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Agronomic and environmental aspects of the cultivation of genetically modified herbicide-resistant plants

A joint paper of BfN (Germany), FOEN (Switzerland) and EAA (Austria)

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EXECUTIVE SUMMARY

Conservation of biodiversity is high on the agenda of international and national environmental policies though not very present in public awareness. The need to protect biodiversity and stop the loss was acknowledged in the Convention on Biological Diversity (CBD), internationally agreed on in 1992, and underscored by relevant decisions since then.

It has been known for some time that intensive high input farming is one of the main drivers of ongoing biodiversity losses in agricultural landscapes. An indicator for such losses is the diversity and abundance of weed flora. Transgenic crops resistant to the herbicides glyphosate (accounting for the great majority) and glufosinate have first been cultivated commercially in the nineties of the last century. Since then, a wealth of information has been collected on use patterns and on impacts of herbicide-resistant (HR) crops. There are concerns that HR crops will help to further intensify farming and may therefore increase pressure on biodiversity. The need to study potential environmental consequences of changes in herbicide usage due to transgenic HR plants has recently been underlined by the Council (of Environment Ministers) of the European Union (EU[1]). This paper summarizes the lessons that can be learned from the experience up to now.

Impacts on agricultural practice and agronomy

Like any significant change in crop choice, HR crops can have various impacts on the agricultural practice and agronomy, including weed control, soil tillage, planting, crop rotation, yield, and net income. Glyphosate- and glufosinate-resistance allows previously sensitive crops to survive application of the complementary broad-spectrum systemic herbicide. HR crops facilitate weed control, *i.e.* they enable the usage of broad-spectrum herbicides and thus an easier choice of products as well as an extension of the time window for spraying, giving the farmer more flexibility. HR crops also allow post-emergence application of herbicides instead of the routine pre-emergence application in conventional crops.

Results on yields of HR crops compared to conventional crops are actually mixed. In general, there has been little, if any contribution of HR crops to increase the yield.

The herbicide usage in HR crop systems and their impacts is difficult to compare to conventional crop management because different herbicides are applied at different rates and the specific environmental impacts may vary. There is some agreement in the literature that with the introduction of HR crops in the US lower amounts of herbicides (as active ingredient per hectare) were applied during the first years (from 1996 onwards), compared to conventional crops. Based on United States Department of Agriculture (USDA) statistics, the trend turned in 2000 and already in 2004 more herbicides were applied to HR crops than to conventional crops. In the following years, the difference rose progressively and led to an estimated amount of 239 million kg additional herbicides in the whole period of 1996-2011, with HR soybean accounting for two thirds of the total increase. Most of the increase was due to the rising dependence on glyphosate. In Argentina, herbicide use, and in particular glyphosate

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[1] See Council of the European Union (2008) under under references.

use, increased enormously in line with the introduction of glyphosate-resistant soybean. In case HR crops would be authorized for cultivation in Europe, projections predict that herbicide use would increase significantly in the EU as well.

Mechanical weed control decreased with the introduction of HR varieties. Conservation tillage, often recommended to reduce soil erosion and to save costs and energy, expanded and might even further expand if more HR crops are grown as they are well adapted to tillage systems without or reduced mechanical weed control. In regions where HR crops are widely adopted, less crop rotation and crop diversification takes place. There is a clear trend towards monoculture of HR crops, which enhances disease and pest pressure although, in theory, high weed control levels in HR cropping systems would allow to include crops with higher weed infestation and to broaden crop rotation.

However, crop rotations in HR systems may change due to volunteer problems. HR volunteers which survive pre-seeding herbicides can cause undesirable effects in less competitive crops and require that different or further herbicides are applied.

Reasons for farmers to adopt HR systems are, besides simplification of weed control, reduced production risks, the currently low herbicide prices and expected lower costs of HR systems (*e.g.* in combination with conservation tillage and other production factors such as less labor and fuel consumption). It is not increased yields that are cited in first place as reason for HR crop adoption. Overall, the higher flexibility rather than the crop yield and the final economic success (costs vs. returns) are the decisive factors for adopting HR systems.

Changes in weed susceptibility

In general, increased dependence on herbicides for weed control leads to a shift in weed species composition. Less sensitive species and populations will survive sprayings and subsequently grow and spread, whereas more sensitive species disappear. Although glyphosate was not considered to be a high-risk herbicide with regard to the development of resistances, at least 24 glyphosate-resistant weed species, comprising more than 150 populations, have been found. Today, they infest millions of hectares of HR crops and conventional crops. Some of the resistant weed biotypes are cross-resistant to other herbicides. Glyphosate-resistant weeds can withstand up to 19-fold the dose tolerated by ordinary sensitive weeds and exhibit a great diversity of molecular and genetic resistance mechanisms. Recently, two weed species resistant to glufosinate have been described as well.

Weed scientists recommended for years farmers should implement an integrated weed management approach, comprising a combination of a number of weed management methods ranging from crop rotation, herbicide rotation and mechanical weeding to cover crops, intercropping and mulching. But continuous glyphosate-resistant cropping became common in the Americas, and farmers often simply resorted to higher herbicide doses and other herbicides. Increasingly, companies develop and sell transgenic crops with stacked HR traits, which combine glyphosate-resistance with resistance to glufosinate and/or resistance to other herbicides such as synthetic auxins like 2,4-D or ALS (acetolactate synthase)-inhibiting herbicides. However, a number of hard to control weeds is already resistant to synthetic auxins and even more so to ALS-inhibitors. In addition, merely rotating herbicides may exacerbate resistance problems by selecting for more generalist resistance mechanisms in weeds.

In particular, crops with characteristics such as shattering and seed persistence, *e.g.* oilseed rape, are likely to emerge as volunteers. Seed spill can also occur outside the fields and along transport routes potentially leading to HR feral plants. Oilseed rape volunteers with resistance to glyphosate and glufosinate have already been detected in fields where HR crops have not been planted previously. Oilseed rape plants with multiple herbicide resistance genes not commercially sold have also been found, providing evidence of novel transgene combinations in the wild. Thus, the HR trait can spread both spatially and temporally. More HR plants might show up, if outcrossing from HR crops into the same or related species occurs. The transfer of HR genes to wild relatives should be particularly taken into account and avoided in centers of crop origin and regions where interfertile and weedy hybrids occur.

Impacts on biodiversity

Farmland biodiversity is an important characteristic when assessing sustainability of agricultural practices and is of major international concern. The environmental impacts of a particular HR crop are difficult to assess as they depend on a range of factors that vary from region to region. These factors encompass the whole agricultural management, *e.g.* the complementary herbicide compared to the conventional herbicides, the dose, time and frequency of herbicide applications, and additional management features of the HR crop and of other crops in rotation with it. The receiving environment plays an important role.

Growing HR crops is associated with the use of broad-spectrum herbicides that have long been perceived as less hazardous. For herbicides, specific legal frameworks regulating the approval procedures and assessment criteria are established. While glufosinate, due to its reproductive toxicity, is expected to be phased out in the EU in 2017, glyphosate is presently evaluated for renewed approval in the EU. Due to the adoption of HR crops almost twenty years ago, glyphosate is today by far the herbicide most widely used in the world. In light of the great number of glyphosate-resistant crops that are authorized or in the pipeline, glyphosate will likely remain one of the most used herbicides for the next decade.

Data collected within the last years indicate that glyphosate and glyphosate-based herbicides, apart from being toxic to plants, can also be toxic to other life forms. There are adverse effects on mammals, some invertebrates, aquatic species and the soil microflora. Glyphosate-based herbicides are particularly toxic to amphibians. Glyphosate impacts plants also by binding minerals which can lead to an undersupply of necessary micronutrients and thereby decrease their disease resistance.

Growing HR crops facilitate the operation of reduced/no-tillage systems and consequently may support their expansion. Long-term experiences with reduced tillage indicate that weed populations shift to perennial and grass species and the diversity and abundance of broadleaf plants may decrease further when reduced/no-tillage systems are combined with HR crops.

The Farm Scale Evaluations have provided ample evidence that, compared to conventional farming, weeds are removed more efficiently in HR systems, leading to a further reduction of flora and fauna biodiversity and abundance in farmland. This includes direct effects, such as depletion of the weed seedbank and low weed density and diversity, as well as indirect effects, *e.g.* impacts on animals feeding on weeds and on predators of these animals. Thus, farmland birds may be particularly affected. The significant reduction in monarch butterfly populations in the US has been linked to the widespread cultivation of HR crops in the Midwest which drastically reduced the population of milkweed, the feeding plant of monarch larvae.

As agricultural intensification and pesticide use are among the main drivers of biodiversity loss, agreement on farming practices is required, that are more environmentally friendly and less dependent on pesticides. According to the experience in countries adopting HR crops, where herbicide use was increased instead of reduced, it is highly questionable whether HR systems comply with measures to stop the loss of biodiversity on farmland or can be managed in a sustainable way without further adverse impacts on biodiversity. From a nature protection perspective HR crops seem to be no option for a sustainable agriculture focussing also on protecting biodiversity.

1 INTRODUCTION

Since the first commercial use of herbicide-resistant transgenic crops in the nineties of the last century a wealth of information on patterns of use and impacts of specific applications has been collected. The purpose of this document is to review the available information on the cultivation of herbicide-resistant transgenic crops. The document will cover agronomic and environmental impacts (direct and indirect effects), but focuses on impacts on biodiversity.

There is scientific consent that biodiversity is endangered and its protection is urgent (*e.g.* Rockström *et al.* 2009). For this reason, conservation of biodiversity has become an important issue of international and national environmental policies. Its importance is underscored by the Convention on Biological Diversity (CBD) internationally agreed at the 1992 Rio Earth Summit and the relevant decisions since then[2]. The main aims of the CBD are conservation of biodiversity, sustainable use of its components and both access to genetic resources and sharing of the benefits arising out of their utilization. The Strategic Plan for Biodiversity 2011-2020[3], accepted in 2010, aims at stopping the loss of biodiversity, while finding out the underlying causes for it, including production and consumption patterns. To achieve this, countries should develop national strategies and action plans. In December 2010 the United Nations (UN) General Assembly declared 2011-2020 the United Nations Decade on Biodiversity.

One of the important decisions of the parties to the CBD is the Cartagena Protocol on Biosafety (CPB), adopted in 2000, that seeks to protect biological diversity from the potential risks posed by living modified organisms (LMO)[4]. The protocol establishes a Biosafety Clearing House to facilitate information exchange on LMOs and procedures to ensure that countries can make informed decisions before they agree to the import of LMO (advance informed agreement AIA). It also refers to the precautionary approach. Today, 192 nations plus the EU are parties to the CBD and 165 to the Cartagena Protocol.

The importance of biodiversity protection is underscored by initiatives such as the UN Millennium Declaration in 2000, where all UN member states and important international organizations agreed to achieve by 2015 eight Millennium Development Goals (MDG), among them No. 7 “Ensure environmental sustainability” and, more precisely, Target 7B “Reduce biodiversity loss, achieving by 2010, a significant reduction in the rate of loss”[5]. In 2007 the G8+5 Group launched the related TEEB[6] initiative in order to promote a better understanding of the true economic value of ecosystem services and to contribute to more effective policies for biodiversity protection.

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[2] http://www.cbd.int

[3] http://www.cbd.int/sp/elements

[4] The CPB uses LMO instead of GMO, restricting the scope to only living modified organisms.

[5] http://www.un.org/millenniumgoals

[6] The Economics of Ecosystems and Biodiversity. See interim report, TEEB (2008).

The EU Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (GMOs) aims to protect human health and the environment and to control risks from the deliberate release of GMOs, reference to the precautionary principle is made. According to the Directive, the requirements of the Cartagena Protocol on Biosafety should be respected, monitoring of potential cumulative long-term effects should be considered as a compulsory part of the monitoring plan, and the diversity of European ecosystems shall be taken into account. The principles for the environmental risk assessment (Annex II) require to analyse direct and indirect, intended and unintended as well as cumulative long-term effects relevant to the release and the placing on the market comprehensively. This is in terms of human health and the environment, including inter alia flora and fauna, soil fertility, soil degradation of organic material, the feed/food chain, biological diversity, animal health and resistance problems in relation to antibiotics.

The term biodiversity is used for the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems (CBD, Article 2. Use of Terms). Biodiversity in agricultural landscapes can be characterized by composition (which and how many species/genotypes), structure (dominance), and function (Duelli 1997). Composition and structure can both affect its function (Duelli 1997, Büchs *et al*. 2003).

Intensive high input farming is one of the drivers of ongoing biodiversity losses in agricultural landscapes (Krebs *et al.* 1999, Robinson & Sutherland 2002, Secretariat of the CBD 2005, Foley *et al.* 2011). Farming intensity affects the diversity and abundance of the within-field weed flora which can be regarded as an indicator for it (Hawes *et al.* 2010). Herbicide-resistant (HR) crops will help to further intensify farming and may therefore increase pressure on biodiversity. Herbicide resistance in crops can result from two different breeding procedures: traditional and genetic engineering. These breeding techniques use dissimilar strategies to achieve herbicide resistance, but environmental effects may be comparable. It has been argued, therefore, that almost all of the effects of GM HR plants apply accordingly to non-GM HR plants (Tan *et al.* 2005), and that impacts on biodiversity are linked to the introduction of new crops for intensive management, irrespective of how the variety was developed (Sutherland *et al.* 2006). However, the wide in-crop use of broad-spectrum herbicides such as glyphosate and glufosinate was only made possible by genetic engineering.

The need to study the potential consequences for the environment of changes in the use of herbicides caused by transgenic HR plants has been underlined by the Council of (Environment Ministers) of the EU (2008). The Council also emphasised the need of competent authorities involved in the implementation of Directive 2001/18/EC and of Council Directive on pesticides 91/414/EC (meanwhile replaced by Regulation (EC) 1107/2009) to coordinate their action as far as possible[7]. The vast majority of commercialised HR crops is resistant to either glyphosate or glufosinate, and increasingly both traits are combined in one crop, especially in maize, cotton and soybean[8]. GM crops with resistance to other herbicides such as

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[7] http://www.consilium.europa.eu/ueDocs/cms_Data/docs/pressdata/en/envir/104509.pdf

[8] *E.g.* maize Bt11 x GA21, MON 89034 × 1507 × NK603, cotton GHB614 x LL25 x MON 15985, soybean DAS44406-6.

imidazolinone, sulfonylurea, dicamba, 2,4-D[9] and HPPD[10]-inhibitors are mostly still in the regulatory pipeline (Stein & Rodríguez-Cerezo 2009). In 2013, the first stacked glyphosate and 2,4-D-resistant maize is planned to enter the market and in 2014 the first stacked glyphosate and dicamba-resistant soybean (Bomgardner 2012). A soybean triple stack with resistance to Balance (HPPD), glufosinate, and glyphosate is announced for 2015-2016[11]. For the time being resistance to glyphosate and glufosinate will remain the most important HR traits. Hence, this paper focuses on the agronomic and environmental aspects of cultivating genetically engineered HR crops resistant to glyphosate and glufosinate. Both are non-selective (broad-spectrum) herbicides (Tab. 1). "HR" refers to these two resistances in the context of this document.

