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Working Group on the Harmonisation of Regulatory Oversight in Biotechnology

MODULE III: DRAFT CONSENSUS DOCUMENT ON GENERAL INFORMATION CONCERNING AGRONOMIC AND ENVIRONMENTAL ASPECTS OF THE CULTIVATION OF GENETICALLY MODIFIED HERBICIDE RESISTANT PLANTS

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The first draft of this consensus document was prepared by Germany as the lead country, and presented at the 15th meeting of the Working Group in June 2004. Since then, this document has been revised on several occasions, taking into account comments from the Working Group.

The lead country prepared this latest draft taking account of comments from Australia, Canada, the Unites States, and BIAC. The document will be considered for declassification after those delegations come to agreement on the contents.

ACTION REQUIRED: ***The Working Group is invited to review this draft and provide input as appropriate.***

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INTRODUCTION

It has been more than ten years since the first commercial use of herbicide resistant transgenic crops, and a wealth of information is available on patterns of use and impacts of specific applications. The purpose of this document is to review the available information on the use of herbicide resistant transgenic crops in order to serve as a resource for risk assessors and decision makers. The document will cover the scale and area of commercial adoption, observations on how the crops are being used in agriculture and their impact on agricultural practices, as well as observations of environmetal responses (direct and indirect effects) to the changes in the agricultural environment. This includes changes in biodiversity (weed species, wild plant and animal species and populations, microbes) and other environmental changes like e.g. soil erosion or compaction.

1. OECD's Working Group in Harmonisation of Regulatory Oversight in Biotechnology aims to ensure a harmonisation concerning environmental risk/safety assessment of genetically modified organisms especially in agriculture. Consensus documents are one instrument which represents a common understanding among the member states.

2. These documents should contribute to the risk / safety analysis of transgenic organisms and promote international harmonisation activities. The activities are based on the characteristics of the organism, the introduced trait, the environment into which the organism is introduced, the interaction between those, and the intended application (OECD, 2005). This has been done for genetically modified herbicide resistant plants (HR plants).

3. Descriptions of resistance genes commercialised prior to 2009, the metabolic pathways of the herbicides in plants and the residue situation have been published as modules I and II of a consensus document for transgenic herbicide resistant plants.

4. Module III of the document on herbicide resistance focuses on agronomic and environmental aspects. Some of these aspects are not exclusively relevant for genetically modified herbicide resistant varieties. The changes in conventional herbicide resistant varieties are not within the focus of this document and are not as widely documented either. They are also only adopted in a few OECD contries, but they can be considered as part of the baseline for a comparative assessment.

5. Agronomy is the science of crop production. It incorporates several sciences / scientific fields into an applied science which is the foundation for most agriculture. The definition for environment is: All living and non-living components by which an organism is surrounded and affected (Frey, 2005).

6. The adoption of herbicide resistant varieties affects several agronomic and environmental subjects and some *vice versa* influence the degree of adoption. The choice of crop species (parent lines/varieties, crop rotation), growing region (wild plant abundance, climate, cropping history, soil) and tillage system (non-till, reduced tillage, conventional tillage) affect the occurrence of volunteers, resistant

weeds, biodiversity and herbicide application patterns. A new trait will have numerous interwoven effects on these agronomic and environmental subjects.

7. Conservation of biodiversity is an important issue of international environmental politics and is underscored by the Convention on Biological Diversity (www.cbd.int). Intensive high-input agriculture accounts for ongoing biodiversity losses (Krebs *et al*., 1999; Robinson and Sutherland, 2002). The importance of biodiversity for agriculture has to be considered taking note of the interrelationship of agriculture with biodiversity (Secretariat of the CBD, 2005). Inappropriate reliance on monoculture, over-mechanisation, and misuse of agricultural chemicals diminish the diversity of fauna, flora and micro-organisms, including beneficial organisms (Secretariat of the CBD, 2005). Biodiversity losses reported after 1980 are associated with the intensification and industrialisation of agriculture.

8. However, different nature conservation practices (as well as management systems and agricultural systems) in different countries have to be considered. Countries with a high number of exotic species, e.g. Australia, have to regulate exotic weeds in agricultural areas more in order to conserve the endemic biodiversity. Countries with mainly indigenous weeds can manage weed populations within agronomic areas. Nevertheless, the goal of biodiversity management is different from the goal of intensive agriculture and must be determined by each country's context of acceptable risk and risk/safety assessment endpoints.

