between *Avr* and *R* genes has been a vexing challenge, races of *P. infestans* have now overcome all the 11 *S. demissum* derived *R* genes introgressed in commercial potato varieties in most potato growing regions of the world (Haltermann et al., 2008; Vleeshouwers et al., 2011).

Another challenge in resistance breeding is that traditional breeding methods, such as somatic fusion, are considered to be laborious, particularly since large numbers of undesirable traits (linkage drag) from the wild species must be removed through several generations of backcrosses to the commercial potato variety. Cooke et al. (2011) report that cultivars with increased resistance

to P. infestans are generally not grown on a large scale in western Europe, because these cultivars usually do not perform well when it comes to commercially important traits such as quality, yield and earliness.

2.3.1 Development of GM potato with increased resistance to P. infestans

Novel, information and biotechnology-driven approaches to plant breeding that intend to overcome some of these challenges are currently practised. Both the genome of S. tuberosum and of P. infestans are now sequenced (Potato genome sequencing consortium, 2011; Haas et al., 2009). Markerassisted selection (MAS) is applied to speed up the identification and selection of R genes (Pankin et al., 2011; Sokolova et al., 2011). Genetic modification is suggested as an approach to overcome the problems of linkage drag (Vleeshouwers et al., 2011). Moreover, breeders now work with R genes that have proven to confer resistance to a broad spectrum of *P. infestans* isolates. These broad spectrum R genes have been identified in various wild potato species including S. bulbocastanum, S. stoloniferum, S. venturii and S. mochiquense (Vleeshouwers et al., 2011). Using genetic engineering tools to 'stack' (i.e. insert in tandem) several broad-spectrum R genes (from various sources and with different specificity to P. infestans isolates) in the genome of commercial potato varieties, is suggested as an effective approach to achieve more durable resistance to *P. infestans* (Vleeshouwers et al., 2011).

Box 2: Key Concepts

Gene technology involves techniques that enable isolation, characterization, modification and insertion of genetic material into living cells or viruses.

A genetically modified organism (GMO) is defined any organism that possesses a novel combination of genetic material obtained through the use of gene technology.

Transgenesis implies that the GMO has received artificially produced genes or genes from donor organism(s) that is sexually incompatible.

Cisgenesis implies that the GMO has only received gene(s) from sexually compatible organisms, i.e. the recipient organism and donor organism(s) are naturally crossable.

Conventional breeding is improvement of farm animals and cultivated plants through deliberative interbreeding of related individuals and application of genetic principles, using other techniques than those defined as gene technology.

Marker assisted selection (MAS) is an indirect selection process for plant and animal breeding where a trait of interest is selected, not based on that trait itself, but on a genetic marker linked to it. Both GM and conventional breeding may use MAS.

Several European research institutes and companies are currently involved in the development of GM potato with increased resistance to *P. infestans*. Sixteen notifications of field trials of such GM potato lines are recorded in the European commission's GMO register (European Commission, 2011). 13 of these notifications involve field trials of GM potatoes developed by the chemical company BASF Plant Science GmbH, while the remaining three notifications are filed by Wageningen University (the Netherlands), University of Ghent (Belgium) and the Sainsbury Laboratory (United Kingdom). Both BASF, Wageningen University and the Sainsbury Laboratory aim to develop commercial varieties for market launch (Storck et al., 2011). BASF Plant Science has developed a GM potato with the two R genes Rpi-blb1 and Rpi-blb2. This variety, marketed as 'Fortuna', has been tested extensively in Europe and is probably closest to commercialisation (Storck et al., 2011), for more details see Box 4, pp. 11. Wageningen University is running the DuRph project (Haverkort et al., 2008; 2009) which particularly aims to develop a marker free P. infestans potato variety through a cisgene approach, where several R genes (up to three to four) from different wild potato species (S. bulboscatanum, S. demissum, S. stoloniferum and S. venturi) are transferred. Wageningen University conducted the first field trials of GM potatoes in 2007 with trials located at different sites in the Netherlands and one in Belgium. The Sainsbury Laboratory conducted the first field trial with GM potatoes of the commercial variety Desiree in the United Kingdom in 2010 (The Sainsbury Laboratory, 2010). This variety contains *R* genes from the wild potato species *S. venturii* and *S. mochiquense*, as well as a kanamycin resistance nptII gene (used as a marker).

Box 3: Molecular data needed to evaluate risks of virulence development

An ERA of GM potato with increased resistance to *P. infestans* should include an evaluation of the risk for virulence development in the *P. infestans* population. This is crucial in a Norwegian context, given the strong adaptive potential in the Norwegian *P. infestans* populations. This assessment requires information about:

- The molecular characterization of the GM potato, i.e. description of the transgene construct, including promoters, terminators and in-planta sequence data.
- Tissue- and stage specific transgene product expression, i.e. transgene product minimum expression levels in all relevant tissues that can potentially be infected, during the time that *P. infestans* infection may occur.

2.3.2 Broad-spectrum R genes from S. bulbocastanum

The wild potato species *S. bulbocastanum* was originally considered to be highly resistant to all known races of *P. infestans* (Vleeshouwers et al., 2011) and has therefore been of particular interest to potato breeders. Two broad-spectrum *R* genes from *S. bulbocastanum* (*Rpi-blb1* and *Rpi-blb2*) have been identified, successfully cloned (Song et al., 2003; van der Vossen et al., 2005) and introduced to commercial potato cultivars using GM approaches (Vleeshouwers et al., 2011).

The *Rpi-blb1* gene is described as an ancient *R* gene which is predicted to have evolved along a slow evolutionary trajectory. So far, this *R* gene has not been detected outside of Mexico and even there it is limited to just a few *Solanum* species. Despite its broad resistance, Champouret and coworkers (2009) recently identified two isolates of *P. infestans* (found in central Mexico) that are virulent against the *Rpi-blb1*. Haltermann and coworkers (2008) tested the performance of several GM potato cultivars with the *Rpi-blb1*gene to investigate whether introduction of the *R* gene had any effect on tuber size and yield. No significant effects were detected. They did however find that the GM potato plants were only resistant to foliar late blight infection, but had no tuber resistance (even though the *Rpi-blb1*gene was expressed in potato tubers). Besides this, studies have shown that the expression of *Rpi-blb1* in cultivated GM potato shows a lower degree of resistance to the same strains of *P. infestans* compared to *S. bulbocastanum* (Bradeen et al., 2009; Champouret et al., 2009; Kramer et al., 2009). This means that using only the *Rpi-blb1* gene may not be sufficient in a GM strategy, but more importantly it shows the difficulty of predicting the function of a genetic construct when moved into a new host, even when the host is a closely related species.