Tab. 1: Glyphosate- and glufosinate-resistant crops approved for unconfined environmental release in North America

Herbicides	**Crops**
Glufosinate	Canola, maize, cotton, soybean, rice
Glyphosate	Soybean, canola, cotton, maize, sugar beet, alfalfa

Modified table published by Duke 2005, current status on http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml

Glyphosate interferes with normal plant metabolism through inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). EPSPS is an enzyme of the shikimate pathway for biosynthesis of aromatic amino acids (phenylalanine, tryptophan and tyrosine) in plants and microorganisms. It is not present in animals. As a consequence of the inhibition of aromatic amino acid biosynthesis, protein synthesis is disrupted, resulting ultimately in the plant's death (OECD 1999a). Disruption of the shikimate pathway leads also to a lack of phenolics, including defence molecules such as phytoalexins, lignin derivatives and salicylic acid that functions as signal molecule (Powell & Swanton 2008). Also, glyphosate is a strong systemic metal chelator (Toy & Uhing 1964) which probably adds to its herbicidal activity (see chapter 5.3.1). Glyphosate was authorized in 1997 and last reviewed in 2002 (EC 2002). A new review was due in 2012, but postponed until 2015 (Antoniou *et al.* 2011).

Glufosinate ammonium is an equimolar, racemic mixture of the D- and L-isomers of phosphinothricin (PPT). L-PPT inhibits glutamine synthetase of susceptible plants and results in the accumulation of lethal levels of ammonia (OECD 1999b). Because of its reproductive toxicity, use of glufosinate will be phased out in the EU until September 2017 (EC 2011).

Glyphosate and glufosinate resistance genes allow previously sensitive crops to resist herbicide formulations containing glyphosate or glufosinate. Various HR crops contain *epsps* genes from *Agrobacterium* spp. encoding a glyphosate-insensitive EPSPS protein, some additionally the *gox* gene from *Ochrobactrum anthropi* encoding the glyphosate-degrading

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[9] 2,4-dichlorphenoxyacetic acid.

[10] Hydroxyphenylpyruvate dioxygenase.

[11] http://www.gene.ch/genet/2012/Oct/msg00109.html

glyphosate oxidoreductase (GOX). The more recently used *gat* gene confers glyphosate resistance by an enzyme that modifies glyphosate[12]. Many crops have also been transformed with one of the two bacterial genes *pat* or *bar* from *Streptomyces* spp. Both genes encode the enzyme phosphinothricin acetyl transferase (PAT) which detoxifies L-PPT thereby conferring resistance to glufosinate (L-PPT).

Also new types of HR crops are being commercialised. Among them are soybean with resistance to both glyphosate and ALS-inhibiting herbicides, *e.g.* sulfonylureas and imidazolinones (USDA/APHIS 2007), soybean with resistance to aryloxyalkanoate herbicides (such as the synthetic auxin analogue 2,4-D) or isoxaflutole (a HPPD inhibitor) and maize with resistance to both 2,4-D and certain aryloxyphenoxypropionate (AOPP) herbicides (Stein & Rodríguez-Cerezo 2009, EFSA 2011).

Glyphosate and glufosinate use are not unique to HR cropping systems, but glyphosate and glufosinate may be used in HR crops at other application rates, dosages and/or crop life stages, compared to other cropping systems.

Throughout this document the terms "herbicide resistance" and "herbicide tolerance" are used as defined by the Weed Science Society of America (WSSA 1998):

- "*Herbicide resistance* is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In a plant, resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis."
- "*Herbicide tolerance* is the inherent ability of a species to survive and reproduce after herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant."

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[12] Glyphosate acetyltransferase (GAT).

2 SCALE AND AREA OF APPLICATION

HR crops have been adopted in several countries. Since the adoption rate is dynamic and the number of countries changes, this chapter can only give an overview about the global area cultivated with HR crops. Also the increasing number of crops with stacked traits makes it difficult to get reliable data and a comprehensive picture.

2.1 Commercial cultivation

Many transgenic glyphosate and glufosinate-resistant crop species have been globally tested in field experiments, but only four have been widely commercially grown as approved varieties: maize, cotton, canola and soybean (Brookes & Barfoot 2011). According to James (2012), in 2012 transgenic crops have been grown in 28 countries, in 18 of them on more than 50,000 hectares. Together, herbicide resistance and insect resistance make up almost 100% of the introduced transgenic traits.

Global HR area

Herbicide-resistant crops are by far the most widely planted GM crops. Of the 170.3 mio ha transgenic acreage worldwide, about 59% (100.4 mio ha) were planted with HR varieties and another 25.6% (43.6 million ha) were planted with crops with stacked traits (basically HR/insect resistance stacks) (James 2012). Hence in 2012, 84.6% of the GM crops carried herbicide resistance genes (144 mio ha). The remaining 15.4% (26.3 mio ha) were planted with varieties carrying insect resistance only, which is less than the 25.6% of stacked crops with double and multiple traits. The adoption of stacked crops grew faster than single HR varieties between 2010 and 2012 (James 2012).

44.7% of the global area of the four crops soybean, cotton, maize, and canola is currently planted with HR crops (James 2012). The current share of the HR crop areas per global acreage of these four crops is shown in Tab. 2.

Tab. 2: HR acreage as percent of global crop acreage for principal crops (2012)

Crop	Global area in mio ha*	Biotech crop area in mio ha	HR area in mio ha**	HR area in % of global crop area**
Soybean	100	80.7	80.7	80.7
Cotton	30	24.3	1.8 (5.5)	6.0 (18.3)
Maize	159	55.2	7.8 (47.7)	4.9 (30.0)
Canola	31	9.2	9.2	29.7
Sum	**320**	**169.4**	**99.5 (143.1)**	**31.1 (44.7)**

* Data from James (2012).

** Only HR. In brackets HR/insect-resistance (stacked) included; modified table cited in James 2012.

From the data can be deduced that HR soybeans are by far the most widely planted HR crop: 80.7 mio ha, of 170.3 mio ha for all crops in 2012 representing 47.4% of the global biotech crop area. HR soybeans are the dominant biotech crop grown commercially in countries such as USA, Argentina, Brazil, Paraguay, Canada, Uruguay, Bolivia, South Africa, Mexico, Chile and Costa Rica (James 2012). All biotech canola is herbicide-resistant and grown on about 30% of the global canola area. In the past, most transgenic cotton plants (77.4%) were insect-resistant, a smaller portion (15.2%) had stacked insect resistance/HR traits and 7.4% carried only HR traits (James 2012). Herbicide resistance traits are also important in maize, often combined with insect resistance genes.

Additional HR crops such as alfalfa, sugar beet, and creeping bentgrass are already approved or under development (APHIS 2012). HR sugar beet, cultivated for the first time in 2008 and grown in 2012 on 0.5 mio ha (James 2012), has reached a share of 95% in US sugar beet production in 2012 (http://www.sugarindustrybiotechcouncil.org/sugar-beet-news/). HR alfalfa, the first perennial HR crop, fully deregulated in the US in 2012 (Cowan & Alexander 2012), has been grown in the same year on about 0.4 mio ha (James 2012). Together with the area of 99.5 mio ha for the major cash crops soybean, cotton, maize, and canola (Table 2), the global crop area with HR varieties thus reached 100.4 mio ha.

3 IMPACTS ON AGRICULTURAL PRACTICE AND AGRONOMY

Herbicide-resistant transgenic crops are adopted mainly as a component of agricultural practices and weed management methods. Some of the agricultural practices associated with HR crops are better predictable (i.e. the use of the herbicide to which the crops are resistant) than others, which may be less obvious (i.e. the association of HR soybean with reduced tillage practices). In many cases, it may not be possible to precisely differentiate between direct effects of the adoption of HR crops, indirect consequences, and coincidental impacts from some other cause. Other factors, including government policy and incentives, fuel prices, and even weather conditions can influence farmers' decisions on a yearly basis. Looking at the experience of the last 15 years or so when HR crops were cultivated enables us to see what changes in agricultural practices have occurred over time. While these observations may not result directly and necessarily from HR crop cultivation only and observations from one country may not apply to others, it is important for decision makers to understand how these crops may have affected agricultural practices. Informed decisions related to national policy goals may thus be possible.

3.1 Reasons why producers adopt HR crops

The acreage of HR crops has significantly increased world-wide during the last years. A couple of surveys and analyses investigated the reasons why farmers choose to grow HR varieties instead of conventional crops. "Improved weed control" was the most often stated reason in the published surveys performed within the first years of HR crop adoption (presented in Tab. 3), followed by "cost reductions" (calculated by Sankula *et al*. (2005) and Sankula & Blumenthal (2004) for average weed control costs for conventional and HR crops canola, maize, cotton and soybean – not included or considered in Tab. 3).

Soybean producers (who wanted better weed control) also adopted glyphosate-resistant varieties because glyphosate allowed control of ALS-inhibitor resistant weeds that had become increasingly a problem (Shaner 2000). Price reductions for glyphosate have been a driving factor for the adoption of the corresponding HR crops too (Freudling 2004).

In Argentina, the main reasons to grow HR crops were low glyphosate prices, fewer expenses on labour, fuel and machinery, increased awareness of the synergy between no-till and HR soybean and the possibility to plant soybean earlier (Pengue 2004, Trigo & Cap 2006). Lack of patent protection for GM seeds made the introduction of HR soybean easier and cheaper as seeds could be saved for planting and resale which promoted their adoption in South America (Schnepf 2003).

Yield and returns had less priority for Canadian and US-farmers. Canadian (Manitoba) respondents did not indicate increased yields as important, but mentioned reduced dockage as an important factor (Mauro & McLachlan 2003).

In general, there is a strong desire to reduce production risks (Kalaitzandonakes & Suntornpithug 2001, Fernandez-Cornejo & Caswell 2006). In case of HR cotton and canola, further reasons to adopt these varieties are increased flexibility (extended time window for spraying), simplicity in weed control and less labour (CEC 2000, Firbank & Forcella 2000). In contrast, neither biodiversity nor weed resistance management have been significant considerations to farmers (Owen 2000, Hin *et al*. 2001, CEC 2000).

Tab. 3: Published farmer surveys on reasons to adopt HR crops

Reasons to adopt HR	**Percentage of the respondents who stated the reason, by crop**			
	Canola	**Maize**	**Cotton**	**Soybean**
Improved weed control	**50**	**94.3**	**76.3**	**97.5**
Cost reduction	10	44.3		60.7*
Labour reduction		47.9		48.5
Enable no-till planting/planting flexibility	3	42.1	1.8	41.3
Yield increase		45.6		29.6
Decrease pesticide inputs			18.9	**72.5**
Better returns	19			
Clean up fields	3			
Reference	Canola Council of Canada 2001	Van der Sluis & Grant (2002)	Klotz-Ingram *et al.* (1999)	Van der Sluis & Grant (2002)
Specification of the survey	1.600 farmers in western Canada	1000 farmers in South Dakota	696 farmers in 8 US-States	1000 farmers in South Dakota

Percentages ≥ 50 are in bold.
* But 34.8% were not satisfied with economic returns (other respondents did not state whether they were satisfied with returns).

The perception of the importance of weed resistance management might have changed, as in recent years both the number of glyphosate-resistant weeds and the area inflicted by them has been growing. In a 2010 survey of US corn, cotton, and soybean growers with continuous RR systems for 5-9 years, more farmers reported problems with resistant weeds, compared to a 2005 survey, but 30% of them did not consider such weeds to be a problem on-farm yet (Prince *et al.* 2012 a,b).

3.2 Weed control patterns and herbicide use

3.2.1 Factors influencing the time and the mode of applications

In non-HR farming with crop rotation, farmers can choose to apply a sequence of herbicides with different modes of action or tank mixtures to control weeds. Some of these herbicides can only be applied before crop emergence and are therefore often routinely applied for preventive reasons. Weeds may survive these control measures because they are nonsusceptible to certain herbicides or because they emerge after application of a non-residual herbicide.

HR crops allow the post-emergence application of a single herbicide with a broad activity spectrum, such as glufosinate or glyphosate. Moreover, both herbicides can be used alone, in combination with other herbicides (*i.e.* pre-emergence herbicides to provide soil residual control), or with mechanical weeding.

The appropriate time span for post-emergence weed control in conventional farming is usually 3-5 weeks after crop emergence. The time may vary depending on the herbicide, crop, weed abundance and weather. In HR crop farming, glyphosate and glufosinate provide the option to delay post-emergence application compared to many selective herbicides (Kalaitzandonakes & Suntornpithug 2001, Pallutt & Hommel 1998), whose late applications are sometimes risky and not economically sound (Dewar *et al*. 2000).

3.2.2 Herbicide amounts, herbicide application frequencies, and mechanical weeding

Changes in overall amount of herbicides used are difficult to assess because different herbicides are applied at different rates. For example, in Canada glyphosate is applied with glyphosate-resistant crops at rates ranging from 0.6 L/ha (500 g a.i./L, *i.e.* 0.3 kg a.i./ha) in canola to 2.5 L/ha (360 g a.i./L, *i.e.* 0.9 kg a.i./ha) in maize to as high as 5 L/ha (360 g a.i./L, *i.e.* 1.8 kg a.i./ha) in soybean, whereas atrazine is applied in maize at a rate of up to 3.2 L/ha (480 g a.i./L, *i.e.* 1.5 kg a.i./ha) (Anonymous 2011). Additionally, each of these herbicides differs from each other with regard to their environmental behaviour and toxicological profile. High-effective low dose herbicidal compounds are used in less amounts, but their environmental impact may be comparable to high dose herbicidal compounds. This means that a change in the overall amount of herbicide applied must be considered in terms of the change in the environmental impact of applied herbicides (reviewed in Kleter *et al.* 2008). A change in amounts does not necessarily imply a change in side-effects or number of applications.

Calculating herbicide use is far from simple (USDA/ERS 2000), and most studies have been performed in the US. USDA/ERS used three different statistical approaches and its analyses for 1997 and 1998 ranged from no significant effect to a reduction of 10% (Hin *et al*. 2001). Duke (2005) concluded, based on his review of several studies, that herbicide amounts (weight per unit area) used in conventional and HR varieties did not substantially differ from each other.

There is some agreement in the literature that with the introduction of HR crops such as maize, soybean and cotton in the US less amount of herbicides (as active ingredient per hectare) were applied during the first years (1996 and 1997), compared to conventional crops. According to Benbrook's calculations based on USDA statistics (2003, 2004), the trend turned in 2000 and in 2004 more herbicides were applied to HR crops than to conventional crops. In the following years the margin of difference rose progressively in the US, reaching 0.72 kg/ha more herbicides in HR cotton in 2008 (Benbrook 2009). Between 1996 and 2008 the average amount of herbicides applied to HR soybean hectares increased from 0.99 kg active ingredient per hectare to 1.84 kg, while in conventional soybean it dropped from 1.33 kg to 0.54 kg per hectare (Benbrook 2009). According to Benbrook (2012a), the three HR crops soybean, maize, and cotton increased herbicide use in the USA by an estimated 239 mio kg in the 1996-2011 period, with HR soybean accounting for 70% of the total increase. Most of this increase was due to the rising reliance on glyphosate.

According to Brookes & Barfoot (2005, 2006, 2011), herbicide use was generally reduced for several countries between 1996 and 2005 and up to 2009, although results varied by region and tillage system. However, the calculations of Brookes & Barfoot are based on data from the first years when HR crops were introduced (mainly 1996 to 2001). This holds true also for their most recent publications from 2011 and 2012.

In Argentina, soybean production is dominated by Roundup Ready (RR) varieties. According to Qaim & Traxler (cited by Trigo & Cap 2003), the introduction of RR soybean has reduced herbicides with the higher toxicity classes II and III by 83% and 100%, respectively. However, the number of herbicide sprays and the amounts applied per hectare increased in reduced tillage systems planted with RR soybean from the beginning (Qaim & Traxler 2005). The total amount of glyphosate used in Argentina increased from 1 mio L in 1997 to 160 mio L in 2002/2003 (Pengue 2004). More recently, the use of about 200 mio L of glyphosate on RR soybean has been reported, on an area that steadily increased within the last years (Lopéz *et al.* 2012, Catacora-Vargas *et al.* 2012).

