Roundup Ready Soybean – Reapproval in the EU?

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1 Introduction

Since May 1994, Monsanto's soybean GTS 40-3-2, also known as Roundup Ready Soybean, which is resistant to the herbicide Roundup, has been granted non-regulated status in the US. This means that it can be cultivated in the US without further restrictions (APHIS 1994). Two years later, in April 1996, the Roundup Ready (RR) soybean was granted approval for placing on the market in the European Union (EU). This is restricted to the importing and processing of the soybean into food and feed products, and cultivation is not permitted. The approval was brought into effect under the Directive 90/220/EWG, which was valid at that point in time, on the deliberate release into the environment of genetically modified organisms (GMO). Thereby, no monitoring following marketing approval was designated. The RR soybean was licensed based on the documents presented by Monsanto to the British Advisory Committee on Novel Foods and Processes (ACNF) in 1994.

After the new regulations on genetically modified food and feed products (1829/2003) as well as on the traceability and labelling of genetically modified organisms and resulting food and feed products (1830/2003) came into force, previously approved GMOs were permitted to remain on the market as “existing products”.
There was one precondition: The products had to be put through a notification procedure, i.e. the provision of safety appraisals, standardised verification procedures and proposals for labelling and traceability.

It is possible to file a request for an extension from the EU Commission (Reg. 1829/2003, Art. 8) within nine years after the date on which the GMO / GMO products were first put into circulation, but not earlier than three years after the start of validity of the new regulations (April 2004). Among other information, “all additional new information concerning the evaluation of safety when using the food, and the risks that the food poses for consumers or the environment” as well as information on later monitoring must be included in this extension request (Reg. 1829/2003, Art. 11). It can therefore be expected that the Monsanto Company will file an extension request for the RR soybean GTS 40-3-2 in 2007/2008.

2 The herbicide-resistant Roundup Ready Soybean

The herbicide resistant Roundup Ready (RR) soybean GTS 40-3-2 from Monsanto is resistant to glyphosate, the active component of the Monsanto product Roundup. Glyphosate was developed as a complexing agent, which captures and binds ions. Apparently, this major herbicidal effect wasn’t discovered until later (Hobom 2007). Glyphosate is an inhibitor of 5-Enolpyruvylshikimate 3-Phosphate Synthase (EPSPS), an enzyme on the pathway for the synthesis of aromatic amino acids in plants. The inhibition of EPSPS halts the creation of aromatic amino acids and ultimately leads to a termination of the protein synthesis and zero growth. The shikimate metabolic pathway plays an important role not only in the creation of the amino acids phenylalanine, tyrosine and tryptophan, but also in the metabolic pathways leading to vitamins and secondary metabolites. This adversely affects the plants’ growth rate and stress reaction, as well as the formation of lignin. Hence, phytoalexins, substances that act against fungi, are synthesised over the shikimate metabolic pathway (Kremer et al. 2005). Glyphosate is absorbed via the surface of a plant and distributed in the whole plant (systemic effect). As a general rule, this leads to visible toxic effects within a week, and the plants wither and finally die. The resistance against glyphosate in the RR soybean was achieved through the expression of a bacterial EPSPS enzyme, which is not inhibited by glyphosate (APHIS 1994).

The transformation was achieved by the vector PV-GMGT04, which was introduced into the soy cells via particle bombardment. The EPSPS gene from the *Agrobacterium* sp. strain CP4 is under the control of the 35S CaMV promoter (with a duplicated enhancer region) and linked to a chloroplast transit peptide sequence derived from *Petunia hybrida* as well as the termination sequence T-Nos (Nopaline synthase 3 terminator from *Agrobacterium tumefaciens*). The chloroplast transit peptide effectuates the transport of the synthesized EPSPS protein into the chloroplasts, where the enzyme activity and the effects of glyphosate are taking place. According to Monsanto, line GTS 40-3-2 contains only one copy of this construct in the nuclear genome and the transgenes show dominant inheritance, following Mendel's laws (APHIS 1994).

According to the publicly available application documents, the vector PV-GMGT04 contains various bacterial genes: two CP4 EPSPS genes controlled by different promoters (35S CaMV and CMoVb), the GUS marker gene (colour reaction with β-glucuronidase) as well as the nptII gene (neomycin phosphotransferase), which provides resistance to the antibiotic Kanamycin and related substances (Müller 2004). Line 40-3-2 was selected from the progeny of the transformed lines as it exhibited the highest glyphosate resistance in the field. Neither GUS activities nor the GUS gene were detected in this line.

The vector construct PV-GMGT04 is not in its entirety present in the RR line GTS 40-3-2. During the transformation via particle bombardment, fragments of the construct were presumably integrated into different genomic positions. Following segregation, only the insert responsible for high resistance to glyphosate remained in the line GTS 40-3-2 isolated from the progeny of the reference line (Müller 2004). However, it seems that a detailed characterisation of the insert and bordering genomic DNA was not undertaken at that point in time, even though it has been known for a long time that the integration of foreign DNA can influence the activity of adjacent genes and that comprehensive rearrangements of the integration sites can occur (Wilson et al. 2006, 2004, Latham et al. 2006). The possible integration of superfluous DNA sequences and genomic rearrangements that accompany integration increase the genetic instability and the probability of both position effects and the inactivation or activation of endogenous genes in the transgenic plants. For this reason, Smith et al. demanded as early as in 2001 that the transformed lines should be subject to closer scrutiny, in particular if they were developed for commercialisation.

Following a request from the British regulatory authority, Monsanto provided further data in 2000, which revealed that the RR soybean apparently contained additional DNA sequences: A 250-base pair (bp) fragment of the EPSPS gene that was joined (downstream) to the 3'end of the NOS terminator, as well as a separate 72 bp sequence from the EPSPS genes. However, in the enhancer domain of the promoter, part of the sequence is missing (http://www.food.gov.uk/multimedia/pdfs/monsantodossier.pdf). According to this document, transcription of these sequences is extremely unlikely, as the newly discovered sequences do not have a promoter or terminator. In 2001, data published by independent scientists showed that, besides the designated EPSPS fragments, a further previously unidentified 540 bp fragment was present that did not exhibit any sequence homology to conventional soy DNA (Windels et al. 2001). Consequently, the deletion and/or rearrangement of DNA sequences at the integration point could not be ruled out.

In 2002, Monsanto communicated that the rearranged DNA sequences would in fact be transcribed. A 7400 bp RNA transcript and other less common transcripts could be shown to be present. These RNA transcripts seem to begin at the 35S CaMV promoter and to be products of an incomplete termination (read through) through the 3'NOS polyadenylic sequence, meaning that they also contain genomic sequences (http://www.food.gov.uk/multimedia/pdfs/TranscriptFinalcleanMSL17432.pdf). However, according to Monsanto, the new findings about sequence rearrangements at the integration site do not affect the safety assessment of the RR soybean. Rang et al. (2005) reported that at least 150 bp of the 250 bp DNA fragments joined to the NOS terminator are transcribed. The read-through transcript is processed into four different RNA molecules, in which the transcribed NOS sequence is entirely missing. So-called open reading frames are formed, which could encode for unknown EPSPS fusion proteins. This indicates that the NOS terminator does not stop the transcription in either case and may promote DNA recombination.

These data show that, before approval, the RR soy line GTS 40-3-2 was insufficiently examined and that minimum standards regarding the characterisation of the integrated DNA sequences, the integration sites, the RNA transcripts produced were not met. Likewise the absence of superfluous DNA sequences was not proven. Should these additional DNA sequences and RNA transcripts indeed lead to the expression of fusion proteins, uncertainty as to the safety of the RR soybean would be increased. Müller (2004) also stressed that, according to recent research, small RNA molecules can play a major role in the regulation of gene activity and that DNA/RNA fragments could intervene in the cells' control processes.

The 35S promoter from the cauliflower mosaic virus (CaMV 35S) that controls the expression of the bacterial EPSPS gene contains two enhancer sequences. This promotor is used very often in transgenic plants but has been criticised on a number of occasions. Certain sequence regions in this promoter might enhance DNA recombination and thus genetic instability. It was recently demonstrated that the CaMV 35S promoter is not only active in plants, yeast and in the intestinal bacteria *Escherichia coli* (Ho et al 2000), but also in cultivated human intestinal cells (Myhre et al. 2006). The promoter is considered to be constitutive, which means that it is active in all (green) plant cells, independent of external influences. However, its activity can be influenced by various factors, for example stress (Brinker 1999). This could lead to altered expression of the transgenes and possibly the additional DNA sequences found. Furthermore, enhancers such as those present in promoters can influence the transcription of other endogenous genes (Quist et al. 2007).

According to Monsanto, line GTS 40-3-2 does not exhibit any other altered characteristics, with the exception of its resistance to herbicide. Monsanto claimed that, during the field trials conducted before US approval, they did not find any change in the susceptibility of RR soybean to diseases or pests or to plant pathogenic nematodes (APHIS 1994). On the basis of the documents presented by Monsanto, APHIS declared that the RR soybean GTS 40-3-2 is substantially equivalent to traditional soybeans.

3 Health effects

3.1 Toxic effects of glyphosate

The agro-biotech industry has often claimed that glyphosate and the formulated herbicide Roundup are less harmful to health and the environment than other herbicides. Consequently, its use would serve to protect both public health and the environment. They also argue that the EPSPS enzyme, target of glyphosate, does not exist in human and animal cells and that for this reason glyphosate would hardly be toxic for humans and animals. Glyphosate is claimed to be non-carcinogenic, non-mutagenic and non-teratogenic (disturbing the embryonic development). However, an increasing amount of data has been collected in previous years that show that the toxic effects of glyphosate are not limited to plant cells (and microorganisms) and that, therefore, the active component and the formulating agents are by no means as safe as portrayed to the public.

Monsanto markets the herbicidal active ingredient, glyphosate, under the trade name Roundup in a number of variations. The formulated products contain additives that facilitate the spreading and moistening of the plants' surfaces and stabilise the ingredients. These product formulations ease absorption of the active component not only into plant cells, but also into the cell membranes of animal and human cells, and they exhibit toxicity of their own. In general, kind and composition of the product formulations are retained as trade secrets and are not published by Monsanto (and other companies that sell herbicides containing glyphosate). Some of the formulating agents are categorised in a higher toxicity class than glyphosate itself (Isenring 2004). Müller (2004) criticises that a large proportion of the studies concerning glyphosate and/or Roundup conducted by private companies are confidential and therefore not open to the public.

Humans, as well as farm and other animals, come into contact with glyphosate and Roundup in a number of different ways: directly through its spreading or indirectly through the herbicidal residues found in food and feed products. Users of the herbicide can be exposed to high doses while handling and spraying, putting them at particular risk. According to reports from several countries, acute poisoning symptoms such as eye irritations, rising blood pressure, swellings and breathing problems have occurred due to exposure to glyphosate (Isenring 2004). Even people who do not use Roundup themselves are affected when the herbicide spray drift is carried to inhabited land. In particular, aerial spraying, as is common in Latin America, will lead to unwanted glyphosate deposition on non-target areas. In Brazil, Roundup was found to be the principal cause of poisoning (Andrioli 2007). Indirect contact with Roundup, the active component glyphosate, and its main metabolite aminomethylphosphonic acid (AMPA) can result from the consumption of RR plant material containing corresponding residues, or in some circumstances even from contaminated water.