It is assumed that *Rpi-blb2* gene has evolved more recently than *Rpi-blb1*. Even though *Rpi-blb2* was also considered to be highly resistant against all races of *P. infestans*, infection of GM potato plants containing the *Rpi-blb2* gene has been reported in the Netherlands (G.Kessel, unpublished, reported in Vleeshouwers et al., 2011). Genetic analyses have also shown that the *Rpi-blb2* and the *Mi-1* gene from tomatoes have 82 % sequence similarity and are located in the same region of the genomes. *Mi-1*, when expressed, shows resistance to attack from nematodes, aphids and white flies (Milligan et al., 1998; Nombela et al., 2003; Rossi et al., 1998) which are organisms with important ecological functions. The effect of *Rpi-blb2* when expressed in cultivated potatoes on the same species is unknown. Moreover, *Avrblb2* (the target *Avr* gene in *P. infestans* isolates) belongs to a multigene family with many (at least 7) duplicated copies in the *P. infestans* genome. The protein is highly polymorphic with a high mutation rate. Little is known about the potential to gain virulence due to point mutations or deletions in the *Avrblb2* gene (Vleeshouwers et al., 2011).

Box 4: Fortuna

'Fortuna' is the brand name of a GM potato developed by BASF Plant Science GmbH. It has been under development by BASF Plant Science for several years with the first field trials conducted in the United Kingdom and Germany in 2006, followed by field trials in the Netherlands and the Check Republic in 2007. Currently, field trials are taking place in Belgium and Sweden (European Comission, 2011). As the event is still under product development, very little information about its characteristics is currently publicly available. The brief description given in Table 1 is based on the information provided in the notification report posted on the European Commission webpage (European Commission, 2011).

Table 1: Brief description of GM potato with increased resistance to P. infestans (Fortuna)

Host organism	Solanum tuberosum (potato)			
Transformation method	Plasmid DNA was introduced into the potato lines by Agrobacterium-mediated gene transfer technology using a binary vector system			
Introduced genes	Gene	Origin	Purpose	
	T-DNA borders, pTiT37	Agrobacterium tumefaciens	Allowing incorporation of the construct into the plant genome by agrobacterium	
	ahas gene Arabidopsis Thaliana Imidazoline tolerance (a a marker gene) ? A. tumefaciens Promoter and terminato from the nopaline synthase gene Rpi-blb1 Solanum bulbocastanum Resistance to P. infestans			
	Rp1-blb2 S. bulbocastanum Resistance to P. infestans			
Intended effect	 improved resistance to <i>Phytophthora infestans</i> tolerance to Imidazolinone herbicides, mediated by the <i>ahas</i> gene as selectable marker gene to identify transgenic cells in tissue culture 			
Intended use	Potato production for human consumption			
Involved companies	BASF Plant Science GmbH			

3. Introduction to the test species selection procedure

The purpose of test species selection procedure is to identify the ecologically most relevant test species and methods for assessing ecological impacts of GM plants on non-target organisms (Hilbeck et al., 2008; 2011). The development of the procedure is motivated by a recognition of shortcomings in the current implementations of ERA of GM plants in Europe, which largely follows the ecotoxicological testing strategy developed for pesticides – for instance, by only requiring testing of isolated bacteria-produced novel proteins and selecting test species from a standardised list (Hilbeck et al., 2008, 2011). Hence, this procedure, which was recently included, at least in parts, into the newly revised guidance document on ERA of GMOs in the EU (EFSA, 2010), is an attempt to improve upon currently practiced testing procedures. An essential feature of this procedure is that it places the whole GMO in its receiving environment at the center of the assessment. Test species and methods are selected on a case-by-case basis (using a functional approach to biodiversity), and later subjected to a step-wise selection procedure to identify the ecologically most relevant test species. It allows the ERA to focus on species with critical ecological roles and limits the range of test species to a practical number (Hilbeck et al., 2008). The rational is that if these species are adversely affected by the GM plant, it may result in severe impacts on the entire agro-ecosystem. Importantly, the procedure is carried out in a transparent manner through a participatory approach (i.e. during workshops).

This procedure is part of the Problem Formulation and Options Assessment (PFOA) framework (for further details see www.gmoera.umn.edu). "The PFOA process emphasizes engagement with stakeholders in an iterative series of stages, from identification of the problem(s) through comparison of multiple technology solutions that could be used in the future with their relative benefits, harms, and risks" (Nelson et al., 2009). The PFOA methodologies have been used and refined in many countries including assessment of Bt corn in Kenya (Hilbeck and Andow, 2004), and Bt cotton in Brazil (Hilbeck et al., 2006) and Vietnam (Andow et al., 2008). In 2005, a book-writing workshop was organised, which included discussing how PFOA can be applied to environmental risk assessments of GM fish (Kapuscinski et al., 2007). Our attempt here is to apply this procedure to GM potatoes. Here, we will present a brief summary of the species selection procedure. For a more detailed introduction please refer to Hilbeck et al. (2008).

3.1 Description of the steps in the species selection procedure

The species selection procedure is based on a comprehensive description of the 'case' to be assessed (in this case GM potato with increased resistance to *P. infestans*). Table 2 describes the three elements that constitute the case² and suggests information that should be compiled for each of these elements. Describing the case in this comprehensive manner helps to clarify what the problem is in the first place, and how the proposed GMO intends to solve it. Hence, this exercise is fundamental for determining the scope of the whole assessment.