In general, not the application frequency, but the number of different herbicides (active ingredients) has been reduced within the first years of growing HR varieties, as glyphosate was frequently applied at pre- and post-emergence in resistant varieties (Benbrook 2001, Hin *et al.* 2001, Duffy 2001). In western Canada, the pre-seeding application was a two-herbicide mixture (glyphosate plus 2,4-D or MPCA) (Van Acker *et al*. 2003). According to Gianessi (2008), the number of active ingredients used on at least 5% of the US soybean hectares has declined from 19 in 1996 to only one (glyphosate) in 2005.

Since 1999, a number of weed species have become more troublesome first in the US Cotton Belt, later also in soy and maize growing regions, *e.g.* the abundance of glyphosate-resistant horseweed (*Conyza canadensis*), which is well-adapted to no-till systems, increased tremendously (Heap 2008, 2012). Tank mixtures (Clarity [dicamba] or Kixor [saflufenacil] and glyphosate), autumn burndown herbicides such as Valour [imazethapyr plus pendimethalin], or the additional use of pre-emergence herbicides (*e.g.* 2,4-D, Clarity) were recommended against horseweed (Freudling 2004, Deterling 2003, Waggoner *et al.* 2011). Furthermore, for other troublesome weeds such as teaweed (*Sida spinosa*), sicklepod (*Senna obtusifolia*) and morning glory (*Ipomoea*), tank mixtures with glyphosate and Harvade 5F [dimethipin] or Cadet (fluthiacet) are recommended (Deterling 2002, FMC Corporation 2012). Currently the stacking of different herbicide resistant traits is seen as an option to mitigate resistant weeds (Mortensen *et al.* 2012).

For some parts of Europe, extrapolations from field trials let Phipps & Park (2002) conclude that the number and the amount of herbicides (and of active ingredient) per ha may be reduced in HR varieties of oilseed rape, maize and sugar/fodder beet. Likewise, Dewar *et al.* (2005) and Champion *et al.* (2003) deduced from the Farm Scale Evaluation (FSE) that generally one herbicide active ingredient per crop, later and fewer sprays and less active ingredient (for beet and maize) than in the conventional treatments would be necessary. However, if glyphosate-resistant crops (maize, soybean, sugar beet) would be authorized in the EU, Benbrook (2012b) expects a significant rise in total herbicide use: a lower amount of other herbicides would become more than surmounted by more glyphosate which would account for 65% of total herbicide use. In his report, Benbrook projected the likely changes in herbicide use for the three crops through 2025, compared to the 2011 baseline, under three adoption scenarios: (1) If the status quo remains (no approval or planting of HR crops), overall herbicide use would decrease by 1%. (2) With unlimited adoption (following the US pattern of HR crop adoption in 1996-2010), the 31% fall in use of other herbicides would be surmounted by the explosive 824% growth of glyphosate leading to a net 72% increase in overall herbicide use. (3) With targeted adoption (binding commitments to resistance management), total herbicide use would still rise by 25%, caused by a 409% increase in glyphosate use.

3.3 Tillage and planting

Conservation tillage can help to prevent soil erosion, reduce soil compaction and save fuel. Many factors can influence adoption of reduced tillage practices, including government programs, declining costs of pre-emergence herbicides, and improvements in seeding technologies (Zentner *et al*. 2002). Reduced tillage may or may not be used with conventional cropping systems (Diercks und Heitefuss 1990, Kees 1990). However, HR varieties and reduced tillage are often adopted together, whether as a result or a consequence of each other.

Reduced tillage practice on US-soybean acreage increased already from 25% to 48% before the introduction of HR varieties, afterwards it varied from 50% to 60% (Fernandez-Cornejo & McBride 2002). Farmer surveys indicate that 2% to 40% of soybean and cotton and 3% of canola farmers planted HR varieties in order to reduce tillage (Ward *et al*. 2002, Klotz-Ingram *et al*. 1999, see Tab. 3 in Canola Council of Canada 2001, Fernandez-Cornejo & McBride 2002, Kalaitzandonakes & Suntornpithug 2001). In Canada, no-till (Low Disturbance Seeding “LDS”) was practiced on about 40% of the cropping area in Saskatchewan in 2002 (Van Acker *et al*. 2003) and among canola growers 37% to 44% practiced no-tillage with HR varieties compared to 25% with conventional ones (Canola Council of Canada 2005). With no-tillage, farmers apply glyphosate and sometimes another herbicide before seeding in the spring. Also, 44% of the HR canola growers said they are seeding earlier in the spring, because post-emergence herbicide application is possible with HR varieties (Canola Council of Canada 2001). In Argentina, many farmers who adopted HR soybean also reduced tillage, with 42% of conventional fields and 80% of HR fields practicing reduced tillage (Qaim & Traxler 2005).

In Europe, no transgenic HR crops are grown and no data are available to correlate no-till systems and HR cropping systems. Furthermore, no-till practice and mulch planting is not very common in many parts of Europe. In Germany, however, cover crops are more frequently adopted by the farmers (Lütke-Entrup *et al*. 1995). Cover crops with a high competitive ability (*e.g* legumes or mustard) can suppress weeds in no or reduced till production systems. Traditional herbicides can be used (Kees 1990, Heitefuss *et al*. 1994, Auerswald *et al*. 2000), but they are not always necessary when cover crops with a high competitive ability are planted (Petersen & Hurle 1998).

3.4 Crop rotation options in HR crops

According to Davis *et al.* (2012) crop rotation helps to maintain high productivity by reducing pesticide use and fertiliser input. The latter is especially true when legumes are planted. Crop rotation can also facilitate no-till production as shown in maize-soybean systems: soybean stubble and autumn-killed sod crops make excellent no-till seedbeds, and rotation reduces the inoculum for diseases such as grey leaf spot (*Cercospora zeae-maydis)*, which can be severe in continuous no-till maize. With crop rotation, farm work may be more evenly distributed than without.

Some herbicides including imidazolinone in soybean persist in the soil and can damage subsequent crops. In case of Pursuit plus (imazethapyr + pendimethalin), waiting periods of up to 40 months before planting other crops are recommended in the USA (Rohm & Haas 1998 cited in Carpenter & Gianessi 1999). As glyphosate and glufosinate are perceived to have a low residual activity, carryover restrictions are low with these two herbicides. Thus in HR crops, rotation options are increased in principle (Carpenter & Gianessi 1999), but the experience of the last 15 years shows otherwise (Mortensen *et al.* 2012).

In Argentina, HR soybean displaced about 4.6 mio ha of land planted to cotton, maize, orchards, sunflower, horticulture, as well as fallow and pasture land within the years 1999-2004 (Pengue 2004). Fallow seasons that have been used to grow cattle pasture were replaced by continuous agriculture. HR soybean is also planted in more sensitive areas and on virgin land of the north and north-east (*e.g.* rainforest “Yungas”, “Great Chaco” and the Mesopotamian Forest) (Pengue 2004). Within a couple of years a noticeable homogenisation of production and landscapes has taken place. While the option to use glyphosate in-crop may have paved the way, the development was mainly driven by the increasing demand for soybean on the world market.

3.5 Yields

Whereas yield data from HR cropping regions mainly result from surveys or statistical reviews and less from field tests, corresponding data from non-GM cropping countries are always gained by field tests. Field tests attempt to deliver results under similar conditions and with greater reliability. They include a number of growing seasons and typical locations as results can vary from year to year and depend on local conditions. Yield differences of HR relative to conventional crops may be due to the scale and region of growing, but they may also be due to other reasons, *e.g.* site, farm size, soil, climate, tillage system, weed abundance, varieties, crop management practice, weed control practice, and the education of the farm operators (Zentner *et al*. 2002, Fulton & Keyowski 1999).

According to early reports about the yield potential of HR cotton, ‘there has been little, if any, positive or negative contribution’ of the HR input traits to the overall yield potential of the transgenic varieties (National Cotton Council 1999). In HR canola, yield increases by 10% have been reported compared with conventional farming (Canola Council of Canada 2001), in particular under high weed densities (Cathcart *et al.* 2006). According to Phillips (2003), yields of conventional varieties were higher than of glyphosate-resistant varieties, whereas glufosinate-resistant varieties yielded the same in 1999. For Manitoba farmers increased yield was not an important reason for adoption of HR crops (Mauro & McLachlan 2003).

Holtzapffel *et al.* (2008) reported an increase in yield with the adoption of HR canola in Australia, due to the replacement of lower yielding conventional varieties (TT oilseed rape tolerant to triazine). Therefore, comparisons of yields between HR and conventional varieties must also consider their respective genetic background.

The EU FACTT Project (Familiarisation and Acceptance of Crops incorporating Transgenic Technology) compared the agronomic performance of glufosinate-resistant with conventional oilseed rape and found equivalent or lower mean yields for the transgenic varieties (Förster *et al*. 1999, Greenadas & Boothsack 1999). HR varieties showed less grain mass, but a higher seed number/pod and more variable yields (Greenadas & Boothsack 1999). Differences in ramification (branching structure) and pod numbers/plant in HR versus non-HR oilseed rape were not found (Förster *et al*. 1999). An early reported “small yield increase” for HR soybean (USDA/ERS 1999) may have been due to different production factors such as farm size, planting higher-priced crops on better lands, the experience of farm operators and narrow row production (Carpenter & Gianessi 1999, USDA/ERS 1999, Gianessi & Carpenter 2000). Many field tests of HR varieties and conventional varieties under similar conditions proved otherwise.

Elmore *et al*. (2001) showed that (backcross-derived) non-HR soybean lines outyielded their HR sister lines by 5%. The observed yield drag might be due to the present resistance gene in first generation HR lines or, when glyphosate was applied, to reduced nodular nitrogen fixation and a weaker defence response (King *et al*. 2001). A second generation HR soybean (RR2Y, MON 89788) is claimed to have a yield increase, compared to the former one (RR 40-3-2). According to Gurian-Sherman (2009) this was due to a superior recipient line (A3244 instead of A5403) plus a newer insertion method to avoid the yield drag in RR 40-3-2. However, when tested in the greenhouse, different cultivars of RR2Y performed less well than RR 40-3-2 (Zobiole *et al.* 2010). According to Cerdeira & Duke (2006), effects of glyphosate applications on microbes in HR crops are transient. Nodule number and mass (which have been correlated with nitrogen fixation) were not affected by the genetic modification itself (Powell *et al.* 2007, King & Purcell 2001, van Berkum *et al.* 1985). Data from more than 10 years of US HR soybean production show that HR crop yields are, on average, not higher and sometimes lower than yields of conventional varieties (Gurian-Sherman 2009). He also found that HR corn did not provide any consistent yield advantage over conventional systems.

3.6 Conclusions on impacts on agricultural practice and agronomy (Chap. 3)

Like any significant change in crop choice, HR crops may have various impacts on the agricultural practice and agronomy including weed control, soil tillage, planting, crop rotation, yield and net income. However, because the adoption of HR crops correlates with several other production factors, it is nearly impossible to attribute statistically evaluated differences to the adoption of herbicide-resistant plants alone. Particularly the results from different studies on herbicide amounts and application frequencies, yields, and net returns are often not consistent.

In HR farming, post-emergence herbicide applications increased with most crops, while pre-seeding applications decreased in some cases and regions, in particular in the first years of HR crop adoption. In the US Midwest and in western Canada, farmers resorted to two glyphosate applications (pre- and post-emergence) and occasionally admixed further herbicides in early planted soybean and in no-till systems.

No homogeneous picture about changes in weed control patterns can be drawn, as they depend on regional differences and are quite diverse. In addition, they are not documented for all regions and crops.

Reports about application frequencies of herbicides for HR soybean and HR cotton are conflicting for the first few years of their adoption. For the following years, a significant increase in herbicides in terms of amount or applications was found in soybean and cotton growing regions in the US and in Argentina, where HR crops are intensively cultivated.

Changes in overall amount of herbicide use and their impacts are difficult to assess because different herbicides are applied at different rates and their specific environmental impacts may vary. A change in amounts does not necessarily imply a change in side-effects or number of applications.

Mechanical weed control decreased with the introduction of HR varieties. HR varieties are often adopted in conjunction with low tillage practice which can help to prevent soil erosion. Adoption of low-tillage practices can be influenced by a variety of factors. In the first years of adoption the level of weed control increased in HR crops in most cases.

Results on yields of HR crops relative to conventional crops are mixed. In general, there has been little, if any contribution of HR crops to the overall yield.

In theory, HR farming provides more options for crop rotation, because glyphosate and glufosinate are thought to have low residual activity and low carryover restrictions, but the experience indicates otherwise. When HR crops were introduced, they often replaced rather than widened the rotation, which was beforehand used to control weeds. This might be due to their broad-spectrum weed control or rotational constraints.

“Improved weed control” was the most often stated reason to adopt HR crops, followed by “cost reductions”. In general, reasons for the adoption of HR crops are the reduction of production risks and the increased flexibility in weed control.

4 CHANGES IN WEED SUSCEPTIBILITY

Weed control is an important agricultural management tool that aims to preserve crop yield by reducing weed pressure. Any change of the weed management strategy will change weed populations as well. The planting of HR crops is also connected with several changes in weed control measures. Where HR crops are planted continuously, weeds will be under higher selection pressure from fewer herbicidal modes of action than before. In addition, the trend to no-tillage or less soil cultivation is of greater relevance for HR crops. This will impact weed communities and populations. The effectiveness of weed control in HR crops can be undermined by less susceptible or resistant plants. Due to the reliance on herbicides for weed control and their increased use the number of weeds resistant to various modes of action rose steeply within the last decades. Experience with HR crops shows that there is no exception to this rule. This chapter highlights the mechanisms that lead to shifts in weed susceptibility and that have to be considered in risk analysis and environmental assessment of HR crops.

Weeds occur in agricultural production fields and are commonly regarded as pests because they compete with the crop for water, light, and nutrient resources and can cause harvest or quality problems. Weed control is an important agricultural management tool that aims to preserve crop yield by removing weeds or by reducing weed pressure during important growth stages of the crop. But weeds offer considerable benefits for the agroecosystem as well. They support a range of organisms, in particular arthropods, among them decomposers, predators, and parasitoids, providing food and shelter for them (Marshall *et al.* 2003). The application of selective and/or non-selective herbicides, possibly in combination with mechanical weed control practices (including hand-pulling, hoeing, mowing, and tillage) are commonly adopted tools in conventional agriculture across all crops and by farmers across the world. The widespread planting of HR crops is connected with various changes in weed control and agricultural activities such as seeding, tillage, or land use. Some of these changes are related to the associated herbicide spray regimes, while others are due to government incentives or driven by the world market. Where HR crops are planted in sequence, weeds will be under selection pressure from fewer herbicidal modes of action than before.

Both non-selective herbicides glyphosate and glufosinate are effective on a wide range of annual grass and broadleaf weed species, with glyphosate showing the broader spectrum. Glyphosate is said to control over 100 weed species, glufosinate has a somewhat smaller range. As glufosinate, contrary to glyphosate, is not translocated down into the root system, it is not active on perennial structures of weeds.

Target plants differ in their sensitivity to herbicides. There is also considerable intraspecific biotype variability in susceptibility at the whole plant and cellular level. Weed biotypes with a higher tolerance or a resistance may contribute to shifts of the weed flora spectrum.