It ist known that glyphosate accumulates in seeds. In particular with repeated glyphosate applications, RR Soybeans can contain high levels of residues. Within the approval framework for RR soybeans, the highest permissible residue level (maximum residue limit, MRL) of glyphosate in Australia and New Zealand was increased from 0.1mg/kg to 20mg/kg (= parts per million, ppm), amounting to a 200-fold increase. Since 1996, the glyphosate MRL for soybeans in the EU is 20mg/kg. This high value is not permitted for any other pesticide in the EU or for any other produce. In general, maximum residue levels for pesticides in soybeans are between 0.01 and 0.1mg/kg, with a few exceptions, such as Endosulfan (http://ec.europa.eu/food/plant/protection/resources/mrl_crop.pdf). Müller (2004) cites the following defined MRLs in the USA: 20mg/kg for soybeans, 100mg/kg for pulses, and 200mg/kg for hay. The Food and Agriculture Organisation of the United Nations (FAO 2005) likewise recommend an MRL for soybeans of 20mg glyphosate per kg.

According to the FAO, soybeans from the USA were found to contain up to 5.6mg of glyphosate per kg and a total residue level of up to 17mg/kg. Sandermann (2006) points out that no maximum allowable residue level exists for the main metabolite AMPA, although the concentration of glyphosate found in beans (up to a few mg/kg) was minimal compared to the concentration of AMPA, which was up to 25mg/kg. Thus, a consumption of the MRL of 20mg of glyphosate per kg would clearly result in a higher exposure to AMPA, which remains, however, unstudied.

In recent years, studies have provided data about negative effects of glyphosate and/or Roundup to the kidneys of mice, to pregnant rats, and to sperm production in rabbits (Benachour et al. 2007). As statistically significant effects of glyphosate Müller (2004) quoted increased occurrences of testicular cell tumours and pancreatic adenomas, and a rise of gastritis and kidney pathological effects in young animals. Depending on the dosage, increased DNA strand breaks and alterations to the cell nucleus of erythrocytes (red blood cells) were observed in goldfish (Cavas & Könen 2007). Marc et al. (2004) described negative effects caused by a glyphosate formulation (Roundup 3plus) on DNA synthesis and cell division in sea urchin embryos. Cellular and genetic toxic effects were also found in studies of human cells, such as increased chromosome aberrations (Monroy et al. 2005, Lioi et al. 1998). Recently published research showed that both glyphosate and the formulated product “Roundup Bioforce” damage human embryonic cell lines as well as placental cells, and, indeed, do so in concentrations that are clearly under the recommended values for agricultural use (Benachour et al. 2007). Glyphosate alone was less toxic, which points to the induced additional / synergetic toxicity of the product formulation. The addition of serum delayed the toxic effects by approximately 1 to 2 days. In addition, Roundup and glyphosate blocked the aromatase enzyme, to which an important role is attributed: the production of steroids and thus the formation of germ cells and reproduction. According to Benachour et al. (2007), Roundup is suspected of interfering with human reproduction and embryonic development. Moreover, the toxic and hormonal effects of the formulations have been so far underestimated.

Bacteria also have the shikimate metabolic pathway, and their EPSPS enzymes are not generally unsusceptible to glyphosate (see Chapter 4.1). Therefore, glyphosate residues in RR soybeans can possibly interfere with the microflora in the gastrointestinal tracts of humans and animals. It is still unknown, if and to what extent health effects are caused by this.

3.2 Possible effects of the RR soybean

The genetic engineering of plants is by no means an exact process. Neither the number of copies nor the integration sites of the inserted transgenes can be predefined. Duplications, deletions, and inversions of transgenic sequences are frequently observed. Upon closer analysis, integration sites appear to be largely complex structures, which consist of a mixture of transgenic sequences and endogenous DNA sequences. This can affect the genetic stability and genetic activity of the transformed plants. In addition, the newly produced proteins can intervene unexpectedly with the plant metabolism (Traavik et al. 2007, Wilson et al. 2006, 2004, Latham et al. 2006). Therefore, aside from the intended formation of new proteins/products in genetically engineered plants, other effects can be expected that cannot be directly clarified by the expression of the newly inserted gene, but may be due to mutations or position effects (due to the integration site) or the occurrence of pleiotropic effects (side effects of the new products). There are numerous examples of such unexpected effects in the literature concerning GMOs. For example, in RR soybeans, higher lignin content was found, which is possibly the reason for the splitting of stems observed in high soil temperatures (Coghlan 1999).

Genetic engineering can affect the safety of food and feed produced from transgenic plants. To assess the safety of genetically produced food and feed products, the concept of substantial equivalence is very often applied. According to this concept, transgenic plants and/or the products thereof are equivalent to the respective conventional plants and/or the food and feed products resulting from them. Only the newly produced proteins/products will be considered. Substantial equivalence is generally determined by chemical-analytic comparisons, as well as agronomical and morphological characteristics. However, this concept has been criticised for its fundamental presuppositions as well as its means of practical implementation, so that the conclusions arrived at using substantial equivalence are of minor significance (Millstone et al. 1999, Novak & Haslberger 2000, Spök et al. 2002a, 2003, Müller 2004, Traavik et al. 2007, Traavik & Heinemann 2007).

The central points of criticism made of the concept of substantial equivalence are:

- Given that new allergens, toxins or anti-nutritional substances can appear or become stronger in transgenic plants, the mere chemical-analytic comparison of specific substances does not permit conclusions to be made about the safety of the transgenic plants and the resulting products.
- With existing methods, not all the ingredients in plants and foods can be analysed, compared, and examined for their toxicological and allergological safety. The targeted choice of parameters and the choice of base lines and/or products are possibly inappropriate for secondary effects to be recognised with sufficient plausibility.
- Concrete applications are controversial. In practice, there appear to be great discrepancies in the quality of data, and the traceability and conclusiveness of research and conclusions drawn. The environmental impacts of ingredients in transgenic plants are often insufficiently considered.

Furthermore, in toxicological and allergological research, the newly produced proteins in transgenic plants are often not included, but rather those produced in bacteria. This was also the case in the studies concerning the safety of the RR line GTS 40-3-2: an EPSPS protein produced in *Escherichia coli* was used, which is said to be comparable in molecular weight, amino acid sequence and enzymatic activity to the protein produced in RR soy plants. Consequently, position effects and pleiotropic effects cannot be measured. Müller (2004) further criticised that the protein produced in *E. coli* was not compared to the one from the RR line GTS 40-3-2, but was actually compared to one from another line (event 61-67-1), for which further development was discontinued. Thus, the actual protein produced in the GTS 40-3-2 line was not analysed in detail. Since every transformation event is singular and leads to respectively individual DNA integration patterns and subsequent resulting effects, research should be carried out using the actual lines that are intended for commercial use. To what extent the production method and site of a protein can affect its characteristics is currently a matter of intense discussion in the case of so-called “biosimilars”, which concerns the imitation of genetically produced protein drugs for which patent protection has expired (Ledford 2007).

Monsanto based its 1994 application documents on research that analysed ingredients, allergenicity, toxicity, and feed conversion of RR soybeans, which, taken together, are intended to demonstrate the safety to health. Related articles from Monsanto employees appeared later in scientific newspapers (Padgette et al. 1996, Burks & Fuchs 1995, Harrison et al. 1996, Hammond et al. 1996).

Müller (2004) analysed the publications in detail and challenged the conclusions drawn by the authors' claim that the RR soy line GTS 40-3-2 is equivalent in composition to the conventional soybeans (Padgette et al. 1996), that no changes to endogenous and allergenic proteins were observed (Burks & Fuchs 1995), that the expressed EPSPS protein is rapidly digested and not acutely toxic (Harrison et al. 1996) and that the soy feed value for rats, chickens, cows, and fish is not changed by glyphosate tolerance (Hammond et al. 1996).

The most important points of criticism (Müller 2004):

- Data in publicised works differ from data in approval applications.
- The analysis of ingredients was conducted on mixed material, which originated from different cultivation regions/years – a direct comparison between RR soy and non-genetically modified soy (isogenic line?) from the same location is lacking.
- Not all of the parameters required by the OECD for the analysis of GM soybeans were examined; for example, an analysis of amino acid values in roasted soy flour was missing.
- Only acute toxicity tests were performed with bacterial EPSPS proteins– therefore, substantial equivalence cannot be proved
- Chronic tests, such as tests for carcinogenicity and reproduction toxicity of EPSPS proteins, were not carried out.
- Data were inconsistent or missing and the feed studies showed methodological faults, for example, the feed quality for fish and rats.
- Observed differences (lower weights and lower feed consumption with male rats and fish, higher kidney/testicle weight in rats, increased milk fat value in cows) between those fed with RR soybeans and those with the control diet were generally interpreted as being within biological variability and were not interpreted as being biologically meaningful
- Histological investigations of the gastrointestinal tracts of the examined animals are missing

Generally striking is that the studies performed by Monsanto scientists featured diverse methodical inconsistencies and that significant discrepancies were liberally interpreted in favour of the RR soybeans. Müller (2004) criticized the statistical validation of the study's outcomes as unsatisfactory. The fact that in vitro digestion studies cannot fully detect the potential allergenicity of new proteins and, therefore, are of limited significance as regards the potential of allergens, has already been criticized by Spök et al. (2002b). In most cases, RR soybeans were tested without glyphosate treatment, although it has not been conclusively clarified to what extent glyphosate influences the synthesis of plant substances. For example, the application of glyphosate in transgenic glyphosate-resistant oilseed rape plants leads to changes in amino acid profiles (Nandula et al. 2001). Under stressful conditions, Duke et al. (2003) observed increased concentrations of daidzein isoflavones in RR soy plants, but they attributed these effects not to glyphosate. Reduced concentrations of isoflavone, particularly that of genistein, were also described in RR soybeans (Lappe et al. 1999, cited in Müller 2004) – daidzein and genistein belong to the group of phytoestrogens that can bind oestrogen receptors and create oestrogen-like effects.

Müller (2004) concluded that the present data do not guarantee the safety of RR soybean GTS 40-3-2 line. He and Seralini (2006) demand further studies based on the toxicological examination of pesticides, namely:

- Sub-chronic toxicity tests over 90 days (three mammal species)
- Chronic toxicity studies
- Carcinogenicity studies over 24 months
- Reproduction toxicity studies over two generations
- Neurotoxic studies

In their analysis of published studies up to the end of 2002, Pryme & Lembcke (2003) found a total of only ten peer-reviewed studies concerning the safety of genetically produced food, two of which were about RR soybeans. However, neither study was classified as an independent investigation. The studies, which claim to have found no significant effects originated from Monsanto employees (Hammond et al. 1996, see above) as well as from non-independent parties, according to the authors (Teshima et al. 2000).