Table 2: Elements to describe a case

Elements of a case	Information needed
The crop plant	Characterisation of the biology and ecology of the crop plant, including spatio-temporal agronomic use and limitations of use
The novel trait and its intended effect	The novel trait: Molecular characterization of the GM plant, its introduced genetic material, tissue-specific expression of the novel proteins, Intended use: Data on the problem to be solved with the GM plant, efficacy data of the GM plant demonstrating the ability to solve that problem, the severity of the problem, how widespread it is and who is most affected.
The receiving environment	Characterization of the existing biodiversity and ecological processes that might be affected and from which the candidate testing species will be selected

² This definition of a 'case' is based on provisions by the Directive 2001/18/EC and by the Cartagena Protocol (2003) on Biosafety.

The selection procedure involves a series of steps (see Figure 1) which allow, in a funnel-like process, to reduce the (potentially quite high) number of candidate test species and functions in a systematic, transparent and step-wise fashion, to a relevant and also practical number of species/processes to be tested. In the following, we will give a brief description of each of the steps.

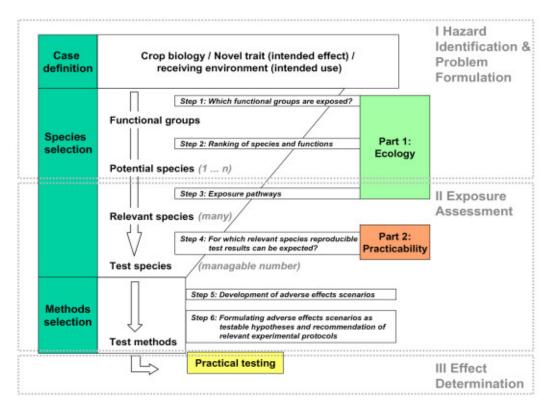


Figure 1: Species and method selection procedure for ecotoxicity testing of GMOs (from Hilbeck et al., 2011)

Step 1: Identification of functional groups of species

The first step in the selection procedure is to identify the most important functional groups that exist in the receiving environment of the respective GM plant, based on the information generated when describing the case. The step involves generation of lists of non-target species known to exist in the receiving environment (considering both organic and conventional production systems), that belong to the identified ecological functional groups.

Step 2: Ranking of species or functions

The purpose of this step is to narrow the initial list of species to those that are ecologically most relevant. All the non-target organisms initially listed are systematically evaluated and ranked based on five ecological criteria: (i) Geographic distribution (degree of overlap in geographic distribution of the crop plant and the non-target species), (ii) Habitat specialisation (degree of association between the non-target species and the crop habitat), (iii) abundance (average or typical density where the species is present), (iv) phenology (degree of temporal overlap of non-target species with the crop plant) and (v) ecological significance (degree of non-target species specialisation on the crop (including both herbivores degree of feeding specialisation to the crop and predators feeding specialisation to the prey/host of the crop)).

Step 3: Determination of possible exposure pathways

The goal of this step is to differentiate the species into those that are possibly exposed and those unlikely to be exposed to the transgene product (including their metabolites), any other altered composition of metabolic compounds or to the corresponding measure necessary for the intended effect of the GM plant (e.g. application of pesticides). It involves conducting an exposure analysis which is case-specific to the GM crop and based on information about the phenotypic pattern of transgene expression and any induced pleiotropic changes in the various parts of the GM plant over the whole growing season.

Step 4: Applying practicability criteria

In this step, species that are not suitable for obtaining meaningful data (e.g. due to low abundance) or reproducible test results are removed. This is done by assessing whether the selected species fulfils the practicability criteria such as: Abundance, easy to keep and breed, quick succession of generations, moderate sensitivity to stress factors, measuring parameters (whether different parameters can be measured during one test run), documentation or experience (whether scientific expertise exists and is documented regarding the behaviour of the organism in testing conditions), broad ecological tolerance and protection status.

The outcome of these four steps is a list of selected test species and ecological functions that are determined to be of greatest ecological importance, most extensively exposed to the GM plant and its transgene product(s) and considered suitable for testing. Importantly, all the steps focus on identification of gaps of knowledge. The next steps of the selection procedure involve identification of potential adverse effects and development of testing programs.

Step 5: Adverse effect scenarios and research hypotheses

Possible adverse effect scenarios are identified. This part ends with the formulation of a testable adverse effect hypothesis, particularly including hypotheses that address critical gaps of knowledge, for which experiments/tests can be selected or developed.

Step 6: Developing the testing program

Adverse effect hypotheses are formulated using information from step 3 and 5. The information synthesised during the previous steps also guide the development of ecologically meaningful experiments in terms of protocols, feeding strategies and food types to be used etc.

The species selection procedure has been applied in workshops to assess a number of real case examples (see Hilbeck and Andow, 2004; Hilbeck et al., 2006; Andow et al., 2008; Hilbeck and Römbke, 2009). In the project reported here, as will be described in more detail in the next section, we applied the procedure to select test species for impacts on non-target organisms from the production of GM potato with increased resistance to *P. infestans* in Norway.

4. The Expert Workshop: Introducing and initiating the test species selection procedure

In August 2011, we conducted a one-day workshop with invited researchers with an expertise in biodiversity on flora and fauna associated with potato agro-ecosystems in Norway. The aim of the workshop was to introduce the species selection procedure to the experts and apply the first two steps of the selection procedure (as outlined in section 3). These steps included, from above: (i) identifying the most important ecological functions in Norwegian potato fields and generate species lists for these ecological functions and (ii) to rank these species according to ecological criteria. The expected outcome of the workshop was to agree on a list of the species that have the most important ecological role in potato agro-ecosystems in Norway. The work is based on the assumption that if these species are adversely affected by GM potato with increased resistance to P. infestans, it could potentially result in a significant adverse environmental effect on the entire agro-ecosystem (Hilbeck and Römbke, 2009). Importantly, the outcomes of the workshop are by no means final, and the species identified in this phase of the project will need to be subjected to further selection steps to arrive at a final list of the most relevant test species to include in an ERA of GM potato with increased resistance to *P. infestans*. However, the exercises conducted and the species lists generated in this phase of the project constitute the entry point of any ERA. Consequently, the results reported here are fundamental to determine the scope and structure of an ERA of GM potato with increased resistance to *P. infestans*.