In general, the simplicity and effectiveness of weed control in HR crops can be undermined in three different ways:

- shifts in weed communities and populations resulting from the selection pressure of the applied complementary herbicides (see chapter 4.1),

- escape and proliferation of transgenic plants as weedy volunteers (see chapter 4.2),
- hybridisation with – and HR gene introgression into – related weedy species (see chapter 4.3).

4.1 Selection of resistance and weed shifts

Within the last decades, herbicide resistance in weeds has increased dramatically. Up until November 2012 a total of 393 herbicide-resistant weed biotypes have been recorded. They belong to 211 species (124 dicots and 87 monocots) and occupy over 680,000 fields worldwide (Heap 2012). Weeds can resist herbicides through several mechanisms including target site insensitivity, overproduction of the target protein, amplification of the target protein gene, herbicide detoxification, reduced herbicide entry, reduced herbicide translocation, and changes in the intracellular accumulation of herbicides. Herbicide resistance in different weed populations (against any herbicide) may occur because the resistance spread from a few initial sites or because it evolved independently several times (Mortimer 1993, McNaughton *et al.* 2005). Neve (2007) points out that low herbicide doses have the potential to rapidly select for high levels of resistance, as observed in rigid ryegrass (*Lolium rigidum*) in Australia. However, resistance can also spread through out-crossing, as proven by the successful hybridisation of the glyphosate-resistant horseweed (*Conyza canadensis*) with the related species *Conyza ramosissima* (Zelaya *et al.* 2007), and by transport of resistant seeds through *e.g.* farm equipment, animals, wind, and floods (Norsworthy *et al.* 2008).

4.1.1 Resistance to glyphosate

Weed population shifts and the evolution of herbicide resistance are inevitable consequences of the HR cropping system, *i.e.* cultivation of HR crops and application of their complementary herbicide(s). Their relative economic importance, however, will depend on the specific agroecosystem (Owen & Zelaya 2005). Glufosinate and glyphosate have long been considered to be low risk herbicides in terms of the evolution of resistant weed populations (Beckie 2006) for several reasons, amongst them the timing of application, low occurrence of mutants, and the genetic background for glyphosate resistance (Neve *et al*. 2003). The chemical structure, mode of action, and limited metabolism of glyphosate in plants as well as the perceived lack of soil persistence, lack of residual activity, limited uptake from the soil (Böger 1994), and its application pattern were thought to be further reasons as to why resistance to glyphosate may evolve rather slowly (Baylis 2000, Jasieniuk 1995).

The first reports on glyphosate-resistant weed species did not appear until the mid-nineties (rigid ryegrass (*Lolium rigidum*) found in 1996 in Australia in conventional crops), even though the herbicide had been released on the market in 1974. In addition to the reasons cited above, late appearance of glyphosate-resistant weeds was thought to be favoured also by the fast degradation of glyphosate, its limited adsorption to the soil, and its particular mode of action (Johnson *et al.* 2009). Furthermore, before glyphosate-resistant crops were introduced, glyphosate was mostly used in alternation or in combination with other herbicides reducing selection pressure to some extent (VanGessel 2001). As non-selective herbicides can be applied in HR cropping systems before and after planting and during the growing season, selection can take place at all times of the growing season, which was previously not the case for most selective herbicides (Darmency 1996).

To date, at least 24 cases of glyphosate-resistant weed species (more than 150 populations) have been confirmed, observed on millions of hectares, at many different locations and in various countries, and increasingly associated with RR crop[13] cultivation (Heap 2012). Most reports stem from the US, where the true area infested likely amounts to 20-25 mio ha (Benbrook 2012a). Multiple resistances have been observed too: 28 glyphosate-resistant populations[14], members of 11 species, express also resistance to other herbicide classes, such as ALS inhibitors, ACCase inhibitors, PPO inhibitors, or paraquat. Glyphosate-resistant palmer amaranth (confirmed first in 2005) increasingly creates control problems in glyphosate-resistant crops and poses a major economic threat to US cotton production (Benbrook 2012a). Recently, the first weed population resistant to both glyphosate and glufosinate has been confirmed: rigid ryegrass (*Lolium rigidum*) found in Oregon in 2010 (Heap 2012).

Increasing numbers of glyphosate-resistant weeds, such as horseweed (*Conyza canadensis*), Johnson grass (*Sorghum halepense*), hairy fleabane (*Conyza bonariensis*), and *Euphorbia heterophylla*, have also been reported from Argentina and Brazil (Vila-Aiub *et al.* 2008). In Europe, although no glyphosate-resistant crops are authorized for cultivation at present, glyphosate use has increased significantly (*e.g.* in low-till agriculture and for desiccation). Thus, it may not come as a surprise, that, according to Heap (2012), resistant weeds (14 biotypes, belonging to five species) have developed in European countries as well: Spain, Greece, Italy, Portugal, France, the Czech Republic, and Poland. Spain is the most afflicted country, where horseweed (*C. canadensis*), Italian ryegrass (*Lolium multiflorum*), rigid ryegrass (*Lolium rigidum*), hairy fleabane (*C. bonariensis*), and Sumatran fleabane (*Conyza sumatrensis*) have infested hundreds of hectares.

The Australian glyphosate-resistant *Lolium rigidum* biotype is 9- to 10-fold more resistant to glyphosate and also acquired a 3-fold higher tolerance to diclofop-methyl relative to susceptible biotypes. This biotype acquired resistance after 15-20 years of glyphosate use (Pratley *et al.* 1999), Chilean populations (*L. multiflorum*) after 8-10 years (3 applications a year), and the *E. indica* population in Malaysia after 10 years (8 applications a year, first report in 1997). All these resistances were obtained in conventional cropping systems (Lee & Ngim 2000, Heap 2012). Resistant weeds can withstand up to 19-fold the glyphosate dose tolerated by herbicide sensitive plants (VanGessel 2001, Jasieniuk *et al.* 2008, Legleiter & Bradley 2008). Palmer amaranth was shown to have an LD50 (lethal dose to kill 50% of plants) up to 115-fold greater than that of sensitive biotypes (Norsworthy *et al.* 2008).

The molecular and genetic mechanisms of resistance to glyphosate are very diverse and can co-occur (reviewed by Perez-Jones & Mallory-Smith 2010, Zelaya *et al.* 2007, Bostamam *et al.* 2012). Mutations inside the critical amino acid sequence (target site) of the EPSPS enzyme (Kaundun *et al.* 2008, Simarmata & Penner 2008), increased EPSPS mRNA levels (Dinelli *et al.* 2008) and amplification (up to 160-fold) of the EPSPS gene (Gaines *et al.* 2010) have been described. Resistance may also be conferred by delayed translocation of glyphosate from the leaves to other plant parts (Preston & Wakelin 2008, Shaner 2009) or by in-

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[13] RR crops are crops from Monsanto with resistance against the herbicide Roundup Ready, containing glyphosate as active ingredient. RR crops are widely adopted and cultivated, *e.g.* RR 40-3-2 soybean.

[14] Glyphosate can be found under G (glycines) as herbicide site of action on: Heap (2012).

creased sequestration of glyphosate in plant cell vacuoles (Ge *et al.* 2010). Resistance mechanisms not based on target site mutations are considered particularly problematic, as they could favour evolution of resistance to other herbicidal modes of action (Yuan *et al.* 2007). Resistance is mainly propagated by semi-dominant or dominant inheritance of single-gene mutation, but sometimes multiple genes are involved (Christoffers & Varanasi 2010). In general, fitness penalties can occur in resistant weeds, but their probability, frequency and significance are not well understood. The fitness of resistant biotypes is not always lower than of susceptible biotypes. For example, no fitness difference between susceptible and resistant biotypes of *Lolium rigidum* could be detected, but differences in competitiveness can occur at different life stages (Pedersen *et al.* 2007). Target site overexpression (EPSPS overproduction in case of glyphosate) or detoxification are likely to have a significant cost of resistance, especially when extra gene expression is involved and constant. These biotypes might disappear when the herbicide is changed to forego selection pressure.

4.1.2 Resistance to glufosinate

There seems to be little selection for glufosinate resistance as up to now only a small share of transgenic crops is resistant to glufosinate. Harker (2005) assumes also that there is a very low frequency of resistant biotypes in unselected populations. For many years, no glufosinate-resistant weed biotypes have been recorded though weed species with lower sensitivity to glufosinate such as dead nettle (*Lamium purpureum*), common fumitory (*Fumaria officinalis*), or violet (*Viola arvensis*) are known (Nap & Metz 1996, Jansen *et al.* 2000, Hommel & Pallutt 2000, Champion *et al.* 2003, Heard *et al.* 2003b). Recently two glufosinate-resistant weed biotypes were found, namely goosegrass (*Eleusine indica*) in Malaysia in 2009 and Italian ryegrass (*Lolium multiflorum*) in Oregon, USA, in 2010 (Heap 2012). In both cases estimates are that the resistant biotypes infest 2-5 sites and 20 to 40 hectares each. The areas are expected to increase.

The Italian ryegrass population is also resistant to glyphosate (Heap 2012). The biotype requires 2.8-times higher glufosinate rates to reduce growth by 50%), caused by a single amino acid exchange in the target enzyme glutamine synthetase (Avila-Garcia *et al.* 2012). According to the authors, this is the first reported glufosinate resistance in weeds based on an altered target site.

4.1.3 Weed species shift

The tremendously increased glyphosate use has promoted species shift among the weed flora (reviewed by Reddy & Norsworthy 2010). Glyphosate does not affect all weed species to the same extent and not all plants are coated in the same way. Less sensitive species and populations can survive sprayings and subsequently grow and spread, whereas more sensitive species disappear. Weed species may be naturally tolerant to glyphosate or avoid glyphosate by late-season or continual emergence. If early germinators have grown quite tall, they may not be fully eliminated and be able to re-germinate and set seed. Soil nitrogen status could influence survival rates too: under low nitrogen, glyphosate effectiveness on velvetleaf (*Abutilon theophrasti*) and common lambsquarter (*C. album*) was reduced (Mithila *et al.* 2008).

In the Southern United States, a major change in the prevalence of the most troublesome weed species in cotton and soybean has occurred in the interval from 1994/1995 to

2008/2009, parallel to the rapid adoption of HR crops (Webster & Nichols 2012). Some monocot weeds, *e.g.* Johnson grass (*S. halepense*), foxtail (*Setaria* spp.), Italian ryegrass (*L. multiflorum*) and broadleaf weeds such as ragweed (*Ambrosia* spp.), waterhemp (*Amaranthus* spp.), lambsquarter (*Chenopodium* spp.), horseweed (*C. canadensis*), morningglory (*Ipomoea* spp.), and dayflower (*Commelina* spp.) are becoming problematic weeds in glyphosate-resistant crops (Johnson *et al.* 2009, Reddy & Norsworthy 2010). Prevalence of weeds such as waterhemp is also favoured through increased no-tillage and reduced tillage practices (Nordby *et al.* 2007). Weed shift has also been reported for Argentina: After only a few years of RR soybean cultivation, 37 weed species have gained in significance, while only 18 species have decreased (Vitta *et al.* 2004).

4.1.4 Cross resistance and multiple resistance

Cross resistance is defined as the expression of one genetically-endowed mechanism conferring the ability to withstand herbicides from different chemical classes (Powles & Preston 1995), whereas multiple resistance is defined as the expression of several mechanisms within individuals or populations. In both cases plants or populations are resistant to several herbicides with different modes of action.

Multiple resistance is presumed to develop through accumulation of resistance mechanisms as a result of gene flow between individuals with different resistance mechanisms or by selection following extensive use of two or more herbicides with different modes of action. Multiple-resistant weeds have been reported in several regions, including Europe. The evolution of a multiple-resistant rigid ryegrass biotype in Chile may serve as an example showing triple resistance to ACCase-inhibitors[15], ALS-inhibitors[16], and glyphosate (Heap 2012). Multiple-resistant *Brassica napus* volunteers in Canada were due to pollen flow between adjacently-planted resistant varieties (Hall *et al.* 2000).

4.1.5 Resistance management

For years, weed scientists recommend farmers should implement a long-term plan to reduce the selection pressure placed on weeds by glyphosate. The simplest way to do so is to avoid using glyphosate as the only weed management tool and to combine and rotate a number of weed management methods from crop rotation, mechanical weeding to covercrops, intercropping and mulching. (*e.g.* Wolfe 2000, Buhler 2002, Beckie 2006, Powles 2008, Vencill *et al.* 2012, Norsworthy *et al.* 2012).

Despite these recommendations, continuous glyphosate-resistant cropping is common in the Americas, and farmers often simply resort to increased herbicide doses and other herbicides (Prince *et al.* 2012c). They focus rather on short-term weed control than on preventive integrated pest management practices (Wilson *et al.* 2008, Sanyal *et al.* 2008, Norsworthy *et al.* 2012). Many farmers do not scout their fields for problematic weeds and are not concerned about glyphosate-resistant weeds (Johnson *et al.* 2009, Johnson & Gibson 2006). Within the last few years, the situation may have changed somewhat, as more farmers surveyed in

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[15] Herbicides inhibiting acetyl CoA carboxylase.

[16] Herbicides inhibiting acetolactate synthase, *e.g.* sulfonylureas and imidazolinones.

2010 compared to 2005 recognized the importance to manage glyphosate-resistant weeds (Prince *et al.* 2012d). In soybean, among others, paraquat and synthetic auxins[17] are recommended in tank mixtures or in rotation with glyphosate (Beckie 2006). However, 30 of the 393 listed herbicide-resistant weed populations are already resistant to paraquat, among them three with resistance to glyphosate (rigid ryegrass, horseweed, and hairy fleabane). Another 28 populations are resistant to synthetic auxins (Heap 2012). Merely rotating herbicides for weed control may exacerbate resistance problems by selecting for more generalist resistance mechanisms in weeds (Neve 2007).

Many farmers may rely on the development of a new herbicide within the next few years, but most experts disagree because of the challenge to find substances that are suitable and comply with the stricter requirements for new chemicals (Johnson & Gibson 2006, Rüegg *et al.* 2007, Service 2007). In addition, development costs have increased dramatically. Industry rather tends to modify well-known active ingredients and to stay, for instance, in the class of the ALS- or ACCase-inhibitors. According to Vencill *et al.* (2012) the search for new herbicide chemistry slowed to the extent that producers can no longer count on “the next new herbicide” to control resistant weeds.

Biotech companies have acknowledged the increasing problems to control glyphosate-resistant weeds and have set up websites to inform farmers about resistance management strategies and herbicide solutions. Monsanto (Gustafson 2008), for instance, recommends to use new commercial seed free from weed seeds, to rotate to other RR crops and to occasionally include other herbicides in RR cropping systems. A pursued solution is to develop new transgenic crops which can resist higher glyphosate doses without damage (Service 2007).

The trend is to develop transgenic crops with stacked herbicide resistance traits that should offer a new solution to control herbicide-resistant weeds (Behrens *et al.* 2007). Biotech companies expect that the era of the single herbicide resistance trait will soon be replaced by stacked traits conferring resistance to glyphosate plus other active ingredients, such as glufosinate, ALS inhibitors, ACCase inhibitors, synthetic auxins, and others (Green *et al.* 2008). The “SmartStax” corn, combining resistance to glyphosate and glufosinate, together with six Bt insecticidal toxin genes, has already been commercialized in 2010, other stacked traits are supposed to follow.