9. 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 (Convention on Biological Diversity, Article 2. Use of Terms). Biodiversity in agricultural landscapes can be characterised by composition (which and how many species / genotypes), structure (dominance), and function (Duelli, 1997). Composition and structure can affect its function (Duelli, 1997; Büchs *et al*., 2003).

10. Module III, 'Agronomic and Environmental Aspects of the Cultivation of Genetically Modified Herbicide-resistant Plants', is subdivided into the four sections 'Scale and area of cultivation', 'Changes in weed susceptibility', 'Changes to agricultural practice and agronomy' and 'Impacts on Biodiversity'.

11. Information on these aspects has been retrieved by literature, internet searching, and contacting experts. Results from national studies and surveys are cited beside international publications in order to highlight certain aspects. Most environmental and agronomic field tests are conducted on a regional scale. International papers generally have a wider geographic focus.

12. Herbicide resistance in crops can result from two different breeding procedures: traditional and genetic engineering techniques. These breeding technologies use similar strategies to achieve herbicide resistance and therefore cause comparable effects on the environment. Almost all of the effects of HR-GMOs apply accordingly to non-GM HR plants (Tan *et al.,* 2005). Impacts on Biodiversity occur with introduction of new crops for intensive management irrespective of the variety constructed with conventional breeding or genetically modification (Sutherland *et al.,* 2006).

13. The 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.

Table 1.

Table 2. Table 1. Glyphosate- and glufosinate-resistant crops approved for unconfined environmental release in North America

Herbicides	**Crops**
glufosinate	canola, maize, cotton, soybeans, rice
glyphosate	soybeans, canola, cotton, maize, sugar beet

Modified table published by Duke 1999, Duke 2005, current status on http://www.aphis.usda.gov/brs/not_reg.html

14. Since the termination of the Monsanto patent, the number of products containing glyphosate has greatly increased (but differs from country to country). Glufosinate is marketed by BayerCrop Science.

15. Glyphosate is widely used as a broad-spectrum weed control agent. It interferes with normal plant metabolism through inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS, OECD, 1999b).

16. Glufosinate ammonium is an equimolar, racemic mixture of the D- and L-isomers of phosphinotricin (PPT). L-PPT, inhibits glutamine synthetase of susceptible plants and results in the accumulation of lethal levels of ammonia (OECD, 1999c).

17. Glyphosate- and glufosinate-resistance genes allow previously sensitive crops to resist glyphosate or glufosinate. A variety of crop plant species have been transformed with genes encoding a glyphosate-insensitive EPSP synthase derived from *Agrobacterium* spp., some in combination with the *gox* gene from *Ochrobactrum anthropi* encoding the glyphosate-degrading glyphosate oxidoreductase (GOX). Many crop plants have also been transformed with one of the two bacterial genes *pat* or *bar* encoding the enzyme phosphinothricin acetyl transferase (PAT) which detoxifies L-PPT in order to confer glufosinate (L-PPT) resistance. Also new types of HR crops are being commercialised e.g, soybeans with modified glyphosate acetyltransferase (GAT 4601) and acetolactate synthase (ALS) proteins, imparting resistance to both glyphosate and ALS-inhibiting herbicides e.g., sulfonylureas and imidazolinones (USDA/APHIS, 2007).

18. Glyphosate and glufosinate use is 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 (Anonymous, 2009) than glyphosate or glufosinate can be used in other cropping systems.

19. The official WSSA (Weed Science Society of America) definitions of "herbicide resistance" and "herbicide tolerance" (WSSA, 1998) are used throughout this document[1]:

- "*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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[1] The OECD Consensus Document No. 25 “Module II: Phosphinothricin” only uses the term “tolerant” for genetically modified plants.

SECTION I SCALE AND AREA OF APPLICATION

HR crops have been adopted in an increasing number of countries. Since the adoption rate and the number of countries is very dynamic, this section can only give an overview about the global scale of adoption and area of cultivation. Adoption of biotech crops by a country are changing year by year and can therefore only give a broad indication. Also the increasing area planted with crops having stacked traits makes a clear distinction between HR area and other biotech crops difficult.

I.1 Field trials

20. For the USA currently more than 4000 approved releases for HR plants are listed (http://www.isb.vt.edu/cfdocs/fieldtests1.cfm). For Europe current releases are listed on (http://gmoinfo.jrc.ec.europa.eu/).

21. Small-scale trials provide opportunities to evaluate the performance of plants in the natural environment (instead of in a lab or greenhouse) and are an important component of tiered approaches to data collection, but the conclusions from small-scale data cannot always be extrapolated to a commercial scale. In particular, effects on biodiversity from the use of herbicides in HR crops and the magnitude of gene flow between neighbouring crops and from transgenic crops to feral populations and wild relatives are recognised to be scale and time dependent (DETR, 2000).