4.1 Preparations for the expert workshop

The preparatory phase of the workshop primarily involved selecting a GM potato case example to discuss at the workshop, identifying the expertise that we wished to have at the table, and inviting researchers who hold this expertise.

4.1.1 The GM case example and selection of participants

The GM potato with increased resistance to *P. infestans* developed by BASF Plant Science GmbH and known under the brand name 'Fortuna' was chosen as the case example for the workshop. It is likely that BASF will be one of the first companies to seek approval for a GM potato with increased resistance to *P. infestans* (BASF, 2010, Storck, 2011) (for further information see Box 4, pp.11). Therefore, this event constitutes a highly relevant case example for the workshop. The workshop participants received general information about GM potato with increased resistance to *P. infestans*. and about this event in particular, prior to the workshop as well as in presentations at the workshop. We wanted to invite researchers with expert knowledge about the biodiversity of flora and fauna associated with Norwegian potato agro-ecosystems to the workshop. Expertise on species belonging to the four taxonomic groups; fungi, bacteria, nematodes and insects were considered to be most relevant. Additionally, we wanted experts with knowledge on potato production in different regions of the country as well as different potato production systems (i.e. conventional and organic). In an attempt to avoid that the species identified and selected in the workshop were limited to only those recorded as beneficial and pest organisms in potato production, we searched for an ecologist/ entomologist with knowledge on biodiversity in potato agro- ecosystems and surrounding semi natural and natural habitats, who is not working directly with agriculture. Finally, we wished to have experts with different institutional affiliations at the table.

Based on these criteria, we recruited researchers to the workshop by contacting different universities, research institutes and farmers' advisory bodies in Norway, as well as by reviewing scientific literature in the field. Through this process, we experienced that there is only a limited number of researchers working in this field in Norway, of which most are based at Bioforsk³. For instance, we did not manage to recruit ecologists/ entomologists with sufficient knowledge about the agro-biodiversity associated with potato production, but not working directly with agriculture. As a result, four researchers from Bioforsk were invited to the workshop of which all wanted to participate (see Table 3). We consider them as the most competent experts in this field in Norway. In addition to the invited experts, four GenØk employees participated in the workshop. Some acted as facilitators, but all participated in the discussions.

Table 3 Workshop participants

Names	Area of expertise	Affiliation
Arne Hermansen	Potato pathogens (fungi and bacteria)	Bioforsk, Plant Health and Plant Protection
Richard Meadow	Potato pests (insects)	Bioforsk, Plant Health and Plant Protection
Ricardo Holgado	Potato pests (nematodes)	Bioforsk, Plant Health and Plant Protection
Tor J Johansen	Potato pests (insects), Potato production, Artic Agriculture	Bioforsk, Artic Agriculture
Odd-Gunnar Wikmark	Molecular biology and Microbiology	GenØk - Centre for Biosafety
Angelika Hilbeck	Entomology, impact on non-target arthropods of GMOs	Swiss Federal Institute of Technology/ GenØk
Frøydis Gillund	Natural resource management, science and technology studies	GenØk - Centre for Biosafety
Thomas Bøhn	Ecology, Gene ecology	GenØk - Centre for Biosafety

³ Bioforsk is a Norwegian research institute which conducts applied and specifically targeted research linked to multifunctional agriculture and rural development, plant sciences, environmental protection and natural resource management (for more information see www.bioforsk.no).

4.1.2 Restrictions and foci of the workshop

Prior to the workshop we had to make some choices with regard to its restrictions and foci. Since one of the main intentions with the workshop was to conduct a trial run of the selection procedure, and introduce this methodology to a group of researchers who could possibly help to facilitate a larger follow-up workshop, we decided to invite a small number of researchers (4). Only terrestrial invertebrate non-target organisms, that have been reported as beneficial or pest organisms in the potato agro-ecosystem, were included in the assessment. There was special focus on pathogenic soil microorganisms (i.e. fungi, bacteria and nematodes) and insects in potato production (i.e. pests and natural enemies), as this was the area of expertise among the invited experts. Vertebrates like farmland birds or small mammals and aquatic invertebrates were excluded due to time limitations. We wanted the assessment to cover all potato producing regions in Norway.

4.2 Results of the application of the initial steps of the species selection procedure

During the workshop, we completed the first two out of the six steps that compromise the species selection procedure (as outlined in section 3). The working tools for these two steps are guidance tables for selection of ecological functional categories (Step 1, see Annex 1) and a matrix for species ranking based on ecological criteria (Step 2, see Annex 2 and 3). Prior to conducting the exercises, the participants were introduced to the complete species selection procedure. Here, we present the results for each of the two steps undertaken during the workshop.

4.2.1 Step 1: Selection of functional categories using guidance tables

The participants were asked to fill in guidance tables in order to identify the most important ecological functions in potato agro-ecosystems in Norway. The guidance tables are designed to allow for a more comprehensive risk assessment of GM plants and are structured on the basis of the elements describing the case GMO, i.e.; (i) the biology of the crop and its agronomic requirements for production, (ii) the novel trait related to the intended effect and (iii) the receiving environment relating to the intended use (see Table 2 pp. 12).

This step was done as a joint exercise where all workshop participants discussed the questions raised in the guidance tables (the filled-in guidance table can be found in Annex 1). Based on the discussions generated when filling in the guidance tables, we identified four functional categories that are important to include in an ERA of GM potato with increased resistance to *P. infestans* (Table 4).

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Table 4: Ecological functional	Categories.	UI Zamsims anu	DI OCCSSES IUCHUHCU.

Ecological functional category	Organisms and processes
Herbivory & disease transmission	Herbivores and pathogens (Fungi, bacteria, viruses and nematodes)
Natural enemies	Predators, parasitoids
Ecological soil processes	Soil organism, soil processes
Pollination	Pollen collectors, pollen feeders, flower visitors

It should be noted that these four categories almost always turn out to be among the most important ones, as reported from similar workshops using this selection procedure (see Hilbeck and Andow, 2004; Hilbeck et al., 2006; Andow et al., 2008; Hilbeck and Römbke, 2009). Among these four functional categories pollination was considered to be the least important, primarily because cultivated potato varieties produce only small amounts of pollen and pollination is rare and not a critical issue for potato production since only tubers are harvested. Producing potatoes for breeding purposes is an entirely different enterprise and therefore outside the scope of this workshop.

