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

Biosafety Report 2013/01
Updated and extended version of Biosafety Report 2011/05

genøk

- Updated and extended version of Biosafety Report 2011/05 –

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

Frøydis Gillund, Angelika Hilbeck, Odd Gunnar Wikmark,
Lise Nordgård and Thomas Bøhn

This report presents the outcome an expert workshop conducted in October 2012. The purpose of the workshop was to identify ecologically relevant testing species for assessing impacts on non-target organism from cultivating of GM potato with increased resistance to *Phytophthora infestans* in southern Scandinavia. The workshop was a follow-up of a pilot workshop conducted in August 2011 which is presented in Biosafety Report 2011/05 (Gillund et al., 2011). This report is an updated and extended version of that report. The work was commissioned to GenØk – Centre for Biosafety by The Norwegian Directorate for Nature Management.

GenØk – Centre for Biosafety (www.genok.no) is an independent research institute founded in 1998 and situated in Tromsø, Norway. GenØk is engaged in the field of biosafety and gene ecology research on modern biotechnology, nanotechnology, synthetic biology and their emergent biotechnologies. The institution also works with capacity building and advisory activities related to biosafety. GenØk focuses on precautionary, holistic and interdisciplinary approaches to biosafety.

The Norwegian Directorate for Nature Management (www.dirnat.no) serves as an executive and advisory body under the Ministry of the Environment. The main areas of responsibility are outdoor recreation and the conservation and sustainable use of biodiversity. The Directorate assists the Norwegian Government in its environmental protection work at national and international level and is responsible for implementing the Government's environmental policy, and for identifying, preventing and dealing with environmental problems.

Acknowledgements
This work was partly funded by the Norwegian Directorate for Nature Management. We would like to thank the workshop participants for their willingness to participate in the project, fruitful discussions during the workshop and also valuable comments to drafts of this report. Thanks also to Georgina Catacora, Anne Myhr, Fern Wickson and David Quist (our colleagues at GenØk) for constructive feedback to the report.

Summary

Infections of potatoes with *Phytophthora infestans* result in the most devastating potato disease worldwide, known as 'potato late blight'. Its occurrence causes 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*, using a variety of approaches. Due to the remarkable ability of *P. infestans* to overcome resistance, conventional potato breeders have not 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 which are expected to have more durable resistance. Several European companies and research institutes are involved in this research and field trials have taken place in several localities in Europe since 2006.

The mandate for the project reported here was to apply a procedure developed to select ecologically relevant testing species for assessing potential impacts on non-target organisms of GM-potato plants with increased resistance to *P. infestans*. This was done during an expert workshop involving 11 Scandinavian researchers with an expertise on terrestrial invertebrates, soil fungi and ecology in Scandinavian agro-ecosystems. The workshop was a follow-up of a pilot workshop conducted in August 2011, where the initial steps of this selection procedure were applied (reported in the Biosafety Report 2011/05 (Gillund et al., 2011)). This report is an updated and extended version of that report. It concludes with the following recommendations for follow up research and analysis:

- **Identify tissue- and developmental stage specific transgene expression levels in the GM potato plant, i.e. transgene product expression levels in all tissues and secretes of the GM potato plant. Expression levels should be measured in GM potato grown in the receiving environment, i.e. the Scandinavian biogeographical region, during different stages of the growing season.**

- **Increase funding for baseline studies to generate background knowledge about the current level of species diversity of fauna and flora in potato agro-ecosystems in Scandinavia, particularly with regard to soil organisms and the presence of species that are not known to be pests or beneficial organisms from an agronomic point of view.**

- **Conduct a workshop to evaluate risk for virulence development in the *Nordic P. infestans* populations and suggest a resistance management program. This should involve experts on evolutionary genetics, quantitative population genetics, resistance evolution and fungal diseases.**

- **Facilitate a full Problem Formulation and Option 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.**

Content

Summary 3
1. Introduction 5
2. Background 7
2.1. Potato production in Norway 7
2.2. Prevalence of potato late blight disease in Norway 7
2.2.1. Epidemiology and population characteristics 8
2.2.2. Control strategies 10
2.3. Breeding for increased resistance to *P. infestans* 10
2.3.1. Development of GM potato with increased resistance to *P. infestans* 12
2.3.2. Broad- spectrum R genes from S. bulbocastanum 13
3. Introduction to the species selection procedure 16
3.1. Description of the steps in the species selection procedure 16
4. The Expert Workshop: Introducing and applying the testing species selection procedure 20
4.1. Preparations for the workshop 20
4.1.1. The GM case example and selection of participants 20
4.1.2. Restrictions and foci of the workshop 21
4.1.3. Defining the biogeographical region 22
4.2. Results of applying the species selection procedure 22
4.3. Discussion 29
5. Conclusions and recommendations 34
References 35
ANNEXES 40

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 Gene Technology 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 not possible to include all species that can be exposed to the GM plant as testing species. Hence, the basis for selecting testing 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 testing 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 testing species for an ERA or monitoring of GM plants (Hilbeck et al., 2008; 2011; submitted). By adopting a functional approach to biodiversity, this procedure aims to identify the ecologically most relevant testing 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 et al., submitted), and was recently, at least in part, included in the revised guidance document on ERA of GMOs in EU (EFSA, 2010a,b).

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

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

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; Halterman 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, 2013). In October 2011 BASF Plant Science Gmbh applied to the EU for food and feed uses, processing and cultivation of this type of GM potato (marketed as 'Fortuna') (BASF, 2011a), but in January 2013 the company announced that they will withdraw their application and no longer seek approval of this GM event in Europe (BASF, 2013). BASF have however not yet formally notified EFSA about this decision or withdrawn the application from their register. Hence, Fortuna is in the process of regulatory approval in EU at the time this report is written.

The mandate of the project reported here was to apply the proposed procedure (Hilbeck et al., 2008; 2011) for selecting testing species to assess impacts on non-target organisms, using GM potato with increased resistance to *P. infestans* as a case. The selection procedure was applied during a workshop conducted in October 2012, involving 11 Scandinavian researchers with an expertise on terrestrial invertebrates, soil fungi and ecology in agro-ecosystems. 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 testing species selection procedure, before we describe the expert workshop 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 cultivated crops globally (along with wheat and rice). The global production of potatoes approached 370 Megatons in 2011, with Asia and Europe representing the regions of the globe with the largest areas of potato production (FAOSTAT, 2012).

Potato is an important food crop in Norway. In total, 297600 tons potato were produced in Norway in 2011, on an area of 12890,5 ha cultivated land (Statistics Norway, 2012). This includes 133,7 ha which is approved as organic potato production (Debio, 2011) and 888,8 ha which is used to produce certified seed potatoes (Norwegian 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., 2008). 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. Almost half of all the potatoes produced in Norway are cultivated in the areas around Lake Mjøsa (Statistics Norway, 2012). 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 5,16 ha in 2011 which is an increase of 0,38 ha as compared to the previous year. There are however big regional differences in terms of farm size; in Hedmark the average size the potato production area on a potato farm was 11,7 ha in 2011, while it was only 1,6 ha in Troms (Møllerhagen, 2012).

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., 2011).

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).

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 spores. If the spores are spread to potato leaves or stems and conditions are cool and wet they form secondary sporangia which 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 latency period (at optimal conditions as short as 3 days). New sporangia may form in the border between healthy and injured tissue and spores 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, spores 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 infested soil and infected 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 prolonged periods 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, 2008; Fry et al., 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 mating types were present in 40 % of the fields sampled (Brurberg et al., 2011). Sexually produced 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).

The Nordic *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 Nordic *P. infestans* population has a strong adaptive potential. This may influence the durability of the resistance to *P.infestans* in GM potato, if cultivated in this region.

The various studied populations of *P. infestans* in the Nordic countries have similar patterns of 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., 2011; 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 (Goméz-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 in Norway (Cooke et al., 2011; Sæthre et al. 2006). No fungicides for post-harvest 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, 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 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 (Halterman 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). Marker-assisted 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).

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*. 21 notifications of field trials of such GM potato lines are recorded in the European commission's GMO register (European Commission, 2013). 13 of these notifications involve field trials of GM potatoes developed by the chemical company BASF Plant Science GmbH, while the remaining notifications are filed by Wageningen University (the Netherlands), University of Ghent (Belgium), the Sainsbury Laboratory (United Kingdom), The agriculture and food development authority in Ireland (Teagasc) and Vesa Velhartice (Chez republic). Among these, BASF Plant Science seem to be closest to commercialisation of a GM potato with two inserted *R* genes *Rpi-blb1* and *Rpi-blb2*, as the company applied to the EU for food and feed uses, processing and cultivation of this GM potato marketed as 'Fortuna' in October 2011 (BASF, 2011a) (for more details on this event see Box 4). 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).

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., 2003; 2005) and introduced to commercial potato cultivars using GM approaches (Vleeshouwers et al., 2011).

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 Scandinavian context, given the strong adaptive potential in the Nordic *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 developmental 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.

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 co-workers (2009) recently identified two isolates of *P. infestans* (found in central Mexico) that are virulent against the *Rpi-blb1*. Halterman 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). The loss of resistance phenotype may be due to instability of the R protein or an ability of *P. infestans* to circumvent the R mediated resistance during the tuber infection process (Halterman et al., 2010). Genetic evidence suggest that some *R* genes in potato have the ability to confer both foliar and tuber resistance and other results indicate the contrary, where there is no correlation between foliar and tuber resistance (Platt and Tai, 1998; Kirk et al., 2001; Park et al., 2005).

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.

It is debated whether or not these *R* genes will result in durable resistance, or can be deployed in a durable way at all, by e.g. pyramiding broad spectrum *R* genes, constructing multi-lines and/or sequential individual use, targeting molecules that turn off *R* genes etc. (Fry, 2008; Halterman et al., 2010; Chen et al., 2012; Zhu et al., 2012). However, whether any of these strategies results in durable resistance, do also depend on the biology of *P. infestans* and its ecological biotrophic behavior.

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, which shows some of the difficulties of predicting the function of a genetic construct when moved into a new host, even when the host is a closely related species.

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).

The broad spectrum activity associated with *Rpi-blb1* and *Rpi-blb2* has motivated the use of genetic engineering to produce plants carrying a combination of *Rpi-blb1* and *Rpi-blb2* by companies (BASF, 2011b).

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 and Belgium and Sweden in 2011(European Commission, 2013).

In October 2011 BASF applied to the EU for food and feed uses, processing and cultivation of Fortuna (BASF, 2011a,b). In January 2013 the company announced that they have decided to withdraw the application, and no longer seek to have this GM event approved for the EU market (BASF, 2013). BASF have however not yet formally notified EFSA about this decision or withdrawn the application from their register. Hence, Fortuna is in the process of regulatory approval in EU at the time this report is written. Two *R* genes; *Rpi-blb1* and *Rpi-blb2* and their native regulatory elements (isolated from the wild potato *S. bulbocastanum*) are inserted into Fortuna (see Table 1). By combining these two *R* genes the producer expects that the resistance of this potato should be broad and durable and that the fungicide use on potato fields should be significantly reduced, resulting in several benefits for both farmers and the environment.

At the time of the workshop, very little information about its characteristics was currently publicly available. Little is known about the molecular characterisation of the event, particularly whether the *R* genes (or their metabolites) are expressed in all tissues (foliage and tuber) as well as secrets, including phloem and root exudates of the GM potato. The brief description given in Table 1 is based on the information provided in a summary of BASF's application for commercial release of Fortuna to the EU (BASF 2011b) which is publically available on the European Commission webpage.