Meanwhile, inconsistent accounts have appeared on the negative effects of RR soybeans. Malatesta et al. (2002a,b) described ultra-structural changes in the pancreatic cells of mice fed with RR soy and found irregularly formed cell nuclei and nucleoli in liver cells, in which they saw indications of an increased metabolism. Ermakova (2005) gave an account of the reproductive toxic effects of RR soybeans, according to which the progeny of female rats that were fed RR soy frequently exhibited lower weight, smaller and lighter organs, and higher mortality. In an article published in September 2007 in Nature Biotechnology, the results and conclusions reported by Ermakova were criticised by scientists, who emphasised the methodical shortcomings of her study (Marshall 2007). To substantiate their criticism, they referred, among others, to the study by Hammond et al. (1996), which was certified by Müller (2004) as methodically inadequate, as well as the study by Teshima et al. (2000), which was judged by Pryme & Lembcke (2003) to be a non-independent investigation.

As different works have pointed out, ingested DNA is not immediately and completely decomposed, but is able to enter the gastrointestinal tract and the cells of different organs and possibly also microorganisms. DNA fragments as big as several hundred to over one thousand base pairs that are found in food are able to survive not only the gastrointestinal passage, but can also enter the peripheral leukocytes, spleen and liver cells, and even overcome the placental barrier and reach the cells of foetuses and new-born mice. The ingested tagged DNA was found in cell nuclei, partly bound to chromosomes (Schubbert et al. 1997, 1998, Doerfler 2000). It is largely unknown whether transgenic promoter sequences are active in the case of DNA ingestion by cells in mammalian systems. The focus here is on the 35S CaMV promoter, which controls transgene expression in the RR soybeans as well as in most of the GMOs authorised for commercial use. At the very least, it is active in cultivated human intestinal cells, as Myhre et al. (2006) have recently demonstrated. However, research into the possible activity of these promoters in vivo is lacking, as Traavik & Heinemann (2007) determined in their critical study.

Simulated digestion trials with RR soybeans show that a part of the ingested transgenic DNA can also survive the passage through the human gastrointestinal tract (Martin-Orue et al. 2002). Netherwood et al. (2004), using individuals with colostomy bags, observed that, following the consumption of RR soy products, up to 3,7% of the transgenic DNA survived the passage through the small intestine. However, this percentage diminished during the passage through an intact gastrointestinal system and the final intestine. In three of the seven test subjects using colostomy bags, evidence was even found of the transfer in bacteria of the gastrointestinal tracts of the transgenic EPSPS sequence, which, according to the authors, can’t have taken place during the test, but following a previous consumption of RR soy products. Consequently, the stability of transgenic DNA following consumption, as well as horizontal gene transfer, should be given greater attention (Heritage 2004, Traavik & Heinemann 2007).

4 Ecological effects

Over 80 % of the genetically modified plants cultivated worldwide are resistant to herbicides. Most of these are resistant to glyphosate, the so-called Roundup Ready (RR) plants. RR soy makes up the majority of these. Thus, over 80 % of US soy fields and almost 100 % of Argentinean soy fields are cultivated with RR soy. The cultivation of RR soybeans began several years ago in Brazil and other neighbouring Argentinean states too (they were often smuggled into the country). Herbicide resistant plants are propagated with the argument that cultivating them reduces the use of plant protecting agents (pesticides) and that GMOs pose no threat to the environment. Furthermore, growing herbicide resistant crops is supposed to aid weed control and reduce the number of required sprayings and therefore also reduces the mileage of agricultural machines and thus climate-relevant CO_2 emissions. It is also argued that less toxic herbicides need to be used, and that this is a contribution to improved environmental protection. Moreover, it is claimed that harvests increase and that soil erosion is reduced.

However, the reality in the countries with widespread cultivation of RR soybeans, such as the US and Argentina, does not match the brightly painted picture. Negative environmental effects due to the cultivation of herbicide resistant plants have been proven many times over. On the one hand, the active ingredient and the formulating agents lead to direct toxic effects on various organisms. On the other hand, the elimination of the wild plant flora endangers numerous organisms that are dependent on the flora as a source of nutrition and habitat. Genetically modified plants can also display unexpected characteristics that alter their interactions with the environment. As experience has shown, the selection pressure exerted by the herbicide inevitably leads to the evolution of herbicide resistant wild plant species. If the relevant broadspectrum herbicide does not have its intended effect, additional amounts of herbicides are normally used. The herbicide use does not decline, as predicted, but in fact increases – which, in turn, reinforces the negative effects of the herbicide use.

4.1 Effects due to glyphosate

Behaviour of glyphosate in the soil

The decomposition of glyphosate ($C_3 H_8 NO_5 P$, N- (phosphonomethyl)-Glycine) in the soil is predominantly performed by microorganisms. The active ingredient is moderately persistent; the half-lives are between 3 and 215 days, depending on soil conditions and temperature (Viehweger & Danneberg 2005, FAO 2005, Schuette 1998). Other scientists refer to the fact that in many soils, glyphosate can't be decomposed rapidly as this is also dependent on the mineral composition (Strautman 2007). Glyphosate is not readily decomposed by light. Although the active ingredient binds well with soil particles, the Danish National Pesticide Monitoring Program have proven that glyphosate and its main decomposition product aminomethylphosphonic acid (AMPA) are washed out of the root zone of clay-rich grounds in concentrations that exceed the acceptable quantities for drinking water of 0,1 µg/l, with maximum values of over 5 µg/l (Kjaer et al. 2004). In the process, glyphosate is washed out within the first months following use, whereas AMPA can remain in the soil for considerably longer. Glyphosate and AMPA were also detected in surface water and ground water in Germany and France (Sturm & Kiefer 2007, www.pan-europe.info/newsletter/news31.htm). The formulating agents used for an improved moistening of the plant surface in Roundup exhibit an independent toxicity, in particular for aquatic organisms (Relyea 2005a). If *Bacillus thuringiensis* compounds are used together with glyphosate, the persistence of the herbicidal active ingredient increases (Accinelli et al. 2004). Glyphosate decomposition in water is usually slower than in the soil, which is possibly due to the smaller number of microorganisms; half-lives of 35 - 63 days were reported (Schuette 1998), the main part appears to be stored in the sediment.

Side effects of glyphosate on RR soybeans

The complexing agent glyphosate easily binds trace elements (such as iron and manganese) in the soil and almost entirely prevents their transportation from the roots up into the shoots. If glyphosate concentrates in the root tips of treated plants, the roots seem to find it difficult to extract the necessary trace elements from the barely soluble glyphosate-metal complexes (Strautman 2007, Hobom 2007). To what extent a scarcity of trace elements leads to an undersupply of the plants with micronutrients depends on the prevailing soil conditions. To date, Israel is the only country in which glyphosate can only be reapplied on sandy grounds following a longer waiting period. In Brazil it has been observed that glyphosate treatment leads to a considerable yield decline in soybean crops in the following years. In the US, the widespread application of glyphosate on transgenic RR plants is apparently partly accompanied by a lack of manganese, which has already lead to a yield decline in crops. In order to cure this defect, the additional use of manganese is encouraged (McLamb 2007). However, if manganese is applied together with glyphosate, RR soybeans show a reduced resistance to glyphosate. Furthermore, it reduces the availability of other trace elements (Hobom 2007).

Manganese plays an important role in numerous processes in plants, such as photosynthesis, nitrogen and carbohydrate metabolism and defence against diseases. If plants are undersupplied with manganese, they can become more vulnerable to pathogens (Strautman 2007, Neumann et al. 2007). A connection between a low manganese level and vulnerability to diseases and degree of disease has been shown for numerous plants and diseases. With regard to RR soybeans, the new varieties appear to have a reduced ability to absorb and transport manganese (Fixen et al. 2005). The authors pointed out that a sufficient supply of micronutrients is of great importance, in particular for protection from Asia rust, a fungal disease that is appearing increasingly in the soy-growing areas in Latin and North America and which strongly reduces the harvest.

In general, glyphosate spreads quickly within the plant (systemic effect) and is only partially metabolised. However, in soybean cell cultures, 50 % of glyphosate is transformed into the main metabolite AMPA (Sandermann 2006). In RR soybeans, only a small percentage of the applied glyphosate binds to EPSPS, the larger part ends up in metabolic sinks such as roots and seeds. Not only do RR soybean roots accumulate glyphosate, they also release active ingredients into the soil; significant amounts were released as many as 16 days after glyphosate treatment, (Kremer et al. 2005). Furthermore, the authors observed that RR soybeans release more soluble carbohydrates (sugar) and amino acids than non-transgenic control plants. Interestingly, also non-treated RR plants released more sugars and amino acids than the control plants, which points towards a (pleiotropic) effect of genetic modification.

At any rate, if the genetically engineered resistance to glyphosate does not develop fully, for example as a result of an altered expression of the bacterial EPSPS enzyme under stress conditions, the metabolic pathway to aromatic amino acids in treated RR soybeans is compromised. A reduced content of the amino acid phenylalanine in the RR soybean, as reported by Benbrook (2001), could be explained in such a way. Moreover, Kremer et al. (2005) point out that the new bacterial EPSPS enzyme is possibly less efficient than the EPSPS of the plant – the shikimate metabolic pathway could also be influenced as a result of this. Due to the fact that this metabolic pathway and aromatic amino acids play an important role in many metabolic processes in plants, and in the stress and pathogen defences such as the formation of defensive substances like phytoalexins, the stress and pathogen tolerance of RR plants would be impaired as a whole. Such an effect would be reinforced yet more by a reduced absorption of trace elements like manganese. Plants with weakened defences are more likely to fall victim to pests and pathogens. Benbrook (2005) also reports on an increase in pest infestation and plant diseases in RR soybeans in Argentina.

It has not been clarified whether the higher lignin content observed in RR soybeans, which makes the stems go brittle and therefore encourages them to break open with higher soil temperatures (Coghlan 1999), are related to the effects of glyphosate on the shikimic acid cycle or are a consequence of other effects of the genetic modification. Unexpected effects of glyphosate treatment in RR plants were also described for RR cotton, whereby glyphosate accumulated in the plant's sprouts and blossoms (Pline 2001). If the resistance against glyphosate was less intense in exactly these tissues, this would offer an explanation for the reports of a reduction in pollen viability and boll dropping in RR cotton (Pline et al. 2002, Yasuor et al. 2006).