Besides identifying the most important functional categories, the guidance tables also help to identify limiting factors for potato production. For instance, potato is highly sensitive to some pests and diseases, in all stages of the growing season, but depending on the disease or pest. Moreover, competition from weeds could be a limiting factor in early growth stages. Potato is also sensitive to water logged soil and frost. In an ERA of GM potato with increased resistance to *P. infestans*, it

is particularly important to investigate whether (and how) any novel trait could interfere adversely with these limiting factors. For instance, it would be important to assess whether resistance to *P. infestans* in the potatoes may open a niche for other potato pathogens, and what the current measures to combat these pest might be.

The guidance tables also identified agricultural practices which may influence the ability of the transgene(s) to spread to surrounding environments. For instance; after harvesting and processing, potato residues are commonly disposed of at informal sites close to the farm or packing facility. Germination of potato plants from culled tubers may occur in the following season. These informal sites could be located in semi-natural or natural habitats, and consequently non-target organisms present there could be exposed to the GM potato. The role of humans as a vector for spreading the transgene was also discussed, particularly when potatoes are grown in small areas or in private gardens, which is commonly practised in Norway. It was commented by the workshop participants that since many private consumers use potatoes bought at the supermarket as seed tubers, it will in principle be impossible to control where GM potato may be further grown if they become commercialised. Again, this may influence the type of organisms exposed to the transgene, as well as challenges related to co-existence with conventional or organic potato production.

These are some examples of information generated during the discussion that would be important to take into consideration when evaluating the data provided for an ERA of GM potato with increased resistance to *P. infestans*. For this workshop, however, we focused on the functional categories identified. The next step of the selection procedure involves filling these categories with species known to contribute to the respective function, and rank these organisms based on ecological criteria. Given the expertise at the table during the workshop, we decided to only include herbivory & disease transmission and natural enemies as the two functional categories to be subjected to the next selection step in the selection procedure.

4.2.2 Step 2: Species lists and selection of test species based on ecological ranking

Prior to the workshop the experts were asked to prepare a list of terrestrial invertebrate species⁴ associated with potato fields in Norway. The lists of species generated by each expert reflected their specific area of expertise and consisted of (i) beneficial and pest insects, (ii) pathogenic fungi and bacteria and (iii) pathogenic nematodes. These species were categorised according to the functional categories; herbivory & disease transmission and natural enemies. As seen from Table 5, the initial lists generated by the experts included totally 31 species, of which 24 belonged to the herbivory & disease transmission and 7 belonged to the natural enemies functional group.

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Table 5: Number	or species	llisted accord	ning to	tiinctional	caregory

Functional category	Taxonomic groups	Numbers of species listed
Herbivory & disease	Insects	8
transmission	Fungi	6
	Bacteria	2
	Nematodes	8
Natural enemies	Insects	7

It is important to note that these species lists were generated on a voluntary basis, and it is likely that more species would have been included if more time was available or more experts took part in the process. For instance, if the entire species selection procedure is to be completed in the future, lists of species representing ecological soil functions and pollination would have to be included. Moreover, it was noted by the workshop participants that the expertise at the table was not complete, and it was particularly mentioned that we lacked an expert with knowledge on species associated

⁴ Some of the lists (see Annex 2 and 3 and Table 6) included genus or families of species, rather than individual species. Hence, in some cases groups of organisms belong to these taxa were ranked rather than the individual species.

with potato agro-ecosystems beyond those that are recorded as pest or beneficial organisms. It was however also recognised among the experts that there are limited data, and consequently, huge gaps of knowledge about the invertebrate fauna associated with agro-ecosystems in Norway, particularly concerning species that are not directly pest or beneficial organisms.

Nevertheless, the species lists used during this workshop do include the most important beneficial and pest species present in Norwegian potato agro-ecosystems, and it was a suitable number of species to work with during the time available for this workshop. However, this focus, on pest species in particular, is important to take into consideration when designing experiments to test for adverse effects of GM potato with increased resistance to *P. infestans*. These are species that are selected for withstanding the chemicals used against *P. infestans*, and consequently the hardiest and most robust species. Therefore, the outcomes of environmental safety testing of these organisms may not adequately reflect the range or average susceptibility of these species found in low-input, small scale intercropping systems, organic farming systems or urban gardens (Hilbeck and Römbke, 2009).

Each of the species were ranked (by the most competent expert) according to five predefined ecological criteria, using the matrix tool (the filled-in matrices can be seen in Annex 2 and 3). These criteria relate to the potential exposure of the non-target organism/ process to the crop plant and the significance that a possible adverse effect could have on their ecological functions (and are explained in some more detail in the description of Step 2, pp. 13). The ranking exercise implied that each species was ranked for each criterion, along the qualitative scale: 'high = 1', 'medium =2' and 'low = 3'. Based on this, an overall ranking was estimated for each species, both by description and as a numeric mean (see Annex 2 and 3 for further details). Below (Table 6) is a list of the species that were given an overall 'high' rank – meaning that the species were typically abundant in geographical regions where potatoes are grown and highly specialised to and with life cycles overlapping temporally with the period of potato cultivation. Consequently, these were the species that were identified as having the closest association with the potato crop and the most significant role in the functioning of the potato agro-ecosystem.

