Inventory of observed unexpected environmental effects of genetically modified crops

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Applied Plant Research
Research Unit for Arable farming, Field production of vegetables and Multifunctional agriculture (PPO-AGV)
November 2010

CGM 2010-08, PPO no. 3250165700

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PPO project nr.: 3250165700

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Samenvatting

In de Europese Unie is een bedrijf dat genetisch gemodificeerde gewassen op de markt brengt, verplicht om na toelating te monitoren op het optreden van onverwachte negatieve milieueffecten. Aangezien er weinig ervaring is met het monitoren op deze onverwachte milieueffecten van GM-gewassen, is er een inventarisatie gemaakt van de onverwachte milieueffecten die zijn opgetreden in landen waar reeds gedurende de laatste 10 à 15 jaar genetisch gemodificeerde gewassen zijn geteeld. Het doel was om na te gaan of de verkregen informatie bruikbaar is voor het opstellen van monitoringsprotocollen. In de inventarisatie is in de eerste plaats aandacht besteed aan de gewassen die voor Nederland van belang zijn, met name maïs, suikerbieten, aardappelen, koolzaad en luzerne. Daarnaast is de inventarisatie, zij het op beperktere schaal, ook uitgevoerd voor sojaboon en katoen. De inventarisatie heeft zich uitdrukkelijk beperkt tot effecten op het milieu tijdens de teelt, de opslag na de oogst en het transport naar de verwerkende industrie. Effecten die eventueel kunnen optreden tijdens en na de verwerking of eventuele effecten op de volksgezondheid zijn buiten beschouwing gebleven. De inventarisatie is als volgt uitgevoerd: Er is uitgebreid gezocht op internet, vervolgens is de wetenschappelijke literatuur geraadpleegd en tenslotte is er informatie verzameld uit gesprekken met vertegenwoordigers van toelatingsinstanties en onderzoekers, m.n. tijdens een studiereis naar de Verenigde Staten. Tijdens het verzamelen van deze informatie bleek dat de monitoring na toelating van een GM-gewas zich in de Verenigde Staten vooral heeft gericht op agronomische aspecten en slechts in veel mindere mate op milieueffecten. Voor de indeling van milieueffecten in "verwacht" en "onverwacht" is als uitgangspunt gekozen of het effect al dan niet vermeld werd in de eerste Environmental Assessment Reports van de betreffende modificatie, die opgesteld zijn door het Animal and Plant Health Inspection Service (APHIS) van de USA. Bij de eindbeoordeling of een effect echt onverwacht was, is ook meegewogen of er ten tijde van de toelating wetenschappelijke informatie beschikbaar was waaruit bleek dat het effect destijds reeds verwacht werd.

Er zijn twee typen genetische modificaties die in een aantal gewassen op grote schaal gebruikt worden, m.n. in de Verenigde Staten, maar daarnaast ook in diverse andere landen: herbicidetolerantie en resistentie tegen insecten. Herbicidetolerante rassen worden gebruikt in de teelt van maïs, sojabonen, koolzaad, suikerbieten en katoen. Het gaat vooral om tolerantie voor glyfosaat (ca. 95%). Deze tolerantie-eigenschap wordt vaak aangeduid met Roundup Ready. Daarnaast worden ook rassen gebruikt met tolerantie voor het herbicide glufosinaat-ammonium (Liberty Link). Resistentie tegen insecten wordt op grote schaal gebruikt in maïs en katoen. Bij aardappelen zijn gedurende een beperkt aantal jaren op kleine schaal rassen geteeld met insectenresistentie. In maïs wordt Bt-gebaseerde insectenresistentie ingezet tegen de Europese maïsboorder (*Ostrinia nubilalis*) en de Maïswortelkever (*Diabrotica virgifera*). In katoen wordt de Bt-modificatie gebruikt om het gewas te beschermen tegen de belangrijkste plaag in dit gewas, de Katoendaguil (cotton bollworm, *Helicoverpa armigera*).

Herbicidetolerante gewassen

Bij maïs en bij sojaboon is in een beperkt aantal gevallen geconstateerd dat de opname van micro-nutriënten (m.n. Mn en Fe) door glyfosaattolerante rassen slechter is dan bij conventionele rassen. De slechtere opname is niet een direct effect van de genetische modificatie, maar is een indirect effect. De beperktere opname is een gevolg van het glyfosaat zelf. Dit effect was niet verwacht blijkens de Environmental Assessment Reports en de wetenschappelijke literatuur van die tijd. Het effect lijkt echter slechts in beperkte mate op te treden. In sommige gevallen zal een additionele bemesting moeten worden uitgevoerd. Dit heeft een beperkt negatief effect op het milieu.

Bij maïs, suikerbieten en sojaboon is gevonden dat de gevoeligheid voor ziekten bij glyfosaat-tolerante rassen na bespuiting met glyfosaat groter is dan bij conventionele rassen. Dit effect was onverwacht. In sommige gevallen maakte toegenomen vatbaarheid voor ziekten een extra ziektebestrijding noodzakelijk. Dit heeft een negatief effect op het milieu.

Op basis van veldproeven zijn er aanwijzingen dat glyfosaatbespuitingen bij glyfosaat-tolerante sojabonen

leiden tot een verminderde stikstofbinding door stikstofbindende bacteriën die in symbiose met de sojaboon leven. Dit effect was onverwacht. Tot nu toe zijn er echter geen aanwijzingen dat dit effect dusdanig groot is dat dit in commerciële teelten geleid heeft tot het uitvoeren van extra stikstofbemestingen.

Uit Engels onderzoek (Farm Scale Evaluations) waarbij nauwkeurige vergelijkingen zijn uitgevoerd tussen herbicidetolerante gewassen en conventionele gewassen blijkt dat de biodiversiteit wordt beïnvloed. Deze effecten zijn niet vermeld in de Environmental Assessment Reports van APHIS. Ze waren echter wel te verwachten, omdat, zoals in de wetenschappelijke literatuur toen ook al bekend was, elke wijziging in teelt- of onkruidbestrijdingstechniek dit soort effecten op de biodiversiteit kan hebben. In de vergelijking tussen glufosinaat-ammonium-tolerante maïs en conventionele maïs, waarin het onkruid voornamelijk bestreden werd met atrazin, werd vastgesteld dat er in de herbicide-tolerante maïs meer onkruiden voorkwamen, evenals meer insecten en onkruidzaden die als voedsel konden dienen voor vogels. Op akkers met bieten met glyfosaattolerantie en koolzaad met glufosinaat-ammoniumtolerantie werden daarentegen juist minder onkruiden, insecten en onkruidzaden waargenomen. De gevonden verschillen hangen o.a. samen met verschillen in effectiviteit van de betreffende onkruidbestrijdingsmiddelen en de momenten waarop deze middelen worden toegepast.

Voor de gebieden in de VS met intensieve maïsteelt is met behulp van modelstudies aangetoond dat de toepassing van herbicidetolerante maïs geleid heeft tot een kwaliteitsverbetering van het oppervlaktewater. Voorheen werden veel onkruidbestrijdingmiddelen gebruikt die in vergelijking met glyfosaat een negatiever effect op het milieu hadden en die ook in grotere hoeveelheden in het oppervlaktewater terecht kwamen. Dit effect werd niet genoemd in de Environmental Assessment Reports, maar werd door landbouwkundigen al wel verwacht.

Een belangrijk effect dat bij alle herbicidetolerante gewassen wordt gevonden, is de uitbreiding van het areaal waarop "reduced tillage"-systemen (minder intensieve grondbewerking) worden toegepast.
Het toepassen van "reduced tillage" systemen is ook mogelijk zonder de toepassing van herbicidetolerante gewassen, maar deze gewassen hebben zeker een bijdrage geleverd aan de uitbreiding van deze systemen. "Reduced tillage" systemen hebben in veel situaties een positief effect op het milieu. Erosie wordt tegengegaan en er is vanwege de verminderde grondbewerking een positief effect op de fysische, chemische en biologische toestand van de bodem. Het uitvoeren van minder grondbewerkingen levert ook een energiebesparing op en hiermee ook een beperking van de broeikasgasemissies. Ten tijde van het opstellen van de eerste Environmental Assessment Reports, werd in de landbouwkundige literatuur reeds voorspeld dat uitbreiding van het areaal met "reduced tillage" mede mogelijk zou zijn door het toepassen van herbicide-tolerante gewassen. Het effect wordt niet vermeld in de eerste Environmental Assessment Reports, maar het werd dus wel verwacht door verschillende deskundigen.

In de Environmental Assessments Reports van APHIS werd er rekening mee gehouden dat het gebruik van herbicidetolerante gewassen herbicideresistente onkruiden kon opleveren en dat er herbicidetolerante opslagplanten in volggewassen zouden worden aangetroffen. Als gevolg van de grootschalige introductie van herbicidetolerante maïs, sojaboon en katoen, die in sommige regio's ook in rotatie met elkaar geteeld worden, hebben zich meer herbicidetolerante onkruiden ontwikkeld dan was voorzien. Hetzelfde geldt voor het optreden van herbicidetolerante opslagplanten in volgteelten. Zowel het optreden van herbicideresistente onkruiden als het optreden van herbicidetolerante opslagplanten heeft geleid tot de extra inzet van onkruidbestrijdingsmiddelen. Herbicidetolerante opslagplanten kunnen, indien ze niet voldoende worden bestreden, ook de vruchtwisseling verstoren, waardoor soms extra ziektebestrijdingen nodig zijn. Bij koolzaad dat in Canada in een ruime vruchtwisseling met granen geteeld wordt, blijken er vanwege de lage selectiedruk geen glyfosaatresistente onkruiden te ontstaan. Wel werden hierbij verschuivingen in het soortenspectrum van onkruiden vastgesteld. Bij de introductie van herbicidetolerante gewassen werd een effect op het herbicidengebruik verwacht. Door de grootschalige introductie is dit effect ook opgetreden. Bij maïs, sojaboon, koolzaad, suikerbiet en katoen heeft de vervanging van de voorheen gebruikte herbiciden door glyfosaat een verlaging van de milieu-belasting tot gevolg gehad.

Bij suikerbieten en koolzaad werd verwacht dat op beperkte schaal herbicidetolerantie genen zouden uitkruisen met verwante soorten en ferale populaties. Bij koolzaad is uitkruising met verwante soorten op beperkte schaal geconstateerd. Daarnaast is de verspreiding van herbicidetolerantiegenen bij koolzaad naar in het wild voorkomende koolzaadpopulaties op grote schaal vastgesteld. Bij suikerbieten is de teelt van herbicidetolerante rassen dusdanig recent dat er nog geen gevallen bekend zijn. Wel is uit grootschalige veldproeven gebleken dat uitkruising met onkruidbieten op grote schaal mogelijk is.

Bt-gewassen

Zowel bij maïs als bij katoen is er veel discussie geweest over onverwachte effecten op andere insecten dan de insecten waartegen Bt-resistentie wordt ingezet. Deze discussie had vooral betrekking op de Monarch vlinder en water- en bodemorganismen. Op basis van uitgebreide wetenschappelijke analyses van de beschikbare data is in een aantal publicaties geconcludeerd dat er geen eenduidige negatieve effecten op andere insecten zijn gevonden, afgezien van insecten die nauw verwant zijn aan de doelinsecten. Bij deze verwante insecten zijn echter de effecten van het gebruik van insecticiden groter dan het Bt-effect. Er zijn ook effecten van Bt-gebaseerde insectenresistentie vastgesteld op parasieten en predatoren van het insect waartegen de resistentie wordt ingezet. Het blijkt echter dat deze effecten toegeschreven moeten worden aan de afname van het aantal insecten waarvan deze parasieten en predatoren leven en ook aan de afname van de voedingskwaliteit van deze insecten.

Zowel in maïs als in katoen is gevonden dat de inzet van Bt-resistentie leidt tot het optreden van secundaire plagen. In de VS is sinds de introductie van Bt-maïs de schade door de western bean cutworm (*Striacosta albicosta*) toegenomen. Ook in katoen zijn er situaties bekend waarbij er in Bt-katoen meer insecten gevonden werden die zuigschade veroorzaken dan in conventioneel geteelde katoen. Het optreden van secundaire plagen werd niet vermeld in de eerste Environmental Assessment Reports. Op basis van ervaringen met de inzet van insecticiden kon het optreden van secundaire plagen wel verwacht worden.

In maïs heeft de inzet van Bt-resistentie tegen de Maïswortelkever als neveneffect gehad dat het gewas minder gevoelig is voor droogte en legering. Deze effecten zijn een logisch gevolg van het feit dat het wortelstelsel minder beschadigd wordt. Het effect was dan ook niet geheel onverwacht. Bt-resistentie tegen de Europese maïsboorder heeft tot gevolg gehad dat er minder schade door schimmelziekten, m.n. Fusarium in maïs optreedt, wat ook tot gevolg heeft dat er minder mycotoxinen in het geoogste product voorkomen. Dit effect is te verklaren door de afname van beschadigingen aan de maïsplant, waardoor schimmelziekten minder gemakkelijk kunnen optreden. Op het moment van toelating werd het effect niet beschreven in de Environmental Assessment Reports van APHIS en, voor zover bekend, waren er op dat moment ook geen publicaties beschikbaar waarin het effect werd vermeld.

In de VS is gevonden dat het grootschalige gebruik van Bt-maïs er toe heeft geleid dat op regionaal niveau de populaties van de insecten die bestreden worden dusdanig zijn afgenomen dat er ook in andere gewassen, zoals sojabonen en groenten, minder bestrijdingen van insecten nodig zijn. Ook in maïs zonder Bt-resitentie was minder insecticide nodig. Deze effecten zijn wel verklaarbaar, maar zijn niet beschreven in de eerste Environmental Assessment Reports van APHIS en, voor zover bekend, waren er op dat moment ook geen publicaties beschikbaar waarin deze effecten werden vermeld.

Zowel in maïs als in katoen is gebleken dat de groeiomstandigheden een effect hebben op de expressie van het Bt-toxine. In katoen is gevonden dat dit het geval was na perioden met hoge temperaturen. Bij maïs zijn er aanwijzingen dat dit het geval is bij het optreden van stikstoftekort. In een onderzoek werd aannemelijk gemaakt dat bij glyfosaattolerante opslagplanten van maïs in sojavelden, waarop geen stikstofbemesting wordt uitgevoerd, het Bt-toxine in mindere mate geproduceerd wordt, waardoor de Maïswortelkever minder goed bestreden wordt. Dit zou de ontwikkeling van resistentie van dit insect tegen Bt kunnen bevorderen. Dit effect is echter niet op grote schaal gevonden in de commerciële teelt.

In Bt-aardappelen met resistentie tegen Coloradokever kwamen meer predatoren voor van luizen dan in aardappelen waarin de Coloradokevers bestreden werden met insecticiden. Dit effect werd niet vermeld in de eerste Environmental Assessment Reports van APHIS. Het effect is echter niet onverwacht, omdat in de literatuur reeds beschreven was dat vergelijkbare effecten ook optreden bij de toepassing van insecticiden

die een specifieke werking hebben op bepaalde insecten.
Bij aardappelen met Bt-resistentie is gevonden dat de gemodificeerde gewassen ook in andere eigenschappen verschilden dan in Bt-resistentie, o.a in resistentie tegen nematoden. Dit is een onverwacht effect. Het effect is ontstaan doordat bij de selectie van Bt-resistente lijnen de resistentie tegen nematoden verloren is gegaan. Indien de selectie zorgvuldiger zou zijn uitgevoerd, zou dit effect niet opgetreden zijn.

Zowel bij maïs als bij katoen werd verwacht dat de insecten waartegen de Bt-resistentie wordt ingezet, resistent zouden worden tegen het Bt-toxine. Bij beide gewassen is dit echter maar in zeer beperkte mate voorgekomen. Het gebruik van Bt-resistentie heeft bij beide gewassen het gebruik van insecticiden verlaagd, zoals ook verwacht werd op het moment van toelating.

Algemene conclusie

In het algemeen kan gesteld worden dat er in de grootschalige commerciële teelten geen grote onverwachte milieueffecten van herbicidetolerante gewassen en gewassen met insectenresistentie zijn gevonden. Het is mogelijk dat bepaalde effecten pas na een periode die langer is dan 10 -15 jaar, zichtbaar worden. Tot nu toe zijn hiervoor echter geen aanwijzingen.
Naast de rapportage van de Farm Scale Evaluations (FSE) zijn er geen andere rapporten gevonden van onderzoek waarin monitoring van effecten van genetisch gemodificeerde gewassen op de biodiversiteit in akkerranden en omringende ecosystemen heeft plaats gevonden.
Directe onverwachte effecten van de genetische modificatie zijn zowel bij de herbicidetolerante gewassen als bij de Bt-gewassen niet gevonden. Indirecte onverwachte effecten zijn wel geconstateerd bij de herbicidentolerante gewassen, nl. een effect op de opname van micronutriënten en een effect op de ziektegevoeligheid. Deze effecten zijn echter zeer specifiek voor glyfosaattolerante gewassen. Algemene conclusies die bruikbaar zijn voor het opstellen van monitoringsprotocollen zijn hieruit niet te trekken.

Summary

In the EU, the consent holder of a particular GM crop is obliged to monitor the occurrence of unexpected adverse effects after its release on the market. As there is only limited experience with monitoring of unexpected environmental effects of GM crops, a literature inventory was performed of the unexpected environmental effects observed in countries where GM crops have been grown already on large areas in the last 10 – 15 years. The objective of this inventory was to find clues for developing protocols for monitoring environmental effects during post-release growing of GM crops. The inventory primarily focused on crops grown in the Netherlands: maize (*Zea mays*), sugar beet (*Beta vulgaris*), potato *(Solanum tuberosum*), oilseed rape *(Brassica napus*) and alfalfa (lucerne, *Medicago sativa*). In addition, information was also gathered about soybean (*Glycine max*) and cotton (*Gossypium* spp.). The inventory was restricted to field cultivation, post-harvest storage and transport to the processing industry. Effects during and after processing or effects on human health (food safety), and effects only observed under laboratory conditions were not considered. The inventory was executed as follows: scientific literature retrieved from databases was studied, additional searches for reports etc. were performed on internet, and information was gathered by interviewing representatives of the authorities responsible for releasing GM crops and scientists, in particular during a study visit to the USA. During the study it became clear that post-release monitoring of a GM crop in the USA was performed primarily on agronomic aspects and only to a lesser degree on environmental effects. Classification of environmental effects into "expected" and "unexpected" was in the first place based on the information available from the first Environmental Assessments Reports made by the Animal and Plant Health Inspection Service (APHIS) of the USA. The criterion for classification an effect as "unexpected" was that it was not mentioned in these reports. Subsequently, we evaluated to which degree an unexpected effect - according to this criterion - was really unexpected. Some effects not mentioned in the first Environmental Assessment Reports were already expected at that time by scientists, as was apparent from scientific literature.

Two types of genetic modifications have now already been in use on a large scale in a number of crops, especially in the USA, but also in various other countries: herbicide-tolerance and Bt resistance against certain insect pests. Herbicide-tolerance is used in maize, soybean, oilseed rape, sugar beet and cotton. Tolerance to glyphosate (Roundup Ready) is the most important of the herbicide-tolerance traits; it is incorporated in 95% of all herbicide-tolerance crops. In addition, the most common alternative is provided by crop varieties with tolerance to glufosinate-ammonium (Liberty Link). Bt resistance against insects is used in maize and cotton and in the past it has been used for a few years in potato cultivars that since have disappeared from the market. In maize, Bt resistance is used against the European corn borer and the western corn rootworm. In cotton, Bt resistance is used against the cotton boll worm.

Herbicide-tolerant crops

Sometimes it has been reported for maize and soybean that the uptake of micro-nutrients (especially Mn and Fe) was lower in glyphosate-tolerant varieties than in conventional varieties. This was probably caused by the glyphosate application coming with the herbicide-tolerant crop cultivation. This effect was not expected by anyone. However, the effect seemed to occur on a restricted scale. Additional fertilization may occasionally be required to compensate for a reduced nutrient uptake. This could have a negative effect on the environment.

In maize, sugar beet and soybean there were observations that glyphosate-tolerant varieties were more susceptible to some diseases than conventional varieties. This effect was not expected by anyone. In some cases, an extra spraying against diseases was needed. This would have a negative effect on the environment.

Some studies indicated that glyphosate applications on glyphosate-tolerant soybean varieties reduced nitrogen fixation by bacteria in the nodules on the roots. This effect was not expected. However, in

commercial fields the effect was small and has not resulted in additional nitrogen applications as far as we are aware.

In large-scale comparisons between herbicide-tolerant crops and traditional crops (the Farm Scale Evaluations, performed in the United Kingdom), effects on biodiversity were observed: in glufosinate-ammonium-tolerant maize varieties generally more weeds, accompanying invertebrates and weed seeds important to granivorous bird species were occurring than in maize varieties grown in a weed control system with atrazine. When glyphosate-tolerant sugar beet and glufosinate-ammonium-tolerant rapeseed were compared to traditional varieties, this was the other way around. Such effects were not mentioned in the first Environmental Assessment Reports. However, these effects were not completely unexpected, because different herbicide regimes will have different weed control efficacies with their secondary effects on animals dependent on weed plants.