However, weeds resistant to herbicides other than glyphosate infest millions of hectares already. Of the 393 listed herbicide-resistant weed populations 127 are resistant to ALS-inhibitors, 42 to ACCase-inhibitors, and 30 to synthetic auxins such as 2,4-D (Heap 2012). Quite a few of them exhibit multiple resistance, *e.g.* including to glyphosate. For this reason, stacking of herbicide resistance traits in transgenic crops is unlikely to reduce selection pressure on weeds and to lower herbicide amounts applied.

Against this background there is a growing number of voices recommending crop rotation as an important weed management option. In a long-term comparative field evaluation, Davis *et al.* (2012) showed that a four year crop rotation scheme not only helped to reduce herbicide applications and fertiliser input, but also provided similar or even better yields and economic

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[17] 2,4-dichlorophenoxy acetic acid (2,4-D) and dicamba.

output. Cropping systems that employ an integrated weed management (IWM) approach, including crop rotation, cover crops, competitive crop cultivars, the judicious use of tillage, and targeted herbicide applications, are indeed competitive with regard to yields and profit to systems that rely chiefly on herbicides (Mortensen *et al.* 2012).

4.2 Seed escape and proliferation of transgenic plants

Volunteers are crop plants in the field emerging from the previous crop. They can be undesirable when the following crop is a different species or a different variety of the same species. If volunteers are resistant to the same herbicide as the crop species, alternative herbicides or mixtures are needed. Also preventive measures, *e.g.* to reduce harvest loss or to widen rotation, can be useful (Darmency 1996, Bjerregaard *et al*. 1997, Van Acker *et al*. 2003).

While some crops are ready volunteers and easily build up feral populations in off-field habitats, *e.g.* oilseed rape, because of its high seed production, high seed losses and secondary dormancy, other crops (such as cotton) hardly act as volunteers at all (Bjerregaard *et al*. 1997). In general, volunteers and feral populations of crops which are not native to a region tend to have a lower chance of surviving and cause fewer problems.

Oilseed rape readily produces volunteers and feral plants. Its reproductive rate, growth habit and germination ecology is similar to typical weed species (Kloepffer *et al*. 1999). In Canada, average yield losses of about 6% of the crop seed yield, equalling approximately 20 times the normal seeding rate of 4-5 kg/ha, have been observed (Gulden *et al.* 2003). Volunteer oilseed rape occurs as a residual weed in about 10% of all wheat and barley fields in Alberta, Canada (Hall *et al*. 2000). Secondary dormancy varies between cultivars (Gruber *et al.* 2004, Gulden *et al.* 2004, Momoh *et al*. 2002, Thöle & Dietz-Pfeilstetter 2012). In a French study, 35% to 40% of the observed feral populations resulted from seed immigration from neighbouring fields, mainly during harvest time. Around 15% of the populations were attributed to seed transport. The other half was recruited from the seedbank (Pivard *et al*. 2008). Knispel & McLachlan (2009) studied the proliferation of escaped oilseed rape in Canada and concluded that escaped populations were persistent at large spatial and temporal scales and their findings suggest that anthropogenic dispersal processes are sufficient to enable persistence despite limited natural seed dispersal. Glyphosate-resistant feral oilseed rape plants have been found along transport routes in Switzerland and Japan although GM plants are not cultivated in these countries (Schoenenberger & D’Andrea 2012, Kawata *et al.* 2009). Volunteer and feral management should therefore be a multi-scale approach and has to extend over considerable time spans, as oilseed rape seeds can survive in soils for at least 10 years if not more, as shown by D’Hertefeldt *et al.* (2008). Although the field of an experimental HR oilseed rape release had been checked regularly for volunteers, they still found rape plants after 10 years, 40% of which were herbicide-resistant.

US farmers who rotate both glyphosate-resistant maize and soybean already use an additional herbicide (*e.g.* “Select” with clethodim as active ingredient) to control volunteer maize in soybean (Hartzler 2003). Maize plants can survive outside the field, *e.g.* on road sides, in warmer climates – it thus remains an exception in most parts of Europe – but they show no tendency of invasiveness (de Kathen 1999). Maize seeds are not dormant and germinate readily under favourable conditions. Volunteer HR soybean must be controlled with other herbicide mixes at post-emergence.

4.3 HR gene flow to volunteers or interfertile weeds

4.3.1 Variability of gene flow

In recent years, extensive data has been collected from field experiments with regard to gene transfer frequencies. The frequency of outcrossing depends on the crop species in question and its pollination system, the distance to simultaneously flowering volunteers or relatives and a range of variables such as pollinators, weather conditions, and size of pollen donors and receiving populations. Recently, Andersson & de Vicente (2010) reviewed gene flow of several crops including maize, oilseed rape, soybean, cotton and rice. Mallory-Smith & Zapiola (2008) dealt with the different pathways for gene flow of all existing glyphosate-resistant crops. Other reviews focused on single crops such as oilseed rape (Hüsken & Dietz-Pfeilstetter 2007), maize (Czarnak-Klos & Rodríguez-Cerezo 2010), rice (Lu & Snow (2005), sugar beet (Darmency *et al.* 2009), and soybean (Lu 2005).

Outcrossing frequencies vary considerably depending on local and climatic conditions, prevailing pollinators, the size of donor and acceptor populations, their genotypes and so forth (Gliddon 1999, Ford-Lloyd 1998, Simpson *et al*. 1999, Vigouroux *et al.* 1999, Van Acker *et al*. 2003). Even self-pollinating plants[18] do cross-pollinate at low or very low levels.

Pollen flow can extend to distances over several kilometres (Rieger *et al.* 2002) and was up to 26 km for oilseed rape (Ramsay *et al*. 2003). Pollen beetles may contribute to this long distance pollen dispersal. The hypothesis of a negative correlation between distance of pollen receiving plants and cross pollination was mainly disproved when insect pollination occurs and seems not always appropriate for gene flow at a commercial field to field scale (Rieger *et al*. 1999). Most former experiments were done with small pollen sources, where decay curves of cross pollination rates are frequent. Large pollen sources, such as crop fields, seem to interact on a regional scale and will increase gene flow. Thus, according to Shaw *et al.* (2006), isolation distances should be much larger for large fields than for small ones. Squire *et al*. (1999) recommend that gene flow should be considered at the landscape level.

4.3.2 General relevance of HR gene flow to volunteers and ferals

Double or multiple resistance against herbicides can occur in volunteer plants (Hall *et al*. 2000). Some of these plants emerged with unwanted herbicide traits due to seed lot impurities or due to former outcrossing events between fields. Transgenic oilseed rape plants carrying multiple herbicide resistances not commercially planted have been found along roads in North Dakota, USA, providing evidence of novel combinations of transgenic forms in the wild (Schafer *et al.* 2011). Similarly, Knispel *et al.* (2008) reported about stacking of single HR genes in escaped Canadian oilseed rape populations.

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[18] In general, plants are called self-pollinating, when the level of cross-pollination does not exceed 10%.

4.3.3 General relevance of HR gene flow to interfertile weeds

Weeds can also become resistant to herbicides through hybridisation with compatible HR crops, followed by backcrosses and introgression. In centres of crop origin and regions where interfertile weeds are present, as might be the case *e.g.* with oilseed rape (*Brassica napus*) and its close relative field mustard (*Brassica rapa*) in some regions of Europe (Jørgensen *et al.* 2009), gene flow from crop to weeds should be particularly taken into account. Cross pollination is a prerequisite for hybridisation. Generally, frequencies of hybridisation, *i.e.* production of viable progeny, are lower than cross pollination frequencies between individuals of the same species.

Spontaneous hybridisations occur in nature, but are difficult to detect, reliable data is therefore lacking and the number of hybrids within an area can only be estimated.

Genetic compatibility (survival rates and relative fitness of resulting hybrids and of the progeny of backcrosses) and synchronicity of flowering are the most important factors for the introgression of genes or transgenes from crop plants to wild species.

The frequency of pollen transfer from crop to crop or to interfertile weeds depends on a variety of factors, including distance, temperature, humidity, time of the day, wind speed and direction, abundance and foraging behaviour of insect pollinators, and population size of the pollen donor and the recipient (Chèvre *et al.* 1999, Kloepffer *et al.* 1999, Dietz-Pfeilstetter & Kirchner 1998, Darmency 2000, Jørgensen *et al.* 2009, Czarnak-Klos & Rodríguez-Cerezo 2010, Andersson & de Vicente 2010).

Once (trans-) genes conferring herbicide resistance move into weeds, their frequency within local weed populations will increase if there is positive selection pressure (*i.e.* if the corresponding herbicide is applied).

According to Colwell (1994), a „rare“ hybridisation event between crop and weed may be a starting point for the escape of the transgenic trait into the population of a weedy relative. Furthermore, hybrids do not need to be particularly fit as long as they are able to backcross with the weedy relative which can result in competitive progeny, a capacity many interspecific hybrids have.

When the positive selection is missing, a negative selection is possible, because F1 and F2 hybrids often are less fit and the transgene itself can cause fitness losses. Such fitness costs could be caused by pleiotropy, physiological costs of the tolerance[19] trait and could be different in crops and in weeds due to different genetic backgrounds (Snow & Jørgensen 1999). The fitness of hybrids should be assessed from species to species. But even genotypes with a lower fitness may survive if the pollen flow is steady and the source is large (Gliddon 1999).

4.4 Conclusions on changes in weed and volunteer susceptibility (Chap. 4)

The change in weed control (and agricultural practice) of the HR cropping system will provoke changes in weed communities and populations. In general, the more often a specific

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[19] The term “tolerance” is used by the cited authors, but it may be resistant biotypes.

herbicide is applied on the same field, the more likely the weed spectrum will shift towards less susceptible species. Effects of the same transgenic HR crop can vary depending on the agricultural ecosystem.

In HR crops, the observed decrease in numbers of herbicide modes of action and the trend to less soil cultivation can augment selection pressure on weed communities. Changes of the weed community structure (due to selection of resistance in weeds and volunteers and shifts to resistant species) have already resulted in altered weed control patterns in HR crops in many regions. In a crop rotation with soybean and maize or cotton, all crops being glyphosate-resistant, the selection pressure on weeds is very high and weed shifts are most likely.

The data presented make it reasonable to assume that even more glyphosate-resistant/tolerant weeds will develop in future. The same applies to glufosinate-resistant/-tolerant weeds if this herbicide is used frequently enough in high numbers of crop fields.

In particular in the US, Brazil, and Argentina, resistant weed species have caused considerable control problems within the last years, connected to the frequent use of glyphosate in non-HR and HR varieties and to changed agricultural practice. Weed control costs have increased. For years, many weed scientists have recommended to use a diversity of control measures, among them rotation of crops and use of additional herbicide modes of action. A combination and rotation of weed management methods is essential to delay resistance evolution in weeds.

Crops with characteristics such as easy shattering and persistence of seed are likely to emerge as volunteers. Problems with HR volunteers will be more common with crops such as oilseed rape, a ready volunteer that exhibits high outcrossing rates. Volunteer management is not a new problem specific to HR crops, but it will be more demanding for HR crops.

In most cropping areas, the risk of changes in weed susceptibility due to selection and gene flow from HR crops to volunteers is considered to be higher than changes due to gene flow to weeds. However, in regions where highly interfertile weeds are abundant, management priorities may be different. The transfer of HR genes to relatives should be particularly taken into account in centres of crop origin and regions where interfertile and weedy hybrids occur.

5 IMPACTS ON BIODIVERSITY

The conservation and protection or even restoration of native biodiversity is a very important internationally acknowledged goal. Impacts of HR crops on biodiversity, be they direct or indirect, are related to agricultural practices such as herbicide use and cropping system. The aim of this section is to review information about potential impacts of HR cropping systems on biodiversity and to give an overview on additional aspects that have to be considered. These include baseline comparators, direct toxic effects of the herbicides applied, effects that can be attributed to HR crop cultivation, as well as further aspects of sustainable agriculture and potential mitigation of environmental effects.

The importance of biodiversity is internationally recognized and both the implementation of modes to preserve it and its sustainable use are widely shared goals as expressed in the Convention on Biological Diversity (CBD[20]). At the Conference of Parties in 2010 (CBD-COP 10) the agreed goal to stop the loss of biodiversity was phrased as follows: “By 2020, the rate of loss of all natural habitats, including forests, is at least halved and where feasible brought close to zero, and degradation and fragmentation is significantly reduced.”[21]

Most countries, including the EU (EC 2008), now have action plans for the conservation of biodiversity (CBD Factsheet 2010), the United Kingdom was the first country to produce one back in 1998 (JNCC 2012). In regions where most of the land is under cultivation, it is especially important to stop or to reverse the decrease of biodiversity in agriculture. In Germany, for example, agricultural and forested lands make up about 80% of the total area and in the UK around 75% of the land is farmed (Robinson & Sutherland 2002). This means, according to Johnson (1999), that Europe should be farmed in a way that allows biodiversity to thrive within farmland, alongside or within crops, unlike in the US, where intensively farmed areas are often quite separate from large protected wildernesses. This is all the more important, as agriculture has been described being a major force driving biodiversity loss and other environmental impacts beyond the “planetary boundaries” (Rockström *et al.* 2009).

The decrease in farmland biodiversity, indicated *e.g.* by the decrease of farmland birds, is an important issue in Europe, but also in the US and in Canada. The significance and relevance of biodiversity to agriculture and the impacts of agricultural intensification on biodiversity have been widely assessed (El Titi & Landes 1990, van Emden 1990, Wijnands & Kroonen-Backbier 1993, Lewis *et al*. 1997, Firbank *et al.* 2008). Agriculture relies to a large extent on ecosystem function and ecosystem services including pollination, biological pest control, maintenance of soil structure and fertility, nutrient cycling and hydrological services (Tscharntke *et al.* 2005, Power 2010, Garibaldi *et al*. 2011, Foley *et al.* 2011). Management practices strongly influence the diversity and abundance of the within-field seedbank and the weed flora – with an intensity gradient from farms with high agrochemical inputs and winter cropping to those with no inorganic inputs, spring cropping and mixed farming practices (Hawes *et al.* 2010). Weeds play an important role in supporting biological diversity in arable

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[20] http://www.cbd.int

[21] http://www.cbd.int/sp/targets/rationale/target-5/

farmland as they provide food and shelter for arthropods, including pollinators, and birds (Marshall *et al.* 2003). Many potential pests are still controlled by natural antagonists, and decreasing the latter could increase pesticide inputs to substitute them (Van Emden 1990, van Lenteren 1993). That is why integrated management concepts have been developed and improved (see, *e.g.*, OECD background paper Levitan 1997). However, in countries with numerous exotic species in the agricultural environment, the management goals for conserving native biodiversity might be different (*e.g.* Australia).

Herbicide resistance may not increase the fitness and invasiveness of plants in semi-natural or natural habitats (*e.g.* Dale *et al*. 2002), as long as herbicides are not applied or spray drifted. Therefore, direct and indirect impacts of HR crops on biodiversity are related to farming practices in general. The cultivation of HR crops may change farming practices in terms of crop rotation, crop planting and spacing, soil tillage, pesticide application, use of fertilisers and so forth. In addition, the assessment of species diversity within an agricultural field depends on the type and choice of species used as baseline comparators. Off-field habitats can be affected as well, when herbicide-resistant weeds develop and spread. Additional herbicides may be used in such a case and change the weed community composition further.

In general, when performing risk analysis or environmental risk assessments of HR crops, the following aspects should be considered:

- already existing effects of conventional agriculture (chapter 5.1) and the chosen baseline comparators,
- indirect effects of changes in agricultural practice (chapter 5.2),
- direct toxic effects of the herbicides glyphosate and glufosinate (chapter 5.3),
- direct and indirect effects that can be attributed to the growing of HR crops (chapter 5.4),
- further aspects of sustainable agriculture and possible mitigation of environmental effects (chapter 5.5).