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 (as a marker gene)
	?	*A. tumefaciens*	Promoter and terminator from the nopaline synthase gene
	Rpi-blb1	*Solanum bulbocastanum*	Resistance to *P. infestans*
	Rpi-blb2	*S. bulbocastanum*	Resistance to *P. infestans*
Parts of the plant where insert is expressed	Rpi- blb1 protein was only detectable in mature tubers. Rpi- blb2 protein was not detectable in any of the tissues		
Intended effect	- Improved resistance to *Phytophthora infestans* - Tolerance to Imidazolinone herbicides, mediated by the *ahas* 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 species selection procedure

The purpose of species selection procedure is to identify the ecologically most relevant testing species and methods for assessing ecological impacts of GM plants on non-target organisms (Hilbeck et al., 2008; 2011; submitted). 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 testing species from a standardised list, i.e. species that are not necessarily present in the potential receiving environment (Hilbeck et al., 2008; 2011; submitted). Hence, this procedure, which is, at least in parts, included into the newly revised guidance document on ERA of GMOs in the EU (EFSA, 2010a,b), is an attempt to improve upon currently practiced testing procedures. An essential feature of the procedure is that it places the whole GMO in its receiving environment at the center of the assessment. Testing 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 testing species. It allows the ERA to focus on species with critical ecological roles and limits the range of testing 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 involving competent experts).

This procedure is part of the Problem Formulation and Option 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 potato with increased resistance to late blight. Here, we will present a brief summary of the species selection procedure. For a more detailed introduction please refer to Hilbeck et al. (2008; submitted).

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 case[2] and suggests information that

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

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

The selection procedure involves a series of steps (see Figure 1) which allow, in a funnel-like process, to reduce the (potentially high) number of candidate testing 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.

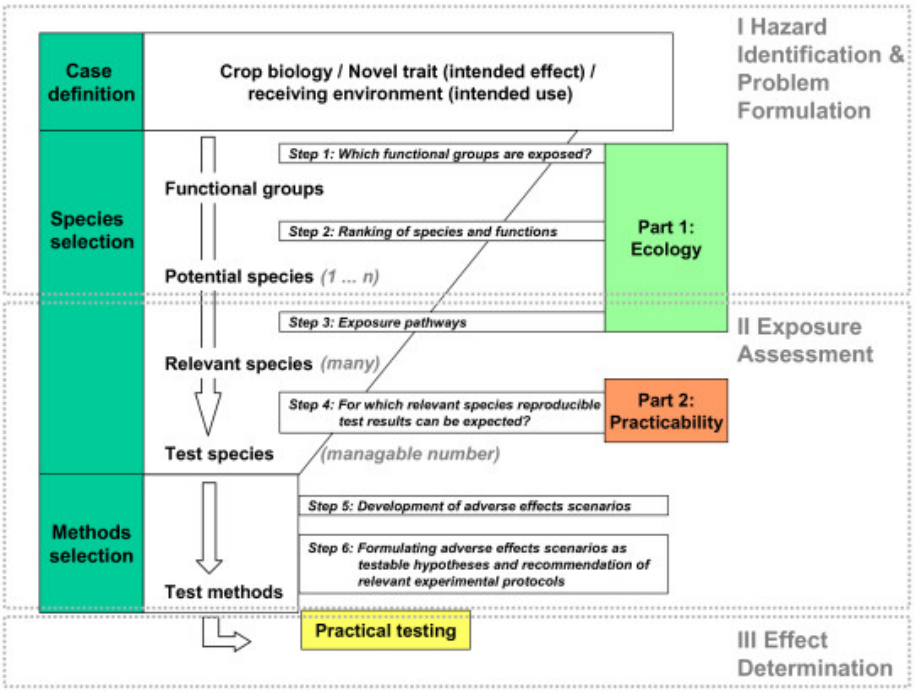

Fig 1: Species and method selection procedure for ecotoxicity testing of GMOs (from Hilbeck et al., 2011; sumitted)

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, by ranking all the species according to a series of ecological criteria. This ranking exercise consists of three parts. First the species are ranked according to the spatial-temporal coincidence of the non-target organism with the GM plant (i.e. criteria such as (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). Then the species are ranked based on an estimation of the functional and trophic association of the non-target organism with the GM plant, i.e. their significance in carrying out their respective ecological function. Finally, the species are ranked according to nature conservation considerations, i.e. in this case their status in the 2010 Norwegian Red List for species.

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. The first task in Step 3 is to provide background information concerning the biology and feeding preferences of the species. This is not a ranking exercise, but the information identified serves to clarify whether the species is likely to be exposed to the transgene or its metabolites. Then the species are ranked in order to evaluate the likelihood that they are exposed to the transgene or its metabolites, and if exposure is considered likely, the species are ranked according to degree of exposure.

Step 4: Applying practicability criteria

The goal of this step is to differentiate between those organisms for which it is possible to obtain reproducible tests results in the laboratory trials and those that may only be testable in field trials. 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.

Working tools have been developed for each of these steps during previous applications of the selection procedure (Hilbeck et al., 2008; 2011; submitted). This includes a guidance tables for selection of ecological functional categories (Step 1, see Annex 1) and a matrices for species ranking based on ecological criteria (Step 2, see Annex 2,3 and 4), exposure analysis (Step 3, see Annex 5,6, 7 and 8) and Step 4 (practicability ranking).

For the steps 2 to 4 the species are ranked for a series of predefined criteria, along the scale: 'high = 1', 'medium =2' and 'low = 3'. A final rank is estimated for each species (by calculating both the arithmetic mean and median) at each step. Based on this total rank the participants select which species that should be taken to the next step in the procedure (the species that are ranked high or medium are typically selected for). Species that cannot be ranked for a given criterion due to insufficient knowledge is reported as '?'. The participants determine how to deal with uncertainties in the selection process. If adopting a precautionary approach to uncertainty, which is typically done, uncertainties are initially treated as high ranks (e.g. '1').

The outcome steps 1 to 4 is a list of selected testing 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 et al., submitted). In the project reported here, we applied the procedure to select testing species for assessing impacts on non-target organisms of GM potato with increased resistance to *P. infestans* planned for cultivation in southern Scandinavia. We planned to complete steps 1 to 4 of the procedure during the workshop.

4. The Expert Workshop: Introducing and applying the testing species selection procedure

In October 2012, we conducted a two-day workshop with 11 Scandinavian researchers with an expertise on terrestrial invertebrates, soil fungi and ecology associated with agro-ecosystems in Scandinavia. The aim of the workshop was to introduce the species selection procedure to the researchers and apply the first four steps of the selection procedure (as outlined in section 3). 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 Scandinavia, that are likely to be exposed to the transgene or its metabolites and suitable as testing species for laboratory testing. The work was based on the assumption that if these species are adversely affected by GM potatoes with increased resistance to *P. infestans*, it could potentially result in a significant adverse environmental effect on the entire agro-ecosystem (Hilbeck et al., 2008).

4.1. Preparations for the 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 around the table and deciding on the scale of bio-geographical region for the exercise.

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. At the time of the workshop this GM event was in the process of regulatory approval in the EU (BASF 2011a,b), and was therefore considered 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 expertise on species diversity and ecology among terrestrial invertebrates and soil fungi associated with Scandinavian potato agro-ecosystems to the workshop. Additionally, we wanted experts with knowledge on 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 ecologists/ entomologist/ soil scientist with knowledge on species diversity in potato agro-ecosystems and surrounding semi-natural and natural habitats, who were not working directly with agriculture. Finally, we wished to have experts with different institutional affiliations, both from Norway, Sweden and Denmark. This resulted in the following group of participants at the workshop:

Table 3 Workshop participants and facilitators

Name	Affiliation	Expertise
INVITED RESEARCHERS		
Atle Wibe	The Norwegian Institute for Agricultural and Environmental Research (Bioforsk)	Agro-ecology, entomology, beneficial plants.
Richard Meadow	The Norwegian Institute for Agricultural and Environmental Research (Bioforsk)	Entomology, beneficial and pest insects, member of the GMO panel in the Norwegian Scientific Committee for Food Safety
Eline Hågvar	Norwegian University of Life Sciences	Entomologist
Peter Esbjerg	University of Copenhagen, Denmark	Entomologist
Barabara Ekbom	Swedish University of Agricultural Sciences	Entomologist
Dennis Jonason	Swedish University of Agricultural Sciences	Entomology, biodiversity and agriculture
Camilla Winqvist	Swedish University of Agricultural Sciences	Entomology, biodiversity and agriculture
Ricardo Holgado	The Norwegian Institute for Agricultural and Environmental Research (Bioforsk)	Nematodes
Theo Ruissen	The Norwegian Institute for Agricultural and Environmental Research (Bioforsk)	Soil fungi, symbiotic microorganisms, mycorrhiza, agro-ecology, plant pathology
Ragnhild Nærstad	The Norwegian Institute for Agricultural and Environmental Research (Bioforsk)	Fungal diseases on potato and vegetables
Heidi Sjursen Konestabo	University of Oslo	Soil ecology, member of the GMO panel in the Norwegian Scientific Committee for Food Safety
FACILITATORS		
Lise Nordgaard	GenØk – Centre for Biosafety	Molecular biology and micro biology, ERA of GMOs
Thomas Bøhn	GenØk – Centre for Biosafety	Ecology, Gene Ecology, ERA of GMOs
Frøydis T. Gillund	GenØk – Centre for Biosafety	Natural resource management, science and technology studies, participatory approaches to ERA

4.1.2. Restrictions and foci of the workshop

Only terrestrial invertebrate and soil fungi non-target organisms were included in the assessment, while farmland birds, small mammals and aquatic invertebrates were excluded due to time limitations. There was a particular emphasis on pathogenic soil microorganisms and decomposers (i.e. fungi, bacteria, nematodes and microarthropods) and terrestrial invertebrates in potato production systems (i.e. pests and natural enemies).

4.1.3. Defining the biogeographical region

The procedure for selecting relevant testing species that interact with a given crop species, here GM potato, requires some consideration of *variation* in space and time. The community of species that *exist* in one field will never be exactly the same as in the next, and *sampling* of that community will at best be a seasonally representative sub-sample of what actually is there.

In order to decide which species to include in the selection procedure and do the subsequent ranking, we needed to define a meaningful geographical area to work with. The workshop participants decided that this area should have the following properties; i) potatoes must be grown there, preferably at a large commercial scale, as that emphasizes both ecological and economic relevance, ii) *P. infestans* must be found in overlap with the potato and cause a problem for the potato production, iii) the community or diversity of species must not differ too much within the region – otherwise, comparing and selecting representative species would be impossible, iv) the climatic condition must not vary too much within the region and v) the defined area must be relatively open to migrations and movement of species, i.e. no large natural borders or barriers (like mountains) that effectively stop distributive movements in the testing species. Based on these criteria we set our focus area to be the southern part of Norway (including Trøndelag), the southern part of Sweden, and the northern part of Denmark. The workshop participants agreed that this was an area that could be handled with a sufficient homogeneity of biological diversity in the relevant organisms.

4.2. Results of applying the species selection procedure

Here we present the results for each of the steps undertaken during the workshop.

Step 1: Selection of functional categories and making initial species lists

The most important functional groups to include in an ERA of GM potato with increased resistance to *P. infestans* were identified at a pilot workshop that took place in August 2011 (Gillund et al., 2011). The outcome of the pilot workshop was set as a starting point for the present workshop. Four functional categories were identified (Annex 1 and Table 4).

Table 4: Ecological functional categories, organisms and processes identified.

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 et al., submitted). 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. Hence, at this workshop the participants focused on species belonging to the functional categories of herbivory and disease transmission, natural enemies and ecological soil processes.