Table 6: Species selected based on ecological criteria ranking

Hervibory and disease transmission	Natural enemies
Arthropods: Insecta: Empoasca vitis(Potetsikade) Lygus rugulipennis (Håret engtege)	Arthropods: Insecta: Parasitoids (Hymenoptera) (Snylteveps) Staphylinidae (Kortvinge)
Fungi: Rhizoctonia solani Colletotrichum coccodes	Carabidae (Løpebiller) Syrphidae (Blomsterfluer) Chrysopidae (Gulløye) Cassinglidae (Marihane)
Bacteria: Pectobacterium atrosepticum	Coccinellidae (Marihøne) Arachnida:
Nematodes: Pratylenchus crenatus (Rotsårnematoder) P. fallax (Rotsårnematoder) Globodera rostochiensis (Gul potetcystenematode) G. pallida (Hvit potetcystenematode)	Aranea (Edderkoppdyr)

As Table 6 shows, the ranking exercise resulted in a considerable reduction in the initial list of species belonging to the herbivory & disease transmission category (from 23 to 9 species), while there was no reduction in the species belonging to the natural enemy category. However, the taxa in the natural enemy category were highly aggregated at the family level and above. This made it difficult to reduce the number of taxa as each family holds many different species with quite different importance for the functioning of the agro-ecosystem. A full ERA exercise of the procedure would have to break this down at least to the genus level for making reasonable decision making in the selection procedure. The many 'unknowns' identified for taxa in the natural enemies category (see Annex 3) can also be explained by the currently limited understanding of the many multi-trophic relationships these species are involved in. During the ranking exercise 'unknowns' are ranked as

'high' by default as it is considered critical to introduce awareness of both the degree and severity of these gaps of knowledge. Hence, critical gaps of knowledge must be dealt with prior to final estimates and ranking (based on consensual expert judgement).

5. Conclusions and recommendations

The main outcome of the expert workshop was to introduce the test species selection procedure (Hilbeck et al., 2008; 2011) to a group of experts in Norway and conduct a trial run of this methodology with a real-world case example that - if it reaches the market - is likely to be submitted for regulatory approval in Norway. Since this selection procedure has, at least in part, been included in the revised guidance document on ERA of GMOs in EU (EFSA, 2010) it is important to build competence about this procedure also in Norway. This project can be seen as a first step in this process. Secondly, by conducting a first trial run with a real-world GM crop case example, we could simultaneously generate useful information for a potential full ERA exercise at a later stage. A useful outcome was the list of selected test species provided in Table 6. However, these species will need to be subjected to the remaining steps in the selection procedure to identify the ecologically most relevant test species for assessing the impacts on non-target organisms from GM potato with increased resistance to *P. infestans*.

Another important outcome was the recognition of huge gaps of knowledge, reflected both in the discussions during the workshops, the difficulties in filling in the matrices, and in the challenges we encountered when searching for experts to invite to the workshop. Hence, an important conclusion from this work is that there is a need for more biodiversity surveys in Norwegian potato agroecosystems and surrounding semi-natural and natural habitats. This is needed both to secure that species representing important ecological functions are tested for possible impacts of GM potato with increased resistance to *P. infestans* (or any other agricultural technology for that matter) prior to commercialisation, and to be able to detect possible unintended consequences after its introduction into the environment through monitoring. For any monitoring, we need to know what is there in the first place.

The exercises undertaken during this workshop particularly highlighted the limited knowledge about beneficial insects, as well as species that are not generally recorded as pests or beneficial organisms in potato production. We recommend that the selection processes initiated at this workshop is followed up so that the entire selection procedure can be completed for all the four functional categories identified (see Figure 1 (pp. 13) and Table 4 (pp. 16)). This would involve organizing a workshop with more participating experts from different fields, including soil biology experts, agro-ecologists, plant physiologists and molecular biologists, over several days (at least 3). However, as the number of experts working in this field in Norway is limited and the lineages of P. infestans found in the Nordic countries probably belong to the same population, we suggest that experts should be recruited from all Nordic countries.

Moreover, from this exercise, we foresee the need to critically examine whether an introduction of GM potato with increased resistance to *P. infestans* is a viable and durable solution to the problems it seeks to address. Experience has shown that potato breeders struggle to keep the pace in the 'evolutionary arms race' between R and Avr genes, even when using GM approaches in plant breeding. Given the high genetic diversity of the *P.infestans* populations in Norway, which contributes to a strengthening of the pathogen's evolutionary potential, one concern is that populations of P. infestans in Norway might adapt to and become virulent against GM potato plants even faster than in other parts of the world. The extended time periods necessary for testing, patenting and regulatory approval of GM potatoes will likely not keep pace with the changing adaptations necessary in such an approach. The outcomes gathered here suggest that the complexities surrounding P. infestans epidemiology and virulence requires a risk assessment with regard to virulence development. This will require both additional experts from relevant fields (evolutionary geneticists, quantitative population geneticists, resistance evolution experts, etc.) and additional information from the developers regarding the molecular characterization of the GM potato (e.g. transgene constructs 19 including promoters, terminators, in-planta sequence data) and data on tissue- and stage-specific transgene product expression (leaves and also roots). Given the current state of knowledge about *P. infestans* resistance genes and the GM potato line 'Fortuna', it is quite likely that the commercial introduction will require a resistance management program to allow for the sustainable use of this GM event.

This also highlights the need to assess the full range of possible solutions to the potato late blight disease. One possibility is to conduct a full Problem Formulation and Options Assessment (PFOA) of potato late blight in Norway. The PFOA framework involves a series of workshops where stakeholders meet to assess the problem (in this case prevalence of late blight) and identify and assess the range of possible solutions to the problem, including GM based breeding approaches. Hence, this comprehensive approach in ERA of GM potato will provide a better understanding of the problem and whether introducing GM potato with increased resistance to *P. infestans* is a suitable option in Norway, or whether there are alternatives that may provide better solutions. To conclude, our recommendations for follow up research and analysis can be summarized as:

- Increase funding for baseline studies in order to generate background knowledge about the current level of biodiversity of fauna and flora in potato agro-ecosystems in Norway, particularly with regard to the presence of species that are not known to be pests or beneficial organisms from an agronomic point of view.
- Conduct follow up workshops involving experts from all Nordic countries to identify the ecologically most relevant test species for assessing impacts on non-target organisms from GM potato with increased resistance to *P. infestans* and to evaluate the risk for virulence development in the Nordic *P. infestans* populations.
- Facilitate a full Problem Formulation and Options Assessment (PFOA) of GM potato with increased resistance to *P.infestans*, to explore whether this approach is a viable solution to the problems of the late blight disease in Norway.

Undertaking these activities will help to meet the provisions of the Gene Technology Act (1993), as they contribute to a more comprehensive ERA, as well as assessments of sustainability and social utility.