Direct and indirect effects on non-target plants

Cultivated crops that are not the target of glyphosate applications can also be subject to the effects of glyphosate. On the one hand, pesticides are often not applied highly accurately; on the other hand, wind, water, and soil particles loaden with glyphosate can lead to transfer of glyphosate into other areas. This drift is influenced by a number of parameters, such as distance, weather conditions, herbicide formulation, drop sizes as well as the type of application and spray equipment. In sunflowers, a simulated glyphosate drift leads to significantly reduced growth, lower chlorophyll content in young leaves and sprout tips and to significantly lower iron and manganese content, with zinc and copper concentrations less influenced (Eker et al. 2006). Glyphosate applications on sunflower leaves inhibited the transportation of iron and manganese from root to sprout almost entirely within a single day.

Thus, cultivated crops in neighbouring areas that do no bear any resistance to glyphosate reveal reduced growth and yield, depending on the amount of glyphosate absorbed and their growth stage, if they do not die off immediately. In the US, the number of claims for compensation by affected farmers has also increased rapidly since the introduction of the Glyphosate resistant plants (Henry et al. 2007). The accumulation of shikimate in leaves of these non-target plants is evidence of damages due to glyphosate.

The latest research even reveals that glyphosate transfers into non-target plants through the soil, independently of an absorption of active ingredient via the leaves. In laboratory experiments with liquid fertilizer and pots, Neumann et al. (2007) showed that after its application on the leaves of RR soybeans, glyphosate quickly gets into the roots and afterwards into the rhizosphere. Here, glyphosate can remain stable for a sufficient length of time to be able to have an effect on neighbouring non-target plants and to lead to an accumulation of shikimate in their sprouts and, more predominantly, in their roots. Non-target plants also absorbed significantly less manganese, an effect observed in acidic sandy ground (Arenosol), but not in calcareous loess soil. The authors discussed whether the reduced absorption of manganese could (also) be the result of damage of manganese-reducing soil microorganisms (such as *Pseudomonas*). In calcareous soil, the calcium might possibly bind the glyphosate so quickly that absorption via the roots of other plants will no longer be possible, which might explain the absence of negative effects. As already mentioned, trace elements such as manganese are highly significant for plants' defences against disease. Therefore, the health of non-target plants could be impaired by glyphosate not only as a result of direct toxicity, but also indirectly, in particular in soils with a reduced availability of micronutrients. For this reason, with an interest in the health of plants and soil in mind, a reassessment of the potential risks of glyphosate applications is urgently demanded (Neumann et al. 2007).

Effects of glyphosate on microorganisms

It was observed very early on that glyphosate influences the microflora in the soil (Roslycky 1982). EPSPS, the target enzyme of glyphosate, is also essential in microorganisms for the biosynthesis of aromatic amino acids. Even though the glyphosate-insensitive EPSPS formed in the RR soybean derives from *Agrobacterium* sp. strain CP4, not all microorganisms contain EPSPS forms that are similarly non-sensitive. Glyphosate is toxic for many organisms that live in the rhizosphere (Strautman 2007), which could have an impact on microbial activity in soils and waters. Kremer et al. (2005) observed a generally reduced bacterial growth in the root exudates of RR soy plants treated with glyphosate. Furthermore, glyphosate excreted by treated plants can be absorbed together with soil particles by the soil fauna and impair their intestinal flora. In waterbodies, the largest proportion of glyphosate is stored in the sediment. Almost no examinations have been carried out on the possible effects of this on microorganisms (Pechlaner 2001). The microflora in the gastrointestinal tract of humans and farm animals is also potentially affected. Since seeds serve as a sink for glyphosate in plants (Duke et al. 2003), higher glyphosate residues in soybeans can be expected (see chapter 3.1). The consumption or feeding of RR soybeans could therefore negatively affect the intestinal microflora. In doing so, residues of AMPA, the main metabolite of glyphosate, are generally not even registered. Although AMPA values that were up to five times higher than those of glyphosate were found in soybeans, no maximum permissible AMPA residue level exists for seeds (Sandermann 2006).

In general, soybeans and other legumes need little or no nitrogen fertilisation, as they accommodate nitrogen-fixing symbiotic bacteria (rhizobia) in root nodules, which assimilate nitrogen from the air and make it available to the plants. Up to 85% of the soybean's nitrogen demand can be met in this way. However, many rhizobia possess a sensitive EPSPS form, among which are also the symbiotic bacteria of the soybean, *Bradyrhizobium japonicum*. These react sensitively to glyphosate, depending on the herbicide concentration and microbial strain (Abendroth et al. 2002, Schütte 1998, Labes et al. 1999). The herbicide enters the root nodules, inhibits their development through *B. japonicum* and reduces both their biomass by up to 28% and the leghaemoglobin, which is important for nitrogen binding in the roots of the RR soybeans, by up to 10% (Reddy & Zablotowicz 2003). Following glyphosate application, RR soy leaves exhibited lower nitrogen content. However, a significant yield reduction was only observed when high doses of glyphosate had been applied (Zablotowicz & Reddy 2007). An altered nitrogen content can influence the formation of amino acids and therefore the protein content. For example, Benbrook (2005) reported lower protein contents and lower levels of important amino acids in Argentinean RR soybeans. In young RR soy plants, glyphosate caused a delayed nitrogen fixation and a reduced growth in roots and sprouts, which can reduce the harvest in less fertile soils, as well as in cases of drought, by up to 25% (King et al. 2001). As a reaction to this, soybeans are increasingly treated with nitrogen fertilizer. As stated in a US patent, there seem to be plans to make rhizobia resistant to glyphosate, either by selecting less susceptible strains, by an induction of glyphosate-resistant mutants or by genetic modification (http://bumperscollege.uark.edu/vision/nov-dec05/20.html).

Alongside bacteria, Roundup might also affect fungi, but the mechanisms that lead to changes in fungal communities (Fernandez et al. 2007b) remain unclear. Fungi that are useful for combating harmful insects react sensitively to Roundup, whereas glyphosate alone did not have a fungicidal effect, but did synergistically reinforce the toxic effects of the formulating agents (Morjan et al. 2002). Kremer et al (2000) observed, that frequent use of glyphosate promoted the appearance of pathogens such as *Fusarium solani*. They concluded from this that glyphosate might possibly increase the sensitivity of plants towards fungal pathogens such as common species of Fusarium. An infestation by Fusarium is undesirable, as they produce toxins that are harmful for both humans and animals. According to Njiti et al. (2003), a Fusarium infestation is not related to glyphosate, but depends on the particular type of soybean. However, in recent works, Kremer et al. (2005) have proven that root exudates of RR soybeans treated with glyphosate significantly promote the growth of various Fusarium strains. For some years there have been reports of an increase of the take-all disease (a common disease in wheat and barley caused by the *Gaeumannomyces graminis* fungus) in the years following a glyphosate application (Strautman 2007). In wheat and barley, various species of Fusarium were also observed in higher quantities following a glyphosate treatment (Fernandez et al. 2005, 2007a,b).

According to Kremer et al. (2005) exuded glyphosate can in some circumstances serve as a source of nutrition for fungi. At the same time, the increased exudation of soluble carbohydrates and amino acids promotes the growth of fungi in RR soybeans treated with glyphosate. Because antagonistic bacteria, which metabolise the exuded substances and thus restrict fungal growth, can be impaired by glyphosate, correcting influences are lacking, with the result of increased growth of pathogenic fungi. Combined with the above-mentioned negative effects of glyphosate on the pathogen defence of plants, this can lead to an increased appearance of pathogenic fungi in crops treated with glyphosate - for which, as a general rule, the answer in conventional agriculture is the use of further pesticides (in particular fungicides).

Decrease in harvest due to RR soybeans

Published studies concerning the yield potential of RR soybeans revealed an unclear picture. Compared to corresponding non-GM species (isogenic lines), RR soybean very often showed a harvest shortfall, which, depending on species and growing conditions, reached up to 20 % and which averaged 5 – 10 % (Benbrook 2001, King et al. 2001, Elmore et al. 2001a,b). It is discussed whether this yield reduction, which many authors have observed, is based on a reduced nodulation and nitrogen fixation (as a result of the toxic effect of glyphosate on the bacterial symbionts) or on unexpected effects that are linked to the insertion of the foreign DNA or the expression of the resistance gene. The impairment of the supply with trace elements such as manganese, due to a binding with the complexing agent glyphosate as well as an increased pathogen infestation, clearly contribute to the yield decline of the RR soybean (Strautman 2007). Other authors refer to AMPA, the most important metabolite of glyphosate, which also develops in plants and has a toxic effect. As a result, AMPA could contribute to the damages on RR soybeans (Zablotowicz & Reddy 2007). The extent of the formation of AMPA and the damage caused presumably depend on the amount of glyphosate, the particular plant genotype and the environmental conditions.

In this context, it is pertinent to note that Monsanto has signalled that it will be launching a second, improved RR soy line, RR2Yield on the US market, starting in 2010. This line is supposed to produce a harvest that is 7 - 10 % higher than the present RR soybean line (http://www.aphis.usda.gov/brs/aphisdocs/06_17801p.pdf). Authorisation for the US was given in late July 2007, and according applications have also been made for the EU (Leonard 2007).

Effects on animals

Even though glyphosate or Roundup is the most common herbicide in the world, comprehensive data is still lacking about its effects on animals that come into contact with the product. Indeed, low toxicity is generally assumed. The low fat solubility reduces the risk of accumulation in animal tissue, and absorbed glyphosate is predominantly excreted (FAO 2005). However, the toxicity in fish is relatively high: The concentration at which 50 % of animals die (LC50), is in some cases around 100 times lower for fish than for rats, ducks and quails (Schuette 1998).

One needs to bear in mind that while glyphosate is often the only applied active ingredient in toxicity studies, the product on the shelves contains additional formulating agents that ease moistening on plant surfaces and thus aid the absorption of the active ingredient. Therefore, data concerning the toxicity of glyphosate alone may conceal undesired effects. According to recent studies that were conducted with using the formulated agents over a longer period of time than is usual (Roundup Weed and Grass Killer Concentrate), amphibians, for example, reacted very sensitively to Roundup. A significant number of the tested tadpoles died when exposed to concentrations that can be found in actual surface waters (0,1 – 2,3 mg active ingredient (AI) per litre) –. The values at which 50 % of the animals died (LC50) were between 0,6 and 2,5 mg AI/l (Relyea 2005a,b, Relyea et al. 2005). Stress inflicted by predators seems to synergistically intensify the toxic effect. The maximum permissible Roundup content (3,8 mg AI/l) reduced the number of amphibians by up to as much as 70 % (Relyea 2005c). Hereby, the formulating agent polyoxyethylenamine (POEA) clearly played an important role. Relyea (2006) objected the claim that the studies had been completed in artificial conditions, and with dosages that couldn't be achieved in water in practice (Thompson et al. 2006): The tested dosages corresponded to the manufacturer's data and lead to concentrations in water that were in accordance with worst-case scenarios and levels found in many ponds. Thus, Roundup was found in corresponding amounts in small aquatic biotopes, which are of great importance, especially for amphibians. It has also been observed that the toxicity of Roundup - for unknown reasons – increases with a higher pH-value and, in particular, that the formulating agent POEA exhibits a high toxicity for amphibians (and fish).