A model study performed for the intensely farmed maize-growing regions in the USA showed that the use of herbicide-tolerant maize has improved the quality of surface water. Before the introduction of glyphosate-tolerant maize, many herbicides were used with a relatively negative impact on the environment that contaminated the surface water as a result of precipitation run-off. This effect was not mentioned in the Environmental Assessment Reports, but it was expected by agronomists from the relatively faster degradation of glyphosate and glufosinate-ammonium.

An important effect associated with herbicide-tolerant crops was the increased adoption of reduced tillage systems. Although the adoption of reduced tillage systems is also possible without the use of herbicide-tolerance traits, the introduction of herbicide-tolerant varieties has contributed to the increase of the area with reduced tillage systems. Reduced tillage systems have in most cases a positive environmental effect. It assists in reducing soil erosion, improving soil physical, chemical and biological properties and it minimizes the environmental costs of ploughing. From an agricultural point of view it is logical that the introduction of herbicide-tolerant varieties has facilitated the adoption of reduced tillage systems. The effect was not mentioned in the first Environmental Assessments Reports, but it was expected by different agronomic specialists.

The first Environmental Assessment Reports of APHIS mentioned the possibility of the development of weed biotypes resistant to herbicides and the occurrence of herbicide-tolerant volunteers in other crops. Due to the large-scale adoption of glyphosate-tolerant maize, soybean and cotton, sometimes grown in rotation with each other, the development of glyphosate-resistant weeds has occurred more frequently than expected. Occurrence of herbicide-tolerant volunteers sometimes has made it necessary to use additional herbicides. Glyphosate and other herbicide usage may also have increased in reaction to development of resistence in some weed species. Herbicide-tolerant volunteers could potentially increase pests and diseases by allowing populations to increase during the year that another crop is growing. Sometimes extra disease control may also be needed. At the moment of introduction of herbicide-tolerant crops, however, a decrease in herbicide use was expected. With the very rapid adoption of these crops, this effect has been reported indeed, at least for the early years. In maize, soybean, rapeseed, sugar beet and cotton, glyphosate replaced several herbicides with a larger environmental impact.

In sugar beet and oilseed rape, gene flow to related species and feral populations was expected on a limited scale. In rapeseed, gene flow to related species has indeed been reported. Gene flow to feral rapeseed (*Brassica rapa*) population was observed on a large scale. Due to the very recent adoption of GM sugar beet no examples of gene flow have been observed until now. From large scale experiments it is known that gene flow to weed beets is possible.

Bt crops

There has been a lot of discussion about the effects of Bt maize and Bt cotton on non-target invertebrates. This discussion was especially referring to the Monarch butterfly, water organisms and soil organisms. From extended scientific analysis of the available information published in scientific articles it can be concluded that no consistent adverse effects were found of the Bt trait on non-target organisms, except for some members of groups to which the targeted plague insects belong. However, among members of these groups, mainly lepidopterans and coleopterans, the effects of insecticide treatments are generally larger than the effect of the Bt trait. There are also effects on some parasitoids and/or predators of the target insect pest, but in general, this can be ascribed to an effective pest control, reducing prey numbers and/or quality.

With Bt crops, the occurrence of secondary pests was reported. In the USA, the introduction of Bt maize caused an increase of damage by the western bean cutworm. In Bt cotton, sometimes greater numbers of sucking insects were observed in Bt fields than in non-Bt fields. The occurrence of secondary pests was not mentioned in the First Environmental Assessment Reports. Based on the experiences with insecticide treatments the occurrence of secondary pest could have been expected.

The use of Bt resistance against the western root worm in maize led to less root damage and consequently, the crop was less susceptible to drought and lodging. Although not expected by APHIS, these are logical side effects of rootworm resistance. Bt resistance in maize against the European corn borer made the crop less susceptible to fungal diseases, especially *Fusarium*. This has generally led to less mycotoxins in the harvested product. This effect could be attributed to reduced damage caused by the corn borer, which gave less opportunities for *Fusarium* infection. Although not expected by APHIS, these are logical side effects of rootworm resistance.

In the USA, evidence was provided of regional suppression of targeted lepidopteran populations caused by the large-scale adoption of Bt maize. This led to pest management benefits in other crops, such as conventional maize, but also soybean and vegetables. Also refuge fields grown with maize without Bt-resistance were suffering less from western corn rootworm and European corn borer. This effect was not mentioned in the first Environmental Assessment Reports of APHIS. The effect is a logical consequence of the use of Bt maize on a large scale. At the moment of the first release of Bt maize no scientific paper was available that was predicting this effect.

In maize and in cotton, the expression of the Bt gene has been shown to depend on crop growing conditions. In cotton, a reduced efficacy was found after periods of high temperatures. In maize, there were indications that the level of Bt toxin production was reduced under conditions of low nitrogen supply. Volunteer maize plants with low levels of Bt toxins when growing under low nitrogen supply in soybean fields could allow survival of western corn rootworm. As a consequence, the development of Bt-resistant western corn rootworm populations may be increased. However, this effect has not been confirmed yet in commercial maize-soybean rotations.

In potatoes with Bt resistance against Colorado potato beetle, higher numbers of aphid predators were found than in potatoes treated with insecticides. This effect was not mentioned in the first Environmental Assessment Reports of APHIS. However, it was not really unexpected as comparable effects occur with the application of specific insecticides.

In potatoes with Bt resistance against Colorado potato beetle, also other characteristics of the crop were reported to have changed, for example, a change in nematode resistance. This was an unexpected side-effect. It could be due to the occurrence of somaclonal variation during the transformation procedure which had passed the selection process undetectedly or not perceived as relevant to variety marketing. This effect could probably have been avoided by a more careful selection methodology.

In Bt maize and Bt cotton, development of resistance against Bt by insects was expected. However, in both crops this has been rare up till now. As expected, the adoption of Bt resistant varieties has reduced the use of insecticides.

General conclusion

In general, it can be concluded that very few clearly unexpected effects were observed during the large scale post-release growing of herbicide-tolerant crops and Bt crops. Of course, one has to keep in mind

that there will always be an element of subjectivity in assessing effects as “unexpected”. Furthermore, it is also possible that certain effects are becoming visible only after a longer period than 10 – 15 years, but no indications for such effects were found in the literature.
Besides the reports about the Farm Scale Evaluations no other reports were found with information about effects of GM crops on biodiversity in ecosysystems surrounding the production fields.
Unexpected effects caused directly by the genetic modification were not found. Only in herbicide-tolerant crops, we concluded to some indirect unexpected effects: the reduced uptake of micro-nutrients and some positive and negative effects on susceptibility to diseases. These effects were specific to herbicide use with glyphosate-tolerant crops. Based on this, it was not possible to draw general conclusions for developing protocols for post-release monitoring of environmental effects.

1 Introduction

The introduction of genetically modified crops in Europe is subject to continuous debate. Until now only a few GM crops have been released in the EU. In practice, there are only two examples that are presently allowed for cultivation, viz. insect-resistant Bt maize and a GM potato with a modification of starch composition (Amflora). In Europe, Bt maize is mainly grown in Spain as well as in some other EU countries. Several countries, notably France and Germany, have enacted a ban on this GM maize, invoking the precautionary principle. Cultivation of the GM potato Amflora has been approved for growing in the EU in March 2010 and is now grown in Sweden. In other countries outside Europe, especially in North America, there is much more experience with the cultivation of GM crops. In the USA for instance, GM varieties of maize (corn), soybean and cotton have already been grown on large areas for 10–15 years.

In the EU the consent holder of a particular GM crop is obliged to monitor the potential occurrence of unexpected adverse effects after its release on the market, the so-called 'general surveillance'. It is no simple task to find such effects without good hypotheses on what could happen and therefore, it would be worthwhile to make an inventory of any unexpected effects that might already have been observed during commercial GM crop cultivation. This information could then be helpful in adjusting the environmental risk assessment or give reason to set up case-specific monitoring.

This report presents the results of an inventory of the occurrence of unexpected environmental effects observed during the cultivation of GM crops after release on the market. The inventory is primarily performed for the USA and Canada, but any available information from other countries has been used as well. The inventory is restricted to environmental effects observed during post-release cultivation of GM crops or in large-scale field experiments. For example, the field experiments of the Farm Scale Evaluation (FSE) project in the UK with GM maize, beet and oilseed rape, and the long-term experiments with GM sugar beet in France are included. Effects only found under laboratory conditions are not reported. Effects occurring after processing or effects on human health (food safety) are not considered as part of this study. Also effects related to the coexistence of GM and non-GM crops, i.e., their cultivation under measures ensuring segregation of both production chains, are not considered.

According to Directive 2001/18/EC, GM events that are released on the EU market should be submitted to post-market monitoring. Post-market monitoring consists of two types of monitoring; 1) Case-Specific Monitoring (CSM) and 2) General Surveillance (GS). CSM is focused on specific uncertainties identified in the risk assessment that may still exist after approval for cultivation, or is meant to further confirm assumptions made in the risk assessment of a specific GM event. CSM is not required for every GM event that is approved for cultivation. In contrast, every GM event requires GS. GS is defined as "to detect unanticipated adverse environmental effects which were not identified in the environmental risk assessment". As GS is directed at observing potential effects of GM events that are not expected based on the risk assessment of the GM event, GS is in principle unfocussed and not related to the specific GM-crop combination. Effects may become manifest in the long term or in the short term, inside or - as a result of spreading of the GMO - also outside the GM cultivation area. Thus, expected adverse effects are effects that have been identified in the environmental risk assessment evaluated by the competent authorities before introduction on the market.

Classification of effects as 'expected' and 'unexpected' is depending on time and authority. In this inventory, classification of effects is based on the information that was available from the Environmental Assessments (EA) of the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture at the time of introduction of the relevant crops, since this is easily accessible information and much of the cultivation experience and publications on this were also gathered in the USA.

The report is focusing on General Surveillance, thus on unexpected effects. However, some expected effects are described as well, if there were unexpected issues related to degree of occurrence causing scientific/societal debate. The described effects are classified as follows:

1. Expected effects that were already mentioned in the first Environmental Assessment Reports of APHIS concerning the genetic modification, but the degree of occurrence was higher or lower than expected.
2. Unexpected effects.

The report is structured according to this classification of effects. However, in Chapter 9 (Discussion) unexpected effects are evaluated with respect to the question whether they really were unexpected according to the above-mentioned definition. Some of the effects not mentioned in the first Environmental Assessment Reports of AHPIS were at that time expected by scientists.

In the report a distinction is also made between direct effects and indirect effects. A direct effect is directly related to the modification, for example a Bt crop can have adverse effects on non-target lepidopterans. An indirect effect is a consequence of using the GM crop, for example an effect of herbicide on uptake of nutrients associated with the cultivation of a herbicide-tolerant crop.

The inventory was started with a literature and internet search, followed by gathering information via personal contacts. In the report the effects are given per crop. The inventory primarily focused on crops grown in The Netherlands with commercial growing of GM varieties in other countries: maize, sugar beet, potato and oilseed rape. In addition, information was gathered on soybean and cotton. This information is included because there is a lot of information available about GM soybean and GM cotton, and because their products are imported into The Netherlands and Europe.

In most countries post-release monitoring is not performed on a structural basis. Reporting about adverse effects on the environment or on human health is required for EPA (Environmental Protection Agency)-registered products in the USA (Schoen *et al.*, 2008). As a condition for registration of certain transgenic crops expressing Bt, the EPA has required post-commercialisation monitoring for evolution of resistance to Bt in target pests and accumulation of the Bt in soil. If new information demonstrates unanticipated effects or plant health risks, the United States Department of Agriculture (USDA) can re-establish the regulated status of the product. Not all information is available to the general public, because it is part of proprietary confidential business information. In Canada post-release monitoring is conducted on an ad-hoc basis (Beckie *et al.*, 2006). This means that the detection of unexpected effects is depending on research projects started by universities, research institutes or NGOs, like the Union of Concerned Scientists in the USA.

The inventory has been executed as follows:

- Searches have been performed on the internet (Web of Science, OvidSP, Google, Google Scholar). Searches were started by general search terms (for example: “Bt corn environmental effects” and “unexpected”, “glyphosate-tolerant canola gene flow”). Later, more specific search terms were used (for example: “weed control glyphosate-tolerant sugar beet”). Both scientific information and “grey information” (i.e. reports without peer review) was found. “Grey information” was checked by looking for confirmation from peer-reviewed scientific journals.
- Information from Greenpeace and GMwatch was also used to find assumed adverse effects. These effects are described in the report if additional information enabling assessment of these effects could be found in scientific literature.
- Expert knowledge was also used. Knowledge originated from scientists (including the authors of this report) who had gathered peer-reviewed papers on GM crops on a regular basis and/or are involved in GM crop research.
- Information was also obtained from personal communication. In June 2010, a study visit was made to Washington and Minnesota. In Washington the authorities responsible for releasing GM crops were visited: Animal and Plant Health Inspection Service (APHIS) and Environmental Protection Agency (EPA). In Washington the Center for Science in the Public Interest was visited as well. In Minnesota a visit was made to the University of Minnesota, Department of Agronomy and Plant Genetics, and the Department of Insect Ecology. A personal report of the interviews can be found in the appendix.

The report is structured as follows. Chapters 2 - 8 are describing the effects for the crops maize, sugar beet, potato, rapeseed, alfalfa, soybean and cotton, respectively. General crop characteristics and the adoption of the GM crop are described in each chapter. Adoption is expressed as the percentage of the crop area grown with GM varieties. This is followed by a description of the effects: first the expected effects that have been occurring more - or less - than expected, and second the unexpected effects. In chapter 9, the effects are discussed in relation to the Dutch conditions, by assessing the relevance of the effects for the Dutch situation. Implications for monitoring systems and environmental risk assessments are given as well.

2 Maize (*Zea mays*)

2.1 Introduction

2.1.1 Crop characteristics

Maize is an annual, monoecious, outcrossing (about 5% self-pollinated), primarily wind-pollinated crop that produces abundant pollen. As the ears are protected by the husks, maize kernels have a low potential for being scattered during harvest. However, any maize grains or cobs left on the field after harvest can result in volunteer plants the following year, depending on climatic conditions, which are unfavourable in northern Europe because of its cold winters.

2.1.2 Adoption of GM maize

Maize (usually called corn in North America) is one of the leading grain crops in the world with 157 million hectares in 2008 and at this moment it is also one of the largest biotech crops worldwide. Genetically modified (in the USA also called genetically engineered (GE)) maize was grown for the first time in the USA in 1997. Since then, GM maize production has expanded to 42 million hectares (26%) worldwide (GMO-compass, 2010). Maize is the leading grain crop in the USA, with 35 million ha planted in 2009 (USDA). In 2009, about 85% of the maize produced in the US was genetically modified. But GM maize is also grown in many other countries in North (Canada) and South America (Argentina, Brazil), Africa (South Africa and Egypt) and Asia (Philippines). Maize is the only GM crop that is commercially grown in the EU. The first GM maize lines were approved in the EU in 1997. Spain became Europe's first country to put it to use. Nowadays, genetically modified varieties are grown on about 80,000 hectares (24%) of Spanish maize. In addition, production of GM maize is now also taking place, albeit to a lesser extent, in the Czech Republic and in Portugal.

Two traits are used in today's GM maize cultivars: insect-resistance (IR, at present based on Bt genes) and herbicide-tolerance (HT). More and more, cultivars are being grown that have these traits simultaneously (stacked genes). Most of the herbicide-tolerant cultivars have been engineered to withstand glyphosate on the basis of the CP4 EPSPS transgene (Roundup Ready or RR cultivars). Another, far less used, type is based on tolerance to glufosinate-ammonium (Liberty Link or LL cultivars). The insect resistant maize cultivars produce a crystalline toxic protein (Cry protein) originally from the soil bacterium *Bacillus thuringiensis* that protects the maize against specific insect pests. There are several types of Cry proteins with different action spectra with respect to pest organisms. The most frequently used ones are Cry1Ab against the lepidopteran pest, the European corn borer (ECB, *Ostrinia nubilalis*) and Cry3Bb against the coleopteran pest western corn rootworm (WCR, *Diabrotica virgifera* subsp. *virgifera*).

The 2000-2009 GM adoption data for the USA (Table 1) were collected as part of the June Agricultural Survey conducted by USDA NASS (National Agricultural Statistics Service) during first two weeks of June. Randomly selected farmers across the United States have been asked since 2000 whether they planted GM maize, soybeans or cottonseed that had been made resistant to herbicides, insects, or both, via genetic modification. Conventionally bred herbicide-tolerant varieties were discarded. Adoption data for 1997-1999 (Table 1) included herbicide-tolerant maize obtained via traditional breeding methods. Therefore, these data cannot be compared with the more recent data (2000-2009) in which these varieties were discarded.

Table 1. **Genetically modified (GM) maize varieties (percentage of all maize planted); United States 2000-2009.**

	Insect-resistant (Bt) only	Herbicide-tolerant only	Stacked gene varieties	All GM varieties
1997				10
1998				25
1999				35
2000	18	6	1	25
2001	18	7	1	26
2002	22	9	2	34
2003	25	11	4	40
2004	27	14	6	47
2005	26	17	9	52
2006	25	21	15	61
2007	21	24	28	73
2008	17	23	40	80
2009	17	22	46	85

Herbicide-tolerant (HT) crops, developed to survive application of specific herbicides that previously would have destroyed the crop along with the targeted weeds, provide farmers with a broader range of options for effective weed control. Based on USDA survey data, the adoption of herbicide-tolerant maize, which was slower in previous years than soybeans and cotton, has recently accelerated, reaching 68 percent of the USA maize acreage in 2009 (Table 1). The economic advantages of herbicide-tolerant maize were not as clear as those of herbicide-tolerant soybeans (Cerdeira and Duke, 2006). The glyphosate-tolerant (GT) type of HT maize was deregulated in the USA in 1997, and, by 2007, 50% of the total USA maize crop was planted to herbicide-tolerant hybrids, with glyphosate-tolerant maize accounting for the majority of the plantings. Glyphosate is particularly effective because of its systemic mode of action (the herbicide is translocated within the weed plant and thus has a stronger effect than contact herbicides that stay on and affect only the surface of the plant). GM maize hybrids with resistance to glufosinate-ammonium have also been released in North America, but are estimated to be planted on only about 5% of the US maize surface area (Beckie & Owen, 2007).

Insect-resistant crops expressing Cry proteins have been available for maize since 1996. Plantings of Bt maize increased from about 8 percent of the USA maize acreage in 1997 to 26 percent in 1999, then fell to 19 percent in 2000 and 2001, before climbing to 29 percent in 2003 and 63 percent in 2009. The increases in acreage share in recent years may be largely due to the commercial introduction in 2003/04 of new Bt maize varieties containing genes (e.g. MON863 and MON88017) encoding the Cry3Bb protein against the western corn rootworm (WCR), which may be more destructive to maize yield than the European corn borer (ECB), which was previously the only pest targeted by Bt maize (e.g. the MON810 event).

2.1.3 Overview of environmental effects of GM maize

Several environmental effects have been studied and published since the release of GM maize on the market. The environmental effects of herbicide-tolerant maize and Bt maize as identified in the inventory are summarised in Table 2 and are discussed below.

Table 2. **Overview of observed environmental effects of GM maize.**

Effect	Expected by APHIS in first EA	Observed in commercial production or in large scale experiments	Scientifically confirmed as a relevant environmental effect
Herbicide-tolerant Maize			
Glyphosate-tolerant maize volunteers	+	+	+
Glyphosate-resistant weeds	+	+	+
Effect on herbicide use	+	+	+
Increased area with reduced tillage systems	-	+	+
Effects on biodiversity (FSE)	-	+	+
Reduced uptake of micronutrients	-	+	+
Increased susceptibility to diseases	-	+	+
Effect on surface water quality	-	+	+
Bt-Maize			
Resistance development against Bt	+	+	+*
Effect on insecticide use	+	+	+
Effect on non-target invertebrates	-	+**	-
Resistance development on maize volunteers in soybean	-	+	+
Decreased susceptibility to Fusarium	-	+	+
Increased damage by western bean cutworm	-	+	+
Reduced lodging	-	+	+
Increased drought tolerance	-	+	+
Pest management benefits in other crops and non-Bt maize	-	+	+

*: on limited scale
**: Concerns members of groups to which the targeted plague insects belong and effects on some parasitoids and/or predators of the targeted pests that can be ascribed to the decrease in pest prey numbers and/or their quality

2.2 Expected effects of herbicide-tolerant maize

2.2.1 Glyphosate-tolerant maize volunteers

The occurrence of glyphosate-tolerant maize as weed in other crops was expected from the moment that glyphosate-tolerant maize was released to the market (APHIS, 1997), but it was not clear how big the problem would be. Volunteer maize as a weed in soybean has indeed become an important problem. The very rapid adoption of glyphosate-tolerant varieties of the major crops soybean, cotton and maize grown in rotation is an important factor in this respect. In the state of Indiana, the prevalence of volunteer maize in soybean increased from an average of 3% of all fields sampled in 2003 to 12% of all fields sampled in 2005. This increase is strongly correlated with the adoption of glyphosate-tolerant maize (Davis et al., 2008). According to Beckie and Owen (2007), maize volunteers are also occurring in Canada. Farmers need to apply additional herbicide treatments specifically to manage volunteer maize. This extra input of alternative herbicides to control volunteer maize counteracts the advantages of the shift towards the use of glyphosate, which has one of the lowest environmental impact values of herbicides in common use. Volunteers can severely reduce soybean yield, up to 56% (Golden Harvest, 2008). Volunteer maize can also physically interfere with the soybean harvest and its grain can commingle with the harvested soybean if it remains in the field until maturity. Management strategies are developed to control these volunteers.