Potential environmental impacts of HR crop cultivation can be assessed by techniques such as life-cycle assessment (Bennett *et al*. 2006), bow-tie risk management (Pidgeon *et al*. 2007) or other conventional system safety techniques (fault tree analysis, casual factor charting, event tree analysis). Because all these techniques involve a certain subjectivity, which cannot be eliminated, assumptions involved and decisions taken should be made transparent.

5.1 Effects of conventional agriculture

Impacts of the new weed control in HR cropping systems most often are compared to effects of agronomic management practices in conventional cropping systems. Over the period of increasing herbicide use (1950-1985), the diversity of associated agricultural flora (measured as number of different species) was reduced by 30 - 70% in Germany (Hanf 1985). In the UK and Denmark the reservoir of viable seeds in arable soils has been reduced by more than half, within the last decades, with losses of >90% for some species (Robinson & Sutherland 2002, Marshall *et al.* 2003).

Many insect species depend on certain plants during early larval stages making each plant species essential for on average of 10-12 insect species in northern Europe (Heydemann 1983). Because of this relationship and the decrease of floral diversity, the epigeal (soil dwelling) arthropod fauna declined by 45% - 85% in terms of species and even further in terms of biomass (Heydemann 1983). Adults of beneficial organisms may lose pollen and nectar sources if weeds are reduced.

In a long-term trial, 12 years of herbicide use in wheat led to a decline of the soil seedbank by 35% - 60% (Pallutt & Haass 1992). Similar declines of farmland species were observed in the UK (Johnson 1999, Guerrero *et al.* 2012). Arthropod numbers (abundance) decreased by 60% - 80% in Sussex (UK) from 1970 to 1989 (Aebischer 1991). The decrease in associated flora (and arthropod) abundance and diversity affected the whole food chain including hares and farmland birds. A similar decrease of farmland birds and other taxa has been reported from most agricultural regions including Canada and the USA (McLaughlin & Mineau 1995, Krebs *et al*. 1999, Robinson & Sutherland 2002).

Mechanical weeding does not reduce the density and diversity of the weed flora and associated flora as effectively as herbicides, but it is more labour intensive. In Germany, the abundance of arable weeds was (on the average of 12 studies) three times higher (range: 0.3-10 times) in mechanically weeded fields compared to fields where herbicides were employed (Schütte 2002). All fields were ploughed.

Organic farming tends to increase abundance and diversity of the weed flora and may support rare species (Marshall *et al.* 2003), although results vary (Hawes *et al.* 2010). Compared to conventional, organic wheat production favoured broad-leaved, insect-pollinated and legume weeds and led to similar diversity of weed species between crop fields and edges (Romero *et al.* 2008). In conventional farming, herbicide treatment particularly affects the inner-field. In organic farming, weed diversity can be up to ten times higher compared to conventional farming, depending on the history of herbicide use of the test sites. Meanwhile, action plans for conservation of biodiversity have been installed in a range of countries (CBD factsheets 2010, EC 2012). In Switzerland *e.g.*, seeding of rare and beneficial wild plants is done for reasons of conservation and financed by public incentives (Herzog *et al*. 2001).

It takes a lot of effort to replenish a seedbank reservoir that has been depleted through decades of herbicide use (*e.g.* Auerswald *et al.* 2000). The aim of weed control in modern agriculture has often been to eradicate rather than to manage weed populations. Threshold models are rarely used. The loss in agrobiodiversity is also due to the depleted number of cropped species, reduced rotation, limited seed dispersal between farms, drainage, and landscape-consolidation. Nevertheless, the field studies mentioned above provide evidence that herbicide use plays a significant role in negatively affecting biodiversity within agricultural ecosystems. Promotion of weed species diversity and reduction of weed seedbanks can be achieved by conservation tillage and crop rotation (Murphy *et al.* 2006). If GM crops provide a new dimension of control over pests, diseases and weeds in a poorly targeted way, they will drive agriculture farther towards monoculture and the excessive control of the agricultural environment (Dale *et al.* 2002) – as has been experienced within the last years in a number of areas of the Americas.

5.2 Effects of changes in agricultural practice

A decline of abundance and diversity of birds over the last 20 - 30 years has been observed in many countries, and many species are endangered (Chamberlain *et al*. 2000, SRU 1996, Marshall *et al.* 2003, Guerrero *et al.* 2012). It is widely accepted that changes in agricultural management practices are responsible for the decline of farmland birds. They are major targets and important indicators of agricultural change (Ormerod & Watkinson 2000).

Agricultural intensification during the post-war period and the concomitant decline in seed density in farmland soils has greatly reduced the amount of food available to foraging birds (Robinson & Sutherland 2002). The seed density in farmland soils fell from, on average, 1750/m^2 in 1900 to about 125/m^2 in 2000 (Leech 2002). Most adult birds feed on weed seeds in winter (Evans 1997), whereas many chicks feed on insects which make both of them complementing biological control agents.

Based on the analysis of multi-year data (1962-1995), Chamberlain *et al*. (2000) not only found a strong correlation between agricultural change and the decline of farmland bird population, but they also observed a time lag of about 6 years implying that effects of agricultural intensification on habitat quality may not become apparent for several years. Across Europe, farmland bird density was negatively correlated with high yields and positively with growing different crops and small fields (Guerrero *et al.* 2012).

The decline of birds is best monitored and analysed in UK. Surveys and Common Bird Census data show that the farmland bird indicator decreased by about 50% between 1970 and 2010[22] and that 13 farmland bird species have declined by over 50% within the 30 years from 1968 to 1998 (Marshall *et al.* 2003). Comparisons showed that birds' decline was most obvious in the farmland compared to other habitats. What caused these developments is often not fully understood, but several factors have been identified (Furness & Greenwood 1993, Evans 1997, Chamberlain *et al*. 2000, Leech 2002):

- Increased use and efficacy of herbicides have helped to reduce the abundance of weeds growing on arable land and therefore to reduce both the availability of seeds to foraging birds and the abundance of invertebrate prey.
- The use of broad-spectrum insecticides has reduced insect numbers important as food for chicks.
- Destruction of hedgerows and field margins has reduced the habitats for seed-producing weed species and invertebrates.
- More efficient harvesting has decreased the amount of spilled grain by factor ten.
- Planting of winter cereal crops has also increased, reducing the amount of fallow land and particularly the area of seed-rich stubble on which birds can forage during the winter.

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[22] http://www.rspb.org.uk/Images/SUKB_2012_tcm9-328339.pdf

5.2.1 Crop rotation

When Argentina started to grow GMO, fallow land, sensitive areas and several crop species (planted in rotation) were replaced by HR soybean. This is one factor why a homogenisation of landscapes and of production has taken place (Pengue 2004).

The options to rotate crops with HR varieties are theoretically numerous because of the lower residual activity of glyphosate and glufosinate, but there is no evidence for it. GM crops provide a new dimension of control over pests, diseases and weeds in a poorly targeted way, thus they have the potential to drive agriculture farther towards monoculture and the excessive control of the agricultural environment (Dale *et al*. 2002) – as has been experienced within the last years in a number of areas of the Americas.

5.2.2 Tillage and Planting

The adoption of reduced tillage in agriculture may lower soil erosion and improve conditions for several soil-dwelling species. In particular, the abundance and diversity of earthworm increases when erosion is lowered as in reduced tillage (Ehlers & Claupein 1994). Large populations of earthworms and of other soil organisms are only found in soils with easily decomposable litter and/or organic fertilisers (Makeschin 1997). This is well provided in systems with reduced tillage where crop residues are not incorporated into the soil and where the level of mineralization is lower. For many soil associated arthropods, however, the amount and diversity/quality of living (see associated flora below) and dead mulch is more important than reduced soil disturbance (Wardle *et al*. 1999, Krück *et al*. 1997).

Effects of reduced tillage are mixed in the case of ground beetles (Kromp 1999, Stinner & House 1990). Populations of beneficial organisms (except spiders to some extent) will not significantly increase in fields with conservation tillage unless plant coverage mitigates cold temperature in winter (Bürki & Hausammann 1993, Stippich & Krooß 1997).

Mechanical weeding had no negative effect on important predatory organisms, such as ground beetles, staphylinids, and spiders (Lorenz 1995). It can have an impact on small arthropods, but does not seem to significantly influence the density of epigeal (ground dwelling) predators (Basedow *et al*. 1991). However, Bitzer *et al*. (2002) showed for HR soybean that soil disturbance can negatively affect abundance of Collembola more than use of herbicides.

Populations of problematic weed types like grasses and perennials often increase in reduced tillage systems (Tab. 2 in Swanton *et al*. 1993), whereas broad-leaved annual plants may decrease in some reduced tillage systems (Knab & Hurle 1986, Belde *et al*. 2000). Belde *et al*. (2000) concluded from their long-term study that abundance and diversity of wild plants increase in the first years of reduced tillage, but will decrease in the long run. In reduced tillage systems, weed seeds will remain closer to the soil surface than in ploughed soil. Hence, germination and elimination may be more probable without ploughing, resulting in a more rapid depletion of the soil seedbank (see also Buhler *et al*. 1997, Swanton *et al*. 1993). However, conclusions on the effects of reduced tillage on weed dynamics are to a certain extent contradictory (Zwerger & Ammon 2002, Swanton *et al*. 1993), and the studies reviewed here have been conducted using selective herbicides only.

Impacts of mechanical weeding on ground nesting birds and hares are likely, depending on the timing. Nesting birds and small mammals are frequently killed or injured by tillage operations. However, as Cowan (1982) showed for spring planted crops, a clear beneficial effect of no-till systems on birds could only be seen, when farmers were careful to avoid crushing nests and cover the eggs during seeding operations. Successful strategies to protect farmland species include analyses of the current abundance of populations, their life cycles and the adaptation of farming practices to life cycles, *e.g.* timing of planting, plant protection, and harvesting operations (McLaughlin & Mineau 1995, Meyer-Aurich *et al*. 1998).

Similar to other cropping systems which use herbicides instead of mechanical weeding, HR soybean and cotton can be planted in ultra narrow rows (Carpenter & Gianessi 1999, Kalaitzandonakes & Suntornpithug 2001). Here, the competitive ability of crop plants is sometimes higher and thus less herbicide may be applied. Nevertheless, the abundance and diversity of the associated weed flora likely decrease in narrow row production due to stronger competition of the crop and herbicide use. Research data indicate that the trend to narrow row production in soybean and cotton negatively influences biodiversity (*e.g.* Duelli 1997, Krebs *et al.* 1999, Wijnands & Kroonen-Backbier 1993, EISA 2004). In rice production, the practice of direct seeding is predicted to increase when HR varieties are available (Gressel 2002). The increase may come at the expense of paddy rice production in wetlands, which are essential habitats for wintering waterbirds such as waterfowls (Ducks Unlimited 2003).

5.2.3 Additional herbicides

Atrazine, acetochlor, dicamba or mixtures of them (Bradley *et al*. 2000, Hamill *et al*. 2000, Owen 2000, Shaner 2000, Stelling *et al*. 2000) have been recommended for use in tank mixtures with glufosinate or glyphosate in various HR crops. In parts of the growing areas for oilseed rape, soybean and cotton, it has become necessary to add 2,4-D and/or other herbicides to glyphosate or glufosinate-based weed control programs (Benbrook 2009). This so called ‘double knockdown’ approach has also been advocated as a tool to address development of weed resistance (Weersink *et al.* 2005).

With the advent of stacked herbicide resistance traits in transgenic crops (APHIS 2012), “old” herbicides such as 2,4-D, dicamba, ACCase- and ALS-inhibitors are coming back. Benbrook (2012a) expects that if corn and soybean resistant to several of these herbicides plus glyphosate and/or glufosinate are deregulated in the US, there will be growing reliance on older, higher-risk herbicides to manage glyphosate-resistant weeds. He estimates 2,4-D use on corn would increase by 2019 over 30-fold from 2010 levels. However, 2,4-D is 75 times and dicamba 400 times more toxic to broadleaf plants than glyphosate (Mortensen *et al.* 2012). The potential for non-target drift damage would increase significantly (Johnson *et al.* 2012).

5.3 Effects of ecotoxicological attributes of glyphosate and glufosinate

For herbicides, specific legal frameworks regulating the approval procedures and assessment criteria are established, varying to some degree in different countries (*e.g.* Regulation (EC) No. 1107/2009 and Regulation 540/2011). While glufosinate, due to its reproductive toxicity, is expected to be phased out in the EU in 2017 (Annex I of Commission Implementation Regulation (EU) No. 540/2011), glyphosate is presently evaluated for renewed approval. Glyphosate, authorized in 2002, should have been re-evaluated within ten years (end of 2012), but this has been postponed until at least the end of 2015 (EC 2010). Already at the time of its approval in 2002, doubts about the environmental safety of glyphosate have been expressed. Since then, more data have been collected indicating that glyphosate, apart from being toxic to plants, can be also toxic to soil life, aquatic organisms and higher organisms. Due to the adoption of HR crops almost twenty years ago, glyphosate is today by far the herbicide most widely used in the world, applied on millions of hectares of glyphosate-resistant crops and increasingly on non-HR crops for desiccation purposes and in non-agricultural settings. In light of the great number of glyphosate-resistant crops that are authorized or in the pipeline, glyphosate most likely will remain one of the most used herbicides for the next decade.

Weed suppression is clearly intensified in most crops and regions where HR crops are planted, because the broad-spectrum herbicides glyphosate and glufosinate are used instead of less effective herbicides and sometimes mechanical weeding. Impacts of changes in weed management are evaluated as part of the ERA. Here we provide a short summary of the ecotoxicological profiles of these herbicides to illustrate possible impacts that should be considered when assessing the environmental risks of HR crop cultivation.

5.3.1 Glyphosate

Glyphosate ($C_3H_8NO_5P$; N-(phosphonomethyl)glycine) is a polar, highly water soluble organic acid (given in acid equivalents a.e.) that inhibits EPSPS. As a potent chelator it easily binds divalent cations (*e.g.* Ca, Mg, Mn, Fe) forming poorly soluble or very stable complexes (Toy & Uhing 1964, Cakmak *et al.* 2009). In addition to the active ingredient that can be present in various concentrations, herbicides usually contain so called inert ingredients (also called adjuvants or surfactants) that facilitate penetration through the waxy surfaces of plants. The best known glyphosate containing herbicides, the Roundup product line, very often contain polyethoxylated tallow amine (POEA) as surfactant (typically 15% or less of the final formulation). POEA exhibits significantly higher toxicity than glyphosate (Cox & Surgan 2006) and is more toxic in alkaline than in acid water (Diamond & Durkin 1997). Data from toxicity studies performed with glyphosate alone and over short periods of time may thus conceal undesired effects. In addition, toxicology studies involving one pesticide at a time may not be appropriate to detect combined effects of exposure to multiple pesticides, but extensive data on natural pesticide concentrations are lacking (Relyea & Hoverman 2006).

Glyphosate is reported to be degraded relatively rapidly with half-lives of up to 130 days, sometimes 240 days (Borggard & Gimsing 2008). Its main metabolite aminomethylphosphonic acid (AMPA) degrades more slowly. Glyphosate reaches aquatic systems resulting in concentrations in surface waters in the µg/L to mg/L range, *e.g.* up to 1,700 µg/L in US pond water (WHO 2005). Glyphosate can also reach groundwater (Sanchis *et al.* 2011).