The next part of step 1 involved filling these identified and chosen functional categories with species known to contribute to these functions. The participants at the workshop started with this exercise. They worked in two different groups: One group worked with herbivores and natural enemies, the other group worked with disease transmitting species, decomposers and other soil organisms. Prior to the workshop the participants were asked to prepare lists of species belonging to the functional groups, limited to species that are present in Scandinavian agro-ecosystems (potato fields and their margins in particular) and which they considered most important. When the participants met at the workshop they agreed on which species to include in an initial list for the selection procedure (see Table 5 for the total number of species included in the initial list). The participants also decided that in cases where species belonging to the same genus had a similar biology and functional role, they would do the selection on genus rather than species level. Similarly, for some of the taxonomic groups (particularly among soil organisms) the selection was done on higher taxonomic units due to lack of knowledge on the species diversity within these taxonomic units. If a genus or higher taxonomic unit were selected for in all the steps of the procedure the researchers would suggest a candidate species for the actual experimental testing at the end of the workshop. Moreover, for decomposers and nematodes, the participants decided to also add the processes (i.e. nitrification and mineralization), in addition to individual species involved in these processes.

It is important to note that besides identifying the most important functional categories, the guidance tables applied in step 1also 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 pests might be.

Step 2: Ecological ranking

Each of the species, genus or higher taxonomic units in the initial lists were ranked according to a series of predefined ecological criteria, using the matrix tool (the filled-in matrices can be seen in Annex 2, 3 and 4). Species, genus or higher taxonomic units that

were identified as 'threatened' (according to the 2010 Norwegian Red List for species) were immediately selected for, but this did only concern one species (*Bombus lapidatius*) belonging to the functional group of herbivores. Species, genus or higher taxonomic units that could not be ranked for a given criterion due to insufficient knowledge (recognised as '?' in the matrices) were automatically treated as a high rank (i.e. '1'), as the participants decided to adopt a precautionary approach to uncertainty.

As seen from the matrices, the total ranking (both in the form of the median and the arithmetic mean) for each of the species, genus or higher taxonomic unit did not vary much (for instance, the final rank (in arithmetic mean) for all herbivores was within the range of 2,3 to 2,8, see Annex 2). It was therefore difficult to differentiate and select among the species based on the final rank only. Consequently, the ranking exercise primarily served to indicate the ecological importance of each species. The final decision on which species to select for and include in the next step of the selection procedure was therefore based on the expert-based judgement by the participants in the groups, where species, genus or higher taxonomic units that were considered to be closest associated with the potato crop, and representing different biological niches and feeding behaviours were chosen.

Table 5: Number of species listed according to functional category

Functional category	Step 1: Numbers of species in initial species list	Step 2: Number of species after ecological ranking	Cut between Step 1 and 2 in percentage
Herbivores	16	8	50%
Natural enemies	49	15	70%
Pathogens	17	10	40%
Decomposers and other beneficial soil organisms/processes	14	11	20%

Step 3: Exposure analysis

Each of the species, genus or higher taxonomic units that were selected for in step 2 were subject to an exposure analysis in step 3, to identify and select the ones that were most likely to be exposed to the transgene from the GM potato plants. As seen in Annex 5, 6, 7 and 8 the participants encountered great challenges and were not able to complete the exposure analysis and ranking. This was due to lack of information about the expression level of the inserted genes and their metabolites in the different parts and secret (e.g. foliar, tuber, phloem, root exudates etc.) of the GM potato plants. As discussed, evidence suggests that the expression of *R* genes, and thus resistance to the disease, vary between different tissues of the potato plant. Some *R* genes in potato have the ability to confer both foliar and tuber resistance and other results indicate no correlation between foliar and tuber resistance (Platt et al., 1998; Kirk et al., 2001; Park et al., 2005). In addition, the genetics, our ability to understand the molecular interactions involved in resistance, and also the occurrence of tissue-specific expression of tuber late blight resistance, have not been as extensively studied as late blight resistance in foliage (Park et al., 2005; Liu and

Halterman 2009; Halterman et al., 2010; Chen et al., 2012). On the background of the information available on Fortuna from the Applicant and the general scientific literature on expression levels of *R* genes, the participants made the assumption that the transgenes and their metabolites were expressed in all plant tissues and tubers, but not in the root exudate from the GM potato. However, when all the technical data is available to do a proper ERA, the results from the ranking done under this assumption will have to be re-evaluated in order to complete the ranking. Due to this lack of information, step 3 could not be completed at this workshop and there was no reduction in the list of species, genus or higher taxonomic units after the groups had conducted this exercise.

Discussions during the workshop, October 2012. Photo: Thomas Bøhn

Step 4: Expert advice on suitable species as testing organisms for the identified species, genus, or higher taxonomic units

In this step, which was the last exercise at the workshop, the experts were asked to give advice on which of the species from step 3 they considered suitable as testing species in laboratory trials. The species were not subjected to a ranking procedure using the developed matrix tool. Rather, the experts where asked to evaluate their suitability based on their knowledge and experience with the species. Their recommendations and comments are provided in the Table 6.

Table 6: Expert based judgment of the suitability of the selected species as testing organisms in laboratory test trials.

Identified species	Expert based evaluation of suitability as testing species
HERBIVORES	
COLEOPTERA	
Leptinotarsa decemlineata (Kolleradobille)	• The larvae are very easy to work with in lab trials • Can be reared without diapause, when day is long and at high temperature • Only suitable for testing with leaves • Can potentially be used as test food for predators
Agriotes spp. (Smeller)	• Larvae of both *A. lineatus* and *A. obscura* can be collected at high numbers in small, old horse grazing areas. • The larvae can be distinguished and kept in either soil or preferably in artificial medium and then fed with potato tubers as test material.
HYMNOPTERA	
Bombus lapidarius (Steinhumle)	• Nesting in boxes is possible, but difficult • *B. terrestris* may be considered as a test substitute, but there are some uncertainties about biotypes
LEPIDOPTERA	
Agrotis segetum (Gråpudret jordfly)	• The species is easy to rear and standardize • Diapause can be avoided • Both leaves and tubers can be tested • Can potentially be used as test food for predators
HEMIPTERA	
Empoasca vitis (Potetsikade)	• No recommendations
Lygus rugulipennis (Håret engtege)	• Suitable to use field collected nymphs • Possible to test effect of leaf feeding and after last moult
APHIDOIDEA	
Myzus persicae (Ferskenbladlus)	• Very easy to work with in lab trials
MOLLUSCA	
Deroceras reticulatum (Nettkjølsnegl)	• The species can easily be kept under lab conditions • Both leaves and tubers can be tested • Juvenile slugs can be used as test food for predators
NATURAL ENEMIES	
ARANEA	
Pardosa spp. (Ulveedderkopp)	• The species *P. agrestis* is suitable as testing species for lab trials
Oedothorax apicatus (Dvergedderkopp)	• No recommendations
COLEOPTERA	
Bembidion lampros (Skyggeløper)	• Adults can be collected and tested, but rearing will be very difficult.
Pterostichus melanarius (Løpebille)	• Difficult to use because of very specific demands for moisture, temperature, hiding places and suitable prey culture
Harpalus rufipes (Markløper)	• No recommendations

Identified species	Expert based evaluation of suitability as testing species
Coccinella 7-punctata (Sjuprikka marihøne)	• Easy to work with in lab trials, but adult diapause in ovaries implies difficulties to breed several generations in lab. • Possible substitute is *C. bipunctata* which is more easy to culture
Tachyporus spp. (Kortvinger)	• *T. chrysomelinus* suggested as a suitable species
Philonthis spp. (Kortvinger)	• Difficult to keep in culture because both larva and adults are very aggressive
Chrysophidae (Nettvinger)	• *Chrysoperla carnea* suggested as a suitable species
DIPTERA	
Syrphus ribesii (Vanlig hageblomsterflue)	• The species is easy to keep in the lab for one generation, but difficult with copulation in lab and thus difficult to keep successive generations in lab • Need cultures of plants, aphids and predator • Possible substitute is *S. corollae* which is very easy to keep in the lab all year around
HYMENOPTERA	
Aphidiidae (Bladlussnylteveps)	• *Aphidius ervi* is a suitable species to work with within this genus • Easy to work with in lab trials, but need cultures of plants, aphids (e.g. *M. persicae*) and parasitoids • Commercially available
NEMATODES	
Steinerma spp. (Nematode)	• *S. feltiae* is commercially available and suggested as a suitable species • Can easily be kept in cultures • Easy techniques for study and work in lab trials
FUNGI	
Trichoderma spp. (Sopp, patogen på annen sopp)	• *T. harzianum* suggested as suitable species
Metarhizium (Sopp, patogen på insekter)	• The species *M. anisopliae* is commercially available and could be tested both on herbivores and predators which have fed on GM
Pandora neoaphidis Entomophthorales (Sopp, pathogen på insekter)	• Possible to keep in culture
DECOMPOSERS	
CLITELLATA	
Aporrectodea caliginosa (Meitemark)	• Suitable
HYMENOPTERA	
Formica spp. (Maur)	• No recommendations
COLLEMBOLA	
Folsomia fimetaria (Spretthale)	• Suitable (OECD standard test, 232)
Isotoma spp. (Spretthale)	• Most species in the genus *Isotoma* spp. are easy to keep in culture • Commonly found in large numbers in agricultural soils, and can be collected in the field

Identified species	Expert based evaluation of suitability as testing species
ACARI	
Oribatida (Midd)	• Oribatida as an entire order is not suitable, the order is very diverse. However, providing a field sample yields high enough numbers of one species/genus, these can be used for testing. Genus commonly found in agricultural soils include members of the family Camisiidae, large oribatids that with some practice can be sorted into genus. They are, however, difficult to keep in culture, so tests must rely on field sampling
Uropodina (Midd)	• Not suitable
BACTERIA	
Streptomyces spp. (Bakterie, ikke de som gir flatskurv)	• Suitable
PROCESSES	
Nematodes in mineralisation processes	• Suitable Mycophage especie *Aphelechus avenae* is easy to keep in culture, and are also commonly found in large numbers in agricultural soils, so they can be collected in the field. • Bacteriophage *Pelodera* spp. and *Plectus* spp. is easy to keep in culture, and are also commonly found in large numbers in soils, so they can be collected in the field.
Mineralisation	• Suitable
Nitrification	• Suitable
OBLIGATE BIOTROPH SYMBIONTS	
Glomeromycota (Arbuscular mycorrizha på potet)	• Arbuscular mycorrhizal development in potato roots can easily be quantified by root staining technology • Degree of colonization can be further specified by differentiation according to: i) intraradical hyphal development, ii) arbuscular formation, iii) presence of vesicles • Techniques are relatively easy, but processing takes time
PATHOGENS	
BACTERIA	
Streptomyces spp. (Flatskurv (bakterie))	• *S. europaeiscabiei* suggested as a suitable species
Pectobacterium atrosepticum (Bløtråte (bakterie))	• Suitable
Rhizoctonia solani Ag3 (Anostomose gruppe 3, Potetspesifikk (Bakterie))	• Suitable
NEMATODES	
Pratylenchus spp. (Rotsårnematoder)	• *Pratylenchus* spp. can easily be kept under lab conditions
Tylenchorhynchus spp. (Stuntnematoder)	• *T. dubius* is suggested as a suitable species • Can be collected and tested, but rearing could be very difficult
Trichodorus spp. (Stubrotnematoder)	• Can be collected and tested, but rearing could be very difficult
Paratrichodorus spp. (Stubrotnematoder)	• *P. pachydermus* is suggested as a suitable species • Can be collected and tested, but rearing will be very difficult

Identified species	Expert based evaluation of suitability as testing species
Globodera rostochiensis (Gul potet nematode)	• Easy to keep in cultures and easy to work with in lab trials • Development in potato roots can easily be studied and quantified. • Cultures need a diapause.
FUNGI	
Colletotrichum coccodes (Sopp)	• Suitable
OOMYCETE	
Phytophthora erythroseptica (Rødråte)	• Suitable

4.3. Discussion

The workshop ended with a plenary discussion about the methodology used, critical knowledge gaps, adverse scenarios, routes for gene flow and recommendation for follow-up research.