Occurrence of maize volunteers could potentially increase maize pest insects or diseases by allowing populations to increase during the year that soybean is grown (Beckie and Owen, 2007). The climatic conditions in Northern Europe are different from the conditions in the USA. In general, maize volunteers are not occurring under Dutch conditions. In recent years, researchers of PPO encountered maize volunteers in two of their research fields, which also contained exotic materials. Occurrence of volunteers in agicultural practice has to our knowledge not been found or scientifically reported in the Netherlands.

2.2.2 Effect on amount of herbicides applied

There has been controversy about the question whether the use of herbicide-tolerant varieties has increased herbicide usage or not. In this discussion, not only the total volume of herbicide is important, but also the environmental damage and toxicological risks. Glyphosate is not a low-dose herbicide, but in comparison to several herbicides used in conventional crops it is considered to be a lower risk herbicide in terms of environmental effects (low Environmental Impact Quotient, EIQ, e.g. Kleter *et al.* 2008). The use of herbicides also depends on the shift to weed species that are becoming more important after application of glyphosate in consecutive years. Sometimes it is needed to increase the number or doses of glyphosate applications. The cost of glyphosate has decreased since 2000 as result of the termination of patent protection. This makes higher application rates economical in some cases. Sometimes the use of other herbicides with more adverse environmental effects is needed (Cerdeira and Duke, 2006). According to Benbrook (2009), herbicide use in herbicide-tolerant crops rose considerably from 2007 to 2008. This statement has been challenged as it is purely based on extrapolations, since no good figures on herbicide usage were available for these years (Sheridan, 2010). However, increased resistance to glyphosate in several weed species may stimulate growers to use increased doses of glyphosate (see also 2.2.3). Monsanto and Syngenta are now offering to give farmers rebates on the order of $ 12 per acre to also spray herbicides that work through a mode of action different from glyphosate (Sheridan, 2010). Monsanto also has a second generation glyphosate-tolerance (RR) trait in the pipeline that allows higher application doses and a longer window of use for killing off difficult perennial weeds (Monsanto website, 2010).

2.2.3 Glyphosate-resistant weeds

The likelihood of the development of resistance to glyphosate in weeds was originally deemed low, also by APHIS in their first environmental assessments (see also Cerdeira & Duke, 2006). Conventional use over several years, e.g. in tree and vine crops, and on railroad tracks (for up to 10-15 years), at the time had hardly led to resistant weeds. Moreover, it proved very hard to select for resistance in crop plants through conventional breeding, including mutagenesis of the target of glyphosate's inhibitory effect in the shikimate metabolic pathway, the EPSPS enzyme, and *in vitro* methods. Thus, the transgenic approach was only effective by using a resistant bacterial EPSPS enzyme (cp4 from *Agrobacterium tumefaciens*) and not so by adapting plant-derived EPSPS (Bradshaw *et al.*, 1997). As a consequence of using glyphosate year after year on the same field, weed species have shifted to those that can more successfully withstand glyphosate and to those that avoid the time period of its application (Cerdeira and Duke, 2006). However, contrary to the previous expectations described by Bradshaw *et al.* (1997), the adoption of glyphosate-tolerant maize, soybean and cotton has also resulted in the development of glyphosate-resistant weeds. This effect is not a direct effect of the genetic engineering of herbicide tolerance *per se*. It is caused by the concomitant use in cultivations of a single herbicide on a very large scale. In line with the problems in engineering glyphosate-insensitive EPSPS, most resistance was reported to be "non-target-site", i.e. not due to any mutation in EPSPS (Yuan *et al.* 2007). For instance, Gaines *et al.* (2010) showed the glyphosate resistance found in pigweed (Palmer amaranth, *Amaranthus palmeri*) populations in Georgia to be related to a large increase in copy number of the EPSPS gene in the genome and not to any genetic change in the EPSPS itself as in the transgenic approach. Thus, pigweed is able to cope with glyphosate applications by overproduction of the EPSPS enzyme.

In the USA, there are large areas with a cropping consisting of glyphosate-tolerant varieties of maize annually rotated with glyphosate-tolerant varieties of soybean. In 2005, this was occurring on 60% of the USA maize acreage (Krupke, 2009). Glyphosate-tolerant cotton is also often grown in a crop rotation with maize and soybean. Cerdeira & Duke (2006) mentioned three weed species that most likely developed herbicide resistance under cultivation of glyphosate-tolerant crops, i.e. *Conyza canadensis*, *Ambrosia* *artemisiifolia* and *Amaranthus palmeri*. There is discussion about the size of the area infested with glyphosate resistant weeds. APHIS estimated that a relatively small percentage of the area planted to the four major crops (wheat, corn, soybean, and cotton) was harbouring glyphosate-tolerant weeds in 2007 (somewhere between approximately 0,1 and 1%, APHIS, Finding of No Significant Impact of Corn DP-098140-6, 2009) According to Benbrook (2009), there are presently nine glyphosate-resistant weeds that collectively infest millions of acres of US cropland. The South of the US is most heavily affected, though resistant weeds are rapidly emerging in the Midwest, and as far north as Minnesota, Wisconsin, and Michigan. Especially glyphosate-resistant *Amaranthus palmeri* has spread dramatically across the South since the first resistant populations were confirmed in 2005, and poses already a threat to USA cotton production (Benbrook, 2009).

In 2005, Johnson *et al.* (2009) conducted a survey among approximately 1200 farmers in six states of the USA, representing major regions growing glyphosate-tolerant crops. Only 30% of farmers thought glyphosate-resistant weeds were a serious problem. A substantial number of farmers underestimated the potential of glyphosate-resistant weed populations to evolve in an agro-ecosytem dominated by glyphosate as the weed control tactic. To compensate for glyphosate-resistance development in weeds, farmers could increase the glyphosate application rate or apply other herbicides. Application of alternative herbicides could be efficiently achieved by the use of crop varieties with different types of herbicide tolerance, such as for glufosinate-ammonium or ALS inhibitors. Soil tillage for weed control or manual weed control could also be needed again. Other options are changes in crop rotation or the use of winter wheat cover crops (Davis *et al.* 2009).

2.3 Unexpected effects of herbicide-tolerant maize

2.3.1 Adoption of reduced tillage systems

The adoption of herbicide-tolerant crops such as glyphosate-tolerant maize and soybean in North and Latin America coincided with a large increase in the area of arable lands under zero tillage, supporting the link between herbicide-tolerant crops and the use of soil conservation systems (Bonny, 2008; Christoffoleti *et al.*, 2008; Cerdeira *et al.*, 2007; Cerdeira & Duke, 2006; Fawcett & Towery, 2002). For instance, no till systems in the United States have increased from 15 million ha to over 25 million ha from 1994 to 2004. As mechanical weed control conflicts with the aim to minimize tillage operations, soil conservation systems usually come with an increased reliance on chemical weed control. Herbicide-resistant crops allow the application of a broad-spectrum herbicide after crop emergence and therefore facilitate the implementation of soil conservation systems. However, the benefits of soil conservation systems are also valid in non-GM cropping systems and the adoption of soil conservation systems started well before the introduction of herbicide-resistant GM crops (Bolliger *et al.*, 2006). Therefore, it is difficult to state how the adoption of soil conservation systems would have proceeded without the availability of herbicide-resistant crops.

Soil conservation tillage in many cases improves the sustainability of farming as it assists in reducing soil erosion, improving soil physical, chemical and biological properties and minimizing the environmental costs of ploughing (Bernoux *et al.*, 2006; Boddey *et al.*, 2003; Follett, 2001; Buschiazzo *et al.*, 1998). Especially soil erosion is associated with very high economic and environmental costs, both on-farm and off-farm (Pimentel, 1995). The adoption of no-tillage systems has also an effect on wildlife. Barnes (2000) reported that farmers in the USA mentioned increased bird and animal sightings when they left the soil undisturbed.

Reduced tillage is however not solely associated with positive agronomic and environmental impacts. Zero tillage in Brazil could under specific circumstances lead to soil compaction, an increased abundance of pests and diseases overwintering in residues, and increased soil acidity due to the reduced opportunities to incorporate lime into the soil (Bolliger *et al.,* 2006). Depending on soil type, climate and land use, these drawbacks are more or less relevant. Moreover, a change from conventional to zero tillage has a large impact on weed abundance due to the change in tillage practices and a change in weed control practices (i.e. usually a stronger reliance on herbicides for weed control). This has been well documented for the

Pampas of Argentina (Ghersa & Martinez-Ghersa, 2000; de la Fuente *et al.*, 2006). At a different level, zero tillage may allow arable farming on lands that were previously considered unsuitable for cultivation due to tillage and erosion problems, such as erosion-sensitive soils on slopes and wetlands. Apparently this was the case in some of the wetter parts of the Argentinean Pampas that are nowadays under arable farming partly as a result of the availability of zero tillage techniques (Bindraban *et al.*, 2009).

2.3.2 Effects on biodiversity

In the largest-ever field trials assessing environmental impacts of GM crops in the world, the so-called Farm Scale Evaluations (FSE), UK researchers compared GM herbicide-tolerant and conventional varieties of maize, winter-sown oilseed rape, spring-sown oilseed rape and beet. The GM maize and rapeseed were tolerant to glufosinate-ammonium. The GM beet was tolerant to glyphosate. By comparison, weeds were more abundant in herbicide-tolerant maize fields. Although more weeds were present in the GM herbicide-tolerant maize fields, the total frequency was not high. As there were more weeds in and around the herbicide-tolerant crops, there was also a higher diversity of crop-associated invertebrates (Haughton *et al.*, 2003; Hawes *et al.*, 2003) and more weed seeds, which could serve as food for, e.g. birds. Gibbons *et al.* (2006) indeed showed that in herbicide-tolerant maize fields the densities of seeds important to 7 out of 17 species of granivorous birds were larger than in conventional fields; for the other species, numbers were similar. Nevertheless, Chamberlain *et al.* (2007) did not observe larger amounts of granivore birds on a subset of GM herbicide-tolerant maize fields of the FSE during crop growth after herbicide application, as compared to conventional maize fields. However, on the stubbles during winter, some bird groups were more abundant on the GM herbicide-tolerant fields. These observations show that growing herbicide-tolerant maize was generally associated with a more abundant wildlife than conventional maize.

The differences found in biodiversity did not arise as a direct effect of the maize being genetically modified. They arose because on these herbicide-tolerant maize farmers used different herbicides and applied them differently. In three-quarters of the FSE conventional maize fields, atrazine was used (Perry *et al*, 2004). At that time in the UK, atrazine was not used as long as in other countries and it was relatively more effective in weed control than other herbicides in oilseed rape and beet. This is probably the reason why herbicide-tolerant maize had a positive effect on wildlife in comparison to traditional maize, while herbicide-tolerant oilseed rape and sugar beet had a negative effect on wildlife as compared to their respective conventional counterparts. In herbicide-tolerant maize, herbicide was applied at a later moment and in comparison to conventional maize crops more weed seeds were allowed to germinate and develop. Atrazine has been banned in the EU since 2004 and nowadays a comparison between herbicide-tolerant maize and conventional maize could lead to different results.

2.3.3 Reduced uptake of micronutrients

Many commercial crop plants have been genetically modified to compensate for the physiological disruption of the shikimate pathway by blocking the EPSPS enzyme targeted by glyphosate so that they tolerate the chemical. Experiments have shown that glyphosate application on herbicide-tolerant maize and soybeans reduces the efficiency of manganese (Mn) uptake and physiological efficiency by 10 to 50 percent, depending on the genetic nutrient uptake efficiency of the particular transformed variety or hybrid (Huber, 2007). However, other physiological effects of glyphosate (e.g. reduced uptake and translocation of Fe, K, and Mn, physiological immobilisation of Mn, drought stress, and early maturity) may go largely unnoticed in commercial practice without a near-isogenic comparison available (Huber, 2007). The greatest impact can be expected on soils with a low micronutrient-availability. Huber (2007) mentioned a significant increase of maize yield following a foliar-application of Zn on glyphosate-tolerant maize. There is still discussion about the effect of glyphosate on the uptake of micronutrients. Based on several publications, Hartzler (2010) concluded that although there has been research indicating glyphosate-tolerant soybean may respond differently to Mn than conventional varieties, the majority of research does not support this observation. According to agronomists of the University of Minnesota reduced uptake of micro-nutrients by glyphosate-tolerant crops is not perceived as an important effect in commercial fields (see appendix).
Reduced uptake of micronutrients is an indirect effect of the introduction of glyphosate-tolerant crops, because it is not a direct consequence of the modification itself, but an effect from the associated herbicide

application. The additional micronutrient fertilization which is needed can have a negative effect on the environment, because an extra operation may be needed.

2.3.4 Increased susceptibility to diseases

Glyphosate-induced Mn deficiency (see previous section 2.3.3) can compromise plant resistance, so that diseases such as take-all, *Fusarium* head scab and *Fusarium* root rot, *Corynesporium* root rot, increase after glyphosate is applied for weed control (Huber, 2007). Kremer and Means (2009) found that *Fusarium* colonization of roots of glyphosate-tolerant maize treated with glyphosate was 3-10 times higher than after an atrazine treatment. Powell and Swanton (2008) have analysed several studies evaluating glyphosate effects on diseases. To their opinion conclusions supporting a causative link between glyphosate and elevated crop diseases associated with *Fusarium* spp require further testing. Observations of microbiology might suggest that the link between glyphosate and fungal desease is context dependent. They proposed more research in which more consideration should be given to microbial ecology in the experiments.

As also observed in the cases above, increased susceptibility to diseases is an indirect effect of the introduction of glyphosate-tolerant crops, because it is application of the herbicide, not the genetic modification itself, that leads to the effects described. If increased susceptibility to diseases makes it necessary to apply fungicides, a negative influence on the environment could be envisaged.

2.3.5 Effects on water quality

In the intensively farmed maize-growing regions in the USA, surface waters have often been contaminated by herbicides, principally as a result of precipitation run-off shortly after application of herbicides to maize and other crops. In general, glyphosate and glufosinate-ammonium are relatively rapidly broken down in the field. A model study in which glyphosate-tolerant maize and glufosinate-ammonium tolerant maize were compared with conventional maize showed that glyphosate and glufosinate-ammonium loads in run-off could be generally one-fifth to one-tenth of those of atrazine and alachlor. This is an indication that the introduction of herbicide resistant maize has reduced herbicide concentrations in vulnerable watersheds (Cerdeira and Duke, 2006).

2.4 Expected effects of Bt-maize

2.4.1 Resistance development against Bt

Overall, resistance development against the Bt Cry proteins by insects in agricultural field situations has been surprisingly rare up till now. A recent review by Tabashnik *et al.* (2009) listed only one reported example in cotton and two in maize: *Busseola fusca* to Cry1Ab maize in South Africa (see also Kruger *et al.* 2009) and *Spodoptera frugiperda* to Cry1F maize in PuertoRico. In the USA, part of the stewardship plan for use of Bt maize crops is to provide refuges for susceptible insects by planting non–Bt-crops as a portion of the total acreage. This plan aims at alleviating selection pressure for resistance development by offering the opportunity of oviposition on a refuge of the non-Bt variant of the crop species itself in the vicinity of the Bt crop. According to Davis (2006) in the northern US, the requirement is 20%, and for cotton growing regions it is 50% because maize is the alternate host of the cotton bollworm. The United States Environmental Protection Agency (EPA) mandates this planting of a refuge for Bt-technology. As a condition of registration of a Bt crop variety by EPA, seed companies are required to conduct IRM (Insect Resistance Management) compliance assessments during the growing season to ensure farmer compliance. Failure to follow IRM guidelines and properly plant a refuge may result in the loss of access to Bt technologies. Monsanto is publishing IRM guides (http://www.monsanto.com/monsanto/ag_products/pdf/stewardship/2009_irm_guide.pdf). Nevertheless, Craig *et al.* (2008) mentioned that a survey in 2000 has shown that almost 30% of the farmers failed to comply with the refuge protocols designed to prevent or delay the emergence of insect resistance to Bt-toxins.

A recent publication by Jongsma *et al.* (2010) has implied, based on modeling, that the lack of resistance development in pest insects may also be related to variation in selection of host plant species for oviposition. Thus, by the opportunity of choosing alternative host plant species, the selection pressure on the pest insect to develop direct resistance to Bt maize itself would be alleviated. This would mean that the observed rarity of resistance development to Bt in pest insects is not necessarily due to the application of the high dose/refuge strategy in stewardship programs.

2.4.2 Effects on the amount of insecticides used

The first released insect-resistant maize provided protection against the European corn borer and a reduction in insecticide use was expected. Fewer non-selective insecticides, such as organophosphates and synthetic pyrethroids, could be used. The introduction of insect-resistant maize reduced the risks associated with the application of some insecticides including risks from exposure to field workers' and consumers, adverse effects on nontarget species, and ground water contamination by insecticides.

When assessing effects on insecticide usage in maize, one needs to take into account that many farmers do not apply insecticides against the European corn borer. The larvae can only be targeted successfully within a specific window of application, as they are hiding within the plant stems. In a study on the impacts of Bt maize cultivation in Spain, Gómez-Barbero *et al.* (2008) did show a decrease in insecticide application, but this was strongly dependent on local situations, probably related to corn borer infestation levels, and was mainly apparent in differences in whether or not and how often insecticides were applied: over all three regions studied, the average number of treatments was 0.32 times per year with Bt maize compared to 0.86 with conventional varieties and 70% of Bt maize growers did not apply insecticides as compared to 42% of conventional growers. According to Benbrook (2009), Bt maize reduced insecticide use in maize culture on average by about 0.1 kg/ha (= about 10%) in the USA.

2.5 Unexpected effects of Bt maize

2.5.1 Bt effects on non-target invertebrates

In the first Environmental Assessments Reports, APHIS stated that the Bt-toxins have shown to be very selective for lepidopteran or coleopteran insects. They are not expected to adversely affect other invertebrates. In the course of Bt crop introduction, much controversy arose on unexpected and/or unpredictable adverse effects on non-target organisms in the field or in intricate soil foodwebs. From two meta-analyses and a recent review (Marvier *et al.*, 2007 and Wolfenbarger et al. 2008, and Naranjo 2009, respectively), the following generalizations of Bt impacts in the field, as compared to non-Bt cultivations, could be gathered. No consistent adverse effects are found for non-target organisms, except for some members of groups to which the targeted plague insects belong and that thus are expected to be to some extent vulnerable to Bt. However, with members from such groups (mainly lepidopterans and coleopterans) the effects of insecticide treatments are generally larger than the Bt effect. There are also effects on some parasitoids and/or predators of the target insect pest, but in general (also based on experimental research), this can be ascribed to the targeted decrease in pest prey numbers and/or their quality by the Bt toxin and not by a direct effect of the Bt toxin on the parasitoids and/or predators themselves. Also, this is an effect that is to be expected with the application of any resistance (GM or not) against plague insects that leads to reduction in their fecundity and/or viability. As illustrations of these general conclusions, some examples that attracted relatively much attention, are discussed below.

Effect of Bt on the Monarch butterfly

A direct effect of Bt on Lepidoptera, including the more conspicuous ones like the Monarch butterfly (*Danaus plexippus*), is not unexpected, since several of the Cry toxins used are aimed specifically at Lepidoptera (moths and butterflies). However, because the Monarch butterfly could be exposed to Bt through maize pollen deposited on leaves of its feeding plant, milkweed (*Asclepias syriaca*), growing in the direct vicinity of maize fields, more extensive field studies have been performed. These studies showed the actual exposure under normal agronomic field conditions to be very low (Stanley Horn *et al.* 2001). In a more recent study, Dively *et al.* (2004) made more specific calculations of overall exposure of the Monarch

butterfly across the USA Corn Belt. This started from the worst case exposure scenario, milkweed growing within a maize field and thus covered by the local maximally possible density of Bt-containing pollen, leading to an average 23.7% reduction of first instar larvae reaching maturity. With an extrapolation across the whole Corn Belt, which represents half of the total distribution area of the Monarch butterfly, growing Bt maize would lead to 0.6% additional mortality in comparison with non-Bt maize. It is questionable whether this would exceed natural variability in mortality rates.
According to Oberhauser (pers. comm., see appendix) the occurrence of milkweed in maize field has been reduced considerably since glyphosate is used on a large scale.

Effect of Bt on aquatic organisms
Another study focussed on the assumption that Bt maize litter may end up in headwater streams in agricultural areas with much maize cultivation and that Bt from this litter could affect aquatic organisms. Laboratory studies by Rosi-Marshall *et al.* (2007) indicated that Bt also had a negative effect on caddisflies (Trichoptera), which could be hypothesized from their close relationship to Lepidoptera. Again, however, this needs further assessment in the field. Another study, published as abstract at a conference (Pokelsek *et al.* 2007) showed no significant effects on caddisfly growth in a field test. A later study by the same research group (Griffiths *et al.* 2009) revealed little difference in decomposition of maize litter in agricultural headwater streams between Bt and non-Bt varieties. They also observed that these streams were depauperate of caddisflies, indicating that such leaf-shredding insects had little influence on decomposition. These findings were in line with a publication by another research group (Swan *et al.* 2009), who also did not find consistent differences in decomposition of maize litter and invertebrate communities between Bt and non-Bt varieties in a two-year study on streams draining agricultural fields.