Impacts on soil life

Glyphosate has been shown to impact the composition of the soil microflora which means that some soil microorganisms can be suppressed while others can be favoured (Roslycky 1982, Kremer & Means 2009). Early studies based on standardised tests indicated that there were no long-term effects on soil microorganisms also at rates that exceed maximum application rates (Sullivan & Sullivan 2000, Cerdeira & Duke 2006). Another long-term (10 years) study, however, revealed shifts in composition and activity of microorganism populations. In particular, beneficial fluorescent pseudomonads, associated with antagonism of fungal pathogens and manganese reduction (to Mn^{2+} that is taken up by plants), have been reduced in the rhizosphere of RR crops (Kremer & Means 2009, Zobiole *et al.* 2011a).

Studies of Zablotowicz & Reddy (2004) and Means *et al.* (2007) indicate the potential for reduced nitrogen fixation in the HR soybean system, although overall yield reductions due to reduced N_2 fixation in early growth stages have not been demonstrated. At above label use rates of glyphosate, nitrogen fixation and/or assimilation in HR soybean were consistently reduced, in particular under soil moisture stress (Zablotowicz & Reddy 2007). Zobiole *et al.* (2011b, 2012) observed negative impacts of increasing glyphosate rates on nodulation, nutrient accumulation and other growth characteristics of first and second generation RR soybean[23].

Studies focusing on glyphosate impacts on fungi have led to contradictory results, possibly depending on factors such as study sites, pathogen inoculum, herbicide timing, soil properties, and tillage (reviewed by Powell & Swanton 2008, Sanyal & Shrestha 2008). Some fungi seem to be sensitive to glyphosate, *e.g.* mycorrhizal fungi (Kremer & Means 2009), others, including rust and blight fungi, can increase under glyphosate application. Root exudates of glyphosate-treated RR soybeans seem to favour growth of pathogenic Fusarium fungi (Kremer *et al.* 2005). Long-term studies indicate that roots of glyphosate-treated RR soybeans and RR maize have several times higher Fusarium numbers (2-5 times in RR soy, 3-10 times in RR maize), compared to untreated plants or plants treated with conventional herbicides (Kremer & Means (2009). Roundup has been described to affect entomopathogenic fungi that combat harmful insects (Morjan *et al.* 2002).

Recent studies reported adverse effects of glyphosate on micronutrient uptake in plants (*e.g.* Eker *et al.* 2006, Tesfamariam *et al.* 2009, Cakmak *et al.* 2009). Being a strong systemic metal chelator, very low levels of residual glyphosate in soil can greatly impede the availability and uptake of Mn, Fe, Cu, and Zn by plants leading to an undersupply with these nutrients and finally to reduced disease resistance and plant growth (Johal & Huber 2009). Together with the weakened defence, the root environment may thus contribute to the herbicidal action of glyphosate.

Impacts on aquatic organisms

The WHO (1994) classified technical grade glyphosate (without surfactants) as slightly to very slightly toxic to aquatic invertebrates and moderately to very slightly toxic to fish.

In worst case scenarios (6-12 mg a.i./L), glyphosate/Roundup affected the structure of phytoplankton and periphyton assemblages. Total phytoplankton decreased in abundance, whereas abundance of cyanobacteria increased significantly, at the expense of diatoms (Pérez *et al.* 2007, Vera *et al.* 2010). Cyanobacteria are known to be particularly resistant to extreme environments and are remarkably tolerant to glyphosate, possibly due to an insensitive form of EPSPS and/or the ability to metabolize glyphosate (Forlani *et al.* 2008). Should glyphosate, which contains phosphate, add to the phosphorous load in surface waters and

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[23] First generation RR soybean is RR 40-3-2 and second generation RR soybean is MON 89788.

lead to a shift in phytoplankton assemblages with an increase of cyanobacteria, harmful cyanobacteria blooms might result (Pérez *et al.* 2007). A single glyphosate addition to the mesocosm produced a long-term shift in the water body typology which, according to Vera *et al.* (2010), is consistent with the regional trend in Argentina where aquatic ecosystems around the Pampean region are at risk of being affected by toxicity and the eutrophication potential of glyphosate.

Many studies suggest that some of the surfactants used in glyphosate formulations have a significantly higher toxicity than the active ingredient, in particular for aquatic organisms. As the toxicity of the individual surfactants varies, toxicity of formulated products to aquatic organisms may differ (Tsui & Chu 2004). In addition, the sensitivity of aquatic organisms to POEA is highly variable. For example, for common carp (*Cyprinus carpio*), $LC50_{96\text{-}h}$ values for glyphosate-containing formulations have been reported ranging from 2.4 to >895 mg/L (WHO 1994, Durkin 2003). For the invertebrate *Daphnia magna*, $LC50_{48\text{-}h}$ values for different glyphosate formulations range from 3 to 676 mg/L and for green algae (*Selenastrum capricornutum*), EC50 values for concentrated glyphosate formulations from 2.1 to 150 mg/L have been reported (Durkin 2003). With regard to POEA, the fairy shrimp *Thamnocephalus platyurus* is significantly more sensitive to this surfactant than *Daphnia magna*, as the $LC50_{48\text{-}h}$ value for the most toxic POEA surfactant was as low as 2.01 µg/L for *T. platyurus* (Brausch & Smith 2007).

Due to their toxicity – some formulated glyphosate products are labelled being toxic to fish and aquatic invertebrates – restrictions may be placed on them. In Germany, for example, these formulated products shall not be applied close to or on wetlands, they should not be allowed to contaminate fresh water and should not be sprayed when rainfall could wash the product away (BVL 2010, Ohnesorge 1994).

In ecotoxicological studies with pesticides usually one stressor, namely the pesticide, is tested. However, in nature, simultaneous exposure to various stressors is common (Relyea 2005, Jones *et al.* 2011). Kelly *et al.* (2010) found that survival of New Zealand freshwater fish *Galaxias anomalus* was affected neither by exposure to a glyphosate-based herbicide at an environmentally relevant concentration (0.36 mg a.i./L) nor by infection of trematode *Telogaster opisthorchis* alone, but simultaneous exposure to infection and glyphosate significantly reduced fish survival. Juvenile fish developed spinal malformations when exposed either to infections or to infections and glyphosate, with a trend towards more severe malformation after exposure to both stressors.

Competitive stress (increased tadpole densities) has been reported to cause declines in tadpole growth but also to make the Roundup formulations significantly more lethal for one (*Rana catesbeiana*) of three amphibian species tested (Jones *et al.* 2011). However, survival and growth of amphibians may not always be reduced if they are exposed to multiple stressors such as glyphosate and strains of the pathogenic fungus *Batrachochytrium dendrobatidis*. Given these two stressors, glyphosate-based herbicide appeared to affect the pathogen more than the host’s immune system, relieving the host from disease-caused effects (Gahl *et al.* 2011).

Amphibians are particularly at risk to be exposed to glyphosate and the formulated products. Shallow temporary ponds, essential to the life cycles of many amphibians, are areas where pollutants can accumulate without substantial dilution (Mann *et al.* 2003). According to

Thompson *et al.* (2004) however, native amphibian species in shallow natural wetlands were not affected by an overspray application of a glyphosate formulation containing POEA at a rate of 2 kg/ha, the maximum rate used in Canada for forestry applications, although laboratory studies had indicated formulations containing POEA exhibited significant toxicity to amphibians. According to the early review of Giesy *et al.* (2000), Roundup (LC50 value 8.1 mg/L for tadpoles of the most sensitive species, *Litoria moorei*) is at best moderately toxic to amphibians and glyphosate non-toxic to slightly toxic. Since then new data have been collected indicating that amphibians may not only be exposed to the herbicide, but that they are also very sensitive to the product.

Plötner & Matschke (2012) recently reviewed numerous studies dealing with potential impacts of glyphosate formulations on amphibians. They concluded that although glyphosate is itself toxic to amphibians, surfactants such as POEA are even more so, but other components of glyphosate formulations may also contribute to toxicity. The reviewed studies suggest that sublethal concentrations of glyphosate and glyphosate-based herbicides can cause abnormal behaviour, teratogenic effects and developmental failures, such as a prolonged larval period or accelerated growth of tadpoles, and reduced size at metamorphosis. Plötner & Matschke (2012) also pointed out that until recently indirect effects of herbicide exposure had not been considered sufficiently. If glyphosate reduces growth of algae and aquatic plants, then the food supply for tadpoles may be limited and, if herbicide use diminishes weed abundance and spectra, adults may have difficulty finding enough invertebrates for food. Knowledge about synergistic, additive or antagonistic effects resulting from interaction between different pesticides is scarce. Data are lacking whether long-term glyphosate applications influence the immune system of amphibians, perhaps by impairing the microbial communities of their skin, making them more susceptible to parasites and pathogens.

Terrestrial organisms

In general, for any conclusion, it is important to distinguish between results of laboratory studies and field studies in a normal environment and seasonality (Hart *et al.* 2009) and with normal herbicide application rates. The data collected refer mostly to studies performed with glyphosate and formulated products, most often Roundup. Effects of the metabolite AMPA (aminomethylphosphonic acid) that degrades much slower than glyphosate (see EC 2002) are rarely studied. AMPA has been shown to be of low toxicity to birds and aquatic organisms (Giesy *et al.* 2000).

The effects of Roundup herbicide have been investigated in a screening level assay with 18 different beneficial land predators and parasites (Hassan *et al*. 1988). Roundup was found to be harmless to thirteen species, slightly harmful to four species and moderately harmful to one species of carabid beetles. Laboratory studies (semifield in one case) provided by industry and reviewed by the EC (2002) tested 11 arthropods. The mortality of half of the species tested was quite high (53-100%) when exposed to a formulated product and reduced when tested with the glyphosate salt only. The Board for the Authorisation of Pesticides, Netherlands, found that formulated products are toxic to predatory mites and moderately toxic to some beneficial spiders and (parasitic) wasps (CTB 2000). Potential effects of glyphosate on hoverflies (*Syrphidae*) which provide a high level of aphid control (Krüssel *et al*. 1997) seem not to have been studied.

The CTB (2000) also stated that formulated products were of low toxicity to earthworms. Glyphosate (tested as the isopropylamine salt) had no effect on growth or reproduction of the earthworm *Eisenia fetida* at rates up to 21.31 mg a.i./kg dry soil. According to Monsanto Canada (2002), formulated glyphosate (Roundup Original® and Roundup Transorb®) is practically non-toxic to honey-bees (LC50: >100µg/bee, contact 48 hours) and earthworms ($LC50_{14\text{-}d}$: >5,000 mg/kg dry soil) in short-term studies. However, locomotor activity of earthworms might be altered by glyphosate-based herbicides, potentially compromising their survival (Verrell & van Buskirk 2004). When exposed to glyphosate for a longer time (100 days), the growth of the earthworm species *Aporrectodea caliginosa* was severely affected (Springett & Gray 1992).

Assessments of acute toxicity to mammals indicate that the mammalian toxicity of glyphosate is lower relative to other herbicides. A simulation based on LD50 indicators suggests that HR soybean technology is more environmentally friendly in terms of acute mammalian toxicity than conventional systems (Nelson & Bullock 2003). Most mammalian feeding studies reviewed by Giesy *et al.* (2000) have been performed with rats. Acute single oral LD50 values lie around 5,000 mg/kg/d for Roundup and range from 2,047 to 5,700 mg a.e./kg/d for glyphosate. According to Monsanto Canada (2002), formulated glyphosate (Roundup Original® and Roundup Transorb®) is thus practically non-toxic to rats via acute oral, dermal and exhalation exposure and also to Mallard Duck (*Anas platyrhynchos*).

More recent work, however, indicates that glyphosate-based herbicides are toxic to human cells and act as endocrine disruptors, with ethoxylated adjuvants playing a significant role in human cell toxicity (Mesnage *et al.* 2012). In rat hepatoma tissue culture (HTC) cells, treatment with low doses of Roundup resulted in increased lysosome density, morpho-functional modifications of nuclei, and modified mitochondrial membranes (Malatesta *et al.* 2008). In human cells, cellular and genetic toxic effects, such as increased chromosome aberrations, have been observed (Monroy *et al.* 2005, Lioi *et al.* 1998). Both Roundup Bioforce® and glyphosate damage human embryonic cell lines and placental cells, and do so in concentrations at or below the recommended values for agricultural use (Benachour *et al.* 2007). Comparable results have been reported for dilutions (10 ppm to 2%, 1-2% is recommended for agricultural use) of four Roundup formulations (R7.2, R360, R400, and R450), glyphosate, POEA, and AMPA, each tested on three human cell types. Within 24h, the treatment caused cell death through inhibition of a mitochondrial enzyme (succinate dehydrogenase) and necrosis (Benachour & Séralini 2009). Again, glyphosate formulations containing surfactants such as POEA were more toxic than glyphosate alone. In a more recent test series with a human hepatoma cell line, treatment with the same four Roundup formulations and glyphosate resulted in cytotoxicity, genotoxicity, anti-estrogenic, and anti-androgenic effects (Gasnier *et al.* 2009). Séralini *et al.* (2012a) reported that in a 2-year study rats fed with Roundup-treated maize (event NK603), untreated NK603 maize, or Roundup-containing drinking water showed more severe effects than control animals fed with the nearest isogenic non-GM maize line. Treated animals developed more often and more rapidly cancer and in treated males liver congestions and necrosis occurred more often. The scientific debate about the significance of these findings is still on-going (Hammond *et al.* 2012, Séralini *et al.* 2012b).

Reports of birth defects in humans from Argentinean regions where HR crops and glyphosate-based herbicides are widely used led Paganelli *et al.* (2010) to investigate the potential effects of Roundup Original® on two vertebrate embryos, namely the African clawed frog

Xenopus laevis and chicken. They found direct negative effects on embryonic development resulting in different malformations in frog and chicken embryos, for instance craniofacial, eye and head defects. The results suggest that glyphosate itself was responsible for the effects observed, rather than a surfactant or other components of the commercial formulation. Paganelli *et al.* (2010) assume that the teratogenic effects observed might be linked to interference of glyphosate with retinoic acid signalling that plays an important role in gene regulation during early vertebrate development. Recently, after reviewing data about potential health effects, Antoniou *et al.* (2012) came to the conclusion that studies published in the peer-reviewed literature have raised major concerns regarding the potential for glyphosate and commercial formulations to cause genotoxic and teratogenic effects and other reproductive problems. They called for a new and transparent re-examination of toxicity data of glyphosate and its commercial formulations.

5.3.2 Glufosinate

Presumably due to the significantly lower use of glufosinate, data about ecotoxicity of glufosinate are not as extensive as those about glyphosate. Glufosinate is labelled as toxic for the aquatic fauna and for fish (BVL 2010, Ohnesorge 1994). It should not be allowed to contaminate fresh water (BVL 2010). The highest concentration (formulated product) expected after applications in agriculture is 0.25 mg/l in small lakes (Dorn *et al*. 1992).

Glufosinate as formulated product is known to be slightly toxic to fish (LC50: 14-56 mg/l, two species tested, Dorn *et al*. 1992) and aquatic invertebrates. Different EC50 values for formula (the same or different products) are published: 0.5-42 mg/l by Ohnesorge (1994) and 15-78 mg/l by Dorn *et al*. (1992). It is also harmful to spiders (Dorn *et al*. 1992). Hommel & Pallutt (2000) referred to an assessment of the cumulative effect of active ingredients of pesticides (Gutsche & Roßberg 1997). Hommel & Pallutt (2000) stated that glufosinate is less toxic to three of four tested groups (all but earthworms – daphnia, fish, algae) compared to the reference herbicide Butisan Top®. The tests did not cover effects on insects and spiders.