4.2 Indirect effects on biodiversity

Agriculture is seen as one of the most important causes of the enormous loss of biodiversity that has been observed world wide in previous decades and which continues unabated. Pesticides play an important role in this decline; alongside insecticides, herbicides have a particularly negative effect. The elimination of wild plant flora due to herbicides, directly on and near fields, deprives the animal kingdom of its essential nutrition and shelter and therefore reduces its ability to survive. In general, the use of herbicides reduces the seed bank of weeds and thus has a long-lasting effect on biodiversity on agricultural land. Modellings based on studies with herbicide resistant plants in England, conducted over several years, showed that this effect is fortified in the herbicide resistance system (Heard et al. 2005). The weed flora also plays an important role in the battle against erosion and pests, as it retains the surface soil, offers nutrition and shelter to beneficial organisms and it can be an alternative source of nutrition for pests (Norris 2005). If differences in the amounts of pest occurrence between conventional and RR soybeans arose in areas treated with herbicides, it tended to follow that more damaging insects were found. However, grasshoppers appeared in greater numbers in areas with a higher quantity of weeds (McPherson et al. 2003). The effects on soil life should not be neglected either, as the soil biota is also dependent on the diversity of the plant flora (Müller et al. 1996).

Studies with glyphosate-resistant sugar beet and glufosinate-resistant rape and corn plants, performed in Great Britain over a time span of three years in over 260 locations, the so-called Farm Scale Evaluations (FSE), proved conclusively that the HR system significantly reduces the biodiversity of plants and animals on and alongside the respective fields. Biomass and seed rain of weed flora were 15 - >30 % lower in areas with herbicide-resistant plants than in the neighbouring control areas, where "conventional weed control" was applied; the seed bank in the soil was reduced by around 20 % (Heard 2003a). A Danish study with glyphosate-resistant fodder beets conclusively confirmed these results, whereby the time of the first application was of great significance: earlier applications lead to an extremely low density and diversity and biomass of weeds (Strandberg et al. 2005). Monocotyledonous species of weeds (grasses) seem to react more sensitively to glyphosate than dicotyledonous species (Heard et al. 2003b).

The lower concentration and biomass of the weed flora also influenced the occurrence of invertebrates, insects in particular, whereby the effects were strongly dependent on the animals' nutrition and mode of life. In general, omnivores and mobile insects were less affected than insects dependent on seeds; springtails were observed even more frequently – possibly due to an initially higher detritus provided by dying herbal biomass following the application of a broad spectrum herbicide (Brooks et al. 2003). Other insect groups reacted particularly sensitively: 27 % less butterflies and significantly less bugs and bees were found in areas with glyphosate-resistant sugar beet (Haughton et al. 2003).

The effects also expanded to the field boundaries, where it was observed that over 30 % less weeds flourished to form seeds and significantly less spiders and up to 18% less butterflies were found to be living (Roy et al. 2003, Haughton et al. 2003). Applied pesticides can be generally observed to drift to surrounding areas. As a result, adjacent fields are effected by the glyphosate applications, which leads not only to damage to neighbouring crop plants (Thomas et al. 2005), but also to much of the surrounding flora (de Snoo & van der Poll 1999). However, field borders are particularly important for the preservation of biological diversity, as they offer habitat and a source of nutrition for numerous animal species (Marshall & Moonen 2002). The many protection schemes for field border strips explicitly recognise their great importance for biodiversity.

When glyphosate is spread from the air, as it is often the case in large-area RR soy-monocultures in the US and Latin America, glyphosate is distributed over vast areas - areas that are home to more than just RR soy plants (Joensen et al. 2005). Damage to human health and the environment have been documented. The broad spectrum herbicide also causes damage if glyphosate enters neighbouring forests and hedges. After a single glyphosate treatment, mosses need four years to begin to recover in density and diversity (Newmaster et al. 1999). The loss of biodiversity in plants leads to immediate effects on the food chain, whereby bird life are a particularly sensitive indicator. The analysis of data collected in Great Britain from 1962 to 1995 has demonstrated a strong correlation between the changes in structure in agriculture and the beginning of a decline in bird populations (Chamberlain et al. 2000). According to the authors, the first effects were observed after around six years, suggesting that changes in agronomic practice are not immediately noticeable through a decline in species numbers, but that negative effects on biodiversity often only become evident after some years. Experience tells us that, by the time this has been observed, it is usually too late to reverse the changes.

The negative effects on biodiversity have also been proven by reports from Argentina. There, the vast expansion of soy cultivation has lead not only to a strong increase in the application of herbicides (see chapter 6) but also the broad exploitation of previously unused or little-used land and forest areas for intensive agriculture (Joensen et al. 2005, Pengue 2004).

5 Evolution of herbicide-resistant wild plants

The key to selecting herbicide-resistant wild plants is a weed control method that is solely based on the use of herbicides. Since the first reports on a herbicide 2.4-D-resistant weed in 1957, the herbicide resistance in weeds has increased dramatically. Financed by the pesticide industry, the Herbicide Resistance Action Committee (HRAC) gives data on resistance development. In August 2007, their website (www.weedscience.org) listed 315 biotypes that show a resistance to at least one herbicide and are found on over 280,000 fields, whereby 110 of the 183 relevant species are dicots and 73 are monocots. The extent of the resistance clearly correlates with the use of the respective herbicides: the more widely spread and the longer the period that the respective herbicide is applied, the more frequent is the development of resistant weeds. Accordingly, most of these biotypes (95) are resistant to the widely-used ALS inhibitors (e.g. chlorsulfuron, imazamox), which inhibit the acetolactate synthase ALS, 66 are resistant to atrazine and related active components and 35 to ACCase inhibitors (e.g. diclofop-methyl), substances that inhibit the acetyl CoA carboxylase.

It was long assumed that glyphosate was an exception to this rule. The first reports on glyphosate-resistant weed species did not appear until the mid-nineties, even though the product had been released on the market in 1974 for use in large areas, and it was known that weeds exhibit a different sensitivity to the active component (Sandermann 2006). The following reasons were given for this: no long-term impact of glyphosate due to fast decomposition; limited absorption through the soil; the type of application and its particular mode of action which, according to the reports, would involve a mutated EPSPS enzyme that is not inhibited by glyphosate and has a negative effect on the plant's performance and may even cause the plant to die (Jasieniuk 1995, Baylis 2000). Furthermore, before glyphosate-resistant plants were introduced, the active component was mostly used in alternation or in combination with other herbicides, which caused a reduced selection pressure (VanGessel 2001). However, since the first report of a glyphosate-resistant weed was released in 1996, the number of glyphosate-resistant weed species has increased continuously. To date, there are already 13 confirmed cases of glyphosate-resistant weed species, most of which have been observed at different locations and in a variety of countries. A number of these weed biotypes have even developed tolerances to other herbicides.

Table 1 shows the country and the year of the first occurrence of the respective resistant species. In general, several other countries are affected, for example China, Columbia, Spain and France. Nevertheless, not all occurrences of glyphosate-resistant weeds are associated with RR soybean cultivation or RR crop cultivation. There are also frequent occurrences in pomiculture and viticulture, where glyphosate has been broadly used as a herbicide since it was licensed.

Amaranthus palmeri
Palmer Amaranth (USA 2005)
Amaranthus rudis
Common Waterhemp (USA 2005)
Ambrosia artemisiifolia
Common Ragweed (USA 2004)
Ambrosia trifida
Giant Ragweed (USA 2004)
Conyza bonariensis
Hairy Fleabane (South Africa 2003)
Conyza canadensis
Horseweed (USA 2000)
Echinochloa colona
Junglerice (Australia 2007)
Eleusine indica
Goosegrass (Malaysia 1997)
Euphorbia heterophylla
Wild Poinsettia (Brazil 2005)
Lolium multiflorum
Italian Ryegrass (Chile 2001)
Lolium rigidum
Rigid Ryegrass (Australia 1996)
Plantago lanceolata
Buckhorn Plantain (South Africa 2003)
Sorghum halepense
Johnsongrass (Argentina 2005)

Table 1: Glyphosate-resistant weed species, as of August 2007
(www.weedscience.org)

In view of the widespread cultivation of RR soybeans (as well as of RR maize and RR cotton) over millions of hectares in the US, Argentina, Brazil and other countries, this list is expected to grow continuously. Among the currently known glyphosate-resistant weed species, horseweed, palmer amaranth and ragweed are the weeds most frequently observed in soybean cultivation, while horseweed poses particular problems to RR soybean farmers in the US. Since 2000, when the first glyphosate-resistant horseweed population was described in Delaware after only three years of RR soybean cultivation, this resistant species has spread on thousands of hectares in 15 US states (Service 2007, Freudling 2004, www.weedscience.org). *Conyza canadensis* is one of the most widespread species and is among the ten most important weeds that have developed tolerances to numerous herbicides. It has highly effective spreading mechanisms and is very well adapted to ploughless soil tillage (Zelaya et al. 2007). At least one of the glyphosat-resistant *C. canadensis* populations is also tolerant to ALS inhibitors. In the US, amaranth species are also regarded as weeds that are difficult to control, as they have developed various herbicide resistances. The occurrence of glyphosate-resistant populations within these species is therefore regarded as an indication for problematic development (Culpepper et al. 2006).

The glyphosate-resistant weed populations have occurred on a larger scale than anticipated by most experts, and with a comparably small interval following the introduction of the RR soybean on the US market in 1996. More recently, there have also been an increasing number of reports on resistance problems with weeds in soybean cultivation in Brazil (www.lobbywatch.org) and Argentina (Joensen et al. 2005). None of the nine more-or-less tolerant species mentioned by Joensen et al. for Argentina (*Commelina erecta, Convolvulus arvensis, Ipomoea sp., Iresine diffusa, Hybanthus parviflorus, Parietaria debilis, Viola arvensis, Petunia axillaris, Verbena litoralis*) are listed in table 1. This leads to the conclusion that the list of confirmed glyphosate-resistant species will soon be expanded significantly. The glyphosate-resistant Johnsongrass in particular has become a major problem in soybean cultivation in Argentina – six provinces covering 120,000 hectares have already been affected (Romig 2007). The RR soybean itself, spreading as a volunteer in follow-on crop cultivations, increasingly turns into a "weed", which, according to Syngenta, should be controlled with its own product Gramoxone (www.syngenta.com).

Previous experiences with herbicide resistant weed populations should have been a warning to farmers, as the herbicides that had been used in soybean cultivation had in previous years become increasingly ineffective. A very high number of the ALS-inhibitor-resistant weed populations developed alongside soybean cultivation. In particular, the problematic common waterhemp (*Amaranthus rudis*) acquired multiple resistances to different herbicides (Mueller et al. 2005).Therefore, market introduction of the RR soybean, which permitted the use of a herbicide that was based on a different mode of action, may have rashly been considered by farmers to be the solution to the resistance problem. Accordingly, the soybean farmers' high willingness to start growing RR soybeans was not only because the weed control with Roundup permitted more flexible spraying times. The farmers were also hoping to be able to control a wider range of problematic weeds with just one substance, which had a new mode of action and was easy to use.