Effect of Bt on soil organisms
Mulder *et al.* (2006) reported enhanced soil respiration during the first 72 hours after addition of Bt maize residues as compared to non-Bt residues in soil microcosms. The effect had disappeared after three weeks and is relatively small as compared to the effects of *e.g.* changing weather conditions. Similar results were reported by Büchs *et al.* (2007). The relevance of the effect for agricultural field conditions as well as its mechanisms are unclear. Digestibility could be a factor and thus, Bt maize varieties have been reported to be higher in lignin content by some studies but the consistency of this effect across various environmental conditions and genetic backgrounds has been challenged. For instance, Saxena and Stotzky (2001) found that Bt maize hybrids had a significantly higher (33-97%) lignin content than their non-Bt isolines with nine combinations tested. On the other hand, Jung & Sheaffer, in a set-up consisting of replicated field trials on four Minnesota sites using six pairs of Bt hybrid cultivars and their isolines, did not find consistent differences in lignin levels. They concluded that differences found in the previous study might be related to suboptimal growing conditions which also led to using immature plant material. In addition, effects of Bt maize on decomposition have not been demonstrated: e.g. Lehman *et al.* (2008) did not observe persistent differences in degradation of maize residues over 384 days in a study in South Dakota. In addition, Icoz *et al.* (2008) did not show any consistent effect of Bt maize in a four-year field study in Minnesota; effects of genetic background of cultivars, sites and seasons were larger. Other authors neither found consistent effects beyond the normal range of variation with agricultural practice (e.g Griffiths *et al.* (2005), for three sites across Europe testing microbial community, protozoa and nematodes; Lawhorn *et al.* (2009) in a 3-year study did not find adverse effects on microbe communities or decomposition rates).

2.5.2 Resistance development on maize volunteers in soybean
Krupke *et al.* (2009) presented an unforeseen consequence of stacking multiple transgenic traits, viz. glyphosate tolerance and Bt. They sampled volunteer maize plants in soybean fields and quantified damage to maize roots to estimate feeding by larvae of western corn rootworm (WCR, *Diabrotica virgifera virgifera*). The plants were also analyzed for the glyphosate tolerance and Bt events. Volunteer maize plants could survive on glyphosate-tolerant soybean fields due to their glyphosate-tolerance transgene. Furthermore, there was no difference in feeding damage between roots expressing the Cry3Bb1 toxin (Bt) and roots without this toxin. Soybean is grown with less nitrogen because soybeans fix their own nitrogen. The authors were expecting a reduced level of Bt production as a result of growing in an environment with low nitrogen supply. The plants with low Bt levels could allow survival of western corn rootworm that are

heterozygous for Bt resistance, while killing susceptible homozygous individuals. In this way the frequency of the resistance allele is increased. As a consequence the development of Bt-resistant WCR populations may be increased by volunteer maize plants stacked with glyphosate tolerance and Bt traits.

2.5.3 Occurrence of a new pest: western bean cutworm

Since 2000, in the USA, Bt maize expressing CryAb1 has been infested by the larvae of the western bean cutworm (*Stiacosta albicosta*). This cutworm was historically confined to very limited regions and caused no major problems in maize crops. For almost all states in the American Corn Belt, damage caused by the western bean cutworm has been documented recently (Eichenseer *et al.,* 2008). The expansion of western bean cutworm across the Corn Belt is a concern to corn producers because larvae damage the crop directly by feeding on the marketable grain (Dorhout & Rice, 2010). It was suggested that pest replacement was one of the reasons of the increasing damage. Cry1Ab is not only active against the European corn borer but also active against the corn earworm (*Helicoverpa zea*). This latter pest feeds not only on maize but is also a competitor to other pest insects such as the western bean cutworm. Suppression of the corn earworm meant that the western bean cutworm partially lost its natural competitor (Dorhout & Rice, 2010). According to Ostlie (pers. comm., see appendix) it is also possible that the western bean cutworm has developed biotypes which are better adapted to wet conditions. This can also explain partly the expansion of western bean cutworm in certain regions.

2.5.4 Decreased susceptibility to fungi and decreased fumonisin content

Brookes (2007) reported that in Spain the percentage of maize plants attacked by fungi was lower in Bt maize than in conventional maize. Also fumonisins (mycotoxins produced by *Fusarium* moulds) were observed in only 17% of Bt plants compared to 100% of the conventional maize plants analysed. In a study in Argentina, Barros *et al.* (2009) showed a lower percentage of infestation by *Fusarium* and lower levels of fumonisin for Bt maize. However, this study revealed no significant differences in another mycotoxin, deoxynivalenol (DON), between Bt and non-Bt maize. Reduced levels of mycotoxins are important to human and animal health. Moreover, this Bt side-effect on fungi could reduce the need of chemical control of fungi, which means an additional positive environmental effect of Bt varieties. According to Andow (pers. comm., see appendix) the decreased susceptibility to Fusarium can be ascribed to the reduction of damage of maize plant caused by corn borer. This gives the fungi less possibilities to infest the plant.

2.5.5 Reduced lodging

Due to less damage caused by rootworm Bt maize with resistance to rootworm is less susceptible to lodging (Andow, pers. comm., see appendix). Yield and harvest capacity are increased by less lodging. A higher harvest capacity results in lower CO_2 emission.

2.5.6 Increased tolerance to drought

Drought tolerance of Bt maize with resistance to rootworm is improved, because the rooting system is not suffering from rootworms (Andow, pers. comm., see appendix) A higher production and a more efficient use of water is a positive environmental effect.

2.5.7 Pest management benefits in other crops

According to Storer *et al*. (2008), trends in blacklight trap monitoring over the past 35 years in Maryland provided evidence of regional suppression of targeted lepidopteran populations as a result of Bt maize use. The numbers are significantly lower during the last 10-15 years, because of the use of Bt-maize. Also data from Illinois are indicating similar dynamics. This regional suppression of populations of pest species has led to pest management benefits in other crops, such as soybean and vegetables as reported by growers, extension agents and pesticide applicators. Also refuge fields grown with maize without Bt and neighbouring fields of farmers growing conventional maize are suffering less from western corn rootworm and European corn borer.

2.6 Summary of effects of GM maize

Based on the classification criterion of not being mentioned in the first Environmental Assessments Reports of APHIS, the following unexpected effects of herbicide-tolerant maize were found:

- adoption of reduced tillage systems;
- effects on biodiversity;
- reduced uptake of micronutrients;
- increased susceptibility to diseases;
- effects on water quality.

The adoption of reduced tillage systems could have been facilitated by herbicide-tolerant maize. The positive effects on biodiversity were found in a comparison between glufosinate-tolerant maize and conventional maize for a large part treated with atrazine; therefore, this result could likely not be generalized to other GM – conventional comparisons in maize. The effects on water quality could be explained as logical consequences of the more easily degradable herbicides used in conjunction with herbicide-tolerant maize. The effect of reduced uptake of micronutrients and of increased susceptibility to diseases was not predicted to our knowledge, but its relevance in commercial field cultivation and the underlying mechanisms have not become completely clear yet. The expected effects of glyphosate-tolerant maize volunteers and development of glyphosate-resistant weeds have been observed more than expected at the moment of introduction of herbicide-(glyphosate)-tolerant maize. They were related to the intensive use of glyphosate year after year in a crop rotation in which often also glyphosate-tolerant soybean and glyphosate-tolerant cotton were grown. The appearance of glyphosate-resistant weeds may also have led to a recent increase in use of herbicides, where the original effect of the introduction of HT crops most likely was a reduction in environmental impacts of herbicide usage.

Based on the classification criterion of not being mentioned in the first Environmental Assessments Reports of APHIS, the following unexpected effects of Bt-maize were found:

- the occurrence of a new pest (Western bean cutworm);
- decreased susceptiblilty to fungi and decreased mycotoxin (fumonisin) content;
- reduced lodging and increased tolerance to drought as a result of less root damage from rootworm;
- pest management benefits in other crops.

In addition to these effects, Bt effects on non-target organisms appeared to be few and small as compared to insecticide treatment. The possibility of resistance development in rootworm on maize volunteers in soybean cultivation was unexpected, but to what extent the effect was occurring in commercial fields has not been reported. Furthermore, the expected development of resistance against Bt in pest organisms has not been found very frequently until now. The expected effect of a decrease in insecticide use was found, but in comparison with cotton the decrease in insecticide use was small, because in maize insecticides were applied on a smaller scale against corn borers.

3 Sugar beet (*Beta vulgaris*)

3.1 Introduction

3.1.1 Crop characteristics

Sugar beet is an outcrossing, wind-pollinated species. The crop is biennial: in the first year it is grown for root production; after vernalisation, it can produce seed in the second year. When sugar beets are grown for root production, normally no flowering is occurring in the first year and growing techniques are aimed at avoiding bolters. If bolters are occurring, they should be removed in order to avoid volunteers and weed beet development.

According to Mallory-Smith & Zapiola (2008), sugar beet does not produce feral populations in the USA, but in California there are two wild relatives, *Beta macrocarpa* Guss. and sea beet *B. vulgaris* ssp. *martima* (L.) Arcang., which are cross-compatible with sugar beet. Bartsch & Ellstrand (1999) confirmed these species to be introductions from Europe. The only other US state with sea beet is New Jersey, a state without sugar beet cultivation. *B. macrocarpa* is a widespread weed in sugar beet fields in the Imperial Valley in California. In some *B. macrocarpa*, evidence was found for gene flow from crop *B. vulgaris* ssp. *vulgaris* (Bartsch & Ellstrand, 1999). According to McGinnis *et al.* (2010), weed beets could have been introduced through seed imports from Europe, but no weed beet problems have been documented in the sugar beet production areas.

3.1.2 Adoption of GM Sugar beet

Glyphosate-tolerant sugar beet was deregulated in 1998 in the USA, but it was not commercialized until 2008 due to the reluctance of the sugar companies to use sugar from GM sugar beet. This first deregulated glyphosate-tolerant sugar beet was produced by Novartis Seeds and Monsanto and was genetically engineered by inserting the CP4 EPSPS gene into the sugar beet genome. The KWS seed company has used the construct of Monsanto for producing the glyphosate-tolerant H7-1 sugar beet, which also has also been filed for cultivation in the EU. Commercial planting of glyphosate-tolerant sugar beet in the USA started in 2008. In the USA the total area of sugar beet grown for root production is approximately 500,000 ha. In 2008, glyphosate-tolerant sugar beet was already grown on 59% of the area (James, 2008) and this percentage was reaching 95% in 2009 (James, 2009). Also in Canada, glyphosate-tolerant sugar beet is grown since 2008. In September 2009, a federal judge of the Federal District Court for the Northern District of California ruled that the USDA should have better assessed the potential impact of GM sugar beet on closely related crops, such as red table beets and Swiss chard. In August 2010, a federal judge revoked approval of glyphosate-tolerant sugar beet cultivation until APHIS has completed an Environmental Impact Statement (EIS).

The sugar beet seed production area in the USA ranges from 1,000 to 2,500 ha. Nearly 100% of the seed production is concentrated in the Willamette Valley of Oregon. In this region, also two conspecific crop types/cultivar groups are grown for seed: red (table) beet and leaf beet or Swiss chard. Most of the sugar beet seed in the USA is not certified. This means that isolation distances in the seed production industry are voluntary. The two sugar beet seed industry organisations, WVSSA and Betaseed, are demanding growers to use increased isolation distances between glyphosate-tolerant sugar beet and vegetable seed production (4828 m and 6437 m, respectively, instead of the 2438 m originally required by the Oregon Seed Certification standards) (McGinnis *et al.*, 2010). But, it is still possible for seed-producing beets to be planted closer than the recommended isolation distances. There are also additional methods mitigating pollen-mediated gene flow, such as the use of a non-transgenic pollinator line with a transgenic male-sterile maternal line in the hybrid seed production. The sugar beet seed companies have established sanitation protocols to prevent physical transfer of glyphosate-tolerant pollen. For example, crop advisors must wear clean overalls before entering a glyphosate-tolerant field and must remove these after leaving the field. To avoid seed commingling seed producers are not allowed to grow conventional and glyphosate-tolerant sugar beet seed on their farms in the same year. Glyphosate-tolerant sugar beet seed and conventional sugar beet seed are also stored and cleaned separately. In 1998 APHIS said that occasionally sugar beets volunteers occurred in fields in the year after harvesting.

3.1.3 Overview of environmental effects of GM Sugar beet

Commercial growing of glyphosate-tolerant sugarbeet has started very recently. This means that long-term effects cannot be assessed yet. Nevertheless, some environmental effects are discussed below because information is also available from large-scale experiments and some effects can be compared with the effects observed in other glyphosate-tolerant crops. Table 3 presents an overview of the effects that are discussed.

Table 3. **Overview of observed environmental effects of GM sugar beet.**

Effect	Expected by APHIS in first EA	Observed in commercial production or in large scale experiments	Scientifically confirmed as a relevant environmental effect
Gene flow to free-living sugar beets and to Beta species	+	-	+
Glyphosate-resistant weeds	+	+	+
Effect on herbicide use	+	+	+
Increased area reduced tillage	-	+	+
Negative effects on biodiversity (FSE en BRIGHT)	-	+	+*
Increased susceptibility to diseases	-	+	+

* confirmed in FSE en BRIGHT

3.2 Expected effects of GM sugar beet

3.2.1 Gene flow to volunteer sugar beet/weed beets and wild relatives

At this moment, there is no information about gene flow to free living sugar beets and relatives during commercial growing of glyphosate-tolerant sugar beet in North America in 2008 and 2009. Glyphosate-tolerant beet is only grown in Mid-Western regions where no feral beet populations have been documented and no wild relatives are found. In the Imperial Valley in California where *B. macrocarpa* occurs, conventional beet is still prevalent (McGinnis *et al.* (2010). APHIS (1998) believed that if and when the glyphosate-tolerance trait moves to other sexually compatible *Beta sp.* this will not have a significant impact, as it will not give any competitive advantage in environments not managed by herbicides, a view also held by the Dutch COGEM. In France, Darmency *et al.* (2007) described the gene flow from sugar beet in a multi-year and multi-crop monitoring study on farmers' fields at two locations which had been running since 1995. Two sugar beet lines were analysed, one line with glyphosate-tolerance and one line with glufosinate-ammonium-tolerance. Sugar beet bolters produced most (86%) of the herbicide-resistant seeds harvested in the field. Direct progeny from pollen flow of sugar beet bolters to weed beets that were growing in the same field as well as in a neighbouring fallow field accounted for 0.4% of the resistant seeds released over the years and locations, and the remaining 13.6% of seeds were produced by transgenic descendants from the sugar beet-weed beet hybrids. The largest recorded distance of transgenic pollen flow was 112 m.

3.2.2 Effect on herbicide use

Kleter *et al.* (2008) are citing analyses of Coyette *et al.* to calculate the potential impact of the introduction of glyphosate-tolerant sugar beet in the EU. The reduction in quantities of combined herbicides applied amounted to 28–43%. In the Farm Scale Evaluations in the UK, quantities were reduced by 36% (Kleter *et al.* 2008). Not only the amount of applied herbicides is reduced but, also the environmental impact, because the negative impact of several conventional herbicides used in sugar beet is larger than the impact of glyphosate.

3.2.3 Glyphosate-resistant weeds

Development of glyphosate-resistant weeds will be further promoted, if glyphosate-tolerant sugar beet is grown in rotation with other glyphosate-tolerant crops (see 2.2.3). A weed control guideline mentions that glyphosate-resistant weeds continue to increase, especially in southern Minnesota (North Dakota State University and the University of Minnesota Cooperative Extension Services, 2010). Glyphosate resistant giant ragweed (*Ambrosia trifida*), common ragweed (*A. artemisiifolia*) and waterhemp (*Amaranthus rudis*) have been confirmed on different locations. Management of glyphosate-resistant waterhemp in sugar beet is difficult. As a consequence, the decreased environmental impact of herbicide usage with glyphosate-tolerant beet (Kleter *et al.*, 2008) may be negated by increased use of glyphosate, when no proper stewardship plans with adapted crop rotations etc. are implemented (see 2.2.3).

3.3 Unexpected effects of GM sugar beet

3.3.1 Adoption of reduced tillage systems

Glyphosate-tolerant sugar beet facilitates the adoption of reduced tillage systems. The traditional and most common tillage method in sugar beet is moldboard ploughing. The availability of glyphosate-tolerant sugar beets makes it possible to switch to strip-tillage when planting into small-grain residues of a previous cereal crop because intensive ploughing and cultivation are no longer needed for weed control (Moore *et al.*, 2009). Strip-tillage contributes to reductions in soil erosion from water and wind, fossil fuel use, air pollution from dust, loss of soil moisture and soil compaction. Reduced tillage also improves soil structure, leading to reduced risk of run-off and pollution of surface waters with sediment, nutrients and pesticides (Cerdeira and Duke, 2006).

3.3.2 Effect on biodiversity

In the Farm Scale Evaluations (FSE) in the UK, decreases in weed biomass, seed shedding (seed rain) and seed banks were observed in glyphosate-tolerant beets as compared to conventional beets (Champion *et al.*, 2003; Heard *et al.* I and II, 2003). In fields with glyphosate-tolerant beets, the abundance of surface-active springtails was generally increased, probably because of the increased weed detritus (Brooks *et al.*, 2003). The incidence of carabid beetles feeding on these springtails had increased as well. Otherwise, invertebrate diversity was generally lower in glyphosate-tolerant beet, in line with the lower weed occurrence (Haughton *et al.*, 2003; Hawes *et al.*, 2003). In glyphosate-tolerant beet fields the densities of seeds important to 17 species of granivorous birds were lower for 16 of these species and the total energy intake from such seeds was smaller for 15 of these species as compared on conventional fields (Gibbons *et al.*, 2006). An additional study on the actual bird populations in a subset of the fields conducted for the FSE showed that granivorous bird species were more abundant in conventional beet fields than in herbicide-tolerant beet fields. During winter, on bare, ploughed fields after sugar beet harvest, granivores were again more abundant in fields with conventional beet (Chamberlain *et al.*, 2007). In another project in the UK, the BRIGHT project, which studied crop rotations with beet cultivation, abundance of weeds in follow-up cereal crops was less markedly influenced; this may be not surprising because cereal crops show higher levels of weed suppression than sugar beet (Sweet *et al.*, 2004). Sugar beet generally did increase the weed seed bank in the applied four-year rotation, regardless of the herbicides used. Modifications of the herbicide regimes in glyphosate-tolerant sugar beet, such as banded spraying leaving unsprayed areas open for weed and insect community development, were suggested as mitigating measures for biodiversity maintenance. This regime had a positive effect on arthropod populations and weed biomass and seeds that can serve as feed for farmland birds. Thus, it was claimed as an advantage of the flexibility in weed control enabled by herbicide-tolerant crops for mitigating adverse effects on biodiversity (Sweet & Lutman 2006). In general, the effects on biodiversity can be related to the efficacy of weed control enabled by the herbicide-tolerant crop (and also appreciated by the grower) and therefore cannot be really regarded as unexpected.

3.3.3 Effect on diseases

Increased susceptibility to Fusarium yellows and *Rhizoctonia solani* has been reported for glyphosate-tolerant beet (Johal & Huber, 2009) They also reported that after glyphosate application, the sugar beet variety resistant to *Rhizoctonia* was equally susceptible to this pathogen as the susceptible variety.

3.4 Summary of effects of GM sugar beet

Herbicide-tolerant sugar beet has been introduced commercially very recently. This makes the observation of many unexpected effects unlikely. Based on the classification criterion of not being mentioned in the first Environmental Assessments Reports of APHIS the following unexpected effects of herbicide-tolerant sugar beet were found:

- adoption of reduced tillage systems,
- effect on biodiversity
- effect on diseases.

The adoption of reduced tillage systems could have been facilitated by herbicide-tolerant sugar beet. Negative effects on biodiversity were found in a comparison between glyphosate-tolerant beet and conventional beet and were in line with the more effective weed control afforded by the glyphosate-tolerant beet. The effect of increased susceptibility to diseases was not predicted to our knowledge, but its relevance in commercial field cultivation has not been reported upon yet. No unexpected aspects have yet been reported for the expected effects discussed: gene flow, effect on herbicide use and the occurrence of glyphosate-resistant weeds.

4 Potato (*Solanum tuberosum*)

4.1 Introduction

4.1.1 Crop characteristics

Potato (*Solanum tuberosum*) is an herbaceous annual that grows up to 1 m tall and produces a tuber - also called potato - so rich in starch that it ranks as the world's fourth most important staple food crop, after maize, wheat and rice. The potato belongs to the Solanaceae - or "nightshade"- family of flowering plants, and shares the genus *Solanum* with at least 1,000 other species, including tomato and eggplant. The potato is the world's number one non-grain food commodity, with production reaching a record 325 million tonnes in 2007 (Anonymus, 2008).

The potato is propagated vegetatively by tubers or tuber cuttings. Thus, the highly heterozygous potato cultivars don't need to be bred to produce homogenous plants from true seed. A major disadvantage of potatoes for breeders is that *Solanum tuberosum* is tetraploid, which makes it difficult to transfer desirable traits between cultivars and having them expressed in progeny. There are numerous species of *Solanum* which provide a rich source of potential traits for breeding into *S. tuberosum*, incuding resistance to insect pests and diseases. Unfortunately, many of these wild *Solanum* relatives are diploid, greatly complicating the breeding process. Thus, insertion of candidate genes by genetic engineering is a particularly valuable process for developing new potato cultivars (Grafius & Douches, 2008; Park *et al.*, 2009).