Glufosinate has also been shown to suppress some soil microorganisms (Ahmad & Malloch 1995, Ismail *et al*. 1995).

Glufosinate-ammonium has the potential to induce severe reproductive and developmental toxicity seen as pre- and post-implantation losses, vaginal bleedings, abortions and dead foetuses in rats and premature deliveries, abortions and dead foetuses in rabbits (EFSA 2005). In the European Union glufosinate-ammonium was classified in Category 2 and 3 of reproductive toxicity with the risk phrases R60 “May impair fertility” and R63 “Possible risk of harm to the unborn child” (European Commission Directive 2009/2/EC, EC (2009)). It is expected that the herbicide glufosinate will be phased out in the European Union at the end of September in 2017 because of its reproductive toxicity (Annex I of Commission Implementation Regulation (EU) No. 540/2011).

5.4 Effects of HR agriculture

5.4.1 Effects on flora and seedbank

Glyphosate and glufosinate are broad-spectrum herbicides and effective on more weed species than other currently used herbicides (Westwood 1997). Weed suppression is clearly intensified in most crops and regions where HR crops are planted, because less effective herbicides and sometimes mechanical weeding have been replaced by glyphosate and glufosinate.

The effects of the HR cropping system on abundance and species diversity were investigated in a large-scale trial (60-75 fields split in half, 3 years) on fields selected to represent the variation of geography and intensity of management across Britain (FSE: Farm Scale Evaluation) (Firbank *et al*. 2003a, Squire *et al*. 2003). No effects were found due to the crop being genetically modified *per se*. However, differences were found in weed flora between different weed management regimes (Heard *et al*. 2003a, Heard *et al.* 2003b, Firbank *et al.* 2003b). In HR sugar beet, HR fodder beet (both glyphosate-resistant) and HR summer oilseed rape (glufosinate-resistant) the density, biomass and seed rain were between one-third and one-sixth lower compared to conventional management. The seedbank abundance (for 19 out of 24 species) was overall 20% lower in the three HR crops (Heard *et al*. 2003a, 2003b). In HR beets, there were 8 species less that emerged than in conventional beets and 6 species less in case of HR oilseed rape. In HR oilseed rape one species more emerged, compared to the conventional crop. The findings on (abundance and) seedbank dynamics (in HR beet and HR oilseed rape) compounded over time would result in large decreases in population densities of the field flora (Heard *et al*. 2003b). Similar results have been found by Bohan *et al*. (2005). Late applications of glufosinate in HR winter oilseed rape led to a decline in dicot and an increase in monocot plant abundance. Numbers of the two pollinator groups included in the study decreased in consequence.

Findings of the FSE with HR maize (glufosinate-resistant; glyphosate-resistant maize was not tested) were different from the ones with HR beets and HR oilseed rape, and showed more diverse weed species with HR maize compared to conventional maize. In the experiments with maize, the conventional fields were sprayed with atrazine, which is highly effective on a broad range of plants, but is no longer approved in the EU since 2004 because of groundwater contamination[24]. Effects of weed management of HR maize should be compared to the weed management practices that are likely to be displaced. According to the assessment of Perry *et al.* (2004), the ban on the triazine herbicide atrazine is likely to reduce but not negate relative benefits of glufosinate-resistant maize compared to conventionally managed maize.

The results of the British FSE are not applicable throughout the world. Nevertheless, the study shows the high complexity of farm-environment interactions.

From other studies it has been deduced that the changed herbicide management of transgenic crops can result in higher weed diversity compared to conventional management (Dewar *et al*. 2003, Strandberg & Pedersen 2002) or in no significant decrease in plant species diversity, as observed in the BRIGHT study (Sweet *et al.* 2004).

In Canada, effects of different rotations with high frequencies of HR crops have been studied for three years (Harker *et al*. 2004 cited in Schütte 2005). The study comprised five rotations with one, two, or even three crops resistant to glyphosate or glufosinate planted at six locations and with different seeding dates and types of tillage. The overall species diversity of

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[24] http://ec.europa.eu/food/plant/protection/evaluation/existactive/oj_atrazine.pdf

weeds declined by 26%, and their density was reduced by 66%. When no-till plots were directly compared to conventionally tilled ones, their weed density was on average 23% lower.

Weed control is intensified in most HR varieties, even though yields are often not clearly increased (particularly not in soybean, the most abundant HR crop). A few highly damaging weed species are the target of “improved” weed control, but many harmless and benign wild plants are killed by the non-selective herbicides too. In this sense, weed suppression has been overdone in many regions by non-targeted measures and is even further “improved” in HR crops. As shown by the findings above, even if fewer amounts or fewer applications of highly effective, broad-spectrum herbicides were applied, as expected for the first years of HR crop adoption, they do not necessarily cause less damage to biodiversity.

In addition, drift of non-selective herbicides to field margins is a concern to nature conservation and biodiversity of many agricultural landscapes (Johnson 1999, Orson 2002, de Snoo & van der Poll 1999). Field margins often harbour rare plant species. The impact of non-selective herbicides on them and on the associated fauna is of particular significance (Mahn 1994). The scorching of vegetation was more than doubled in HR crops (1.6% to 3.6%) compared to conventional crops in the FSE field trials mentioned above (Roy *et al*. 2003). The cover of field margins was 25%, flowering was 44% and seeding 39% lower in HR spring oilseed rape relative to conventional oilseed rape. For beets, flowering and seeding were 34% and 39% lower. Cover (+28%) and flowering (+67%) in margins was higher in HR maize.

Spray drift can also damage hedgerows and trees growing close to arable fields, these habitats being very important for arthropods and birds for food, shelter and nesting (Sweet 1999, Roy *et al.* 2003).

5.4.2 Effects on fauna

The indirect effects of plant suppression and habitat destruction are the key to invertebrate (and vertebrate) biodiversity. Studies in the US found less canopy arthropods and significantly less spiders and green lacewings in HR soybean than in conventional soybean (Buckelew *et al.* 2000, Jasinski *et al.* 2004). However, other studies revealed no significant differences between both types of crops for pest and beneficial insects (Jackson & Pitre 2004, Morjan & Pedigo 2002). In a study of Goldstein (2003) over three generations of Collembola, no effects of RR soybean or RR maize as food source were found.

In the FSE trials in Britain several sampling methods were used to compare the abundance of different arthropod groups (Firbank *et al*. 2003a). In beet and oilseed rape, numbers of within-field epigeal and aerial arthropods were smaller in HR crops due to forage reductions (Haughton *et al.* 2003, Brooks *et al.* 2003), as population densities will be reduced, when forage is short over large HR crop areas (Haughton *et al.* 2003). Herbivores, pollinators (*e.g.* bees, butterflies) and beneficial natural enemies of pests were reduced as well (Hawes *et al.* 2003). The effects were dependent on the relative efficiency of comparable conventional herbicide regimes. The abundance of arthropods changed in the same direction as their resources (Hawes *et al.* 2003). Effects in HR maize were reverse to the results for beet and oilseed rape, but the findings may be due to atrazine use in conventional plots as discussed above.

The importance of the correct timing of herbicide application was shown by Strandberg *et al.* (2005). In summer, arthropod fauna was higher in HR fodder beets than in conventional beets if glyphosate was applied according to recommendation, but weed diversity and biomass was lower. Weed diversity and weed density were extremely low, when glyphosate was applied earlier than recommended.

Recent data from the US and Mexico indicate that, within the last decade, the size of the Mexican overwintering population of the migratory monarch butterfly (*Danaus plexippus*) has declined significantly. During the overwintering season 2009-2010, the total area in Mexico occupied by monarchs reached an all-time low (Brower *et al.* 2012). Most monarch larvae feed on milkweed (*Asclepias syriaca*) that was once widely spread in the Midwest. The rapid adoption of HR crops has led to a drastic reduction of milkweed populations, *e.g.* in glyphosate-treated fields in Iowa by approximately 90% compared to 1999 (Hartzler 2010). Pleasants & Oberhauser (2012) estimate that there has been a 58% decline in milkweed plants in the Midwest landscape and an 81% decline in monarch propagation in the Midwest from 1999 to 2010. As each year the monarch production in the Midwest was positively correlated with the size of the subsequent overwintering population in Mexico, the results suggest that the loss of agricultural milkweeds is a major cause for monarch population decline.

Models from the UK simulated the planting of herbicide-resistant crops on a larger scale and showed that one consequence will be a major loss of food sources for seed consuming farmland birds (Watkinson *et al*. 2000). A model using FSE data also predicts a distribution of more fields with lower weed densities, which could affect animal populations on farmland, if HR crops were grown as in the FSE (Heard *et al.* 2005). Bohan *et al*. (2005) found a decline in dicot plants in HR winter oilseed rape, which may likewise affect taxa at higher trophic levels including some birds dependent on them as a food source.

In a similar study, Butler *et al.* (2007) focused on potential effects on farmland birds, when equivalent conventional crops were replaced by HR crops. Their model predicted only limited effects after nationwide introduction of HR crops in the UK. Species that rely solely on cropped areas are likely to continue declining at their current rate, unless the value of cropped areas is improved. This is true for conventional and HR agriculture.

Wauchope *et al.* (2002) expected that the replacement of atrazine and alachlor in maize by glufosinate or glyphosate reduces run-off loads in watersheds. While the ingredients glyphosate and glufosinate are considered to be less toxic to mammals than atrazine and alachlor, the common glyphosate formulations have been shown to be toxic to amphibians and a number of aquatic organisms. Farmland mammals such as hares might benefit from glyphosate and glufosinate use, if these herbicides are indeed shown to be less toxic to them than other herbicides, and if the weed loss does not reduce their food supply.

5.5 Further aspects of sustainable agriculture

Measures to mitigate environmental effects of herbicides in conventional systems have been developed in some countries, including details about when and where herbicides may be used on farmland. The promotion of unsprayed field margins and unsprayed in-field areas and the control of timing of applications and of maximum doses have allowed weeds and associated biota to develop in some cases.

For HR sugar beet, similar measures have been proposed to restore biodiversity. Little impact on overall crop productivity per hectare is expected because of the anticipated yield increase associated with HR sugar beet (Pidgeon *et al.* 2001, 2007, Beckie *et al.* 2006). To compensate for reduced weed seed and weed biomass production in HR sugar beet, 2 to 4% unsprayed areas in a field would be required. Effects through the tillage of margins could be mitigated by increasing the margin from 0.5 to 1.5 m. In production systems with HR fodder or sugar beet, delayed spraying within a single season increased weed biomass only transiently and only in soils which already had a rich seedbank (Dewar *et al.* 2000, Elmegaard & Pedersen 2001, Strandberg & Pedersen 2002). Freckleton *et al.* (2004) comprehensively assessed that the soil seedbank is reduced in the long-term, even when applications are delayed. An early band spraying of HR beet (May *et al.* 2005) would have positive overall effects and would be in accordance with possible IPM (Integrated Pest Management).

In HR sugar beet, low-dose (row spraying) post-emergence application has been suggested to reduce negative impacts on weed and insect biomass; it can be combined with an economic threshold evaluation to avoid economic losses (Dewar *et al.* 2002, Elmegaard & Pedersen 2001). However, band spraying or patchy weed control with selective herbicides is better for biodiversity than spraying of non-selective ones (Dzinaj *et al.* 1998, Gerhards *et al.* 1998, Lettner *et al.* 2001). But any modification of spraying regimes in this sense is only effective when the seedbank is not already depleted. For decades, weed scientists in the US and in Europe have been recommending weed control up to a level that eliminates potential interference with net returns (economic thresholds). Clean fields or a 95% control are not required to prevent that weeds, non-target or beneficial wild plants compete with crops for nutrient or water (Korr *et al.* 1996, Pallutt 1997, Werner & Garbe 1998). However, the databases on integrated weed management and the corresponding expert systems are rarely used in practice; growers consider other factors to be more important (Owen 2000) and largely attribute the introduction and movement of weeds to factors outside their control (Wilson *et al.* 2008). The increasing number of herbicide-resistant weeds might change this attitude somewhat, as resistance management becomes increasingly important (Egan *et al.* 2011).

5.6 Conclusions on impacts on biodiversity (Chap. 5)

Farmland biodiversity is an important characteristic when assessing sustainability of agricultural practices and high on the agenda of international concern. Scientific data, collected in recent years from various regions, indicate that agricultural intensification and pesticide use are among the main drivers of biodiversity loss. It will be essential not only to stop the loss of biodiversity, but to reverse the development and to increase biodiversity in agricultural ecosystems. For this reason, agriculture will have to develop practices that are more environmentally friendly, including a reduction in pesticide use. Given the actual trends and the experience in adopting countries, HR crops could not reduce herbicide use and, therefore, they can be expected to go along with a (further) loss of agrobiodiversity, in particular, as there is no evidence of increased crop rotation.

Reduced/no-tillage systems are introduced throughout the world for preventing soil erosion to enhance trafficability (passing over of the fields), to increase soil organic matter and to save money. As growing HR crops facilitate the management of reduced/no-tillage systems, they support their expansion. When combined with cover crops and mulching, when farm operations are re-scheduled and adopted to protect wildlife, and when wild plant abundance is not further decreased by highly effective weed control, reduced tillage could be favourable to biodiversity. But experience shows that in systems, which combine reduced or no-tillage with broad-spectrum herbicide application, weed populations shift to perennial and grass weed species and the diversity and abundance of broadleaf plants decrease further together with the accompanying arthropod fauna. From the Farm Scale Evaluations, there is ample evidence that the seedbank, wild flora and whole food webs in agricultural fields will be reduced further, if HR beet and HR oilseed rape are planted and sprayed with broad-spectrum herbicides. The results for HR maize, showing less impact on biodiversity through glufosinate use being a less toxic and persistent herbicide compared to atrazine, seem not to be valid for Europe, as atrazine is no longer approved. In general, ecotoxicity of glyphosate and glufosinate has been considered to be low, compared to some other herbicides. But glyphosate-based herbicides have shown to be highly toxic to amphibians and a range of aquatic organisms. Glyphosate adversely effects micronutrient uptake of plants, soil microflora and plant disease severity. Microbial activity can be suppressed by glufosinate too. In addition glufosinate shows a high reproductive toxicity for mammals. Taken together the long-term (eco-) toxicological profile and longer term impact assessment does not support a benign impact on biodiversity.

Altogether there is quite some evidence that use and management of HR plants will exacerbate the current trend of biodiversity loss less so due to the ecotoxicological profile of their complementary herbicides, but because of the systematic depletion or extinction of indispensable elements of food webs and ecosystem functions in agricultural ecosystems.

The environmental impacts of a particular HR crop depend on various factors of its cultivation, the dose, time and frequency of the applied complementary herbicide as well as of other herbicides, additional management features of the HR crop and of other crops in rotation with it. These factors will vary from region to region, from country to country, and from season to season, depending on weed pressure, soil type, climatic conditions and the forecasted economic return.

Similarly, environmental impacts of the conventional herbicides applied to conventional comparator crops vary because of the same factors. Therefore it is very difficult to establish, in this very dynamic situation, detailed baselines to compare HR systems with conventional chemical based systems.

Against the background of the internationally agreed policy goal to stop the loss and in addition the need to improve and increase biodiversity in agricultural systems the more fundamental question arises, if the current, chemical-based high input systems are the right points of reference to assess the impacts of a new technology.

Comparative assessments are foreseen in most of the international legal frameworks. Up to now a discussion on which comparison may be adequate to not only conserve a highly unsatisfactory and sometimes even threatening situation, but to achieve improvements are not high on the agenda. Out of a nature protection perspective herbicide resistant crops are not part of the solution, but part of the problem.

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