Feedback on the methodology

The participants generally expressed that the selection procedure served its purpose in identifying testing organisms that were locally and ecologically relevant. The participants did however make some adjustments to the procedure. This involved selecting on genus rather than species level for species within the same genus that had more or less similar biology and functional role in the receiving environment. The rational was that this would lessen the workload, as these species would be given similar ranks anyway. If a genus was selected in all the steps of the procedure, the experts would suggest a candidate species for the actual experimental testing at the end of the workshop.

Moreover, the participants decided to use both the median and arithmetic mean when calculating the final rank for step 2, while the manual for the selection procedure recommends using only the median. An argument to use the median is that the arithmetic mean gives a stronger, but false, impression that ranks are precisely and quantitatively determined. Such precision cannot be justified from the expert judgment and qualitative evaluation that the selection procedure is based on. However, calculations based on the median carry much of the same lack of justification, and do in addition mask important variation in the ranking procedure. For instance, a species with obviously higher ranking, say 1,1,2,2,2,2 as compared to a species with rank numbers of 2,2,2,2,3,3 would not differ in their final rank when using the median. The arithmetic mean would do better for the given example. The participants decided to use both the median and arithmetic mean when calculating the final rank. Despite this, we experienced that the final rank did not differ much between the median and the arithmetic mean. We also noted that the final rank between different species, genus or higher taxonomic units (within a functional category) showed relatively small differences. Consequently the final decision on which species to select for the next step, was primarily based on the experts' judgment and experience. Species considered to be closest associated with the potato crop, and representing different biological niches and feeding behaviours were chosen, in order to

allow for diversity in trophic levels, taxonomy and exposure routes. The participants commented that one possible explanation for the lack of clear separation (small range) in the final rank could be that potato contains a high level of toxins and therefore is not a preferred food source for most terrestrial invertebrates and fungi. Hence, there are very few species (or none) that are specialized on potato as a food source or have the potato field as a specialized habitat.

Identified knowledge gaps

The participants agreed that the diversity of terrestrial invertebrate species in potato agro-ecosystems in Scandinavia is mostly well characterised, and that this knowledge was well represented among the experts at the workshop. It was however emphasised that there are currently large knowledge gaps regarding the diversity of soil living organisms in agricultural fields, and no studies have focused on identifying soil invertebrates in potato fields in Norway. Disease transmitting nematodes are typically quite well characterised, but very little is known about the general species diversity of nematodes. It was also mentioned that there is almost no Norwegian data available on arbuscular mycorrizha development and knowledge on species interactions in the rhizosphere in potato fields is limited. For some of the taxonomic groups, particularly beetles, spiders and bumblebees, the experts' knowledge was limited to what is typically found in Sweden and Denmark, and they were uncertain whether the identified species are common also in Norway. Therefore other Norwegian taxonomists were consulted after the workshop. They could confirm that the species in Table 6 are present in Norwegian potato agro-ecosystems, but it was particularly emphasised that no field surveys have yet been carried out to map the diversity of bumblebees in Norwegian potato agro-ecosystems.

It is uncertain (as documented both in the scientific literature and described by the workshop participants) whether the inserted *R* genes are expressed in all tissues (potato foliage, pollen and tuber) as well as secrets, including phloem and root exudates of the GM potato. This information is needed in order to evaluate whether the non-target organisms are likely to be exposed to the transgenic proteins or metabolites. Information on whether the GM potato possesses both foliar and tuber resistant is also critical in order to assess whether the potato will be susceptible to soil borne infection to late blight or not.

Moreover, several workshop participants mentioned that the insertion of novel *R* genes could result in instability of the potato genome and interfere with the expression of endogenous genes (changes in gene expression, activation/silencing of genes) This may lead to unintended physiological and morphological changes of the potato plant, which ultimately may adversely impact non-target organisms.

Possible adverse scenarios

Many of the workshop participants questioned if it is reasonable to expect that the expression of inserted *R* genes or their metabolites would have any adverse effect on non-target organisms. They argued that in the case of this GM potato plant the inserted genes express R proteins that are part of the potato plants' natural defense system and which are present and expressed also in unmodified varieties. Many participants did however emphasize that it is nevertheless important to do experimental trials on the identified

non-target organisms in order to validate or falsify this assumption. Again, it was also emphasized that using genetic engineering to insert these *R* genes into the host genome could lead to instability and unintended changes in the expression of endogenous potato genes which could ultimately also have adverse impacts on non-target organisms.

It is expected that cultivation of this type of GM potato will reduce the need for fungicides. The fungicides currently applied to control late blight do however suppress many other fungal pathogens in addition to *P*. *infestans*, and a possible adverse scenario is that secondary pests will thrive in GM potato fields due to the reduction in fungicide use. Consequently, the GM crop may have to be sprayed with fungicides anyway in order to control these secondary pests.

Some participants were also concerned that this type of GM potato is introduced as a 'silver bullet' for late blight control. If the majority of the potato farmers adopt this technology it would not only reduce the diversity of commercial potato varieties cultivated, but also the resilience of the potato farming system. Increased monoculture and homogeneity in potato varieties could make the famers more vulnerable if the strategy fails (e.g. *P. infestans* breaks the resistance of these GM potato plants).

As a possible beneficial scenario it was mentioned that the inserted *R* genes could potentially also provide resistance to *Phytophthora erythroseptica,* which is another oomycete pathogen and causal agent of pink rot on potato.

Gene flow

The participants identified several possible routes for gene flow, such as volunteer seedlings, seedlings at informal disposal sites (for potatoes that are trashed before processing or sale), and cultivation of potato in private gardens. In the context of this workshop the participants did not discuss possible impacts from gene flow as such, but rather whether it was likely that other non-target organisms (than the ones identified at this workshop) would be exposed to the GM potato if gene flow occurs. The participants commented that the species diversity (particularly for butterflies) is typically richer in gardens and semi-natural habitats. Hence, to include non-target species that could be impacted in the case of unwanted gene flow, a similar exercise would have to be conducted considering these environments. The participants did however emphasize that they considered it most important to focus on species known to exist in commercial potato fields and their margins.

Follow- up work

The workshop participants experienced two major challenges when applying the selection procedure: i) lack of data on mesofaunal and microarthropod diversity and abundance in Scandinavian potato fields (Norway in particular) and ii) lack of or partly contradicting data on tissue and developmental stage gene expression levels of *R* genes in this type of GM potato. Hence, it was recommended to fund field studies to generate baseline data on species diversity of soil fauna of Scandinavian agro-ecosystems, and request further research on *R* gene expression levels of this specific event. When this information is available the choice of soil species included in the initial list of species and the exposure analysis (step 3) should be re-evaluated. Moreover, if the *R* genes are known to be

expressed in the pollen of the potato plant, it would be important to include pollinators as a functional group, including pollen collectors, pollen feeders and flower visitors. It was also recommended to test if aphids or honeydew from aphids contain the transgene product (as they only use the plant phloem as a food source). If the transgene is detected in aphids, species known to be aphid predators should also be included in the species list. It was recommended to include more mobile and higher trophic organisms such as farmland birds, mice and rodents in the selection procedure in future workshops.

It was also emphasized that when assessing impacts from GM potato it is important to consider impacts arising from changes in the agronomic practice (e.g. less fungicide use). Hence, in future workshops assessing this type of GM potato, it is important to include an assessment of impacts from current use of fungicides, and to compare benefits and adverse effects from the two control strategies.

The need to critically examine whether the introduction of GM potato with increased resistance to *P. infestans* is a viable and durable solution to the problems it seeks to address was emphasised. Experience has shown that potato breeders struggle to keep up with 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 the Nordic countries, which contributes to a strengthening of the pathogen's evolutionary potential and responsiveness, one concern is that populations of *P. infestans* in Nordic countries 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 both require involvement of experts from relevant fields (evolutionary geneticists, quantitative population geneticists, resistance evolution and fungal disease experts, etc.) and more information from the developers regarding the molecular characterization of the GM potato (e.g. transgene constructs including promoters, terminators, in-planta sequence data) and data on tissue- and developmental stage-specific transgene product expression.

Another issue, which was not the scope of the work reported here, is the need for co-existence management strategies at all stages of the potato production chain. Regulations on co-existence have been in force in Denmark since 2005 and Sweden since 2007. In Norway an ad hoc expert committee under The Norwegian Scientific Committee for Food Safety (2006) provided recommendations for co-existence management strategies for GM, conventional and organic crops, but formal rules must be in place before the approval of this type of GM potato. In this context it is of particular importance to consider the role of humans as vectors for spreading GM potatoes. The current practise of cultivating potato in private gardens poses large challenges when it comes to uncontrolled spread.

The information compiled in the initial steps of the selection procedure is not specific to late blight resistant GM potato, and could, at least in parts, be applied for other GM potato plants to be introduced in the same receiving environment. Hence, the

information synthesised at the workshop could be archived into a data base and provide a good starting point for future applications of the selection procedure. Moreover, this information is valuable for the general understanding the biodiversity of Scandinavian agro-ecosystems. In particular, it gives an overview over the functional groups, the most important species in the system and their interactions. This knowledge represents a first step to create a database with baseline data on species diversity of terrestrial invertebrates and soil fungi in Scandinavian agro-ecosystems. Such a baseline is necessary and crucial for understanding potential change in the systems, whatever the cause might be (e.g. climate change, pesticides, GM, etc.). A good baseline for existing biodiversity must be in place in order to make monitoring or surveillance activities meaningful. Based on the work reported here we consider virulence development in the Nordic *P. infestans* population, impacts on non-target organisms (identified at this workshop), secondary pest development and gene flow as priority risk areas for a monitoring plan if this type of GM potato is to be cultivated in Scandinavia.

5. Conclusions and recommendations

The main outcome of the expert workshop was to introduce the testing species selection procedure (Hilbeck et al., 2008; 2011; submitted) to a group of Scandinavian experts and use it to identify ecologically relevant testing species for GM potato with increased resistance to *P. infestans*. Since this selection procedure has, at least in part, been included in the revised guidance document on ERA of GMOs in EU (EFSA, 2010a, b) it is important to build competence about this procedure also in Scandinavia. This project can be seen as a first step in this process. A concrete outcome of the project was the list of ecologically relevant and suitable testing species presented in Table 6. The selection procedure could however not be completed at this workshop, due to insufficient knowledge on tissue- and developmental stage specific transgene expression levels in the GM potato plant. The species identified in Table 6 should therefore be subjected to further selection once this information is available. It is important to note that this is the first time this selection procedure has been applied for a disease resistant GM plant and with a particular emphasis on non-target organisms among pathogenic soil microorganisms and decomposers and other beneficial soil organisms (i.e. fungi, bacteria, nematodes and microarthropods). The workshop illustrates some of the challenges ERA researchers encounter when faced with knowledge gaps and insufficient data, e.g. on the molecular characterisation of the GM event. Nevertheless, the species identified at this workshop are known to be locally relevant, contribute to important ecological functions and suitable as testing species. Hence, the identified species represents an important starting point when deciding on which species to include in experimental trials for ERA of non-target organisms or monitoring of this type of GM potato. To conclude, our recommendations for follow up research and analysis can be summarized as:

- **Identify tissue- and developmental stage specific transgene expression levels in the GM potato plant, i.e. transgene product expression levels in all tissues and secretes of the GM potato plant. Expression levels should be measured in GM potato grown in the receiving environment, i.e. the Scandinavian biogeographical region, during different stages of the growing season.**

- **Increase funding for baseline studies to generate background knowledge about the current level of species diversity of fauna and flora in potato agro-ecosystems in Scandinavia, particularly with regard to soil organisms and the presence of species that are not known to be pests or beneficial organisms from an agronomic point of view.**

- **Conduct a workshop to evaluate risk for virulence development in the *Nordic P. infestans* populations and suggest a resistance management program. This should involve experts on evolutionary genetics, quantitative population genetics, resistance evolution and fungal diseases.**

- **Facilitate a full Problem Formulation and Option 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.**

References

Andow, D.A., Tuat, N.V. and Hilbeck, A. (Eds.) (2008). *Environmental risk assessment of genetically modified organisms, Vol 4*. CABI Publishing, Wallingford, UK. ISBN: 9781845933906.