Some resistant weeds can tolerate up to a twelve-fold quantity of the glyphosate dose tolerated by a herbicide-sensitive plant (VanGessel 2001). The glyphosate-resistance in weeds is based on differing molecular and genetic mechanisms. The resistance mechanisms so far confirmed for glyphosate-resistant weeds are a lower sensitivity of the target enzyme EPSPS (5-enolpyruvyl shikimate 3-phosphate synthase) and a modified transport of glyphosate in the plant. Accordingly, various weed types are proven to have undergone a mutation inside the critical amino acid sequence (target site) of the EPSPS enzyme that is affected by glyphosate. The exchange of one amino acid with another may modify the electric charge and/or folding of the target site in such way that the enzyme is no longer or less inhibited by the herbicidal component. In the resistant Malayan goosegrass and certain populations of the rigid ryegrass, the replacement of proline at position 106 of the EPSPS with serine or threonine seems to be responsible for the glyphosate resistance (Powles & Preston 2006, Wakelin & Preston 2006a). However, in other ryegrass populations, the transport of glyphosate from the leaf to other parts of the plant, including the root, is slowed down (Wakelin et al. 2004). This also applies to the horseweed populations from Delaware and Tennessee (Feng et al. 2004). While the resistance is predominantly inherited as a nuclear single-gene mutation in semi-dominant or dominant inheritance (Powles & Preston 2006, Wakelin & Preston 2006b), it may also be related to multiple genes (Simarmata et al. 2005). Resistance mechanisms that are not based on a target site mutation, such as the modified transport of the active component, are considered extremely problematic as they often lead to the development of resistances to other herbicidal modes of action simultaneously (Yuan et al. 2007).

Of course, resistances may also be spread through out-crossing, as proven by the successful hybridisation of the glyphosate-resistant Canadian horseweed *C. canadensis* with the related species *Conyza ramosissima* (Zelaya et al. 2007). Furthermore, the offspring from these out-crossings have shown an ability to adapt very well to ploughless soil tillage. Species related to the horseweed, which grow effectively in other agricultural ecosystems, can thus acquire resistance genes within a short period of time. In particular, in cross-fertilised weeds such as the rigid ryegrass, out-crossing may quickly lead to the combination of different resistance mechanisms against glyphosate or other active ingredients, which makes herbicidal control even more difficult.

Some experts believe that species shift among the weed flora, which is caused by the selection pressure of the herbicides, is another very important aspect. Glyphosate does not affect all weeds to the same extent, or to a similar extent following an equivalent dose, and not all plants are coated in the same manner. Therefore, less sensitive species may survive sprayings and subsequently accumulate, whereas more sensitive species may disappear. Some weeds also adapt their growing cycles so that they only germinate after the usual spraying date. Other species germinate over a longer period of time or are persistent species that shoot continuously. If early germinators have already grown quite tall, they may not be entirely killed off and are able to re-germinate and create seeds. Reports on problematic weeds that are hard to control with glyphosate although they have not developed a true resistance, have existed for a longer time (Pengue 2004, Benbrook 2005, 2004). The white goosefoot (*Chenopodium album*) in RR soybean fields in the US serves as an example: It germinates over a longer period and the late-sprouting plants remain unaffected by the glyphosate's impact (Scursoni et al. 2007). Reports on weed shift have also come from Argentina: After just a few years of RR soybean cultivation, 37 weed species have gained in significance, while only 18 species have decreased (Vitta et al. 2004).

6 Adaptation strategies and herbicide use

6.1 Adaptation strategies for glyphosate resistance in weeds

Although there is ample information that such effects intensify when RR crops are planted over a longer period, farmers pay comparatively little attention to the problem of glyphosate resistance in weeds and weed shift. But it is increasingly being discussed whether the appearance of herbicide resistant weeds also impairs the (lease) value of agricultural land, as they reduce the productivity and long-term value of the land. According to a survey of US agricultural experts carried out by Syngenta, the lease prices can diminish on average by 17% (www.mindfully.org/GE/GE4/Glyphosate-Resistant-SyngentaDec02.htm). As a result, landowners are now securing agreements from tenants that they will implement resistance management strategies. Data from Australia suggests that 15 to 50% less rent is paid for land with herbicide resistant weeds, depending on the specific resistances and how widespread the problem. Accordingly, resistances to ACCase inhibitors and ALS inhibitors drive the price down by 15% and an additional 10%, respectively. If glyphosate resistance is also present, the lease price falls by 50%.

On the other hand, the results of a survey of RR soy and corn farmers in the US state of Indiana showed that two thirds of the farmers were not particularly worried about glyphosate-resistant weeds and only one third were confronting the problem (to some degree), although glyphosate-resistant weed populations are common (Johnson & Gibson 2006). Farmers that managed more than 800 hectares were more concerned than farmers with less land. Glyphosate selection pressure was rarely perceived as a cause in the development of resistance. Even in Delaware, where the first glyphosate-resistant Canadian horseweed population in RR soybean fields was discovered in 2000, and where 38% of farmers have reported the presence of such plants on their land, RR soy farmers appear to be - continuously and to a large extent - relying on glyphosate (Scott & VanGessel 2007). Just over one half of the Delaware farmers that were affected by resistant horseweed reported additional costs of $5 to $17 per hectare for prevention measures. 28% of the farmers reported costs over $17/ha. Both surveys state that the most important adaptation strategies in the development of resistance in weeds are: 1) The application of another herbicide – alternatively or together as a tank mixture with glyphosate; 2) Additional applications of glyphosate, as well as 3) Changing to a crop other than corn. Other mentioned strategies include moving away from ploughless tillage as well as detecting resistant plants earlier.

According to Mueller et al. (2005), the occurrence of glyphosate-resistant Canadian horseweed plants in cotton-soy-corn crop rotation in Tennessee (USA) cost the local farmers $30 per hectare per year. If resistant amaranth populations arise in the corn-soy crop rotation in Illinois, costs of $44 per hectare and year can be anticipated. This figure includes the cost of more herbicides, additional travel on and over the land, and work hours. As the authors want to ensure that glyphosate retains its role as an effective herbicide for the longest possible time, they recommend a pro-active strategy, i.e. an early adaptation of weed control to the development of glyphosate-resistant weeds. In Argentina, glyphosate-resistant johnsongrass could double herbicide costs and increase the price of soy production by 160 to 950 million dollars per year (Romig 2007).

In most cases, the obvious responses and suggestions from outsiders concerning the evolution of herbicide resistant weeds are not considered. These are, namely, a move towards alternative methods of cultivation and weed control. Instead, the surveys show that higher doses and additional applications of glyphosate, as well as tank mixtures with other herbicides, are being implemented. These are procedures that inevitably serve to increase the glyphosate selection pressure. Monsanto scientists argue, for example, for a high-dose-strategy, so that the weeds with low resistance levels are destroyed (Sammons et al. 2007). Evidently, in order for RR soybeans to be able to withstand higher doses without damage, plants with a higher resistance to the active ingredient are being developed (Service 2007).

For years, experts have recommended, little short of preached, that the evolution of herbicide-resistant weeds can only be prevented, or at least slowed, by diversity on the field and the combination of different approaches to control weeds. For that reason, glyphosate-resistant monocultures and the repeated application of glyphosate should be avoided (Beckie 2006, Hartzler 2005, Buhler 2002, Ghersa et

al. 2000). Diversity is of paramount importance, says Stephen Powles, one of the world's best-known experts on herbicide resistance in weed flora (Bennett 2005). Even companies such as Monsanto have set up their own Internet sites, where farmers can inform themselves about resistance management strategies (www.weedresistancemanagement.com).
Syngenta warns farmers away from the repeated cultivation of RR plants and the excessive use of glyphosate. However, for resistance management they also recommend, "completely unselfishly", their own herbicidal products (http://www.plantmanagementnetwork.org/pub/cm/news/2004/agtech/, www.mindfully.org/GE/GE4/Glyphosate-Resistant-SyngentaDec02.htm).

Necessary measures recommended by scientists:

- Crop rotation, which changes the weed population
- Reducing herbicide use and rotating the herbicidal mode of action in order to reduce selection pressure
- Rotating control measures in order to reduce the dependence on herbicides
- Changing the sowing times, in order to provide cultivated plants with a head start on weeds
- Integrated pest management – specifically adapted to weeds
- Increased scouting of weeds in order to improve knowledge of weed communities
- Ploughing in low light in order to suppress light-induced germination
- Cleaning harvesting machines in order to avoid the spreading of weed seeds
- Other measures: for example cover crops, mixed cropping, fallow land

However, this collection of strategies for handling herbicide resistant weeds is apparently not strictly adhered to. Instead, many farmers hope to be able to solve the problem by means of increased herbicide doses and resorting to other, often "old", herbicides. 80% of the surveyed Delaware farmers expressed the hope that a new herbicide will be discovered within the next five years (Scott & VanGessel 2006). This opinion is far from common among experts. For example, Syngenta employees have stated that it is becoming increasingly difficult to find suitable herbicidal active ingredients that are compatible with the intensified requirements of new chemicals. In addition, development costs are increasing dramatically (Rüegg et al. 2007). They state that industry tends to modify already well-known active ingredients and to stay, for instance, in the class of the ALS or ACCase inhibitors. Other experts have also expressed doubts that new active ingredients that fulfil health and environmental safety requirements will in fact appear on the market soon enough before the RR system collapses (Service 2007, Johnson & Gibson 2006, Kudsk & Streibig 2003).

A particularly "innovative" procedure is genetic engineering with the objective of creating resistance to other herbicides. For instance, Behrens et al. (2007) announced that they have succeeded in transferring a resistance to Dicamba into soybeans. Dicamba belongs to the herbicide class of synthetic auxins. It exhibits negative effects on the reproduction of animals and promotes chromosomal instability (Filkowski et al. 2003, http://www.pesticideinfo.org). Dicamba is manufactured by BASF (product name Banvel); a related product, chloramben, is manufactured by Bayer. Monsanto has secured the rights to this new GM soybean and is assuming that it will be ready for the market in three to four years (Service 2007). However, since 25 weed populations worldwide are already exhibiting resistance to Dicamba and related active ingredients (www.weedscience.org), this will hardly be a successful attempt to solve the problem of herbicide-resistant weeds. It is also inexplicable that plants are being developed to be resistant to ALS as well as ACCase Inhibitors (Service 2007) – these mode of action classes, alongside atrazin, are top of the list for herbicide-resistant weeds. Thus, it was recently announced that BASF, together with the Brazilian company Embrapa, have developed a transgenic soybean that is resistant to in-house ALS inhibitors, and they hope to launch it by 2012. BASF expects to attain a portion of 20% of the soy market (www.genet-info.org). In 2006, DuPont-Pioneer have even announced a soybean in the USA that is simultaneously resistant to glyphosate and ALS inhibitors (http://www.aphis.usda.gov/brs/aphisdocs/06_27101p.pdf). Yet, this kind of dual resistance is already present in one of the US horseweed populations and some other resistant weeds!