4.1.2 Adoption of GM potatoes

Commercialization of genetically modified potato cultivars is difficult due to the current unwillingness of Japanese and European markets to accept such cultivars. Largely for marketing concerns, genetic engineering is not a significant part of most of the commercial potato breeding programmes. GM potato varieties were only grown on a minor scale in the US and Canada between 1995 and 2001. The transgenes in the only commercially available GM potato varieties were patented by Monsanto Company and consisted of a Bt trait (Cry3A) that lead to resistance against herbivorous activities of certain Coleoptera species including the Colorado potato beetle. In 1998, a different GM potato was launched providing resistance against Potato Leafroll Virus (PLRV). This PLRV-resistance was imparted by the orf1/orf2 gene derived from PLRV. From 1999 onwards, the two traits were combined and the potato varieties with stacked transgenes were sold under the name "NewLeaf™ potato". In 2001 sales and marketing of the NewLeaf™ potato varieties were suspended because of marketing strategy reasons. However, the products remained fully approved in the US and Canada. From 1999 onwards, also a Bt trait combined with potato Y potyvirus (PVY) was commercialized as NewLeaf-Y™. The PVY-resistance was derived from the coat protein of an ordinary strain of potato virus Y (PVY-O). Insect resistant GM potato was allowed for food and feed in Canada, Japan and the US and the virus resistant variety was approved only in Canada and the US (Parmar, 2004). In other countries than the US and Canada (Australia, Japan, Korea, Philippines, Mexico), there were only approvals for GM potatoes as import fo food/feed, not for cultivation (www.gmo-compass, 2010). Table 4 gives an idea of the acreage of GM potato varieties grown for seed potato certification in the USA in 1999.

Table 4. **Acreage of genetically modified seed potato varieties in the USA in 1999. Source: the Badger Commontater (pers. comm.), a publication of the Wisconsin potato industry).**

Variety	Genetic modification	Hectare
Atlantic	NL (Colorado potato weevil resistance)	249.71
Ranger Russet	NL (Colorado potato weevil resistance)	0.24
Russet Burbank	NL Y (resistance against Colorado potato weevil and Potato virus Y)	12.95
Russet Burbank	NL (Colorado potato weevil resistance)	222.99
Russet Burbank	NL + (resistance against Colorado potato weevil and Potato leaf roll virus)	591.38
Russet Norkotah	NL Y (resistance against Colorado potato weevil and Potato virus Y)	65.07
Russet Norkotah	NL (Colorado potato weevil resistance)	0.01
Shepody	NL Y (resistance against Colorado potato weevil and Potato virus Y)	342.78
Shepody	NL (Colorado potato weevil resistance)	102.79
Snowden	NL (Colorado potato weevil resistance)	29.95
Superior	NL (Colorado potato weevil resistance)	50.59
Total		1668.47

NL: NewLeaf

The total USA area planted to Bt potatoes has never exceeded 20,000 ha, or 4% of the market (Shelton *et al.*, 2002). In 1995, 700 ha were grown across North America. In 1996, 1600-2000 hectare of seed and 5000-5200 hectare of commercial crops approved for human consumption were contracted to processors and packing companies (Anonymous, 1996). Limited production of Bt potatoes has occurred because a foliar insecticide against the Colorado potato beetle, imidacloprid, was introduced as an effective alternative to Bt potatoes. Moreover, in autumn 1999 McCain Foods (the world's largest producer of French fries) decided to no longer buy GM potatoes because of consumer concern. In 1999, GM potatoes with resistance against insects and virus were cultivated in Canada and Romania but cultivation was not continued. According to a publication of the European Commission, GM potatoes represented about 40,000 ha in 1999. Plantings took place in the USA (30,000 ha), Canada (10,000 ha), Romania (1,000 ha) and Ukraine (1,000 ha). This acreage of 30,000 ha differed from the 20,000 ha reported above by Shelton *et al.* (2002) mentioned above. According to Anghel & Popovici (2008), the NewLeaf Bt potato has been approved in Romania, but was a failure in commercial cultivation in 1999 when it was grown on less than 1,000 ha. It has since been withdrawn from the seed varieties register (ANPED, 2003). The reasons for the failure were not mentioned. Until now, GM potatoes are not commercially grown In Western Europe. Recently, however, in March 2010, the European Commission approved the Amflora potato, a genetically modified starch potato patented by the company BASF. The potato has been developed to respond to the demand for pure amylopectin starch and is intended only for non-food industrial purposes. Food use is not foreseen.

4.1.3 Overview of expected and unexpected effects

Expected and unexpected effects of GM potato are presented in Table 5.

Table 5. **Overview of observed environmental effects of GM potato.**

Effect	Expected by APHIS in first EA	Observed in commercial production or in large scale experiments	Scientifically confirmed as a relevant environmental effect
Effect on insecticide use	+	+	+
Variation in other characteristics	-	+	+
Effect on predators and parasites	-	(+)	-

(+) field study on several locations

4.2 Expected effects of GM potato

4.2.1 Quantity of insecticides used

In the United States, 34% of the total insecticide use in potatoes is applied to control the Colorado potato beetle. Currently, organophosphates and pyrethroids account for about 80% of the insecticide use in potatoes. According to a grower survey in 1998, the number of insecticide applications by Bt potato growers was 1.35 times lower while the Bt potatoes required 0.54 kg/ha less insecticidal active ingredients. Based on the estimated 4% market share of Bt potatoes, EPA estimated a benefit to growers of $23 per ha or $500,000 nationally, resulting in 36,000 fewer acre treatments (Shelton *et al.*, 2002).

4.3 Unexpected indirect effects of GM potato

4.3.1 (Somaclonal) Variation in other characteristics than the transgenic trait itself

In some cases, changes in the characteristics of the GM varieties were observed that were different from those expected from the transgenic trait itself. De Jong and Lambert (pers. comm.) mentioned that one of the released lines of NewLeaf Atlantic was no longer resistant to the golden cyst nematode and NewLeaf Superior matured about three weeks later than the original Superior. In these cases, a positional effect of the transgene or, more likely, somaclonal variation having occurred during the transformation procedure apparently had passed the selection process undetectedly, or was not deemed relevant to market introduction of the variety.

4.3.2 Increased populations of predators and parasites

Hoy *et al.* (1998) concluded from a field study in 1993 in Ohio that the conservation of aphid predators in *Bt* potato plots during a critical two-week period apparently prevented the aphid outbreak. In the standard potato plots treated with pyrethroids aphid populations density reached high levels while predator populations remained at very low or undetectable levels. In the untreated Bt potato plots predatory insects were detectable throughout a long period and an aphid outbreak was prevented. Also from other field studies in Wisconsin it was concluded that conservation of predators in Bt potatoes, in absence of broad-spectrum insecticides, did occur consistently.

4.4 Summary of effects of GM potato

GM potatoes were commercially grown during a very short period. Based on the classification criterion of not being mentioned in the first Environmental Assessments Reports of APHIS the following unexpected effects of GM potatoes were found: variation in other characteristics than the transgenic trait itself and the increased populations of predators and parasites. The first effect can be ascribed to failures made during the selection process. The effect on predators and parasites could be explained as consequences of the reduced use of insecticides on Bt potatoes. The observed decrease in use of insecticides on Bt-potatoes was as expected.

5 Oilseed rape (*Brassica napus*)

5.1 Introduction

5.1.1 Crop characteristics

Oilseed rape is an annual, self-fertile and outcrossing species that is both insect- and wind-pollinated. It has the potential to establish outside cultivation. Mallory-Smith and Zapiola (2008) are referring to research in which outcrossing rates were found as high as 47%, and up to 55% was reported by Timmons *et al.* (1995). Pollen dispersal has been reported until 1,5 km, and distances up to 26 km were found using male-sterile bait plants in the UK (DEFRA report by Ramsay *et al.*, 2003). Pollen dispersal by bees is expected to be possible up to 8 km (Mallory-Smith & Zapiola, 2008). Volunteer rapeseed may be an important weed problem in subsequent crops. At harvest, high seed losses are occurring. In general, oilseed rape seedbanks decline quickly but may persist for several years.

In the literature, rapeseed or oilseed rape is very often called canola, but this name actually refers to so-called double zero types, varieties that are both low in erucic acid and glucosinolates. Thus, the name canola can refer to two species: *Brassica napus* L. (Argentinean canola) and *Brassica rapa* L. (formerly *B. campestris* L., Polish canola). In North America, most of the canola grown and all GM canola is *B. napus*. A recent COGEM study has drawn attention to a potential problem in gene flow studies, namely that *B. napus* and *B. rapa* are difficult to distinguish morphologically in the field (COGEM, 2010). It is not clear yet whether this may have hampered studies published previously, but in many gene flow studies, molecular markers and/or ploidy measurements have been used that are helpful in ascertaining species identification.

5.1.2 Adoption of GM oilseed rape

GM rapeseed has been grown in Canada since 1996. The acreage of GM oilseed rape has since increased very rapidly. In 2007, GM oilseed rape was grown on 5.1 million hectares, which made up approximately 87 percent of Canada's canola crop. Less GM oilseed rape is grown in the USA, mainly in North Dakota. GM oilseed rape was deregulated in the USA in 1996 and in 2007 82% of the area (400,000 ha) was GM oilseed rape. GM oilseed rape is also grown in Australia as of 2008, after lifting its ban on GM cultivation. (www.gmo-compass, 2010). Worldwide, GM oilseed rape occupied 20% of the total oilseed rape acreage and 5% of the total acreage of GM crops in 2008 (James, 2008).

In the USA and Canada, four types of herbicide-tolerant varieties have been grown until now: glyphosate-tolerant varieties (RR: Roundup Ready), glufosinate-ammonium-tolerant varieties (LL: Liberty Link), bromoxynil-tolerant varieties and imidazolinone-tolerant varieties (CF: Clearfield). Imidazolinone-tolerant varieties were obtained by chemical mutagenesis instead of genetic transformation and are therefore treated as conventional under an EU regulation exemption. In 2001, over 80 % of the surface area of oilseed rape in Canada was tolerant to herbicides: 47% glyphosate-tolerant, 13% glufosinate-ammonium-tolerant, <1% bromoxynil-tolerant varieties and 20% imidazolinone-tolerant varieties (Beckie *et al.*, 2003). In 2005, over 80% was transgenic: 50% glyphosate and 32% glufosinate-ammonium. According to Mauro and McLachlan (2008), 96% of the 5.25 million ha of canola grown in Canada in 2008 was herbicide-tolerant; approximately 50% of this being RR, 32% being LL and 14% being CF. **Laurate Canola,* a GM oilseed rape with modified fatty acid composition has been cultivated in the USA from 1996 until 2000. The last time, the acreage had been 70,000 hectares. By now cultivation has ended.

5.1.3 Overview of discussed environmental effects of GM oilseed rape

Several environmental effects have been observed after the release of GM oilseed rape. The environmental effects of herbicide-tolerant oilseed rape as identified in the inventory are summarized in Table 6 and discussed below.

Table 6. **Overview of observed environmental effects of GM oilseed rape.**

Effect	Expected by APHIS in first EA	Observed in commercial production or in large scale experiments	Scientifically confirmed as a relevant environmental effect
Gene flow to related species	+	+	+
Herbicide-tolerant oilseed rape volunteers	+	+	+
Development of feral populations with herbicide-tolerance	+	+	+
Effect on herbicide use	+	+	+
Increased area reduced tillage	-	+	+
Weed shifts	-	+	+
Negative effects on biodiversity (FSE)	-	+	+

5.2 Expected effects of GM Oilseed rape

5.2.1 Herbicide-tolerant oilseed rape volunteers in other crops

Oilseed rape can produce large volunteer populations as a result of the large seed losses before and during harvest. According to a study in Canada in 1999 and 2000, average oilseed rape seed loss during harvest operations ranged from 9 to 56 times the recommended seeding rate of oilseed rape (Beckie & Owen, 2007). Hall *et al.* (2000) reported pollen flow between varieties with different herbicide-tolerance traits leading to volunteers with multiple resistance at a field site in western Canada. In a survey performed in Canada in 2002 by Mauro & McLachlan (2008), the occurrence of herbicide-tolerant canola volunteers was an important issue. Many farmers were concerned about a possibly persistent nature of these volunteers. Farmers were using different techniques to control these volunteers, including the use of additional herbicides and tilling. Many of the zero-till farmers in the study actually reverted to tillage to control glyphosate-tolerant volunteers. Beckie *et al.* (2006) are considering this as one of the most significant threats to zero-tillage. A large majority (76%) of the survey respondents who used herbicide-tolerant canola anticipated that herbicide-tolerant volunteers would become “more of a problem in the future”.

At the moment of releasing glyphosate-tolerant oilseed rape to the market, APHIS stated that it was unlikely that the glyphosate tolerance trait would increase weediness of this oilseed rape, unless glyphosate is the only alternative for control of the plant. The likelihood of canola volunteers possessing a combination of two different herbicide-resistance genes and how such volunteers would be managed by growers was a matter of concern. However, glyphosate was not used at any significant degree for the control of canola volunteers. Moreover, Monsanto provided instructions regarding the use of alternative herbicides that could be used to control *Brassica* volunteers or weeds.

5.2.2 Development of feral populations

In Canada, Yoshimura *et al.* (2006) found many glyphosate-tolerant oilseed rape plants along railways and roadways as a result of seed spillage during transportation. In Saskatchewan, 34% of 300 randomly sampled plants were glyphosate-tolerant and 30% were glufosinate-ammonium- tolerant; in British Columbia these figures were 43% and 22% of 81 plants tested, respectively. This will increase the costs of weed control, since it is common for glyphosate to be used to control weeds in these areas. Likewise, Knispel *et al.* (2008) found tolerance to glyphosate in 88%, to glufosinate-ammonium in 81% and to imidazolinone in 31% of populations in verges of agricultural areas in Western Canada. In 62% of the populations, stacked herbicide-tolerance traits due to outcrossing were observed. Knispel and Lachlan (2010) concluded that feral herbicide-tolerant oilseed rape had become a permanent feature of agricultural landscapes in Western Canada. Agricultural transport enables the ongoing establishment of new populations. Given the high proportion of HT traits in escaped populations and the high frequency of outcrossing events, it is to be expected that escaped transgenes cannot be retracted and may persist even if GM oilseed rape cultivation ceases (Knispel & Lachlan, 2010).

At the moment of releasing glyphosate-tolerant oilseed rape to the market APHIS stated that oilseed rape is able to escape from cultivated fields, and form occasional populations. However, oilseed rape was not considered to be a weed in the USA. There will be no selection advantage of glyphosate-tolerant oilseed rape in areas where glyphosate is not applied.

5.2.3 Gene flow to wild relatives

Many studies have reported gene flow via pollen from oilseed rape to wild relatives in experimental field studies using GMHT oilseed rape or indications for such gene flow by population genetic studies using molecular markers (recent reviews by e.g. Mallory-Smith & Zapiola 2008, Warwick *et al.* 2009). Thus, hybridization has been shown to be possible with other *Brassica* species, such as *B. rapa*, *B. oleracea*, *B. nigra*, *B. juncea*, *Raphanus raphanistrum* and -with a remote likelihood- with *Sinapis arvensis*. For most of these species, hybridization rates were low, except for *B. rapa*. This also is the only species for which hybridization with GM oilseed rape (*B. napus*) was reported in commercial cultivation, i.e. in Canada for the first time as of 2001. Later, backcrossed progeny was found and in 2005, one introgressed plant, i.e., having both the normal ploidy level of *B. rapa* and the herbicide-tolerance transgene, was observed (Warwick *et al.*, 2008*).*

At the moment of releasing glyphosate-tolerant oilseed rape to the market, APHIS stated that gene movement to related species is occurring at extremely low levels and that it was unlikely that the gene that codes for glyphosate-tolerance would confer a competitive advantage in these species unless glyphosate is used for their control.

5.2.4 Reduction in herbicide use

Beckie *et al*. (2006) referred to different authors reporting that herbicide-resistant oilseed rape has reduced herbicide use. From 1995 to 2000, the amount of herbicidal active ingredient applied per hectare of oilseed rape declined by 43% and the environmental impact (EI) per hectare, calculated using the EI quotient for individual herbicides and the amounts of active ingredients applied, declined by 37% (Brimner *et al*, 2005), whereas Leeson *et al*. (2006) calculated a reduction of 20% for Canada. The EI quotient is a value denoting the risks of potential impact on the environment and is expressed per kg active ingredient. The environmental impact per ha is calculated by multiplying the EI quotient by the amount of pesticides applied per ha (cf. Kovach *et al.,* 2009). Based on figures from the NCAFP in the USA, Kleter *et al*. (2007) arrived at an EI quotient reduction of 6.2 (42%) for 2004. Before the introduction of herbicide tolerant oilseed rape herbicide options in oilseed rape were limited and weed control was not always optimal. The improved weed control associated with herbicide-tolerant oilseed rape provided an opportunity to reduce herbicide use in following crops (Beckie *et al.,* 2006).

5.3 Unexpected effects of GM oilseed rape

5.3.1 Weed species shifts

Herbicide-tolerant oilseed rape combined with reduced tillage or direct seeding has significantly influenced weed communities. Over time, weed shifts towards species that are more difficult to control within an herbicide-tolerance system have been observed (Beckie *et al*., 2006). For example, species such as stinkweed (*Thlaspi arvense*) declined in relative abundance, whereas green foxtail (*Setaria viridis*), wild oat (*Avena fatua*), and volunteer crops increased in herbicide-tolerant oilseed rape systems as compared to conventional variants. In contrast to certain regions of the USA where glyphosate-tolerant soybean, cotton and maize dominate in rotation on the same fields, development of resistance in weed species did not occur in the most important oilseed rape growing areas in Canada. In the western grainbelt provinces of Canada (Alberta, Manitoba, Saskatchewan), oilseed rape is the only glyphosate-tolerant crop. The other crops rotating with oilseed rape are wheat and barley. It is usual that glyphosate-tolerant oilseed rape is grown on a particular field only once in 4 years. Moreover, not only glyphosate-ammonium-tolerant oilseed rape is used but also glufosinate-tolerant oilseed rape. This means that glyphosate selection intensity on weed species in this Canadian oilseed rape-cereal cropping agroecosystem is much less than in the glyphosate-tolerant soybean, maize and cotton agroecosystems of the USA, Argentine and Brazil. According to Powles (2008), there are currently no known cases of evolved glyphosate-resistant weeds in Canada. The cereals rotating with GM oilseed rape are generally more weed-suppressive (see 3.3.2). In their review, Beckie *et al.* (2006) neither found studies indicating that herbicide-tolerant oilseed rape has reduced weed diversity.

5.3.2 Increased adoption of reduced tillage systems

According to Beckie *et al.* (2006) herbicide-tolerant oilseed rape has reduced fuel consumption in Canada in 2000 by 31 million L (12,6 L/ha) resulting from fewer passes across fields due to less tillage, etc. In these calculations, however, differences in energy consumption elsewhere, such as in the production of herbicides, have not been taken into account. And it is also difficult to assess the relationship between HT systems and no-tillage: for instance, 45% of LL oilseed rape growers applied no-tillage during the first years of this century.

5.3.3 Effect on biodiversity

In the Farm Scale Evaluations, GM oilseed rape was compared with conventional oilseed rape during four years on 67 fields in the UK. There were fewer weeds and seeds in the glufosinate-ammonium-tolerant oilseed rape than in the conventional oilseed rape (Champion *et al.*, 2003; Heard *et al.* I and II, 2003), Thus, many invertebrates, including for instance herbivorous beetles, were less common in herbicide-tolerant oilseed rape than in conventional oilseed rape (Haughton *et al.*, 2003; Hawes *et al.*, 2003). This was not a direct effect of genetic modification but it can be explained by differences between the efficacy of herbicide treatments in both systems. The pre-emergence herbicides used in conventional oilseed rape were not as effective on weeds as the broad-spectrum herbicides used in herbicide-tolerant oilseed rape. Also the way in which the herbicides were applied was different. The broad-spectrum herbicides used in herbicide tolerant oilseed rape were applied later in the season so that the weeds were larger when they were killed. This was giving more decaying and dead weeds in herbicide-tolerant oilseed rape. This explains why some detritivorous species, such as springtails, were more abundant in herbicide-tolerant oilseed rape (Brooks *et al.*, 2003). The numbers of surviving broadleaved weeds were similar in conventional and GM crops, but the plants had a 70% lower biomass in the GM crops. Seed rain was also lower, with 80% fewer broad-leaved weed seeds. Overall, the weed seedbank was smaller following GM crops. After one year the seedbank of broad-leaved weeds had doubled in the conventional spring rape fields with only a slight increase in the GM equivalent. Butterfly numbers were higher in the fields and field margins of conventional spring rape crops, attracted mainly by the greater numbers of flowering weeds in and around the crop. Most other insect groups, including bees, were found in more or less similar numbers in the GM and conventional fields (Burke, 2003).