BASF (2013) *Press release*. Available online http://www.basf.com/group/pressrelease/P-13-133. Accessed 01.02.2013.

BASF (2011a) *Press information*. Available online: http://www.basf.com/group/corporate/en/function/conversions:/publish/content/products-and-industries/biotechnology/images/2011-10-31_PI_Fortuna_P488-11.pdf. Accessed 23.01.2013.

BASF (2011b) Application for Authorisation of Phytophthora Resistant Potato PH05-026-0048 for Food and Feed Uses, Processing and Cultivation according to Regulation (EC) No 1829/2003. Available online: http://www.transgen.de/pdf/zulassung/Kartoffel/PH05-026-0048_application.pdf. Accessed 13.02.2013.

Bradeen, J.M., Iorizzo, M., Mollov, D. S., Raasch, J., Kramer, L. C., Millet, B. P., Austin-Phillips, S., Jiang, J. and Carputo, D. (2009). Higher copy numbers of the potato RB transgene correspond to enhanced transcript and late blight resistance levels. *Molecular Plant-Microbe Interactions*, 22, pp. 437-446.

Brurberg, M., Elameen, A., Le, V., Nærstad, R., Hermansen, A., Lehtinen, A., Hannukkala, A., Nielsen, B., Hansen, J., Andersson, B. and Yuen, J. (2011). Genetic analysis of *Phytophthora infestans* populations in the Nordic European countries reveals high genetic variability. *Fungal Biology*, 115, pp. 335-342.

Cartagena Protocol (2003). Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Available online: www.cbd.int/biosafety. Accessed 05.06. 2010.

Champouret, N., Bouwmeester, K., Rietman, H., van der Lee, T., Maliepaard, C., Heupink, A., van de Vondervoort, P.J.I., Jacobsen, E., Visser, R.G.F.,van der Vossen, E.A.G., Govers, F. and Vleeshouwers, V.G.A.A. (2009). *Phytophthora infestans* Isolates Lacking Class I ipiO Variants Are Virulent on Rpi-blb1 Potato. *Molecular Plant-Microbe Interactions*, 22, pp. 1535-1545.

Chen Y., Liy Z. and Halterman D.A. (2012). Molecular Determinants of Resistance Activation and Supression by *Phytophthora infestans* Effector IPI-O. *PLOS Phatogens*, 8,e1002595

Cooke, L.R., Schepers, H.T.A.M., Hermansen A., Bain R.A., Bradshaw, N.J., Ritchie F., Shaw D.S., Evenhuis A., Kessel, G.J.T. , Wander, J.G.N, Andersson, B., Hansen J.G., Hannukkala, A., Nærstad, R. and. Nielsen B.J. (2011). Epidemiology and integrated control of potato late blight in Europe. *Potato Research*, 54, pp. 183-222.

Debio (2011). *Statistikk 2011 – økologisk produksjon, private standarder*. Available online: http://www.debio.no/_upl/statistikkhefte_2011.pdf. Accessed 23.01.2013.

Directive 2001/18/EC (2001). On the deliberate release into the environment of genetically modified organisms.
The European Parliament and the Council, Official Journal of the European Union, L 106, 17.4.2001. Available online: www.biosafety.be/PDF/2001_18.pdf. Accessed 08.12. 2011.

Drenth, A., Tas, I.C.Q. and Govers, F. (1994). DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. *European Journal of Plant Pathology*,100, pp. 97-107.

EFSA (2010a). EFSA Panel on Genetically Modified Organisms (GMO); *Guidance on the environmental risk assessment of genetically modified plants*. EFSA Journal 2010; 8(11):1879. Available online: www.efsa.europa.eu/efsajournal.htm. Accessed 08.12.2011.

EFSA (2010b). Scientific Opinion on the assessment of potential impacts of genetically modified plants on non-target organisms. EFSA Journal 8(11): 1877. Available online: http://www.efsa.europa.eu/en/efsajournal/doc/1877.pdf. Accessed 13.02.2013.

European Comission (2013). *Deliberate releases and placing on the EU market of Genetically Modified Organisms – GMO register.* Available online: http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx. Accessed 23.01. 2013.

FAOSTAT (2012). Data available at: faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor. Accessed 23.01. 2013

Fry, W. (2008). *Phytophthora infestans*: The plant (and R gene) destroyer. *Molecular Plant Pathology*, 9, pp. 385-402.

Fry, E., Grünwald, N.J., Cooke, D.E.L., McLeod, A., Forbes, G. and Cao, K. (2009). Population Genetics and Population Diversity of *Phytophthora infestans*. In: Lamour, K. and, Kamoun, K. (Eds.) *Oomycete Genetics and Genomics: Diversity, Interactions, and Research Tools*. John Wiley & Sons, Inc. ISBN: 9780470255674.

Gene Technology Act (1993). Act of 2 April 1993 no. 38 relating to the production and use of genetically modified organism. Ministry of Environment, Oslo, Norway. Available online: www.regjeringen.no/en/doc/Laws/Acts/Gene-Technology-Act.html?id=173031. Accessed 25.11.2011.

Gillund, F., Hilbeck, A. and Wikmark, O.G. (2011). Genetically Modifi ed Potato with Increased Resistance to *P. infestans* - Selecting Test Species for Environmental Impact Assessment on Non-Target Organisms. Biosafety Report 2011/05. Available online: www.genok.com/news_cms/2012/february/new-biosafety-report-genetically-modifi-ed-potato-with-increased-resistance-to-p-infestans-selecting-test-species-for-environmental-impact-assessment-on-non-target-organisms/152. Accessed 13.02.2013.

Goméz-Alpizar, L., Carbone, I. and Ristaino, J.B. (2007). An Andean origin of *Phytophthora infestans* inferred from mitochondrial and nuclear gene genealogies. *Proceedings of the National Academy of Sciences of the United States of America*, 104, pp. 3306 – 3311.

Goodwin, S.B., Spielman, L.J., Matuszak, J.M., Bergeron, S.N. and Fry, W.E. (1992). Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. *Phytopathology*, 82, pp. 955-961.

Haas, J.B. et al., (2009). Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature*, 461, pp 393- 398.

Halterman, D.A., Chen, Y., Sopee J., Berduo-Sandoval J. and Sánchez-Pérez, A. (2010). Competition between *Phytophthora infestans* Effectors Leads to Increased Aggressiveness on Plants Containing Broad-Spectrum Late Blight Resistance. *PLoS ONE*, 22, pp. 1-10.

Halterman, D.A., Kramer, L.C., Wielgus, S. and Jian, J. (2008). Performance of transgenic potato containing the late blight resistance gene RB. *Plant Disease*, 92, pp.339- 343.

Haverkort, A.J., Struik, P.C., Visser, R.G.F. and Jacobsen, E. (2009). Applied Biotechnology to Combat Late Blight in Potato Caused by *Phytophthora Infestans*, *Potato Research*, 52, pp. 249-264.

Haverkort, A.J., Boonekamp, P.M., Hutten, R., Jacobsen, E., Lotz, L.A.P., Kessel, G.J.T., Visser, R.G.F. and van der Vossen, E.A.G. (2008). Societal Costs of Late Blight in Potato and Prospects of Durable Resistance Through Cisgenic Modification. *Potato Research*, 51, pp.47–57

Hermansen, A., Hannukkala, A., Hafskjold Nærstad, R. and Brurberg, M.B. (2000). Variation in populations of *Phytophthora infestans* in Finland and Norway: mating type, metalaxyl resistance and virulence phenotype. *Plant Pathology*, 49, pp.11–22

Hilbeck, A., Weiss, G., Oehen, B., Römbke, J., Jänsch, S., Teichmann, H., Lang, A., Otto, M. and Tappeser, B.(Submitted manuscript). Ranking matrices as operational tools for the environmental risk assessment of genetically modified organisms on non-target organisms.

Hilbeck, A., Meier, M., Römbke, J., Jänsch, S., Teichmann, H. and Tappeser, B. (2011), Environmental risk assessment of genetically modified plants – concepts and controversies. *Environmental Sciences Europe*, 23, pp. 1-13.

Hilbeck, A., Jänsch, S., Meier, M. and Römbke, J. (2008). *Analysis and validation of present ecotoxicological test methods and strategies for the risk assessment of genetically modified plants*. Federal Agency for Nature Concervation, Bonn-Bad Godesberg (DE): BfN- Skripten.

Hilbeck, A., Andow, D.A., Fontes, E.M.G. (Eds.) (2006). *Environmental risk assessment of genetically modified organisms, Vol. 2.* CABI Publishing, Wallingford, UK. ISBN 1-84593-000-2.

Hilbeck, A. and Andow, D.A. (Eds.) (2004.) *Environmental risk assessment of genetically modified organisms, Vol. 1.* CABI Publishing, Wallingford, UK. ISBN 0-85199-861-5.

Johansen, T.J., Møllerhagen, P.J. and Haugland E. (2008). Yield potential of seed potatoes grown at different latitudes in Norway. *Acta Agriculturae Scandinavica Section B - Soil and Plant Science*, 58, pp. 132-138.

Kamoun, S. and Smart, C.D. (2005). Late blight of potato and tomato in the genomics era. *Plant Disease*, 89, pp. 692- 698.

Kapuscinski, A.R., Li, S., Hayes, K.R. and Dana, G. (Eds.) (2007). *Environmental risk assessment of genetically modified organisms, Vol 3.* CABI Publishing, Oxfordshire,UK. ISBN-13:9781 84593 2961.

Kirk W.W., Felcher K.J., Douches D.S., Niemira B.A., and Hammerschmidt R. (2001). Susceptibility of Potato (*Solanum tuberosum* L.) Foliage and Tubers to the US8 Genotype of *Phytophthora infestans*. *American Journal of Potato Research,* 78, pp. 319-322.

Kramer, L.C., Choudoir, M.J., Wielgus, S.M., Bhaskar, P.B. and Jiang, J. (2009). Correlation between transcript abundance of the RB gene and the level of the RB-mediated late blight resistance in potato. *Molecular Plant- Microbe Interactions*, 22, pp. 447-455.

Milligan, S.B., Bodeau, J., Yaghoobi, J., Kaloshian, I., Zabel, P. and Williamson, V.M. (1998). The root knot nematode resistance gene Mi from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell*, 10, pp.1307–1319.

Møllerhagen P.J. (2012). Norsk potetproduksjon 2011. *Jord og Plantekultur*. Bioforsk fokus 7 (1), pp. 195- 199.

Møllerhagen, P. J. (2011). Norsk potetproduksjon 2010. *Jord og Plantekultur*. Bioforsk fokus 6, pp. 216- 219.

Nelson, C.K., Andow, D.A. and Banker M.J. (2009). Problem Formulation and Option Assessment (PFOA) linking governance and environmental risk assessment for technologies: A method for problem analysis of nanotechnologies and genetically engineered organisms. *Journal of law, medicine and ethics*, pp. 732-748.

Nombela, G., Williamson,V.M. and Muniz, M. (2003). The root-knot nematode resistance gene Mi-1.2 of tomato is responsible for resistance against the whitefly Bemisia tabaci. *Molecular Plant-Microbe Interactions*, 16, pp. 645–649.

Norwegian Food Safety Authority (2011). Mye virus på årets settepoteter. Available online: www.mattilsynet.no/planter/plantehelse/nyheter/mye_virus_p___rets_settepoteter_94101. Accessed 08.12. 2011.