I.2 Commercial cultivation

22. Out of the many transgenic glyphosate and glufosinate resistant crop species globally tested in field experiments four plant species have been widely commercially grown as approved varieties and studied for their potential impacts on the environment: maize, cotton, canola and soybeans (Brookes and Barfoot, 2006). Until 2008, 25 countries have approved and adopted transgenic crops. They are listed relative to the size of transgenic growing areas: USA, Argentina, Brazil, India, Canada, China, Paraguay, South Africa, Uruguay, Bolivia, Philipines, Australia, Mexico, Spain, Chile, Colombia, Honduras, Burkina Faso, Czech Republic, Romania, Portugal, Germany, Poland, Slovakia, Egypt (James, 2008). Herbicide resistance has consistently been the dominant trait for commercialisation.

23. Glufosinate or glyphosate resistance genes can be used alone or in conjunction with other genes, e.g. as selectable markers for plant transformation or as part of a male sterility system in hybrid breeding. Transgenic male sterility systems are currently being employed for variety development and seed production in chicory, maize, and oilseed rape.

24. More than 200 seed companies sell either glufosinate or glyphosate resistant plants, according to the conditions of the patent owners (main owners: BayerCrop Science and Monsanto).

I.2.1 Global HR area

25. Herbicide resistant crops are by far the most planted genetically engineered crops. Of the 125 mio ha transgenic acreage worldwide, about 63 % (78.4 mio ha) were planted with HR varieties and another 23 % (27.1 million ha) were planted with crops with stacked traits (including HR/insect-resistance stacks) (James, 2008). In 2008, the stacked double and triple traits occupied a larger area (26.9 mio ha, or 22% of

global biotech area) than insect resistant varieties (19.1 mio ha, or 15%). The stacked trait products were by far the fastest growing trait group between 2007 and 2008 (James, 2008).

26. One third (33.1 %) of the global area of the main biotech crops (soybeans, cotton, maize, canola) is currently planted with HR crops. 6.8 % (105.5 mio ha) of global crop acreage (1,553.7 mio ha, all arable and permanent crops) is currently planted with HR crops (James, 2006, 2007, 2008; FAO 2008). The current share of the HR crop areas per global acreage of the four most important crops is shown in Tab. 2 and Tab. 3.

Table 2. HR acreage as percent of global crop acreage

crop	global area (mio ha)**	biotech crop area (mio ha)	HR area (mio ha) *	HR acreage as % of global acreage of that crop *
soybeans	97	65.8	65.8	67.8 %
cotton	31	15.5	1.0 (3.6)	3.2 % (11.6 %)
maize	161	37.3	5.7 (30.2)	3.5 % (18.8 %)
canola	30	5.9	5.9	19.7 %
sum	**319**	**124.5**	**78.4 (105.5)**	**24.6 (33.1) %**

* Only HR, in brackets: HR/insect resistant (stacked) included; modified table cited in James 2008
** FAO 2008 hectarage

Table 3. Global growing areas of herbicide resistant crops from 1996 to 2008 (mio ha)

	1996	1998	2000	2002	2004	2005	2006	2007	2008
HR soybeans	0.5	14.5	25.8	36.5	48.4	54.4	58.6	58.6	65.8
HR canola	0.1	2.4	2.8	3.0	4.3	4.6	4.8	5.5	5.9
HR cotton	< 0.1	--	2.1	2.2	1.5	1.3	1.4	1.1	1.0
HR/BT cotton	0.0	2.5	1.7	2.2	3.0	3.6	4.1	3.2	2.6
HR maize	0.0	1.7	2.1	2.5	4.3	3.4	5.0	7.0	5.7
HR/BT maize	0.0	--	1.4	2.2	3.8	6.5	9.0	18.8	24.5
HR sugar beet	--	--	--	--	--	--	--	--	0.3
HR Alfalfa	--	--	--	--	--	--	--	--	0.1
total	0.6	21.1	35.9	48.6	65.3	73.8	82.9	94.2	105.9

CropBiotech Net. 2003, cited in http://www.isaaa.org, James 2004, 2006, 2007, 2008

27. HR soybeans are by far the most planted HR crop. 65.8 mio ha, representing 53% of the global biotech crop area of 125 mio ha for all crops in 2008. It continued to to be the dominant biotech crop grown commercially in ten countries, listed in order of hectarage, they were: USA, Argentina, Brazil, Paraguay, Canada, Bolivia, Uruguay, South Africa, Mexico and Chile (James, 2008).