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GenØk Biosafety Report 2011/05 | Genetically Modified Potato

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Annex 1:
Guidance Table for case-specific selection of important, potentially affected biodiversity functions for ERA of GM potato with increased resistance to P.infestans

Main criteria	Characteristics	Associated ecological function/agricultural practise	Affected organisms/process
I. Crop biology			
Harvested product?	Tuber Certified seed-tuber Non-certified seed-tuber Some seed production for breeding purposes	Herbivory Disease Pest management	Below ground tuber and plant feeders Nematodes Pathogens Insects Slugs
Symbiosis with nitrogen-fixing microbes?	No		
Type of reproduction	Vegetative multiplication	Herbivory Disease Pest management	Below and above ground tuber and plant feeders Nematodes Pathogens Insects Slugs
Sensitive to diseases?	Yes, highly sensitive	Pest management (chemical and agronomic practices)	Bacteria Fungi Viruses Insects (vectors) Nematodes (vectors)
Sensitive growth stage (biotic factors)?	Yes, the sensitive stage is disease-dependent.	Increased humidity favours disease	Pathogens (early and late) Nematodes (early and late)
	Weeds (competition)	Plant competition	Weeds (early)
Sensitive growing conditions (abiotic factors)?	Likes cool growing conditions BUT sensitive to frost.	Frost free periods.	Frost protection (irrigation and cover)
	Sensitive to water logged soil.	Hilling. Drainage. Soil types.	Soil cultivation measures
Input routes of transgenic plant p	arts and transgene products		
Wild relatives	Yes (Solanum nigrum and, Solanum dulcamara)	Gene flow (low probability because of the high ploidity number in commercial potato) Pollination	Pollinators Seed feeders (spread)
What plant parts/ residues are expected and in what quantities before harvest?	Few (flower parts/remnants)	Decemposition	Decomposers
Do they contain transgenes, transgene products or metabolites?	Yes (check expression of promoter)	Decomposition	
What plant parts/ residues are expected and in what quantities after harvest? Do they contain transgenes, transgene products or metabolites?	Volunteers (variable but mostly occasional) Haulm (lot) Yes	Haulm killing Informal disposal of plant Residues after processing. Decomposition	Decomposers

Main criteria	Characteristics	Associated ecological function/agricultural practise	Affected organisms/process
What plant excretions/ exudates possibly contain- ing transgenes, transgene products or metabolites are expected?	Some pollen Root exudates	Rhizosphere Mycorrhiza	Root colonizing micro- and mesofauna and fungi, my-corrhiza microbes
Does the crop form persistent seed banks? (Temporal persistance and spread)	Possible, but seldom Fields can be treated with herbicides before ripening of seeds; germination of seeds possible up to 10 years	Weed management	Volunteer plants
Can whole plants or plant parts survive and regener- ate vegetatively and in what quantities? (Temporal per- sistance and spread)	Yes, in the field and in informal disposal sites (cull piles) but no feral populations.	Weed management Tubers in field can develop volunteer populations	Volunteer plants Decomposers
Is an accumulation over time of residues in soils possible? How long do they contain transgenes, transgene products or metabolites	Residues decompose quickly in field. Some tubers remain in field after harvest and may germinate later, if not treated with herbicides or killed by frost	Harvest Crop rotation	Below-ground tuber feeders
Are whole plants or plant parts expected to spread or to be spread in the field margins and in what quantities? (Spatial persistence and spread)	Informal disposal of residues after processing. Dependent on harvest quality (good quality = few residues and bad quality = a lot of residues. Occasionally, seeds if plants set seeds and are not treated with herbicides	Cull management Harvest	Decomposers Herbivores (if plants develop) Microbes
Are whole plant or plant parts spread or be spread into semi-natural or natural habitats and in what quantities? (Spatial persistence and spread)	Informal disposal of residues far away from field, anywhere in habitat Occasionally, seeds if plants set seeds and are not treated with herbicides	Cull management Harvest	Decomposers Herbivores (if plants develop) Microbes
Degree of spatial spread and persistence?	Small degree Persistence is temperature dependent	Short distance spread	Birds
Potato-associated valued species?	No		

Main criteria	Characteristics	Associated ecological function/agricultural practise	Affected organisms/process
II. Trait – intended effect			
Novel transgene product expressed? If yes, which?	Yes Phytophthora i. resistance genes Rpi-blb1 and Rpi- blb2 imidazolinone resistance Ahas gene	Herbivory food chain Diseases	Above ground herbivores Pathogens Above and below ground interactions of pathogens and beneficial microbes.
Metabolite eliminated or significantly reduced?	No		
Metabolite significantly increased?	No		
Intended effect?	Phytophthora infestans resistance	Disease control	Above ground herbivores Pathogens Above and below ground interactions of pathogens and beneficial microbes
Application of corresponding chemical required? If yes, which?	No		
Antibiotics resistance gene present?	No		
III. Receiving environment – i	ntended use		
a. Region			
Landscape structure? Fragmented hilly to uniform plain	Grows everywhere	Undemanding	
Climate type? temperate to tropical	Cold – Temperate to sub- arctic	Frost protection Altered production cycle?	Altered suits of herbivores & microbes?
Number of potential different production regions?	Number unaffected Five regions identified 1.The areas around Lake Mjøsa 2. Areas around the Oslo fjord 3.Nord Trøndelag 4. Rogaland & Agder 5. Northern Norway		
b. Farming system			
How many crop production cycles?	One; unaffected		
Intended/anticipated scale of release	Unclear, depends on variety, price and trait performance	Public discussion on GMOs	Consumers
Replacing other crops (loss, shift, addition)?	No		
Expanding agricultural production zones (to what degree)?	No		
Cropping system? Large to small, subsistence	Likely unchanged. Segregation systems must be in place	Changing agro-system Pest management Biocontrol	Natural enemies & herbivore prey/hosts