Pankin, A., Sokolova, E., Rogozina, E. Kuznetsova, M., Deal, K., Jones, R. and Khavkin, E. (2011). Allele mining in the gene pool of wild Solanum species for homologues of late blight resistance gene RB/Rpi-blb1. *Plant Genetic Resources: Characterization and Utilization*, 9, pp. 305–308.

Park, T.H., Vleeshouwers, V.G.A.A., Kim, J.B., Hutten, R.C.B., and Visser, R.G.F. (2005). Dissection of foliage and tuber late blight resistance in mapping populations of potato. *Euphytica*, 143, pp.75-83.

Platt, H.W.B. and Tai, G. (1998). Relationship Between resistance to Late Blight in Potato Foliage and Tubers Cultivars and Breeding Selections With Different Resistance Levels. *American Journal of Potato Research.* 75, pp. 175-178

Park, T.H., Vleeshouwers, V.G.A.A, Jacobsen, E., van der Vossen, E. and Visser, R.G.F. (2009). Molecular breeding for resistance to *Phytphthora infestans* (Mont.) de Bary in potato (Solanum tuberosum L.): A perspective of cisgenesis, *Plant breeding*, 128, pp. 109-117.

Potato genome sequencing consortium (2011). Genome sequence and analysis of the tuber crop potato. *Nature*, 475, pp. 189-197.

Regulations relating to impact assessment pursuant to the Gene Technology Act. (2005). Availabe online: www.regjeringen.no/en/dep/md. Accessed 25.11.2011.

Rossi, M., Goggin, F.L., Milligan, S.B., Kaloshian, I., Ullman, D.E. and Williamson, V.M. (1998). The nematode resistance gene Mi of tomato confers resistance against the potato aphid. *Proceedings of the National Academy of Sciences of the United States of America*, 95, pp. 9750–9754.

Sujkowski, L.S., Goodwin, S.B., Dyer, A.T. and Fry, W.E. (1994). Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. *Phytopathology*, 84, pp. 201-207.

Sokolova, E., Pankin, A., Beketova, M., Kuznetsova, M., Spiglazova, S., Rogozina, E.,Yashina, I. and Khavkin, E. (2011). SCAR markers of the R-genes and germplasm of wild Solanum species for breeding late blight-resistant potato cultivars. *Plant Genetic Resources: Characterization and Utilization*, 9, pp. 309–312.

Song, J., Bradeen, J.M., Naess, S. K., Raasch, J.A., Wielgus, S.M., Haberlach. G.T., Liu, J., Kuang, H., Austin-Phillips, S., Buell, C.R., Helgeson, J.P. and Jiang, J. (2003). Gene RB cloned from Solanum bulbocastanum confers broad spectrum resistance to potato late blight. *Proceedings of the National Academy of Sciences of the United States of America*, 100, pp. 9128-9133.

Statistics Norway (2012). Avling av potet- og grovfôrvekstar, 2008-2011. Available online: http://www.ssb.no/jordbruksavling/tab-2012-02-01-01.html. Accessed 23.01.2012.

Sæthre, M.G., Hermansen A. and Nærstad R. (2006). Economic and Environmental impacts of the introduction of Western flower thrips (*Frankliniella occidentalis*) and Potato late blight (*Phytopthora infestans*) to Norway, *Bioforsk Report*, Vol.1, No.64.

Tamm, L., Smit, A.B., Hospers, M., Janssens, S.R.M., Buurma, S., Mølgaard, J.P., Lærke, P.E., Hansen, H.H., Hermans, A., Bødker, L., Bertrand, C., Lambion, J., Finckh, M.R., Schüler, C., Lammerts van Bueren, E., Ruissen, T., Nielsen, B.J., Solberg, S., Speiser, B., Wolfe, M.S., Phillips, S., Wilcoxon, S.

and Leifert, C. (2004). *Assessment of the Socio-Economic Impact of Late Blight and Stateof- the-Art Management in European Organic Potato Production Systems.* Research Institute of Organic Agriculture FiBL, Frick, Switzerland. ISBN 3-906081-54-0.

The 2010 Norwegian Red List for Species (2010) . Artsdatabanken, ISBN-13: 978-82-92838-26-6.

The Norwegian scientific committee for food safety (2006). Vurdering av foreslåtte virkemidler for sameksistens mellom genmodifiserte vekster og konvensjonelt/økologisk landbruk, og rangering av spredningsrisiko av transgener fra relevante genmodifierte planter som kan dyrkes i Norge. Uttalelse fra Faggruppe for genmodifiserte organismer (Faggruppe 3) i Vitenskapskomiteen for mattrygghet 21.12.06. Available online: http://www.vkm.no/dav/bc76186492.pdf. Accessed 13.02.2013.

The Sainsbury Laboratory (2010). *GM trial to reduce pesticides*. Press release, 08 June 2010. Available online: www.tsl.ac.uk/docs/potato_trial_PR.pdf. Accessed: 08.12.2011.

Tuzun, S. (2001). The relationship between pathogen-induced systemic resistance (ISR) and multigenic (horizontal) resistance in plants. *European Journal of Plant Pathology*, 107, pp 85–93.

van der Vossen, E., Gros, J., Sikkema, A., Muskens, M., Wouters, D., Wolters, P., Pereira, A. and Allefs, S. (2005). The Rpi-blb2 gene from Solanum bulbocastanum is an Mi-1 gene homolog conferring broad-spectrum late blight resistance in potato. *The Plant Journal*, 44, pp. 208–222.

van der Vossen, E., Sikkema, A., Hekkert, B., Gros, J., Stevens, P. Muskens, M., Wouters, D., Pereira, A., Stiekema, W. and Allefs, S. (2003). An acient R gene from wild potato species *Solanum bulbocastanum* confers broad spectrum resistans to *Phytophthora infestans* in cultivated potato and tomato. *The Plant Journal*, 36, pp. 867-882.

Vleeshouwers, V.G.A.A., Raffaele, S., Vossen, J., Champouret, N., Oliva, R., Segretin, M.E., Rietman, H., Cano, L.M., Lokossou, A. Kessel, G., Pel, M.A. and Kamoun, S. (2011). Understanding and exploiting late blight resistance in the age of effectors. *The Annual Review of Phytopathology*, 49, pp. 507–31.

Zhu S., Li Y., Vossen J.H., Visser R.G.F. and Jacobsen E. (2012) Functional stacking of three resistance genes against *Phytophthora infestans* in potato. Transgenic Res, 21, pp.89-99.

Annex 1: Guidance table for Step 1: Case specific selection of important and possibly affected environmental functions as part of the ERA of GM potato with increased resistance to late blight.

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. Weeds (competition)	Increased humidity favours disease Plant competition	Pathogens (early and late) Nematodes (early and late) 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 parts 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? **Do they contain transgenes, transgene products or metabolites?**	Few (flower parts/remnants) Yes (check expression of promoter)	Decomposition	Decomposers
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
What plant excretions/ exudates possibly containing transgenes, transgene products or metabolites are expected?	Some pollen Root exudates Factors effecting symbiotic signalling	Rhizosphere Mycorrhiza	Root colonizing micro- and mesofauna and fungi, mycorrhiza microbes

Main criteria	Characteristics	Associated ecological function/agricultural practise	Affected organisms/process
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 regenerate vegetatively and in what quantities? (Temporal persistance 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	---	---
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	---	---

Main criteria	Characteristics	Associated ecological function/agricultural practise	Affected organisms/process
III. Receiving environment – intended 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
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.
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 Soil diseases and pests typical for light soils	Organic matter decomposition rates Soil moisture retention, etc. Nematodes, certain fungi
Organic matter content? High to low	Undemanding	---	---
Prone for soil erosion?	No	---	---

Annex 2: Ecological ranking of herbivores. Green: selected species/genus/higher taxonomic unit. ?: Gaps of knowledge

STEP 2: ECOLOGICAL RANKING OF HERBIVORES																				
	PART 1								PART 2								PART 3			
Species or taxon	Geographic distribution	Habitat specialization	Abundance	Phenology: non-target organism perspective	Phenology: crop perspective	Feeding specialization	**Part 1: Arithmetic mean**	**Part 1: Median**	Potential to become a pest	Significance as a vector (disease)	Significance for other ecological functions	Significance as food (prey/host) for natural enemies	Significance associated with other crops	Significance in natural and semi-natural habitats	**Part 2: Arithmetic mean**	**Part 2: Median**	**Part 3: Protective status**	**Final ranking: Arithmetic mean**	**Final ranking: Mean of median**
COLEOPTERA																			
Leptinotarsa decemlineata	2	1	2	1	1	1	1,3	1	1	3	3	2	3	3	2,4	3	3	2,2	2,3
Agriotes spp.	1	3	2	3	1	3	2,2	2,5	1	3	2	2	2	1	2,4	2	3	2,5	2,5
Amara aulica	1	3	2	2	2	3	2,2	2	3	3	2	3	3	2	3,0	3	3	2,7	2,7
Harpalus rufipes	1	3	1	2	2	3	2,0	2	3	3	2	3	3	2	3,0	3	3	2,7	2,7
HYMNOPTERA																			
Bombus terrestris	1	3	1	3	3	3	2,3	3	3	3	1	3	2	1	2,9	2,5	3	2,7	2,8
Bombus lapidarius	1	3	2	3	3	3	2,5	3	3	3	1	3	2	1	2,9	2,5	2	2,5	2,5
LEPIDOPTERA																			
Agrotis segetum	1	3	2	1	1	3	1,8	1,5	1	3	3	3	1	2	2,4	2,5	3	2,4	2,3
HEMIPTERA																			
Empoasca vitis	1	2	2	2	2	2	1,8	2	1	2	3	2	2	3	2,3	2	3	2,4	2,3
Lygus rugulipennis	1	3	1	2	2	3	2,0	2	2	3	2	2	2	2	2,5	2	3	2,5	2,3
APHIDOIDEA																			
Myzus persicae	1	3	2	2	2	3	2,2	2	1	1	3	2	2	3	2,2	2	3	2,5	2,3
Aulacorthum solani	1	2	3	2	2	2	2,0	2	2	2	3	2	3	3	2,7	2,5	3	2,6	2,5
Macrosiphum euphorbiae	1	2	3	2	2	2	2,0	2	2	2	3	2	3	3	2,7	2,5	3	2,6	2,5
Rhopalosiphum padi (virus vector)	1	3	1	3	3	3	2,3	3	3	1	3	2	1	2	2,6	2	3	2,6	2,7
DIPTERA																			
Tipula spp.	1	3	2	2	2	3	2,2	2	3	3	2	2	2	2	2,7	2	3	2,6	2,3
Xylota segnis	1	3	2	2	2	3	2,2	2	3	3	2	3	3	2	3,0	3	3	2,7	2,7
SLUGS/MOLLUSCA																			
Deroceras reticulatus	1	3	1	2	2	3	2,0	2	1	3	3	2	2	3	2,5	2,5	3	2,5	2,5

Annex 3: Ecological ranking of natural enemies. Green: selected species/genus/higher taxonomic unit. ?: Gaps of knowledge