Among others, the herbicides Paraquat and 2,4 dichlorophenoxy acetic acid (2,4-D) are used and/or recommend for use in tank mixtures or in rotation with glyphosate (Beckie 2006, Freudling 2004). In particular, Paraquat (manufactured by Syngenta) is highly controversial and, since July 2007, is no longer permitted for use in the EU as a result of its high toxicity for humans (http://www.evb.ch/p62.html). 2,4-D, considered to be moderately dangerous to health, also promotes chromosomal instability. Certain formulations are poisonous to fish (http://www.pan-uk.org/pestnews/Actives/24d.htm, Filkowski et al. 2003). Both herbicides are considered dangerous to the environment. Incidentally, 23 of the 315 listed herbicide-resistant weed populations are resistant to Paraquat, and 25 are resistant to 2,4-D (www.weedscience.org).

6.2 Effects on herbicide use

Glyphosate is the world's most widely used herbicide. Its use has increased rapidly through the cultivation of RR plants, particularly in the USA and Argentina. According to data from the National Agricultural Statistics Service (NASS), which belongs to the US Department of Agriculture (USDA), total herbicide use in US soy cultivation increased from 25,654 tons per year in 1995 (before the introduction of RR soy) to 35,085 tons per year in 2005. This is an increase of more than one third. However, over the same period, glyphosate use in US soy cultivation increased from 2,870 tons to 25,600 tons, amounting to a nine-fold increase (http://www.pestmanagement.info). Thus, alongside this general increase in herbicide use, an enormous shift towards glyphosate has become apparent: in 1995, glyphosate accounted for 9% of the total herbicide use in US soy cultivation; in 2005, it amounted to 73% - almost three quarters - of the herbicides applied. Glyphosate was used on an average of 88% of soy cultivation areas in the US in 2005. At the top of the list were the states of Mississippi (98%), Kansas (94%), as well as Arkansas and Tennessee (93% each). These percentages are assumed to roughly correspond to the percentage of RR soy cultivation acreages in the respective federal states.

Hence, arguments that state that the use of herbicide-resistant plants lead to an overall reduction of herbicide use are clearly proven wrong by data from the US Department of Agriculture. Between 1995 and 1998, a shift in herbicide use toward glyphosate had already appeared (Wolfenbarger & Phifer 2000). Data on US herbicide use, published some years ago by Benbrook (2001, 2004), were based on analyses of the official USDA data. These analyses found that approximately 11%

more herbicides were used on RR acreages than on conventional soy fields. It has also emerged that herbicide use initially appeared to decrease after the introduction of RR plants (soy, corn, cotton, and oilseed rape). However, since the end of the Nineties at the latest, it has increased substantially - and this is despite a lower application quantity per spraying due to an increased effectiveness, as was the case for glyphosate for example. Benbrook (2004) calculated that from 1996 to 2004, overall herbicide use increased by 62,500 tons.

Data from Argentina and Brazil likewise prove that herbicide use increased following the introduction of RR soybeans. In Argentina, herbicide use has seen a five-fold increase since 1997 (Pengue 2004), and the use of glyphosate rose from 820 tonnes in 1996/97 to 45,860 tonnes in 2003/04 – a 56-fold leap (Benbrook 2005). On the one hand, this enormous increase is explained by the large scale of ploughless tillage, where glyphosate eliminates weeds, and on the other hand by the rapid expansion of RR soy acreages - a 35-fold increase (from 0.4 million hectares in 1996/97 to 14.1 million hectares in 2003/04). On top of this, over 50% more glyphosate pro hectare is being deployed. The rapid evolution of glyphosate-resistant weeds will lead to a further rise in herbicide application. Thus, attempts to tackle the increasingly prevalent glyphosate-resistant johnsongrass (*Sorghum halepense*) in Argentina could lead to an additional 25,000 tonnes of herbicide being applied per year (Romig 2007).

7 Socio-economic effects

7.1 Expansion of RR soybean cultivation

In the US, Argentina and Brazil, among others, the cultivation of RR soybeans has reached unprecedented levels. According to the ISAAA organisation, which is sponsored by the biotech industry, in 2006, RR soy was cultivated on 58.6 million hectares, which corresponds to 57% of the worldwide GMO acreage (James 2006). In 2005, Roundup was applied on 88% of soy fields (http://www.pestmanagement.info); the current percentage is now likely to be over 90%. In Argentina, the proportion of RR soy is thought to be 98% (Romig 2007); in Brazil well over 20 %. The greater part of the Latin American soy harvest is destined for export and is sent predominantly to the EU (as animal feed) and to China (FoEI 2006).

A common explanation pattern for the increase of RR soy fields is that RR soy increases yields, decreases dependence on herbicides and, on top of all this, saves the environment. However, as the data in the previous chapters revealed, no yield increase can be achieved with RR soy and the use of herbicides does not decrease, but rather increases. And the use of glyphosate is far from being environmentally friendly or compatible with healthy living. On the contrary, glyphosate-resistant weeds and diminishing soil fertility pose an enormous problem for farmers. Furthermore, economic earnings do not seem to be higher than with the conventional system (Fernandez-Cornejo et al. 2002, Freese 2007). According to a study from the Soil Association (2002), the profitability of RR soy was actually lower than that of comparable conventional cultures. On the whole, it is a situation in which other factors obviously play an important role - factors that are not as plain as an increase in yield.

The herbicide resistance (HR) system often offers greater flexibility to farmers than conventional cultivation systems, as they are not required to slavishly follow a spraying schedule. This is said to be an important reason for the high acceptance of HR systems in agriculture systems that are characterised by vast areas, monocultures and intensive use of fertilisers, pesticides and machinery (FoEI 2006). For farmer families, the working hours saved by the use of broad spectrum herbicides also seems to be important, as they are then able to find additional employment (Gardner & Nelson 2007). Ploughless soil tillage, where the sowing is done immediately after seedbed preparation, is closely connected to intensive herbicide use. This cultivation system, which is propagated to preserve the soil and save working hours, saw massive growth in North and South America in the Nineties. The cultivation of glyphosate-resistant plants fits well into this system (Benbrook 2004, Pengue 2004).

In the US in particular, the huge problems that arose in the Nineties with weeds that had developed a resistance to the herbicides that are predominantly used in soy cultivation have probably greatly encouraged the general take-up of RR soybeans. In glyphosate, farmers were hoping to have found a miracle cure against these resistances. As Freese (2007) demonstrated using the example of genetically modified cotton, the cultivation of RR soy could expand yet further if varieties with desirable growth traits are made available exclusively as transgenic varieties. To artificially shorten the supply of conventional seeds in favour of transgenic seeds (FoEI 2006) could be an attractive strategy for seed companies if they control the seed market (or operate in accordance with other companies). For the farmer, this would likely lead to a switch to GMO seeds, even though they are generally much more expensive. In this context, it should be noted that, as early as 2004, over 50% of conventional soy seeds in the US had already been contaminated with transgenic constructs (UCS 2004), meaning that the harvest products could no longer be marketed as non-GM.

Since 1996, Friends of the Earth International has studied the conditions that have surrounded the introduction of genetically modified plants (FoEI 2006). They have discovered that GMOs have not been introduced as a result of their supposed advantages for consumers, the environment and agriculture, but as a result of a massive campaign by the agri-biotech industry. This industry is supported by organisations like the ISAAA (International Service for the Acquisition of Agri-biotech Applications), which is itself extensively financed through the biotech industry and which publishes annual figures on the increasing GMO acreage (James 2006). In particular, Monsanto, producer of the RR soybean and the most important producer of GM seeds worldwide, uses its enormous influence in national and international politics to achieve regulations and outcomes in the company’s interest. Monsanto’s products have even been found in huge quantities in countries where GMOs are banned. This has evidently paved the way for later approval: When GMO contamination has already reached huge proportions, it seems to be difficult to undo. The introduction of the RR soybean in Brazil is an object lesson for this.

The industry’s aggressive approach was facilitated and supported by compliant patent laws in the industrialised countries. These laws declare that (genetically modified) organisms and their offspring are inventions and that they are therefore subject to patent protection. Companies are likely to come to rely on GMOs due to the huge appeal of exclusive marketing rights for each genetically modified plant and its offspring. Thereby, the trend towards genetically modified plants is hugely reinforced. Farmers that knowingly or unknowingly cultivate these patented plants are usually taken to court for patent infringement by the patent owners. Thus, in recent years, numerous proceedings in the US have been decided in favour of Monsanto. In these cases, the farmers had to pay high fines or agree to undisclosed settlements with Monsanto, which could well lead to bankruptcy for the farmers (CFS 2005).

On the other hand, it was the lack of patent protection that facilitated the introduction of the RR soybean in Argentina. This encouraged seed saving and seed exchange. As a result, according to Nellen-Stucky & Meienberg (2006), certified RR soy seeds represent only a fifth of the seeds sowed in the country; just under a third of these are saved, and about half of the seeds are from the black market. Therefore, the cost of RR seeds in Argentina is far below that in the US. Glyphosate, which is not marketed exclusively by Monsanto in Argentina, is also cheaper than in the US. As a consequence, US soy growers complained about a competition distortion. Aware of the legal situation in Argentina, Monsanto have launched RR soybeans in the country, but have also attempted to extend their patent claims to the RR soybeans grown in Argentina, as well as the resulting products (Nellen-Stucky & Meienberg 2006). Whilst referring to European patent laws, the company arranged for ships containing Argentinean soy flour to be detained in European ports, thereby hoping to achieve compliance from Argentinean partners.

7.2 Effects of RR soybean cultivation in Latin America

Developments in the soy-growing regions in Latin America demonstrate that not only the environment and rural agriculture but also a country's entire social structure are threatened if a massive focus is placed on genetically modified agriculture (FoEI 2006, Rulli 2006, Joensen et al. 2005, Benbrook 2005, Pengue 2004). In recent years, the cultivation of RR soy in Argentina has resulted in the emergence of "green deserts", where nothing but soy grows, whilst the biodiversity of plants and animals has declined immensely. The RR soy boom, which has already taken over more than half of the countrywide acreage, has been at the expense of a diverse agriculture. The production of wheat, corn, fruit, vegetables, meat and milk has declined significantly. Furthermore, according to Benbrook (2005), since 1996, 5.6 million hectares of land that had not previously been used for agriculture have been transformed into soy fields. Backed by DuPont and other companies, it is the poor population in particular, that are persuaded to a soy-based diet, a shift that does not reflect Argentinean food patterns (Joensen et al. 2005).