A Canadian study showed that the number of wild bees was lower in glyphosate-tolerant oilseed rape fields than in imidazolinone-tolerant oilseed rape fields, and the number in imidazolinone-tolerant oilseed rape fields was again lower than in organic fields containing *Brassica rapa*. This was accompanied by a lower level of pollination in glyphosate-tolerant and imidazolinone-tolerant oilseed rape fields (Morandin & Winston, 2005). Imidazolione-tolerant varieties are obtained by chemical mutagenesis and are exempted from GM regulation in the EU. Factors other than herbicide use could play a role, for example, the possibly higher occurrence of semi-natural areas in the neighbourhood of organic fields, differences in the use of other crop-protectants, such as insecticides, or the fact that organic fields contained another rape species, *B. rapa*. *B. rapa*, however, would be expected to show more problems with a shortage of pollinators than *B. napus*, since it is an obligate outbreeder whereas oilseed rape is self-compatible. The lower level of bees in HT fields could also be related to differences in weed occurrence. In the Farm Scale Evaluations programme in the U.K., there was a trend of less bees in HT oilseed rape than in conventional oilseed rape (Haughton *et al.* II, 2003), but due to the low absolute numbers, the difference was only significant for all observations along the whole growing season taken together (Hawes *et al.* 2003). The FSE did not show whether there was a relationship with a lower presence of weeds. Naturally, a great part of the bees visited the oilseed rape itself (81% in conventional and 93% in GM, Haughton *et al.* II, 2003).

5.4 Summary of effects of GM oilseed rape

Based on the classification criterion of not being mentioned in the first Environmental Assessments Reports of APHIS the following unexpected effects of GM oilseed rape were found:

- weed species shifts;
- increased adoption of reduced tillage systems;
- effects on biodiversity.

The adoption of reduced tillage systems could have been facilitated by herbicide-tolerant oilseed rape, but the relationship did not appear to be very strong. Negative effects on biodiversity were found in a comparison between glufosinate-tolerant oilseed rape and conventional oilseed rape and were in line with the more effective weed control afforded by glyphosate. The development of feral population was expected and since herbicide-tolerant canola has been adopted on a large scale, feral populations have been widely observed, including plants with more than one HT transgene stacked as a consequence of hybridization. Gene flow to wild relatives was expected to occur at low levels and up till now has also been reported to occur under field conditions at low levels to *B. rapa*. So, there do not appear to be clear aberrations from gene flow expectations in reports thus far.

6 Alfalfa (Medicago sativa)

6.1 Introduction

6.1.1 Crop characteristics

Alfalfa is a perennial, mainly outcrossing, insect-pollinated crop. No compatible wild relatives are known to exist in the USA. However, feral alfalfa populations are common in areas of alfalfa cultivation. In the USA, alfalfa seed is produced primarily in the Western states. Insect-mediated pollination is necessary for alfalfa seed production. Control of pollen movement between seed fields is needed for maintaining genetic purity. Alfalfa seed is small. Hard seeds, which are common in alfalfa, may lie dormant for years before absorbing water and germinating. Dormancy allows alfalfa seeds to persist in the seedbank and become volunteers in subsequent crops. Although alfalfa is not usually considered to be vegetatively propagated, it can be propagated by stem cuttings, and alfalfa crowns can persist and regenerate new plants. Alfalfa crowns can be moved by machinery within and between fields.

6.1.2 Adoption of GM alfalfa

In the USA commercial growing of glyphosate-tolerant alfalfa was started in 2006 on 80,000 ha, representing 5% of the approximately 1.3 million ha seeded in the USA. In 2007, a permanent injunction by the District Court for Northern California prohibited further planting pending the completion of the USDA-APHIS Environmental Impact Statement (EIS) and a decision on the regulation petition. Among the Court's concerns were admixtures with conventional and organic alfalfa, as well as the potential for GT alfalfa to increase the prevalence of glyphosate resistant weeds (APHIS 2009). In June 2010, the US Supreme Court lifted the nationwide ban on GM planting, but remanded the case back to the District Court. Based on the District Court's rulings, APHIS can decide on interim measures during the period of completion of the EIS.

7 Soybean (*Glycine max*)

7.1 Introduction

7.1.1 Crop characteristics

Soybean is an annual, highly self-fertile, self-pollinating species. Pollination occurs either in the bud stage or before the flowers completely open. Soybean pollen is too heavy for wind transport over large distances. Pollination by honey bees (*Apis mellifera* L.) has been shown to increase the yield of some cultivars by assisting pollen transfer between anthers and stigma or by increasing outcrossing. Soybean is not found outside cultivated areas and has no compatible wild relatives in the USA and Europe. Soybean does not produce a persistent seedbank because the seeds lose viability quickly and have no dormancy. The lack of dormancy allows soybeans to germinate and become volunteers if temperature and moisture are adequate. Volunteers can occur in subsequent crops.

7.1.2 Adoption of GM soybean

Farmers in the world's main soybean growing nations – the USA, Brazil and Argentina – adopted GM soybean at a large scale. In the USA, 92% of the planted soybean was GM in 2008; in Brazil this was 65%; in Argentina this was 99% (USDA/FAS, 2009). Up to 2008, the only type of GM soybean commercially grown was the so-called Roundup Ready® soybean (RR soybean) (event MON 40-3-2)). Glyphosate-tolerant soybean contains a trait leading to resistance against the broad-spectrum herbicide glyphosate. While glyphosate may also be used in non-GM soybean as a pre-emergence herbicide, glyphosate-tolerant soybean also allows the application of glyphosate after crop emergence. Glyphosate-tolerant soybean was launched with the aim to facilitate weed control in soybean, as glyphosate application after crop emergence may provide more effective weed control than application of the herbicides used in non-GM soybean. Moreover, glyphosate-tolerant soybean may allow a more flexible timing of herbicide application and a reduction in the number of herbicide applications, providing opportunities to save labour. These advantages were important drivers behind the high adoption rate of glyphosate-tolerant soybean by farmers.

In Northern America, the commercial cultivation of Roundup Ready® 2Yield soybean was initiated at a small scale in 2009 (event MON89788). This soybean line, also marketed by Monsanto Company, has the same glyphosate-resistance as RR soybean, but the resistance has been incorporated into a part of the plant genome of a new line that also contains native soybean genes conferring a higher yield potential. This type of soybean is therefore called Roundup Ready®2Yield (RR2Y) soybean. According to Monsanto Company, RR2Y has a 7-11% higher yield potential than comparable soybean varieties without these traits. This yield increase has not been confirmed through independent field research yet and there is little practical field experience with the cultivation of RR2Y soybean up to now.

The company Bayer CropScience commercially launched a new type of GM soybean, Liberty Link® (LL) soybean, on the northern American seed market in 2009 (events A2704 and A5547). The LL trait in soybean leads to resistance against the broad-spectrum herbicide glufosinate-ammonium. LL soybean has been commercially launched with the aim to facilitate weed control in soybean and can offer the same advantages to farmers as RR soybean. Moreover, LL soybean helps to reduce the reliance on glyphosate that has become the main pillar in weed control for the many soybean farmers that adopted RR soybean. From 2010 the LL trait will probably also be introduced in varieties grown in Latin America.

The company Dupont has recently launched a GM soybean line with an increased oleic acid content (event 260-05). This is the first GM soybean line with altered grain qualities rather than constructs focussed on facilitating the management of the crop. The cultivation of GM soybeans with altered grain qualities aiming at specific beneficial health effects, rather than soybeans with agronomical traits, is still minimal but is likely to grow in importance in the future.

7.1.3 Overview of environmental effects of GM soybean

Six environmental effects of glyphosate-tolerant soybean has been identified (Table 7). Most of these were not mentioned in the first environmental assessment by APHIS.

Table 7. **Overview of observations of environmental effects of glyphosate-tolerant soybean cultivation.**

Effect	Expected by APHIS in first EA	Observed in commercial production or in large scale experiments	Scientifically confirmed as a relevant environmental effect
HT soybean as a weed in rice and cotton	+ *	+	-
Increasing adoption of reduced tillage systems	-	+	+
Herbicide use and herbicide resistant weeds	-	+	+
Reduced nutrient uptake	-	+	-
Nitrogen-fixing symbionts	-	-	-
Plant diseases	-	+**	-

* Identified in assessment by APHIS as a potential problem and expected not to cause any major problem
+**: observed in field trials and on-farm-sites

7.2 Expected effects of GM soybean

7.2.1 HT soybean as a weed in rice and cotton

Soybean has few weedy tendencies and glyphosate-tolerant soybean generally does not give any problems as a volunteer weed in subsequent crops. In the Midwest of the USA soybean seeds can sometimes remain their viability over the winter and occur as a volunteer weed in subsequent maize or cotton, but is not generally considered difficult to manage (Beckie and Owen, 2007). Volunteer herbicide-tolerant soybean can be a rare problem in herbicide-tolerant cotton grown in rotation with soybean, especially when hurricanes destroy the preceding soybean crop and leave many unharvested seeds in the field.

7.3 Unexpected effects of GM soybean

7.3.1 Increasing adoption of reduced tillage systems

The adoption of herbicide-tolerant crops including glyphosate-tolerant soybean in Northern America as well as Latin America is linked to an increased implementation of soil conservation tillage systems, involving reduced tillage or no tillage at all combined with increased soil coverage with crop and weed residues. (Bonny, 2008; Christoffoleti *et al.*, 2008; Cerdeira *et al.,* 2007; Cerdeira & Duke, 2006; Fawcett & Towery, 2002). As mechanical weed control conflicts with the aim to minimise tillage operations, soil conservation systems usually come with an increased reliance on chemical weed control. Herbicide-tolerant crops such as glyphosate- and glufosinate-ammonium-tolerant soybean allow the application of a broad-spectrum herbicide after crop emergence and therefore facilitates the implementation of soil conservation systems. However, the benefits of soil conservation systems are also valid in non-GM cropping systems and the adoption of soil conservation systems started well before the introduction of herbicide resistant GM crops (Bolliger *et al.,* 2006). Therefore, it is difficult to state how the adoption of soil conservation systems would have proceeded without the availability of herbicide-resistant crops. Soil conservation tillage in many cases improves the sustainability of farming as it assists in reducing soil erosion, improving soil physical, chemical and biological properties and minimising the environmental costs of ploughing (Bernoux *et al.*, 2006; Boddey *et al.*, 2003; Follett, 2001; Buschiazzo *et al.,* 1998). Especially soil erosion is associated with very high economic and environmental costs, which occur both on-farm and off-farm (Pimentel*,* 1995).
The adoption of no-tillage systems can affect wildlife. Barnes (2000) reported that farmers in the USA

indicate increased bird and animal sightings when they leave the soil undisturbed.

Reduced tillage is however not solely associated with positive agronomic and environmental impacts. Zero tillage in Brazil could in some circumstances lead to soil compaction, an increased abundance of pests and diseases overwintering in residues, and an increased soil acidity due to less opportunities to incorporate lime into the soil (Bolliger *et al.*, 2006). Depending on soil type, climate and land use, these drawbacks are relevant or not. Moreover, a change from conventional to zero tillage has a large impact on weed abundance due to the change in tillage practices and a change in weed control practices (i.e. usually a stronger reliance on herbicides for weed control). This has been well documented in the Pampas of Argentina (Ghersa & Martinez-Ghersa, 2000; de la Fuente *et al.*, 2006).

7.3.2 Herbicide use and herbicide resistant weeds

When herbicide-tolerant crops were introduced in the 1990s, glyphosate-tolerant soybean and other herbicide-tolerant crops were expected to offer the opportunity to replace a cocktail of herbicides used in non-GM crops with a relatively strong environmental impact by glyphosate that has less impact on the environment.

Evidence from the field confirmed that as USA farmers shifted from non-GM to glyphosate-tolerant soybean, their herbicide use changed dramatically with a strong increase in the use of glyphosate and a strong decrease in the use of more selective herbicides. The impact of glyphosate-tolerant soybean on the total amount of herbicides used was less obvious. Bonny (2008) - based on USDA NASS data - noticed that the total amount of herbicides applied on soybean fields in the USA (kg active ingredients or kg a.i.) in 1996-2006, a period in which glyphosate-tolerant soybean almost entirely replaced non-GM soybean, decreased between 1996-2001 and increased irregularly between 2002-2006. In 2006, the herbicide application level in soybean (around 1.6 kg a.i. ha^{-1}) exceeded that in 1996. A strong reliance on glyphosate for weed control in glyphosate-tolerant crops most likely resulted in a shift in weed species and in the development of glyphosate resistant weed biotypes (Powles, 2008). Since the introduction of glyphosate-tolerant crops in the USA, at least nine weed species developed glyphosate-resistant weed biotypes in the US (Heap, 2009). No glyphosate-resistant weeds were recorded before the introduction of glyphosate-tolerant crops. Although the observed increase in the use of herbicides in soybean cannot be directly attributed to the emergence of herbicide-resistant weed biotypes, it is likely that a reduced efficacy of glyphosate due to herbicide-resistant weeds stimulated glyphosate-tolerant soybean producers to increase the dose of glyphosate or apply other additional herbicides to soybean.

The use of herbicides in soybean in Latin America has not been documented as extensively as in the USA, but it is likely that similar processes occurred (Christofolleti *et al.*, 2008; Cerdeira *et al.*, 2007). Data on herbicide use in GM and non-GM soybean in the main soybean production area of Argentina (Districts of Buenos Aires and Santa Fe) in 2002-2007 suggested that RR soybean received substantially more herbicides than non-GM soybean (an increase of 1.25-2.26 kg active a.i. ha^{-1} relative to non-GM soybean receiving 0.20-2.88 kg active a.i. ha^{-1}) due to high glyphosate application rates in glyphosate-tolerant soybean (Bindraban *et al.,* 2009). Since the introduction of glyphosate-tolerant crops in Argentina, Brazil and Paraguay, six weed species developed glyphosate-resistant biotypes, while none was recorded before their introduction (Heap, 2009).

Soybean with tolerance to glufosinate-ammonium offers opportunities to reduce the reliance on glyphosate as the central pillar in weed control and thereby lower the risk of weed biotypes developing glyphosate resistance. Glufosinate-ammonium has a working mechanism which is different from glyphosate. However, glufosinate-ammonium has a narrower weed control spectrum than glyphosate. If glufosinate-ammonium-tolerant soybean leads to a reliance on a single herbicide for weed control as occurred in glyphosate-tolerant crops, similar problems with herbicide-resistant weeds and gradually increasing herbicide application rates could be anticipated.

7.3.3 Reduced nutrient uptake

One field experiment in the US by Gordon (2008) indicated an impact of the glyphosate tolerance trait on the response to manganese (Mn) applications. In this study, glyphosate-tolerant soybean showed a higher sensitivity to Mn stress than isogenic non-GM soybean lines. It was hypothesised that glyphosate applications in glyphosate-tolerant soybean may interfere with the plant's Mn metabolism and also aversely affect populations of soil micro-organisms responsible for the reduction of Mn to a plant-available form. Increased application rates of Mn corrected the deficiencies in glyphosate-tolerant soybean.

7.3.4 Reduced activity of nitrogen-fixing symbionts

As glyphosate also affects pathways involved in the amino acid production of bacteria, glyphosate applications could affect the activities of nitrogen-fixing bacteria in the nodules of soybean, as glyphosate-tolerant soybean plants can transport foliar-applied glyphosate to the roots from where it may be released as root exudates. In greenhouse experiments in the USA, nitrogen fixation by bacteria in the nodules of glyphosate-tolerant soybean varieties was shown to be reduced when the crop was treated with glyphosate at an early stage (5 and 10 days after emergence) (King *et al.,* 2001). Also nitrogen accumulation and biomass growth in plants were reduced. Plants had recovered however by 40 days after emergence. In growth chamber studies, nitrogen fixation was more sensitive to water deficits in glyphosate-treated plants. Also, it was noticed in field studies that glyphosate formulations can inhibit nodule development in glyphosate-tolerant soybean, but soybean had the potential to recover from glyphosate stress (Reddy & Zablotowicz, 2003).

Kremer and Means (2009) found in their studies that nodulation was always lower on glyphosate-tolerant soybean with or without glyphosate compared with conventional varieties with non-glyphosate or no herbicide. They are referring to a field study reporting that glyphosate significantly reduced nodule mass and nitrogen fixation in glyphosate-tolerant soybean, but it did not affect grain yields. On commercial fields in the USA, glyphosate-tolerant soybean is grown without additional nitrogen applications (Naeve, pers. comm., see Appendix). This means that the negative effect on nitrogen-fixing symbionts is restricted.

7.3.5 Plant diseases

Glyphosate affects an enzyme involved in the synthesis of aromatic amino acids. This pathway is present in plants as well as in fungi and bacteria. Therefore, glyphosate applications associated with glyphosate-tolerant crops could directly affect the pressure of fungal and bacterial diseases above- and belowground. Glyphosate can be released from the roots of glyphosate-resistant crops and thereby affect the activities of rhizosphere bacteria and fungi (Kremer *et al*., 2005). Glyphosate could also weaken the disease defence mechanisms of glyphosate-tolerant crops. Also indirectly, for instance through a reduced availability or uptake of micronutrients, glyphosate could weaken the plant's defence mechanism (Johal & Huber, 2009). Moreover, crop disease pressure could be altered due to a change in the timing of weed control, for instance weed control in glyphosate-tolerant crops may be conducted at a later stage than in non-GM crops when a higher weed biomass is present in the field, which could result in an increased pressure of opportunistic root pathogens (Termorshuizen & Lotz, 2002).

Greenhouse and laboratory studies demonstrated that the applications of glyphosate in glyphosate-tolerant soybeans at levels typically applied in the field suppressed Asian soybean rust caused by *Phakopsora pachyrhizi*, but in field studies in Argentina and Brazil under natural infestations the impact of glyphosate applications was more variable (Feng *et al*., 2005 & 2008).

Glyphosate applications have been associated with increased incidences of certain soil-borne fungal diseases. Glyphosate was also shown to enhance the rate of plant death caused by *Pythium* sp. and *Fusarium* sp. in glyphosate-susceptible beans (Johal & Rahe, 1984; Johal & Huber, 2009). Similarly, glyphosate enhanced the virulence and pathogenesis of *Fusarium solani* f. sp. *cucurbitae* and of *Alternaria cassiae* on glyphosate-susceptible crops (Boyette & Hoagland, 2000). In RR soybean, in-vitro bioassays showed that glyphosate in the root exudates stimulated growth of selected rhizosphere fungi. Also in field studies, it was shown that the frequency of root-colonising *Fusarium* increased in glyphosate-tolerant soybean and maize cultivars, in comparison with non-GM or glyphosate-tolerant cultivars not treated with glyphosate (Kremer & Means, 2009). However, other field studies of soil-borne fungal pathogens (*Fusarium solani* f. sp. *glycines*, and *Sclerotinia sclerotiorum*) in glyphosate-tolerant soybean did not detect any increased disease pressure following glyphosate applications compared with applications of other herbicide treatments on soybean (Lee et al., 2000; Sanogo et al., 2001).

7.4 Summary of effects of GM soybean

Based on the classification criterion of not being mentioned in the first Environmental Assessments Reports of APHIS the following unexpected effects of GM soybean were found:

- weed species shifts;
- increased adoption of reduced tillage systems;
- occurrence of glyphosate-resistant weeds;
- reduced nutrient uptake;
- reduced activity of nitrogen-fixing symbionts;
- effects on plant diseases.

The first three effects could be explained as consequences of the introduction of herbicide-tolerant soybean. This is not true for the last three effects. The occurrence of soybean volunteers was expected, but these volunteers are not occurring on a large scale and they are not difficult to manege. The occurrence of glyphosate-resistant weeds were observed more than expected at the moment of introduction. This is caused by the introduction of glyphosate-tolerant soybean on a very large scale in a crop rotation in which also glyphosate-tolerant maize and glyphosate-tolerant cotton are grown. Glyphosate-resistant weeds have effects on the amounts of herbicides used which was not expected at the moment of releasing the first herbicide-tolerant soybean varieties. The effects of glyphosate-tolerant soybean on the activity of nitrogen-fixing symbionts and on plant diseases detected in laboratory and field experiments were not expected by APHIS, nor by scientists, at the time of the release of glyphosate-tolerant soybean. However, the agronomic relevance of these effects in commercial cultivation appears to be small.

8 Cotton (*Gossypium spp.)*

8.1 Introduction

8.1.1 Crop characteristics

Several different cotton species are grown worldwide, but *Gossypium hirsutum* is commercially the most important species being cultivated on about 90% of the total cotton area. In the USA also *Gossypium barbadense* is grown. Both species are usually managed as annuals and can be either self-pollinated or cross-pollinated by insects, most often bumblebees (*Bombus* spp.). Cotton pollen is sticky, and movement by wind is negligible. There are some wild feral populations of *G. hirsutum* in the USA, but they are limited to Southern Florida, which is not a commercial cotton growing area. In the USA there is a wild relative, *G. thurberi* Tod occurring in Arizona, but it is a diploid species and is not compatible with cultivated cottons which are tetraploids. Therefore, gene flow via pollen to compatible relatives is not considered to be a significant issue in the USA. Cotton seeds are not dormant and do not persist in the environment. Volunteer cotton plants do occasionally occur in subsequent crops, but they generally do not survive winter temperatures in the USA.