STEP 2: ECOLOGICAL RANKING NATURAL ENEMIES																	
	PART 1								PART 2							FINAL RANK	
Species or taxon	Geographic distribution	Habitat specialisation	Abundance	Phenology: non-target organism perspective	Phenology: crop perspective	Linkage and association with the crop	**Part 1: Arithmetic mean**	**Part 1: Median**	Significance as natural enemy in crop	Significance as natural enemy in other crops	Significance as food for other natural enemies	Significance as natural enemy in natural and semi-natural habitats	**Part 2: Arithmetic mean**	**Part 2: Median**	**Part 3: Protective status**	**Final rank: mean of mean**	**Final rank: Mean of Median**
ARANEA																	
Pardosa spp.	1	3	1	2	1	2	1,7	1,5	1	1	3	1	1	1	3	1,89	1,8
Trochosa spp.	1	3	2	2	1	2	1,8	2	1	2	3	2	2	2	3	2,28	2,3
Erigone atra	1	3	1	3	1	3	2,0	2	1	2	2	1	1,5	1,5	3	2,17	2,2
Meioneta rurestris	1	3	2	3	1	3	2,2	2,5	1	2	2	2	2	2	3	2,39	2,5
Oedothorax apicatus	1	3	1	3	1	3	2,0	2	1	2	2	1	1,5	1,5	3	2,17	2,2
Theridion impressum	1	3	2	2	1	2	1,8	2	1	2	3	2	2	2	3	2,28	2,3
Pachygnatha degeeri	1	3	2	3	1	2	2,0	2	1	2	3	2	2	2	3	2,33	2,3
COLEOPTERA																	
Bembidion lampros	2	3	1	1	1	3	1,8	1,5	1	1	2	2	1,5	1,5	3	2,11	2
Bembidion obtusum	2	3	2	1	1	3	2,0	2	1	1	2	2	1,5	1,5	3	2,17	2,2
Bembidion guttula	2	3	3	1	1	3	2,2	2,5	1	?	2	2	1,5	1,5	3	2,22	2,3
Pterostichus niger	1	3	2	2	2	3	2,2	2	1	2	3	2	2	2	3	2,39	2,3
Pterostichus melanarius	1	3	1	2	2	3	2,0	2	1	1	3	2	1,5	1,5	3	2,17	2,2
Trechus quadristriatus	1	3	1	2	2	3	2,0	2	1	2	2	2	2	2	3	2,33	2,3
Trechus secalis	1	3	?	2	2	3	2,0	2	1	2	2	2	2	2	3	2,33	2,3
Anchomenus dorsalis	2	3	3	2	2	3	2,5	2,5	1	1	3	2	1,5	1,5	3	2,33	2,3
Poecilus cupreus	1	3	2	2	2	3	2,2	2	1	1	3	2	1,5	1,5	3	2,22	2,2
Harpalus rufipes	1	3	1	2	2	3	2,0	2	1	1	3	2	1,5	1,5	3	2,17	2,2
Carabus granulatus	2	3	3	2	2	3	2,5	2,5	1	?	3	?	1	1	3	2,17	2,2
Patrobus atrorufus	2	3	2	2	2	3	2,3	2	1	2	3	2	2	2	3	2,44	2,3
Synuchus vivalis	1	3	2	2	2	3	2,2	2	1	2	3	2	2	2	3	2,39	2,3
Loricera pilicornis	1	3	2	2	2	3	2,2	2	1	2	3	2	2	2	3	2,39	2,3
Calathus fuscipes	1	3	2	2	2	3	2,2	2	1	2	3	2	2	2	3	2,39	2,3
Calathus melanocephalus	1	3	2	2	2	3	2,2	2	1	2	3	2	2	2	3	2,39	2,3
Clivina fossor	1	3	2	2	2	3	2,2	2	1	3	3	3	3	3	3	2,72	2,7
Amara aulica	1	3	2	2	2	3	2,2	2	1	3	3	3	3	3	3	2,72	2,7
Amara fulva	1	3	3	2	2	3	2,3	2,5	1	3	3	3	3	3	3	2,78	2,8
Amara plebeja	1	3	3	2	2	3	2,3	2,5	1	3	3	3	3	3	3	2,78	2,8
Coccinella 7-punctata	1	3	3	2	1	3	2,2	2,5	1	1	3	1	1	1	3	2,06	2,2
Tachyporus spp.	1	3	1	2	2	3	2,0	2	1	2	2	2	2	2	3	2,33	2,3
Philonthis spp.	1	3	2	2	2	3	2,2	2	1	3	2	2	2	2	3	2,39	2,3
Staphylinus spp	1	3	2	2	2	3	2,2	2	1	3	2	2	2	2	3	2,39	2,3
DIPTERA																	
Episyrphus balteatus	1	3	2	2	2	3	2,2	2	1	1	3	1	1	1	3	2,06	2
Eupeodes corollae	1	3	2	2	2	3	2,2	2	1	1	3	1	1	1	3	2,06	2
Sphaerpphoria scripta	1	3	2	2	2	3	2,2	2	1	1	3	1	1	1	3	2,06	2
Syrphus ribesii	1	3	2	2	1	3	2,0	2	1	1	3	1	1	1	3	2,00	2

NEUROPTERA																	
Chrysophidae	1	3	3	2	2	3	2,3	2,5	1	2	3	2	2	2	3	2,44	2,5
HYMENOPTERA																	
Aphidiidae	1	3	2	2	2	3	2,2	2	1	1	3	1	1	1	3	2,06	2
Formica ssp	1	3	3	2	2	3	2,3	2,5	1	3	3	2	2,5	2,5	3	2,61	2,7
CHILOPODA	1	3	3	?	?	3	2,0	2	1	3	3	3	3	3	3	2,67	2,7
BACTERIA																	
Bacillus spp.	1	3	1	2	2	3	2,0	2	2	2	2	2	2,0	2,0	3	2,0	2,0
Pseudomonas spp. (except pathogens)	1	3	1	2	2	3	2,0	2	2	2	2	2	2,0	2,0	3	2,0	2,0
NEMATODES																	
Steinernema spp.	?	2	3	?	?	3	1,5	1,9	2	2	3	3	2,5	2,5	3	2,00	2,2
Heterrhabditis spp.	?	2	3	?	?	3	1,5	1,9	2	2	3	3	2,5	2,5	3	2,00	2,2
Mermis spp.	?	3	3	?	?	3	2,0	2,1	3	3	3	3	3	3	3	2,50	2,55
FUNGI																	
Trichoderma spp.	1	3	1	2	2	3	2,0	2	2	2	2	2	2,0	2,0	3	2,0	2,0
Metarhizium	1	3	2	3	2	3	2,3	2,5	1	?	3	?	1	1	3	2,11	2,2
Entomophthorales	1	3	3	2	2	3	2,3	2,5	1	1	3	2	1,5	1,5	3	2,28	2,3
Beauveria bassiana	1	3	2	3	2	3	2,3	2,5	1	?	3	?	1	1	3	2,11	2,2
Lecanicilium	1	3	2	3	2	3	2,3	2,5	1	?	3	?	1	1	3	2,11	2,2

Annex 4 Ecological ranking of soil living decomposers, obligate biotroph symbionts and pathogens Green: selected species/genus/higher taxonomic unit. ?: Gaps of knowledge

STEP 2: ECOLOGICAL RANKING SOIL LIVING DECOMPOSERS, OBLIGATE BIOTROPH SYMBIONTS AND PATHOGENS																	
	PART 1								PART 2						PART 3	FINAL RANK	
Species or taxon	Georgraphic distribution	Habitat specialization	Abundance	Phenology: nontarget organism perspective	Phenology: crop perspective	Feeding specialization	**Part 1: Arithmetric mean**	**Part 1: Median**	Significance for important nutrients in the crop system	Significance as indicator for soil quality	Significance for degradation of organic matter	Significance for other processes	**Part 2: Arithmetric mean**	**Part 2: Median**	**Part 3: Protective status**	**Final rank: Mean of mean**	**Final rank: Mean of median**
DECOMPOSERS																	
CLITELLATA																	
Aporrectodea caliginosa	1	3	2	1	1	3	1,8	1,5	1	1	1	1	1,0	1,0	3	1,4	1,3
HYMENOPTERA																	
Formica ssp.	1	2	1	2,5	2	3	1,9	2,0	2	2	2	2	2,0	2,0	3	2,0	2,0
COLLEMBOLA																	
Folsomia fimetaria	1	3	1	1	1	3	1,7	1,0	1	1	1	3	1,5	1,0	3	1,6	1,0
Isotoma spp.	1	3	1	1	1	3	1,7	2,0	1	1	1	3	1,5	1,0	3	1,6	1,5
Lepidocyrtus cyaneus	1	3	2	1	1	3	1,8	1,5	1	2	1	3	1,8	1,5	3	1,8	1,5
Mesaphorura spp.	1	3	2	1	1	3	1,8	1,5	1	2	1	3	1,8	1,5	3	1,8	1,5
CHILOPODA	1	3	3	1	1	3	2,0	2,5	2	2	2	2	2,0	2,0	3	2,0	2,3
ACARI																	
Oribatida	1	3	1	1	1	3	1,7	1,0	2	1	1	3	1,6	1,0	3	1,6	1,0
Uropodina	1	3	1	1	1	3	1,7	1,0	2	2	2	3	2,3	2,0	3	2,0	1,5
BACTERIA																	
Streptomyces spp. (except for the spp. causing Potato powdery scab)	1	3	1	1	2	3	1,8	1,5	1	1	1	2	1,3	2,0	3	1,5	1,8
PROCESSES																	
Nematodes in mineralising processes	1	3	1	1	1	3	1,7	1,5	1	1	1	1	1,0	2,0	3	1,3	1,8
Mineralisation (process)	2	3	3	1	1	3	2,2	1,0	1	1	1	1	1,0	1,0	3	1,6	1,0
Nitrification (process)	1,1	2	1,1	1,5	3	1,1	1,6	1,0	1	1	1	1	1,0	1,0	3	1,3	1,0
OBLIGATE BIOTROPH SYMBIONTS																	
Glomeromycota	?	2	?	1,5	3	?	1,6	1,2	1	1	2	1	1,3	2,0	3	1,4	1,6

PATHOGENS																		
	PART 1								PART 2							PART 3	FINAL RANK	
Species or taxon	Geographic distribution	Habitat specialization	Abundance	Phenology: nontarget organism perspective	Phenology: crop perspective	Feeding specialization	**Part 1: Arithmetric mean**	**Part 1: Median**	Potential to become a pest	Significance as a vector (disease)	Significance for other ecological functions	Significance as food (prey/host) for natural enemies	Significance associated with other crops	**Part 2: Arithmetic mean**	**Part 2: Median**	**Part 3: Protective status**	**Final rank: Mean of mean**	**Final rank: Mean of median**
BACTERIA																		
Streptomyces spp. Known to cause Potato powdery scab	1	2,5	1	1	1	2	1,4	1	1	3	1	3	1	2	1,0	3	1,6	1,0
Pectobacerium carotovorum	1	1,5	1	1	1	1	1,1	1	1	3	3	3	3	3	3,0	3	1,9	2,0
Pectobacterium atrosepticum	1	1,5	1	1	1	1	1,1	1	1	3	1	3	2	2	2,0	3	1,6	1,5
*Rhizoctonia solani (*Anostomose group 3, potato specific)	1	2	1	1	1	1	1,2	1	1	3	3	3	1,5	2	2,5	3	1,8	1,8
NEMATODES																		
Pratylenchus spp.	1	1	1	2	2	2	1,5	1,5	1	2	2	2	1	2	1,5	3	1,6	1,5
Tylenchorhynchus ssp.	1	1	1	3	2	2	1,7	1,5	1	2	3	2	1	2	1,5	3	1,7	1,5
Trichodorus spp.	2	2	1	3	2	2	2	2	1	1	3	2	1	2	1,0	3	1,8	1,5
Paratrichodorus spp.	2	2	1	3	2	2	2	2	1	1	3	2	1	2	1,0	3	1,8	1,5
Globodera rostochiensis	2	1	1	1	1	1	1,2	1	1	2	1	3	3	2	2,5	3	1,7	1,8
Globodera pallida	2	1	1	1	1	1	1,2	1	1	2	1	3	3	2	2,5	3	1,7	1,8
FUNGI																		
Colletotrichum coccodes	1	2	1	1	1	2	1,3	1	1	3	2	3	2	2	2,0	3	1,8	1,5
Sclerotinia sclerotiorum	1	2	1	1	1	2	1,3	1	2	3	3	3	1	2	2,5	3	1,8	1,8
Boremia foveata	1	1	2	1	1	1	1,2	1	2	3	3	3	1,5	3	3,0	3	1,9	2,0
Spongospora subterranea	1,5	2	2	1	1	2	1,6	1,75	1	1	3	3	2	2	2,0	3	1,8	1,9
Fusarium spp. (specific to potato)	1	2	2	1	1	2	1,5	1,5	2	3	2	3	2	2	2,0	3	1,9	1,8
Phytophthora erythroseptcia	1	2	2	1	1	2	1,5	1,5	2	3	3	3	2	3	2,5	3	2,1	2,0
Pythium ultimum	1	1,5	2	1	1	1,5	1,3	1,25	2	3	3	3	2	3	2,5	3	2,0	1,9