Main criteria	Characteristics	Associated ecological function/agricultural practise	Affected organisms/process
Farming practise? Chemical intensive, integrated, organic?	Yes. Less spray Segregation systems must be in place	Pest management Biocontrol	Natural enemies and herbivore prey/hosts
Pest management type?	Less spraying	Change in agricultural practise	Virus and fungal diseases, insect pests, nematodes Biodiversity indices.
III. Receiving environment – inte	nded use		
b. Farming system			
Use of harvested product	Tubers for human and animal consumption. Seed potatoes?	Storage Transport	Storage pests & diseases
Recycling of plant residues after use	Yes	Compost	Compost organism
c. Soil type			
Soil type (heavy to light)?	Medium to light soils	Soil processes influenced by light soils	Organic matter decomposition rates Soil moisture retention, etc.
		Soil diseases and pests typical for light soils	Nematodes, certain fungi
Organic matter content? High to low	Undemanding		
Prone for soil erosion?	No		

Annex 2: Ranking of taxa belonging to the functional category of Herbivory & disease transmission

OVERALL RANK overall estimate as mean	1-2 (1.6)		1-2	2-3 (2.6)	2-3 (2.6)	2-3 (2.6)	2-3 (2.6)	2 (2.3)	ന
OVERALL RANK: overall estimate in words	Hiah		High	Medium	Medium	Medium	Medium	Medium	Low
Trohpic connection: Connection of species to potato	2	- (Also on other hosts (apple and many others))		ဇ	3	ဇ	3 (Many hosts)	9	3
Phenology: how much of potato season is species present?	<u> </u>	From late spring)	1-2 (All but the earliest part of potato season)	ന	ಣ	ന	ന	1 (Several years as Iarva in soil)	2-3 (Early and late)
Phenology: how much of species life cycle is on potato?	_		-	1 (Parthenogenic reproduction in season)	1 (Parthenogenic reproduction in season)	1 (Parthenogenic reproduction in season)	1 (Parthenogenic reproduction in season)	3 (Larvae)	3 (Larvae)
Abundance: Phenology: On potato how much c crop species life on potato?			1	S	3	3	S	೯	3
Habitat specialization: degree of association with potato habitat	20		2 (Very polyphagous, but potato is among favorites for oviposition and nymphal stages)		3			3 Root feeder, prefers Graminae	င
Geographic distribution (widely distributed?)		(More southern)	-	3 (Inland)	3 (Inland)	3 (Inland)	3 (Inland)	<u> </u>	3 (South)
Species	Insects Empoasca vitis		Lygus rugulipennis	Myzus persicae	Aulocorthum solani	Macrosiphum euphorbiae	Aphis nasturtii	Elateridae (click beetle)	Agrotis segetum

Fungi								
Botrytis cinerea	7-	<u>n</u>	3 (leaves)		2 (Late stages)	3 (wound pathogen)	Medium	2-3
Sclerotinia sclerotiorum	Northern Norway NN (NN) Rest Rest of Norway: 1 Rotational pathogen hinging on	off: 3	NN - Rest: 3	NN Rest:1	2 (Late stages)	5	Medium	2 (2.0 - 2.3)
Rhizoctonia solani	-	1 (Pathotypes – anastomosis group)	5	_			High	-
Verticillium spp.	3 (Southern part) 2	5	ന		2 (Late stages)	n	Medium	2 (2.3)
Colletotrichum coccodes	~	1 (Tubers, stems below)	ന		1 (Damage later)	2	High	1-2
Alternaria solani	3 (Southern part)	5	ဇ		2 (Late, could be there all time but not visible)	<u></u>	Medium	2
Bacteria								
Pectobacterium atrosepticum	<u>v-</u>	-	2		3 (Early season)	·		1-2
Pectobacerium carotovorum	-	<u>~</u>	2		3 (In storage)	8	Medium	2 (1.8)

Nematodes								
Pratylenchus crenatus	7-		5	.		2	High	1 (1.3)
P. fallax	-	_	2	<u></u>		8	High	1 (1.3)
P. penetrans	m	2	, -	-		m	Medium	2 (1.8)
Globodera rostochiensis	2 (Up to Mid Norway, patchy)	-		-	_	-	High	V
G. pallida	3 (South, quarantine pest – 40 years)		y-	-			High	V
Trichodorus spp	(Virus vector)		3 (Vector)	2	\circ	3 (Can transmit virus on other crops too)	Medium	2-3
Paratrichodo spp	(Virus vector)	-	က	m	೮	က	Low	m
Tylenchorhynchus spp.			8	-	3	3	Medium	2

Annex 3: Ranking of taxa belonging to functional category of Natural enemies

Species	Geographic	Habitat specialization:	Abundance: Phenology:		Phenology: how	Trohpic connection:	Phenology: how Trohpic connection: OVERALL RANK: OVERALL RANK	ERALL RANK
	distribution (widely	distribution (widely degree of association	On potato	how much of	much of potato Connection of		overall estimate (rounded): overall	unded): overall
	distributed?)	with potato habitat	plant	species life cycle	season is species species to potato		in words esti	estimate as mean (rank
				is on potato?	present?		1-3	1-3) no decimals
Parasitoids	Unknown	Unknown	Unknown	Unknown	Unknown	3	High uncertainty 1	
(Hymenoptera)						(Third trophic level)		
Staphylinidae	_	3	Unknown	Unknown	Unknown	3	Partial uncertainty 1-2	
						(Third trophic level)		
Carabidae	_	3	Unknown	Unknown	Unknown	3	Partial uncertainty 1-2	
						(Third trophic level)		
Syrphidae		3	Unknown	Unknown	Unknown	3	Partial uncertainty 1-2	
						(Third trophic level)		
Chrysophidae	<u> </u>	3	Unknown	Unknown	Unknown		Partial uncertainty 1-2	
						(Third trophic level)		
Coccinellidae		3	Unknown	Unknown	Unknown	3	Partial uncertainty 1-2	
						(Third trophic level)		
Aranea	_	3	Unknown	Unknown	Unknown	3	Partial uncertainty 1-2	
						(Third trophic level)		
Due to large uncer	rtainties, critical gaps	Due to large uncertainties, critical gaps of knowledge must be closed prior to final estimate and ranking based on consensual expert judgment.	sed prior to fir	nal estimate and rar	iking based on co	sensual expert judgr	nent.	