The ongoing deforestation of the rainforest and other forests in Argentina and Brazil is in large part a result of the soy boom. Joensen et al. (2005) report that the deforestation rate in Argentina almost doubled between 1997 and 2001 when compared to the Eighties. The conversion of forests in Northern Argentina is 3.6 times higher that the global average (Benbrook 2005). These large-scale deforestations involve an incredible loss of biological diversity and lead to the disappearance of numerous (protected) species. As a result of the extensive Roundup spraying and the intensified cultivation, the soil life is under threat (for example as a result of negative effects on microorganisms), which has unquestionably already affected soil fertility. According to Pengue (2004), artificial fertilisation cannot compensate for the loss. He stresses that Argentina exports a significant quantity of nutrients with its agricultural products, which will result in a serious and long-lasting reduction in soil quality, leading in a few decades to soil exhaustion. Furthermore, RR soy has proven to be more sensitive to drought than conventional soy, with harvest losses of up to 25 % during dry years (Altieri & Pengue 2005).

Sprayed Roundup, which is often applied using planes or helicopters, gets into areas, that are not cultivated with RR plants. The herbicide damages plants on the fields and in gardens of peasants and country dwellers, who can't ask for compensation Reports on the intoxication of non-involved persons are accumulating (Joensen et al. 2005). Ultimately, peasants are being increasingly displaced from their land by the powerful landowners and international corporate groups that hope to profit from the soy boom in Latin America. After RR soy cultivation began in 1998, the number of agricultural businesses in Argentina decreased by a quarter within just four years (Altieri & Pengue 2005). Hundreds of thousands of peasants left the countryside and went to look for work in the cities, as the cultivation of RR soy did not create any work (Ruiz-Marrero 2005). Unsurprisingly, poverty and starvation increased (Benbrook 2005). Andrioli (2007) arrived at similar conclusions for Brazil. He based his study on technology and family agriculture in Rio Grande do Sul on interviews with the general population. The situation in Paraguay does not seem to be better: peasant groups lament that powerful landowners and foreign entrepreneurs are even calling on the services of paramilitary groups to assert their claim to land (Rulli 2006). As a result, in vast parts of the population, poverty has increased and migration into the cities has reached new dimensions.

8 Conclusions

The cultivation of herbicide-resistant soybeans certainly fails to offer a sustainable model of agriculture, neither in the US/Canada nor in Latin America, or in any other country. Neither consumers nor peasant agriculture nor rural populations profit from RR soy cultivation, but big businesses, the biotech industry and producers of seeds and herbicides.

The use of RR soybeans is linked to numerous health risks, and economic and socio-economic risks:

- There are many open questions concerning the health effects of genetically modified RR soybean on humans and animals.
- The applied broad spectrum herbicide Roundup is not only toxic for plants, but also for microorganisms and animals. It has a toxic impact on humans and likewise endangers both producers and consumers.
- Glyphosate impairs the ingestion of micronutrients and exhibits a negative effect on soil life and soil fertility.
- Wild plants adapt to the intensive use of herbicides and increasingly develop resistance to the active ingredient glyphosate.

- Herbicide use does not decline, as was argued, but in fact increases considerably.
- Biological diversity is reduced, far beyond the RR soy crop area. Deforestations in favour of RR soy cultivation lead to a loss of protected species. This contradicts the aim of the world community and the EU, to bring the loss of biodiversity to a hold.
- Rural communities are destabilised through large-scale soy cultivation, which leads to strong migration into cities and the impoverishment of large sections of the population.

Therefore, EU approval for the RR soybean must not be renewed.

9 Summary

The genetically modified RR soybean GTS 40-3-2 from the company Monsanto is resistant to glyphosate, the active ingredient of the broad spectrum herbicide Roundup. In 1996, it received marketing approval in the EU. Glyphosate kills plants by inhibiting 5-Enolpyruvylshikimate 3-Phosphate Synthase (EPSPS), an enzyme that is involved in the biosynthesis of aromatic amino acids in plants. The associated metabolic pathway is involved in the formation of vitamins, secondary ingredients and defensive substances, as well as in growth and stress reactions in plants. Resistance was mediated by the transfer of a bacterial gene, which leads to the formation of an enzyme that is insensitive to glyphosate. Approval was granted based upon an insufficient data basis with regard to risks to health and environment and socio-economic risks.

The RR soybean GTS 40-3-2
The RR soybean GTS 40-3-2, which was generated through particle bombardment, was insufficiently tested prior to approval. Minimum standards for the characterisation of integrated DNA sequences and integration sites have not been fulfilled. As a consequence, it was only discovered years later that this line contains additional DNA sequences and that further transcripts can be detected. The 35S CaMV promoter that controls the EPSPS gene is controversial, as it is not only active in plants and is suspected of facilitating DNA recombination.

Health effects
Exposure to Roundup leads to acute poisoning. This can affect both the operator and non-involved persons, who, for example, are exposed to spreading from the air. Glyphosate and its main metabolite, Aminomethylphosphonic acid (AMPA), accumulate in seeds and are taken up when RR soybeans are consumed. The highest permitted residue level for glyphosate has been officially increased many times over. Studies prove that the herbicide has a toxic effect on cells and genes. Negative effects were observed in animal experiments and experiments with human cells. The agent is suspected of disrupting cell division, the formation of germ cells and embryonic development. Formulating agents heighten the toxicity of the active ingredient glyphosate.

The principle of substantial equivalence, which forms the basis of the approval decision for the RR soy line GTS 40-3-2, is contentious: The examinations that were performed before the application was filed are not adequate for a demonstration of the lines' allergological and toxicological safety. Moreover, in the studies, a protein extracted from genetically modified bacteria was used in place of the EPSPS protein that actually forms in transgenic plants. As a result, effects that arise due to the genetic modifications in the plant and possible modifications of the protein are not registered. No examinations were carried out concerning the chronic toxicity, cancerogenicity and reproductive toxicity of the EPSP protein and the RR soybean. Even though it is not clarified to what extent glyphosate influences the formation of secondary metabolites (for example of phytoestrogens), untreated RR soybeans were mostly used in the studies. More recent works give hints about cellular changes in organs and toxic effects on reproduction in animals after feeding with RR soybeans. Transgenic RR soy DNA can possibly survive the gastrointestinal passage, at least the small intestine passage, as studies with human volunteers that are ileostomists have demonstrated. Therefore, more attention must be paid to the stability of transgenic DNA following ingestion, as well as to horizontal gene transfer.

Ecological effects - Direct toxic effects

The herbicide Roundup is not only toxic for plants, but also for animals and microorganisms, and it weakens soil life. The formulating agents exhibit an independent toxicity. The active ingredient glyphosate is moderately persistent. It was detected in soil and in surface waters. Its decomposition depends on variables such as temperature and soil conditions. Glyphosate binds trace elements in the soil and hinders their absorption via plant roots. A scarcity of micronutrients in treated plants can lead to yield declines and a reduced defence against diseases.

RR soybeans accumulate glyphosate in seeds and roots and exude the active ingredient into the rhizosphere via their roots. This possibly has negative effects on soil life and non-target plants. Non-target plants are also affected by herbicide drift: The number of complaints about damage to neighbouring crops, which are not resistant to Glyphosate, is rising. Roundup that is carried into non-agrarian land is generally damaging to plant life and the biodiversity of these ecosystems. Glyphosate is also toxic for animals, whereby fish and amphibians are particularly sensitive.

EPSPS, the enzyme targeted by glyphosate, is essential in microorganisms for the synthesis of aromatic amino acids. However, not all of them possess an insensitive form of the enzyme. As a result, glyphosate can impair the microbial activity in the rhizosphere. When it gets into waterbodies, it can also damage organisms that live in the water. Microorganisms living in the gastrointestinal tract of fauna, farm animals and humans can also be adversely affected by the consumption of polluted plants and deriving products.

Soybeans and other legumes live in symbiosis with nodule bacteria, which assimilate nitrogen from the air and make it available to the plants. However, *Bradyrhizobium japonicum*, a species cohabiting with the soybean, reacts sensitively to glyphosate. This results in a delayed and reduced nitrogen binding and a reduced growth in roots and sprouts, in particular in young RR soy plants. The harvest declines, especially in less fertile soils and under stress of drought. Furthermore, glyphosate also influences fungi and obviously promotes the occurrence of pathogenic species such as

Fusarium. Compared to conventional soybeans, RR soybeans generally produce 5-10 % less harvest.

Indirect effects on biodiversity

The herbicide's effective elimination of wild plant flora destroys sources of nutrition and habitat for numerous organisms and therefore endangers biological diversity. Studies in England have proven that the application of broad spectrum herbicides greatly reduces the biomass and seed bank of wild plants, on and beyond agricultural land. The loss of plant biodiversity has led to a strong decline in insects. This results in further negative effects for higher animals like birds and mammals. An expansion and intensification of RR soy cultivation increases the loss of species. Large-scale deforestations in Latin America in favour of RR soy monocultures exacerbate the situation enormously.

Evolution of herbicide resistant wild plants

A broad use of glyphosate leads to the evolution of resistant wild plants: At least 13 different wild plant species that exhibit glyphosate resistance are already known, the unknown figure is likely to be considerably higher. Hundreds of thousands of hectares in the US and Argentina are affected by this. Weed shift towards less sensitive species can also be observed.

Adaptation strategies and herbicide use

Glyphosate-resistant weeds significantly increase the costs of RR soy cultivation. Experts have been recommending for a long time that diversity on fields is of utmost importance, in cultures as well as in the control methods, and that growers should not unilaterally depend on herbicides. Nevertheless, "problematic" weeds are often tackled with further sprayings and high doses of herbicides, often mixed with old compounds, some of which are highly toxic. The use of glyphosate has increased drastically in all countries with large RR soy fields, nine-fold in the US and more than fifty-fold in Argentina. Total herbicide use in the US increased by three times and in Argentina by five. Further increases are certain to follow, as the evolution of ever more glyphosate-resistant weeds can be expected. As a result, the claims by the agro-biotech industry that herbicide-resistant plants entail a reduction in the use of herbicides, is reductio ad absurdum.

Socio-economic effects

The following reasons are usually given to support the strong expansion of RR soy fields: yield increases, higher flexibility for weed control, reduction in working hours, the benefit of ploughless soil tillage and glyphosate's compatibility with healthy living and the environment. Other reasons that are not so often given are: weeds with resistances towards previously-applied herbicides, the artificial shortage of conventional seeds that is encouraged by seed companies, colossal advertising and PR campaigns and the industry's influence on decision makers, as well as GMO contamination in seeds and the circumvention of state regulations.

Large structures are generally favoured. On the other hand, small farmers in Latin America are severely disadvantaged or even driven away from their fields, either directly or indirectly, because of damage caused to their agricultural crop (and their health) by glyphosate. This results in an increase in migration into cities and the impoverishment of further sections of the population.

Conclusion

The use of the RR soy system does not represent a sustainable future agricultural model. The cultivation of RR soybeans endangers the health of humans and animals, leads to an increased use of herbicides, reduces soil fertility and biodiversity and has a negative effect on peasant agriculture and the rural population. For these reasons, EU approval for the RR soybean GTS 40-3-2 must not be renewed.

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