8.1.2 Adoption of GM cotton

In 2008, 15.5 million ha of GM cotton was cultivated worldwide, which was slightly less than half the total cotton area (ISAAA, 2009). Most GM cotton was cultivated in China, India and the USA with smaller areas in Argentina, South Africa, Australia, Brazil, Mexico, Burkina Faso, Pakistan and Columbia (Table 8). In 2009, GM cotton with traits that lead to insect resistance and herbicide tolerance were commercially cultivated. These traits have been approved for import and processing in the EU (GMO Compass, 2010).

Table 8. **Adoption and area of Bt and herbicide-tolerant cotton in main cotton growing countries in 2007 (Source: Brookes & Barfoot, 2009).**

Land	% Bt relative to the total cotton area	Area Bt cotton (x1000 ha)	% herbicide resistant relative to the total cotton area	Area herbicide-resistant cotton (x1000 ha)
China	61%	3 782		
India	63%	3 698		
US	59%	1 534	70%	1820
Brazil	32%	115		
Argentina	49%	152	38%	118
Australia	86%	55	79%	51
Mexico	48%	29	40%	24
South Africa	76%	8	75%	7

Bt cotton is primarily used to reduce crop losses due to infestation of the cotton bollworm (adults are moths, Latin name: *Heliocoverpa zea*) or to reduce pesticide use against this bollworm. Also the pink bollworm (*Pectinophora gossypiella*) and *Heliothis virescens* can be controlled with the help of Bt proteins. Different genes that lead to the production of various Bt proteins have been incorporated in cotton (among others events 531, 15985, COT102, 281-24-236 and 3006-210-23). Different Bt (Cry) proteins provide different control spectra. Bt cotton has become popular among cotton farmers as it allows them to save on pesticide use and/or reduces yield losses and yield uncertainty. Monsanto Company introduced Bt cotton around 1996 and since then other companies and institutes, among others in China and India, developed their own Bt lines. Recently the Indian Council for Agricultural Research (ICAR), a public institute, released a non-hybrid Bt variety that allows farmers to save their own seed without losing the efficacy of the Bt gene (Karihaloo & Kumar, 2009). Bt cotton is a very popular crop among small-scale farmers in developing countries (Table 8). About 12.1 million small-scale farmers in China, India, Pakistan, and South Africa cultivated Bt cotton in 2008 (ISAAA, 2009). The Bt trait is also popular among large-scale cotton producers in the USA, Australia, Argentina and Brazil.

Various herbicide-tolerant GM cotton lines have been developed with the aim to facilitate weed control in cotton. Roundup Ready® (RR) cotton is tolerant to applications of the aselective, broad-spectrum herbicide glyphosate (events MON 1445, 1698, 88913, GHB 614). Glyphosate-tolerant cotton was introduced in the USA in 1997 by Monsanto Company and is currently cultivated in a large number of countries. Also Bayer CropScience has launched a glyphosate-tolerant cotton line. The use of glyphosate-tolerant cotton however is less widespread than Bt cotton. Liberty Link® cotton has an event that makes the cotton plant tolerant to applications of the aselective, broad-spectrum herbicide glufosinate-ammonium (event LLCotton25). This trait has been patented by Bayer CropScience. It was commercially launched in the USA in 2004 and is now cultivated in various other countries as well. Two more herbicide-tolerant cotton lines have been deregulated in the USA (providing tolerance against sulfonylurea and bromoxynil; events 19-51a and BXN), but these are currently not available in seeds for commercial plantings as far as we are aware of.

The Bt trait in cotton can be combined with herbicide-tolerance traits. A cotton line containing the Bt and glyphosate-tolerance traits is already commercially available. Also a cotton line combining the glufosinate-tolerance and the Bt trait will be launched in the near future.

8.1.3 Overview of environmental effects of GM cotton

Herbicide-tolerant and Bt cotton have had different impacts on the environment and most of them were not expected by APHIS during the first environmental assessment (Table 9).

Table 9. **Overview of observations of unexpected effects of herbicide-tolerant and Bt cotton cultivation.**

Effect	Expected by APHIS	Observed in commercial production or in large scale experiments	Scientifically confirmed as a relevant environmental effect
HT cotton			
Herbicide use	+	+	+
Increasing area under no tillage	-	+	+
Herbicide-resistant weeds	-	+	+
Bt cotton			
Pesticide use	+	+	+
Non-target invertebrates presence in Bt cotton	+	+	+
Bt resistance among pest insects	-	-	-
Increase in secondary pests	-	+	+
Reduced resistance to bollworms during periods of drought	-	+	-

8.2 Expected effects of herbicide-tolerant cotton

8.2.1 Herbicide use

USDA NASS data from the USA suggested that total herbicide use (active ingredients) in cotton decreased between 1997 and 2001 and steadily increased thereafter up to 2008 to levels well above the 1997-level (Benbrook, 2009). As 1997-2008 is the period in which most of the non-GM cotton was replaced by herbicide-tolerant GM cotton, the data suggest herbicide-tolerant cotton initially helped to reduce herbicide application levels, but subsequently led to an increase in herbicide use. This increase in herbicide use was probably related to the emergence of herbicide-resistant weeds, stimulating farmers to use more herbicides to achieve satisfactory weed control. Benbrook (2009) segregated the USDA NASS (National Agricultural Statistics Service) herbicide use data for non-GM and glyphosate-tolerant GM cotton based on farmer survey data. These analyses confirmed that glyphosate-tolerant cotton received less herbicides than non-GM cotton in 1997-2000. Thereafter, the roles reversed with non-GM receiving lower herbicide applications than GM cotton.

8.3 Unexpected effects of herbicide tolerant cotton

8.3.1 Increasing adoption of reduced tillage systems

The availability of herbicide-tolerant crops has been linked with an increasing adoption of reduced tillage practices in the Americas. This is also true for herbicide-tolerant cotton cultivated in the USA (Frisvold *et al.,* 2009; Banerjee *et al.,* 2009). The statistical correlation between the adoption of herbicide-tolerant cotton and the adoption of reduced tillage techniques found in these studies suggests that the relation between the two is less evident than for instance in the case of herbicide-tolerant soybean. It is uncertain whether this is because the amount of data on cotton in relation to tillage practices is small or because the relationship itself is weak.

8.3.2 Herbicide-resistant weeds

The change in herbicide management that herbicide-tolerant crops including herbicide-tolerant cotton allow, resulted in a strong reliance on glyphosate, and to a lesser extent on glufosinate-ammonium, for weed control. The utility of glyphosate-tolerant crops is threatened by shifts in weed species composition and the emergence of herbicide resistant weeds. This is clearly currently also the case for glyphosate-resistant cotton in the southern USA (Webster & Sosnoskie, 2010), where biotypes of obnoxious weeds such as Benghal dayflower (*Commelina benghalensis*) and Palmer amaranth (*Amaranthus palmeri*) have developed resistance to glyphosate.

8.4 Expected effects of Bt cotton

8.4.1 Pesticide use

While the Bt proteins do not provide resistance to all pests in cotton, they do control some of the main Lepidopteran pest insects. When Bt cotton was introduced, it was expected to enable farmers to change pest control strategies and biocide use. Lots of evidence is nowadays available that this has been the case. Studies by Huang *et al.* (2002 & 2003) indicated that insecticide use (expressed as quantity of active ingredients) under 1000 surveyed farmers in China in 1999-2001 was 58-81% lower in Bt cotton than in non-GM cotton. The effect of Bt cotton on pesticide use was very large in certain areas due to an extremely high pesticide use in non-GM cotton, which was related to widely spread resistances among insect pests against the commonly used insecticides. Especially the use of relatively toxic pyrethroids, organophosphates and organochlorides was strongly reduced in Bt cotton. Wang *et al.* (2009) noticed in a study partly based on the same data used by Huang *et al.* an increase in insecticide use in Bt cotton between 1999 and 2004 and a reduction in the insecticide use in non-GM cotton. Thus, the relative difference in insecticide use between Bt and non-GM cotton decreased in that period. In 2004 insecticide use in Bt cotton was only 38% lower than in non-GM cotton, while in 2006 the advantage of Bt cotton above non-GM cotton was larger again. The diminishing advantage of Bt cotton above non-GM cotton in terms of insecticide use in 2001-2004 was probably related to an increase in secondary pests (especially phloeme-sucking bugs, Miridae) in cotton. Bt proteins are not effective against pests belonging to this group. The analysis by Wang *et al.* (2009) suggested this increase in secondary plague insects was primarily caused by particular weather conditions in 2001-2004. A study by Sadashivappa & Qaim (2009) among Indian farmers over 2002-2007 indicated that insecticide use (expressed as the amount of active ingredients) was reduced in Bt cotton by 40%, relative to non-GM cotton. In South Africa, Bt farmers on the Makhathini plains used 40-63% less insecticides than non-GM farmers in 1998-2001, according to Morse *et al.* (2006). The use of all types of insecticides used in cotton (organophosphates, pyrethroids and organochlorides) was reduced in Bt cotton. In a study by Hofs *et al.* (2006) among 20 small-scale farmers in 2002-2004 the use of pyrethroids was strong reduced among Bt farmers (<35% of the use in non-GM cotton), while Bt cotton had no significant impact on the use of organophosphates. A study by Qaim *et al.* (2003) in Argentina suggested that insecticide use (active ingredients) among Bt cotton farmers was on average 57-66% lower than among non-GM farmers. In the USA, insecticide use in Bt cotton in 2007 was 6.7% lower than in non-GM cotton (Brookes & Barfoot, 2009). Also Benbrook (2009) confirmed that the introduction of Bt cotton in the US has resulted in a overall decline in insecticide use. In Australia insecticide use in Bt cotton was 69% lower than in non-GM.

8.4.2 Non-target invertebrates presence in Bt cotton

The Bt proteins in Bt cotton generally have a more selective control spectrum than the insecticides used in cotton, because Bt proteins specifically target Lepidopteran species and because only insects ingesting cotton plant parts primarily come in contact with the Bt proteins. Scientific literature on the effects of Bt cotton on non-target organisms and soil ecosystems has been extensively reviewed by the meta-analysis of Wolfenbarger *et al.* (2008) and by Naranjo (2009), Sanvido *et al.* (2007) and Romeis *et al.* (2006). Field studies to the consequences of Bt crops on the incidence of arthropods in general and beneficial insects in particular indicate that the cultivation of Bt cotton result in a similar or higher field agro-biodiversity in comparison with the cultivation of non-GM cotton (Head *et al.,* 2005; Torres & Ruberson, 2005; Shen *et al.,* 2006; Sisterson *et al.*, 2007; Hofs *et al.,* 2008).

8.5 Unexpected effects of Bt cotton

8.5.1 Bt resistance among pest insects

When Bt cotton was introduced, concerns with regard to the risk of pest insects developing resistance against the Bt proteins were expressed and laboratory tests indeed showed that various pest insects can develop this type of resistance (Van Rie & Ferré, 2000). Surprisingly however, up to now cases of insects developing resistance against Bt in the field have been rare and the Bt proteins have kept their effectiveness against the pests (Naranjo, 2009; Tabashnik, 2009).

8.5.2 Increase in secondary pests

The currently available Bt genes in cotton all lead to resistance to particular species belonging to the group of the Lepidoptera and the Bt proteins produced by Bt cotton have a limited control spectrum. While this specificity is advantageous for the protection of beneficial insect populations, a control strategy depending on Bt proteins could also increase populations of pest insects other than those belonging to the Lepidoptera. If farmers in this case need to apply additional pesticides to control some of these so-called secondary pests, the advantage of Bt in terms of pesticide savings could be partly offset. There is some evidence in China that this could have been the case. In experimental trials carried out in 1999-2001, greater numbers of sucking insects (Miridae) were observed in Bt fields than in non-Bt fields. The numbers of sucking insects exceeded the threshold level at which pesticide application is recommended (Men *et al.*, 2005). Also in farmers' fields in China, a rise in pesticide use for the control of pests belonging to the Miridae was observed between 1999 and 2006 and this increase in pest pressure indeed reduced but not annihilated the advantage of Bt cotton above non-GM cotton in terms of pesticide use (Wang *et al.*, 2009). However, Wang *et al.* suggested this increase in secondary pests was related to particular local temperature and rainfall conditions and not so much to the cultivation of Bt cotton. Lu *et al.* (2010) monitored the occurrence of mirid bugs in field trials in northern China over the last 10 years and noticed progressively increasing populations. More importantly, they identified Bt cotton as a source of mirid bugs and related this to the drop in insecticide use in this crop.

8.5.3 Reduced resistance to bollworms during periods of high temperature

In China poor and variable performance of Bt cotton was found in different regions. In field investigations it was discovered that the reduction of the insect resistant efficacy was found after periods of high temperature. Chen *et al.* (2005) found in experiments that exposure to high temperature in the boll period resulted in a significant decrease in soluble protein content, and significant increases in the activity of protease. These results suggest that high temperature may result in the degradation of soluble protein in the leaf, with a resulting decline in the level of the toxin CryIA. It is believed that this may be the cause of the reduced efficacy of Bt cotton in growing conditions in China, where temperatures during the boll period often reach 36-40°C.

8.6 Summary of effects of GM cotton

The impact of the introduction of herbicide-tolerant cotton in the late 1990s on herbicide use in the US is comparable with that of HT soybean and maize. Total herbicide use (active ingredients) in cotton decreased between 1997 and 2001 and steadily increased thereafter up to 2008 to levels well above the 1997-level. The strong reliance on glyphosate in herbicide-tolerant crops including herbicide-tolerant cotton has resulted in the development of glyphosate resistant weed biotypes. The possibility to use broad-spectrum herbicides such as glyphosate in HT crops including cotton has stimulated the adoption of reduced and no tillage techniques. The effect of HT cotton on the adoption of reduced tillage techniques is less evident than with HT soybean.

Bt cotton has had a well-documented and expected impact on pesticide use. Worldwide pesticide use has declined in cotton as a result of the availability of Bt cotton. As Bt proteins are more specific in their control spectrum than the pesticides used in cotton, and because only insects ingesting cotton plant parts come in contact with Bt proteins, the impact of Bt cotton on the biodiversity of arthropods in cotton fields is neutral or positive. Both these effects were expected by APHIS when Bt cotton was about to be deregulated. So far cases of insects developing resistance against Bt proteins have been rare in the field. This can be seen something unexpected, as insects do develop resistance to Bt proteins under laboratory conditions. Moreover, resistance development is a wide spread phenomenon with many regular pesticides. Wide-scale cultivation of Bt cotton and the associated decline in pesticide use has in some cases resulted in an increase in the presence of secondary pests. This has primarily been documented in cotton-growing areas in China. While this is not a surprising finding from a agro-ecological point of view, the effect was not expected by APHIS when Bt cotton was released. Finally, there are indications that Bt cotton is less effective against insects under hot weather conditions.

9 Discussion and implications for monitoring

9.1 Definition of unexpected effects

In this inventory we first had to operationalize the concept of 'effects being unexpected'. We were aware of the subjective element in judging an effect as 'expected' or 'unexpected', since such judgements are in the end all about making predictions of future occurrences and these always include a certain level of uncertainty. Moreover, more information became available, during the 15-year period of growing GM crops, and, with the processing of such information over time, effects could perceived as expected, in particular when effects appear to be logical with the benefit of hindsight.

In order to achieve a more objective operationalisation, we primarily based our distinction between expected and unexpected effect on the first Environmental Assessments (EA) reports of APHIS in the USA concerning the herbicide-tolerance and Bt traits that are still encompassing the main body of GM crops grown nowadays. In this way, an expected effect was defined as an effect mentioned in these first EA reports. This approach however has some limitations:

- Since the EA reports dealt with risk assessments of GM crops, positive environmental effects (benefits) were by definition not addressed. Therefore, effects on tillage practice and pesticide usage now appear artificially under the heading of unexpected effects in our report. This, however does not imply that these effects were unexpected as for instance, the introduction of Bt traits was clearly meant to replace insecticide application against the targeted insect pests.
- The extent to which effects were unexpected in the EA reports could have been assessed differently by other scientists and therefore may even not fully count as unexpected. This could be more difficult to trace back, but was addressed in our literature search and discussed in the relevant sections of the crop chapters above. An interesting example of this is in the development of herbicide-resistant weeds. At the time of introduction, there were several reasons to believe that overcoming glyphosate toxicity was quite hard for plants as described in Bradshaw *et al.* (1997): experiences in the past and difficulties in changing the target of glyphosate, the EPSPS enzyme, to a resistant form without losing its functionality. Nevertheless, the increase in the amount of cultivations in which glyphosate is the main method of weed control, increases the selection pressure on weeds to develop some form of resistance against glyphosate. Therefore, other publications have pointed at the possibility that this leads to the emergence of glyphosate-resistant weeds (e.g. Bijman & Lotz, 1996; Darmency, 1996; Lotz *et al.,* 1999). Later versions of the EAs have taken this into account even though weed resistance being a mainly agronomical problem is not a prime responsibility of APHIS.

9.2 Assessment of unexpected effects

In order to ensure that we reduced the chance of missing relevant effects as much as possible, we used a multi-step approach. Firstly, unexpected environmental effects were traced by searching in comprehensive databases of the peer-reviewed scientific literature. Subsequently, "grey" literature was searched for any effects missed, mainly by using internet search machines for finding reports by institutions and NGOs. Any effect found in this manner was checked against peer-reviewed scientific literature before inclusion in the inventory. The last step was to check our findings with personal contacts, especially during a study visit to the USA, where interviews were held with experts from the Competent Authorities and from research institutions and universities.
The focus on North America, particularly the USA, was warranted by the availability of the first environmental assessment reports and the extensive experience in large-scale growing of GM crops, but had some limitations. These countries did not put in place extended systems of post-release monitoring.

This meant that information about unexpected effects depended on the interest of research groups in these effects. In addition, the possibility always remains that certain unexpected effects will show up after a longer period than 10 – 15 years. Unexpected effects with an agronomic impact are likely to receive more attention than unexpected effects with impact on organisms with no economic value. The growing of some crops has been too limited to be able to fully assess any effects: GM sugar beet cultivation started a few years ago and GM potatoes have only been grown for a few years.

9.3 Unexpected effects of herbicide-tolerant crops

Basically, all effects described in this study were a consequence of the use of the herbicide accompanying the introduction of herbicide-tolerant crops and not from the transgene encoding the herbicide-tolerance trait as such. Some of the effects were mainly agronomical, such as diminished micronutrient uptake and higher sensitivity to certain diseases as a consequence of herbicide application. Other effects were related to biodiversity. The use of herbicide-tolerant crops probably facilitated the increase in reduced or no-tillage systems and these have several benefits. Soil erosion and the environmental costs of ploughing are reduced. Depending on soil type, climate and land use, physical, chemical and biological properties of the soil are improved as well. This in turn results in no-tillage systems also having a positive effect on wildlife. On the other hand, herbicides do have an impact on weed occurrence and thus on the accompanying wildlife. The first environmental risk assessments did not take into account that the efficacy of herbicide regimes under GM and conventional crops may differ, but when they do, they are expected to have secondary effects on wildlife depending on weeds. The UK Farm Scale Evaluations (FSE) showed that in practice there may be differences in efficacy of weed control between the conventional growing of a crop and herbicide-tolerant versions. In oilseed rape and sugarbeet, weed control turned out to be more effective in the GM crops, whereas this was the other way around in maize, mainly due to the effectiveness of the conventional use of atrazin, which is no longer used in the EU. The resulting differences in weed occurrence generally led to changes in invertebrate diversity depending on these weeds (e.g. Brust, 1990) and e.g., also in the presence of birds feeding on weed seeds (Gibbons *et al.*, 2006). These effects of weeds on biodiversity had already been reported before the cultivation of herbicide-tolerant crops started. Optimal weed control is considered to be a basic agronomical and economical aim of crop growers which might place mitigation of its consequences for wildlife beyond the scope of GM crop EA. It also could take several forms, e.g. the more flexible weed control offered by GM crops could make it possible to leave certain parts of a field available for weed growth while minimizing undesirable infestation levels (BRIGHT) or land could be set aside for this purpose.

We concluded from this literature search that with respect to herbicide-tolerant crops the truly unexpected effects (i.e. not described in the first EA and at that time not reported or hypothesized in literature) are observations of reduced uptake of micronutrients and a possible effect on susceptibility to diseases in crops treated with glyphosate. These effects are not directly caused by the genetic modification itself, but they are indirect effects caused by the application of glyphosate.

9.4 Unexpected effects of Bt-crops

Similar to herbicide-tolerant crops, some of the effects of Bt crops have a mainly agronomical character, such as a regional suppression of pest insect populations, a rise in secondary pest, decreased susceptibility to certain fungi or resistance development against Bt in a pest insect. Resistance development against Bt by insects has been rare up till now. Other effects again relate to biodiversity. Bt has quite a specific action spectrum: it is mainly effective against members of the Lepidoptera (moths) and Coleoptera (beetles). Various Cry proteins also differ in the severeness of the effect between different species of these insect groups. Still, quite some research and debate have revolved around effects on non-target species, particularly from these insect groups but also on other important groups involved e.g. involved in decomposition activities in the soil, and on secondary effects at other trophic levels. Generally, where effects were found on some non-targeted members of the Lepidoptera and Coleoptera in the field, these were less severe than the effects of insecticide application. Effects on predators and parasitoids of pest insects were shown to be no direct consequence of Bt action but to be due to the prey quality and/or numbers being lowered by Bt. This could be regarded as a general (expected) effect of any plant resistance effective in bringing down pest infestation. In Bt cotton, Greene *et al.* (1999) studied sucking insects (stink bugs) as secondary pests for which above certain thresholds, additional insecticide treatment might be needed. They referred to an inventory of such bugs only published in Proceedings on the 1995 occasion of Beltwide Cotton Conferences.