Annex 5 Ranking of herbivores based on maximum likelihood of exposure. ?: Gaps of knowledge

STEP 3: EXPOSURE RANKING HERBIVORES													
	Background information				Part 1: Possibility of exposure			Part 2: Likelihood of exposure					
Species or taxon	Life cycle stage with the herbivore function	Life cycle stage with other functions	Plant tissues/secretions on which the non-target organism feeds	Growth stage of potato when orgaism is presen	Is this feeding important for the herbivore?	Do plant tissues/secretions fed upon express the transgene product?	**Ranking of part 1**	Are transgene products/metabolites detectable after feeding on plant	Probability of non-target organism containing transgene after feeding	Alterations in potato that might affect palatability for nontarget organism?	Changes in behaiviour/feeding preferences that increase/decrease transgene exposition	**Ranking of part 2**	**Final rank**
COLEOPTERA													
Leptinotarsa decemlineata	larvae & adult	no	leaves	all	1	1	1	?	?	? Some insect do not want feed containig Bt			
Agriotes spp.	larvae	yes	tubers	early & late	3	1	2	?	?	?	?		
HYMNOPTERA													
Bombus lapidarius	larvae & adult	yes	pollen, nectare	flowering	3	1	2	?	?	?	?		
LEPIDOPTERA													
Agrotis segetum	larvae	yes	leaves & tubers	all	3	1	2	?	?	?	?		
HEMIPTERA													
Empoasca vitis	juvenil & adult	no	leaves	all	2	1	1,5	?	?	?	?		
Lygus rugulipennis	juvenil & adult	yes	leaves	all	3	1	2	?	?	?	?		
APHIDOIDEA													
Myzus persicae	juvenil & adult	yes	leaf phloem	all	3	3	3	?	?	?	?		
SLUGS/MOLUSCA													
Deroceras reticulatum	juvenil & adult	no	leaves & tubers	all	3	1	2	?	?	?	?		

Annex 6 Ranking of natural enemies based on maximum likelihood of multitrophic exposure via intraguild predation/hyperparasitation. ?: Gaps of knowledge

STEP 3 EXPOSURE RANKING OF NATURAL ENEMIES (MULTITROPHIC EXPOSURE VIA INTRAGUILD PREDATION/HYPERPARASITATION)												
	Background information				Part 1: Possibility of exposure			Part 2: Likelihood of exposure				
Species or taxon	Life cycle stage with the predator function	Life cycle stage with other functions	Main prey/host	Growth stage of potato when organism is present	How important is conspecifics/ higher trophic level prey as food source?	Does higher trophic level prey fed upon contain the transgene product?	**Ranking of part 1**	Are transgene products/metabolites detectable in higher trophic level organisms?	Probability non-target organism containing transgene after feeding	Changes in behaviour/feeding preferences that increase/decrease transgene exposition	**Ranking of Part 2**	**Final rank**
ARANEA												
Pardosa spp.	all	No	generalist	all	2	2	2	?	?	?		
Oedothorax apicatus	all	No	generalist	all	3	3	3	?	?	?		
COLEOPTERA												
Bembidion lampros	all	No	generalist	all	2	2	2	?	?	?		
Pterostichus melanarius	all	No	generalist	all	2	2	2	?	?	?		
Harpalus rufipes	all	No	omnivore	all	2	2	2	?	?	?		
Coccinella 7-punctata	all	No	aphids	canopy	3	3	3	?	?	?		
Tachyporus spp.	all	No	omnivore	all	3	3	3	?	?	?		
Philonthis spp.	all	No	omnivore	all	2	2	2	?	?	?		
DIPTERA												
Syrphus ribesii	larvae	Yes	aphids	canopy	3	3	3	?	?	?		
NEUROPTERA												
Chrysophidae	larvae	Yes	aphids	canopy	3	3	3	?	?	?		
HYMENOPTERA												
Aphidiidae	larvae	Yes	aphids	canopy	3	3	3	?	?	?		
FUNGI												
Entomophthorales		No	homoptera	all	3	3	3	?	?	?		
Trichoderma spp.	all	no, decomposer as indirect effect	fungi	all	3	2	2,5	?	?	?		
Metarhizium		Yes	generalist	all	3	3	3	?	?	?		
NEMATODES												
Steinernema spp.	juvenile	No	generalist	all	2	2	2	?	?	?		

Annex 7 Ranking of natural enemies based on maximum likelihood of tritrophic exposure via feeding on herbivores/decomposers.?: Gaps of knowledge

STEP 3 EXPOSURE RANKING NATURAL ENEMIES, TRITROPHIC EXPOSURE VIA FEEDING ON HERBIVORES/DECOMPOSERS												
	Background information				Part 1: Possibility of exposure			Part 2: Likelihood of exposure				
Species or taxon	Life cycle stage with the predator function	Life cycle stage with other functions	Main prey/host	Growth stage of potato when organism is present	How important is herbivore/decomposer prey as food source?	Do herbivore/decomposor fed upon contain the transgene product?	**Ranking of part 1**	Are transgene products/metabolites detectable in the excretions?	Probability non-target organism containing transgene after feeding	Changes in behaiviour/feeding preferences that increase/decrease exposition of trangene?	**Ranking of part 2**	**Final rank**
ARANEA												
Pardosa spp.	all	no	generalist	all	2	2	2	?	?	?		
Oedothorax apicatus	all	no	generalist	all	2	2	2	?	?	?		
COLEOPTERA												
Bembidion lampros	all	no	generalist	all	2	2	2	?	?	?		
Pterostichus melanarius	all	no	generalist	all	2	2	2	?	?	?		
Harpalus rufipes	all	no	omnivore	all	2	2	2	?	?	?		
Coccinella 7-punctata	all	no	Aphids	canopy	1	2	1,5	?	?	?		
Tachyporus spp.	all	no	omnivore	all	2	2	2	?	?	?		
Philonthis spp.	all	no	omnivore	all	2	2	2	?	?	?		
DIPTERA												
Syrphus ribesii	larvae	yes	Aphids	canopy	1	3	2	?	?	?		
NEUROPTERA												
Chrysophidae	larvae	yes	Aphids	canopy	1	3	2	?	?	?		
HYMENOPTERA												
Aphidiidae	larvae	yes	Aphids	canopy	1	3	2	?	?	?		
FUNGI												
Entomophthorales		no	homoptera	all	1	2	1,5	?	?	?		
Metarhizium		yes	generalist	all	2	2	2	?	?	?		
NEMATODES												
Steinernema spp.	juvenile	no	generalist	all	2	2	2	?	?	?		

Annex 8 Ranking of soil living decomposers, obligate biotroph symbionts and pathogens based on maximum likelihood of exposure. ?: Gaps of knowledge

STEP 3: EXPOSURE RANKING SOIL LIVING DECOMPOSERS, OBLIGATE BIOTROPH SYMBIONTS, PATHOGENS AND NATURAL ENEMIES															
	Background information				Part 1: Possibility of exposure				Part 2: Likelihood of exposure						
Species or taxon	Life cycle stage with the soil organism function	Life cycle stage with other functions	Plant tissues/secretions on which the nontarget organism feeds	Growth stage of potato when organism is present	Is this feeding important for the organism?	Is feeding on potato important when organism lives in potato field?	Do plant tissues/secretions fed upon express the transgene product?	**Ranking of part 1 (Median)**	Are transgene products/metabolites detectable after feeding on plant	Probability of non-target organism containing transgene after feeding	Alterations in potato that might affect palatability for nontarget organism?	Changes in behaviour/feeding preferences that increase/decrease transgene exposition	**Ranking of part 2**	**Final rank**
DECOMPOSERS															
CLITELLATA															
Aporrectodea caliginosa	All	No	Dead plant material, fungi, bacteria, roots	All	3	1	1, feeds on plant tissue/secretions	1	?	?	?	?			
HYMENOPTERA															
Formica ssp.	All	No	Dead and living plant material, other arthropods	All	3	3	1, feeds on plant tissue/secretions	3	?	?	?	?			
COLLEMBOLA															
Folsomia fimetaria	All	No	Dead plant material, fungi, bacteria, roots	All	3	1	1, feeds on plant tissue/secretions	1	?	?	?	?			
Isotoma spp.	All	No	Dead plant material, fungi, bacteria, roots	All	3	1	1, feeds on plant tissue/secretions	1	?	?	?	?			
ACARI															
Oribatida	All	No	Dead plant material, fungi, bacteria, roots	All	3	1	1, feeds on plant tissue/secretions	1	?	?	?	?			
Uropodina	All	No	Dead plant material, fungi, bacteria, roots	All	3	1	1, feeds on plant tissue/secretions	1	?	?	?	?			
BACTERIA															
Streptomyces spp. (except for the spp. causing Potato powdery scab)	All	Yes, natural enemy (outcompetes pathogens)	Dead organic material	All	3	1	1	1	?	?	?	?			

PROCESSES														
Nematodes i mineralising processes	All	Yes, mineralisation processes	Dead plant material, fungi, bacteria, other nematodes, arthropods	All	3	1	1	1	?	?	?	?		
Mineralisation	All	No	Dead plant material	All	3	1	?	1						
Nitrification	All	No	Dead plant material	All	3	1	?	1						
OBLIGATE BIOTROPH SYMBIONTS														
Glomeromycota	All	Probably interacting with host plant immunity.	Receives all its energy from plants through carbon exchange in the root excudates	All	1	? The symbiotic relationship may be influenced	1	1	NA	3, but symbiotic relationship may be severely influenced	?	Suitability for the potato to establish symbiotic relationship may be changed		
PATHOGENS														
BACTERIA														
Streptomyces spp. Known to cause Potato powdery scab	All	Yes, decomposer	Potato plant and dead plant material	All	2 (To a smaller degree if potato is present, survives as saprophyte)	1	1	1	?	?	?	?		
Pectobacterium atrosepticum	All	Yes, decomposer	Potato plant and dead plant material	All	1,5	1	1	1	?	?	?	?		
Rhizoctonia solani (Anostomose group 3, potato specific)	All	Yes, decomposer	Potato plant and dead plant material	All	2 (Can survive as saprophyte)	1	1	1	?	?	?	?		
NEMATODES														
Pratylenchus spp.	All	No	Plant tissue, plant cell content	All	1;2	1	1	1	?	?	?	?		
Tylenchorhynchus ssp.	All	No	Plant tissue, plant cell content	All	1;2	1	1	1	?	?	?	?		
Trichodorus spp.	All	No	Plant tissue, plant cell content	All	1;2	1	1	1	?	?	?	?		

Paratrichodorus spp.	All	No	Plant tissue, plant cell content	All	1;2	1	1	1	?	?	?	?		
Globodera rostochiensis	All	No	Feeds from plant cell content, but influences by root exudates when hatching	All	1	1	1	1	?	?	?	?		
FUNGI														
Colletotrichum coccodes	All	Yes, decomposer	Potato plant and dead plant material	All	2	1	1	1	?	?	?	?		
Phytophthora erythroseptcia	All	No	Potato plant and other host plants	All	2 (Survive on other host plants)	1	1	1	?	?	?	?		