We conclude from this literature search that for Bt crops no truly unexpected effects (so not described in the first EA and at that time not reported or hypothesized in literature) have been found.

9.5 Implications for monitoring

This inventory has been performed for the GM crops that have been grown during the last 10-15 years, viz. crops with herbicide-tolerance and Bt-resistance. Conclusions about implications for monitoring are therefore only relevant for these modifications. Assessment and research on GM crops with other modifications, such as e.g. drought tolerance or fungal resistance, may lead to other conclusions about monitoring.

In general, no major effects were found that could be assessed as unexpected to a large degree after taking into account the scientific literature in addition to the first environmental assessments. Therefore, there are no simple conclusions about aspects that should be covered by monitoring programmes. Many of the effects also were of an agronomical nature, which could have a higher likelihood of being detected because of interest from agronomists and farmers alike and the presence of stewardship programmes, aimed at e.g. early detection of resistance development in pest insects.

A limitation of our approach of mainly investigating North America is that there are many differences in soil and growing conditions, crop rotations and production systems between the Netherlands and North American countries. Therefore, it may for instance be difficult to predict in detail to what extent weed shifts will occur or resistant weeds could develop. It is possible that the recent interest in reduced tillage or no-till systems could be raised by the adoption of herbicide-tolerant crops, but this is depending on several other factors.

Another limitation is that quite substantial resources may be required to detect a change that builds up by small steps over periods that may even be longer than 10- 15 years. For instance, experiences in the USA are showing that it takes time before levels of a secondary pest have risen to an economically relevant level. Sometimes much research is needed to detect how insect populations are changing. It can also be difficult to distinguish changes in insect populations related to the adoption of Bt crops from those related to changes in environmental conditions, such as changes in land use or weather conditions. For instance, Aviron *et al.* (2009) concluded from a study of case-specific monitoring of Bt maize with regard to butterflies that already a large sampling effort would be needed to disentangle Bt maize effects from other environmental variables for abundant species and that it was virtually impossible for rare species.

Monitoring schemes will have to take these aspects into account. This inventory only revealed a few truly unexpected effects. These effects were specific to glyphosate-tolerant crops. Therefore, it was not possible to draw general conclusions for developing protocols for post-release monitoring of environmental effects.

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Appendix

Report of visit to the USA*

June 16, Visit to APHIS
Animal and plant Health Inspection Service
United States Department of Agriculture
Biotechnology Regulatory Services
4700 River Road, Unit 147
Riverdale, Maryland
Contact with: David. S. Heron (Assistant Director Policy Coordination Division)
John M. Cordts (Branch Chief Plant Pests and Protectants Branch)

The mission of APHIS is to protect the health and value of American agriculture and natural resources. APHIS regulates the introduction (importation, interstate movement, or environmental release) of certain genetically engineered (GE) organisms. All regulated introductions of GE organisms must be authorized by APHIS under either its permitting or notification procedures.
The activities of APHIS are based on the Plant Pest Act. As long as it is not clear that a certain transgenic crop will not become a plant pest it will have the regulated status. Following field testing done by the company, a petition for non-regulated status may be submitted to APHIS. A petition is a request that APHIS considers that the crop is not a plant pest risk and that it is no longer needed to be regulated. Before deregulation APHIS conducts an environmental assessment and asks public input during a public comment period. The criteria for deregulation are: Is there a risk that the crop is becoming a plant pest? And is there an environmental risk? The base-line is the conventional crop and the question is if the GE organism is giving more risks than the conventional organism. The company has to deliver results of different tests and the results are evaluated by scientist of APHIS. If there are doubts an Environmental Impact Statement is made. APHIS does not have any system of monitoring during post-release growing.

APHIS does not know any serious unexpected effect which has been occurred during post-release growing of GE crops. They are mentioning only two unexpected positive effects which have been observed: the better drought tolerance of Bt-maize with protection against root worm due to a less damaged rooting system and the decreased aflatoxin levels in Bt-maize. According to APHIS unexpected negative effects with a large impact have not been observed. The observed development of weeds resistant to glyphosate is ascribed to the overuse of glyphosate. This could have been avoided. Some effects are ascribed to the genetic background of the varieties in which the events are introduced (slower decomposition of Bt maize corn straw and reduced uptake of micronutrients in glyphosate-tolerant soybean).

The most important points:

- APHIS is focusing strictly on their tasks according to the law. The organization is not responsible for effects occurring during post release growing of a GE crop.
- AHPIS does not have a system for monitoring effects during post-release growing. Monitoring by companies is not prescribed by APHIS.
- APHIS is agreeing with reports in which it is stated that there is no substantial scientific evidence of adverse environmental effects of GE crops available.

* This report consists of summaries of the visits not authorisized by the interviewed scientists

June 17, Visit to EPA
Environmental Protection Agency
Biopesticides and Pollution Prevention Division
Environmental Risk Assessment Team
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460.0001
Contact with: John Kough (Senior Scientist)
Zigfridas Vaituzis (Senior Scientist)

EPA regulates the release of transgenic crops that have been engineered to express a pesticide (PIP = Plant Incorporated Protectants). Their work is based on the Federal Insecticide, Fungicide, and Rodenticide Act. The developer must register the PIP before commercialization. To register a PIP, EPA must determine that the PIP will not cause “unreasonable adverse effects on the environment” and EPA can place any necessary conditions on its safe use. EPA is working with panels of scientists. The companies are delivering protocols for testing the GE crop. The panel is judging these protocols. If the protocols are approved the companies are testing the GE crop according to the protocol. The companies are presenting the results to the panel and the panel is evaluating these results.

It is difficult to monitor unexpected effects, because it is very expensive and time consuming. There are also statistical problems: how many observations are needed to draw conclusions? Moreover, it is not always easy to prove that an effect is related to GE crops. Agriculture is an ever changing system. Not only GE crops are introduced, also other changes are occurring (choice of crops, herbicides, pesticides, crop rotation systems).

During post-release growing of Bt crops the companies are asked to monitor the development of resistance to Bt. Changes in susceptibility of the target species have to be recorded and also monitoring has to be done on the compliance to the refuge requirements. It appears that companies are doing these tests together or they are asking a third company or university to do this. The aim of this monitoring is to minimize the risk of resistance development. If farmers are not sowing enough refuge fields it will be forbidden for the companies to sell any longer Bt seeds to these farmers.

The most important points:

- EPA is prescribing post-release monitoring on development of resistance and on compliance to the refuge requirements. Monitoring on other effects is not required.
- EPA agrees with several studies published in recent years in which it was stated that Bt crops have not caused long-term negative environmental effects on non-target organisms. Also the accumulation of Cry proteins in the soil is not occurring in a significant way.
- EPA does not know other environmental effects than the effect that are reported in our inventory.
- EPA is comparing the effects of GE crops with conventional production. They are convinced that there are no clear adverse environmental effects of Bt-maize. The effects of different insecticides had more adverse effects than Bt effects on animals or birds are not found.

June 18. Visit to Center for Science in the Public Interest
Biotechnology Project
1876 Connecticut Avenue, N.W.
Washington, D.C. 20009
Contact with: Gregory Jaffe (Director, Biotechnology Project)

In 2009, Jaffe has published about the increase of noncompliance of farmers with EPA's refuge requirements (Complacency on the Farm; Significant Noncompliance with EPA's Refuge Requirements Threatens the Future Effectiveness of Genetically Engineered Pest-protected Corn).

His opinion about unexpected effects: recently a report has been published in which the conclusion was drawn that there are no adverse effects with GE crops introduced in practice until now. The report was written by the Committee on the Impact of Biotechnology on Farm-Level Economics and Sustainability (National Research Council). The report was made by scientist who are known as independent and they are giving a realistic approach. (Impact of Genetically Engineered Crops on Farm Sustainability in the United States, 2010).
The findings are that there are no important unexpected effects. According to the Committee genetic engineering technology has produced substantial net environmental and economic benefits to U.S. farmers compared with non-GE crops in conventional agriculture. Generally, GE crops have had fewer adverse effects on the environment than non-GE crops produced conventionally. The use of pesticides with toxicity to non-target organisms or with greater persistence in soil and waterways has typically been lower in GE fields than in non-GE, nonorganic fields.
There are some points which need attention, but this is comparable with other techniques. The reliance on glyphosate on many fields is giving risks. The problems should be solved by management: crop rotation, rotation of herbicides. Only in this way the advantages of herbicide-tolerant crops could be saved for the future. The same is true for Bt maize and Bt cotton. Farmers should comply to the refuge requirements to avoid loss of resistance of Bt crops.
Another point mentioned in the report is that the adoption of herbicide-resistant crops complements conservation tillage practices, which reduce the adverse effects of tillage on soil and water quality. With respect to Bt maize and cotton the report states that targeting specific plant insect pests with Bt corn and cotton has been successful, and the ability to target specific plant pests in corn and cotton continues to expand. Insecticide use has decreased with the adoption of insect-resistant crops. Also the emergence of insect resistance to Bt crops has been low so far.

Most important points:
- The Centre for Science in the Public Interest agrees with publications in which it was concluded that GE crops have fewer adverse effects on the environment than non-GE crops produced conventionally.
- The Centre is focusing on promoting methods to use GE crops in such a way that the advantages of these crops are maintained for a long period (no overuse of glyphosate, compliance to refuge requirements).

June 21, Visit to University of Minnesota
Department of Agronomy and Plant Genetics
411 Borlaugh Hall, 1991 Upper Buford Circle
St. Paul, MN 55108-6026
Meeting with:

- **Nicholas R. Jordan (Professor Application of plant population ecology to agricultural problems, especially integrated weed management)**
- **Jeffrey Gunsolus (extension weed management maize & soybean cropping systems)**
- **Jeffrey Coulter (maize agronomy)**
- **Gregg Johnson (weed ecology; perennial crop agronomy)**
- **Seth Naeve (soybean management)**
- **Roger Becker (weed management in vegetables and forage crops)**

In general, the agronomists present in this group mentioned some agronomic unexpected effects which were occurring in first period of using glyphosate-tolerant crops (more than one application of glyphosate was needed in soybean and in maize glyphosate should be used earlier than in the beginning was done). They are concerned about the optimal use of herbicide-tolerant crops and they fear overuse of glyphosate. In herbicide-tolerant maize there was a substantial yield loss despite good weed control. The reason was that glyphosate was applied too late. Maize is very sensitive to early season weed interference. There was competition between weeds and maize for nitrogen. It was needed to apply glyphosate earlier or to use other herbicides to control early weeds. This competition effect between maize and weeds was unexpected. There are problems with glyphosate-resistant weeds. These problems are not as big as described by Benbrook. On 85 - 90% of the area there are no real problems, but on a small area problems are increasing. Additional herbicides are needed sometimes. Especially in sugar beet, some herbicide options e.g. for giant and common ragweeds, and waterhemp, affect crop rotation options subsequently. These herbicide-carryover effects are particularly severe in the climate of Minnesota, because of the slow degradation in cold/wet soils.

Most important points:

- It is confirmed that control of glyphosate-tolerant volunteer plants of one crop can give problems (weed problems and disturbance of rotation advantages). Sometimes additional herbicides with adverse environmental effects are needed.
- There are on some fields problems with glyphosate-resistant weeds. These problems should be solved by new strategies (using crops without glyphosate-tolerance or with other herbicide-tolerance traits)
- Reduced uptake of micro-nutrients by glyphosate-tolerant crops is not perceived as a serious effect. It is not occurring on a large scale.
- Reduced fixation of nitrogen in soybean is not occurring to such a degree that the fertilization of soybean is influenced by the adoption of glyphosate-tolerant soybean.
- Reduced tillage systems are promoted by the adoption of glyphosate-tolerant crops. These systems may improve habitat for some wildlife species.
- The adoption of herbicide-tolerant crops makes it possible to take marginal land in production. This will have an effect on wildlife.
- Herbicide-tolerant canola is present in many places. It is not cultivated in Minnesota on a large scale. It is spread into the fields in several ways: from the edges of the roads and with trucks which have transported fertilizers.

June 22, Visit to University of Minnesota
Department of Insect Ecology
219 Hodson Hall, 1980 Folwell Ave.
St. Paul, MN 55108
Contact with: David A. Andow (Professor Insect Ecology)

Western corn rootworm is the most important insect controlled by Bt genes in the Corn Belt. There is also a northern corn rootworm which is less easy to investigate. Both are low dose resistance traits. Root worm is causing lodging. This is giving not only yield reductions but also loss of harvest capacity. Bt-resistance is giving also a vital rooting system. The extra effect is a better drought tolerance. In the past seed was treated with insecticide. This was giving a restricted effect: only the roots in the neighbourhood of the seed were protected.

There is resistance against the western corn rootworm and it could be increased very easily. The situation is rather complicated. Companies have to cooperate to solve the problem of loss of resistance. Lack of compliance to the refuge prescriptions is a very serious problem. If a famer is not planting a refuge he is warned in the first year and he is checked in the next year. The companies have to monitor the compliance and they have to monitor the population on resistance development. For the companies it is a conditional process. If needed a re-evaluation will be done.

In maize the western bean cutworm has become a bigger problem in recent years. In the past it was only occurring in Nebraska. At the end of the 20th century it was not an important pest. Nowadays the insect is spread as far as east as Ohio, while it is present in many states. Spreading is caused by west to east winds. The insect is overwintering in Mexico and Louisiana. In three days it is coming back to the Corn Belt.

It is difficult to monitor effects other than agronomic effects. It is important to have a good research infrastructure. The best possible monitoring is needed. Some people say gathering anecdotes can also help in detecting changes. Farmers and crop consultancy people could play a role. In 2002 the National Research Council has evaluated monitoring. It is important to use every eye which is available (farmers, conservation groups) and training is needed. Monitoring must be followed by research to identify the relations and to develop control strategies.

Landscape effects: There are large effects. For example the refuge maize is much less attacked. In China it is known that suppression of insect in cotton leads to less spraying in vegetables.

Most important points:

- Development of resistance of western corn rootworm against the proteins produced by Bt-maize is a point of concern. The resistance could be increased very easily and companies are using stacked genes to solve this problems.
- Bt maize with resistance to western corn borer is less susceptible to drought and lodging. Yield losses and loss of harvest capacity are avoided.
- Lack of compliance to the refuge prescriptions is a very serious problem. There is a chance that target insect are becoming resistant.
- Secondary pest are observed, for example western bean cutworm, which is giving yield losses in the Corn Belt.
- Effects on non-target insects and other organisms are not easy to detect. Monitoring by the extension service, farmers and other people is needed. Monitoring should be followed by research to investigate the relations between organisms.
- Monitoring of non-agronomic effects is very difficult. Agronomic effects with economic consequences will be discovered earlier than other effects.
- Effects of Bt-crops on birds and on animals are not known, except the effects mentioned in the UK Farm Scale Evaluation project.
- Increased lignin content of Bt maize is found in some varieties, but there are also Bt varieties without an increased lignin content.

- Bt maize with resistance to corn borer is giving reduced levels of aflatoxins, resulting from fewer damaged kernels. However, next to Bt resistance also drought and genetic differences are playing a role.

June 23, Visit to University of Minnesota
Department of Insect Ecology
219 Hodson Hall, 1980 Folwell Ave.
St. Paul, MN 55108
Contact with: Ken Ostlie (Scientist Insect Ecology)

Populations of European corn borer and western corn rootworm are tremendously reduced since the introduction of Bt-maize. In the period 1963 – 1997 there was a serious attack every 4-6 years. Since the introduction of Bt-maize the problem has become less important.
Bt maize with resistance against western corn rootworm is sold also in Northern Minnesota, while western corn rootworm is not a problem in this region.

The western bean cutworm was originally only occurring in Nebraska. It is a disease of the drier areas. During recent years this cutworm is moving more to the eastern part of the country. May be there are biotypes that are better adapted to wetter conditions. This is another aspect to be considered in the discussion about the development of western bean cutworm as a secondary pest in Bt maize.

Wide-spread adoption leads to shift in insect populations. It is not known what is coming. For example in MON810 and BT11 (of Syngenta) in 2008 and 2009 cereal aphids (corn leaf aphids, oat birdcherry aphids, English grain aphids) were found in higher densities than before. Especially the English grain aphids can cause damage. This aphid is found in the ear zone. Sometimes yield depressions were reported. However, it is not clear if this is due to the adoption of Bt maize or that the weather conditions in 2008 and 2009 are playing a role. Research will be needed. The relationship between secondary pest and Bt traits has become more complicated because of the introduction of stacked genes.

Bt maize with resistance to western corn rootworm has an improved tolerance to drought. The whole rooting system is protected while seed treatments are only giving a protection of the roots surrounding the seed.

Farmers' compliance to the refuge prescriptions is reduced. EPA is concerned. It was estimated that the life span of Bt would be at least 15 years. There are different methods for sowing refuges: 4 rows out of 20, seed blends.

The development of resistance to corn rootworm is probably increased by the occurrence of resistant volunteer maize in soybean. There is a 5-fold difference in protein content. The late flowering of these volunteer plants is increasing the problem.

Most important points:

- The effect on non-target organisms is sometimes becoming visible after a great number of years and it costs a lot of research to find the relationships between the involved organisms. Sometimes differences between years in growing conditions and changes in agricultural practices are making it difficult to discover these relationships.
- The adoption of Bt maize has reduced the population of western corn rootworm and European corn borer to a large degree. Refuge non-Bt maize field are suffering less from these insects.
- The introduction of Bt maize has sometimes reduced the choice for the farmer. Sometimes only varieties are available with traits that are not needed and with a high seed price.

June 23, Visit to University of Minnesota
Department of Fisheries, Wildlife and Conservation Biology
219 Hodson Hall, 1980 Folwell Ave.
St. Paul, MN 55108
Contact with: Karen S. Oberhauser (Professor Evolution and Behavior)

In Minnesota research has been done on Monarch butterfly. Oberhauser does not agree with the calculations with respect to temporal overlap between Monarch butterfly and the presence of corn pollen. She thinks the whole population is present during the period of flowering.

In 2002/ 2003 research was started on the effect of Bt on Monarch butterfly. The most important point is that milkweed is not occurring any longer in maize because of the use of glyphosate. Also the milkweed in the rural areas surrounding the maize fields has disappeared, as result of drift. According to Oberhauser Bt has not an effect on Monarch butterfly, because milkweed is not present in the maize fields.

There are many questions left about the Monarch butterfly and it is not known how important milkweed in maize fields is for this insect. Monarch butterfly is overwintering in Mexico in trees. It is not know whether environmental changes in Mexico are playing a role in the population density of the Monarch butterfly.

Most important points:

- Milkweed is not present in most maize fields, because it is controlled very well by glyphosate. This means that Monarch butterfly is not feeding on milkweed and Bt pollen can not have an influence on this insect.
- The knowledge about Monarch butterfly is incomplete and the importance of milkweed in maize fields is not known.

June 23, Visit to University of Minnesota
439 Borlaug Hall, 1991 Upper Buford Circle
St. Paul, MN 55108
Contact with: John Lamb (Extension Soil Scientist)
Jeff Stachler (Extension Agronomist Sugarbeet Weed Science)

The introduction of glyphosate-tolerant sugar beet has made weed control much more easy and reliable. In conventional sugar beet growing herbicides were sprayed only in the rows because of the high costs of the herbicides. Between the rows tillage was done for controlling the weeds. In a survey it was found that the number of these tillage operations has reduced from 1,3 to 0,1.

Volunteer maize and volunteer soybean are occurring in sugar beet. The biggest problem is formed by glyphosate-tolerant canola volunteers in sugar beet. Canola is coming into the field in several ways: from the edges of the roads, in the cover crops (to reduce wind erosion) and also by fertilizer-transporting lorries. It is not easy to find a good herbicide for controlling these volunteer glyphosate-tolerant canola plants. Sometimes the combination of a herbicide with glyphosate does not work, because the herbicide affects the weeds in such a way that the effect of glyphosate is lost. Cost-effective methods are developed to control maize volunteers.

Weed beets are not occurring on a large scale and are therefore not a problem in Minnesota. Bolters are removed from sugar beet fields. In California weed beets are a problem. Therefore herbicide-tolerant sugar beets are not grown in California.

Uptake of micronutrients: It is not sure that Mn deficiencies are occurring. On some soils this may be the case. The situation is not clear.

Effects on disease resistance: The first glyphosate-tolerant sugar beet varieties had a lower resistance to Rhizoctonia and also in other aspects the varieties were weaker (also in yield). It is not clear whether this is caused by glyphosate. Probably there is an effect that Rhizoctonia crown and root rot is less spread over the field due to the reduced numbers of interrow tillage operations.

Most important points:

- The introduction of glyphosate-tolerant sugar beet is a big improvement, because weed control until now was expensive and problematic.
- Glyphosate-tolerant canola volunteer are not easy to control in glyphosate-tolerant sugar beet fields. It is hard to find good herbicides that can be used in combination with glyphosate.
- In Minnesota weed beets are occurring, but not on a large scale. Bolters are removed by the farmers. It is not known whether gene flow between glyphosate-tolerant and weed beet is occurring.
- Large-scale growing of glyphosate-tolerant sugar beet started in 2008. It is too early to drawi conclusions about environmental effects of genetically engineered sugar beets.