28. The second most dominant biotech crop was maize with stacked traits, which occupied 24.5 mio ha, equivalent to 20% of the global biotech area and planted in seven countries: USA, Canada, South Africa, Philippines, Honduras, Argentina, and Chile.

29. The fifth most dominant biotech crop was HR canola, planted in Canda, USA, Australia and Chile on 5.9 mio ha. The sixth most dominant crop was HR maize, planted in USA, South Africa, Argentina, Canada, Philippines, Honduras and Chile. The seventh was stacked cotton, planted in USA, Australia, Colombia and Mexico.

30. HR crops, such as alfalfa, sweet maize, sugar beet, rice, wheat and a lot more are already approved or under development (www.nbiap.vt.edu/cfdocs/fieldtests1.cfm; http://www.aphis.usda.gov/brs/status/relday.html; Gianessi *et al*., 2002; James, 2006, 2008). HR sugar beet was grown for the first year 2008 in USA and Canada on 0.3 mio ha, HR alfalfa in USA grown on 0.1 mio ha.

I.3 Conclusions on scale and area of application (Section I)

31. Until 2008, 25 countries have approved and adopted transgenic crops.

32. Herbicide resistant crops are by far the most planted genetically engineered crops.

33. One third (33.1 %) of the global area of the main biotech crops (soybeans, cotton, maize, canola) is currently planted with HR crops.

34. HR soybeans are by far the most planted HR crop.

SECTION II IMPACTS ON AGRICULTURAL PRACTICE AND AGRONOMY

Herbicide resistant transgenic crops are adopted in some countries 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 soybeans with reduced tillage practices). In many cases, it may not be possible to precisely differentiate between what is a direct effect of the adoption of HR crops, what is an indirect consequence, and what is a coincidental impact from some other cause. Other factors, including government policy and incentives, fuel prices, and even weather conditions can influence farmers' decisions on an annual basis. It is possible, however, to take a look at what has been observed during the last decade or more of commercial use of HR crops to see what changes have occurred over time in agricultural practices. While these observations may not be the direct and necessary result of the use of HR crops and observations from one country may not be relevant for the other, it is important for decision makers to understand how these crops may have affected agricultural practices to inform decisions related to national policy goals.

II.1 Reasons why producers adopt HR crops

35. The acreage of HR crops has significantly increased world-wide during the last years (see Tab. 3). The main reasons farmers choose to grow HR varieties instead of conventional crops have manifoldly been analysed and surveyed. "Improved weed control" was the most often stated reason in the published surveys presented in Tab. 4, followed by "cost reductions", which were calculated by Sankula *et al.* (2005) as well as Sankula and Blumenthal (2004) for average weed control costs in terms of conventional and HR crops canola, maize, cotton and soybean (not included in table).

Table 4. Published farmer surveys on adoption reasons of HR crops

adoption reasons for 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 *et al.* (2002)	Klotz-Ingram *et al.* (1999)	van der Sluis *et al.* (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

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

36. Soybean producers (who wanted better weed control) also adopted glyphosate resistant varieties because ALS resistant weeds can be controlled with glyphosate (Shaner, 2000). Price reduction for glyphosate has been a driving factor for the adoption of the corresponding HR crops too (Freudling, 2004).

37. In Argentina the main adoption reasons were low glyphosate prices, fewer expenses on labour, fuel and machinery, increased awareness of the synergy with no-till and HR soybean and the ability to plant soybean earlier (Pengue, 2004; Trigo and Cap, 2006).

38. Yield and returns were ranked lower in priority by Canadian and US-farmers. Canadian (Manitoba) respondents did not indicate that increased yields were important, but did mention reduced dockage as important (Mauro and McLachan, 2003).

39. In general, the surveys indicate a strong desire to reduce production risks (see also Fernandez-Cornejo and Caswell, 2006). This outcome is supported by Kalaitzandonakes and Suntornpithug (2001): The main adoption reasons for HR cotton are the reduction of production risks and the increased flexibility (extended time window for spraying) in weed control. Firbank and Forcella (2000) also emphasised the importance of flexibility (in timing) and simplicity (of control). In some cases, e.g. in canola, lower returns seem to be accepted by farmers because of other "convenience" effects such as the flexibility in timing, easy control, and less labour (CEC, 2000). Those and other benefits of HR canola generated an additional farm income (Brookes and Barfoot, 2005).

40. The option to save labour as well as the simplicity and flexibility of weed control may be of particular interest for large farms and for part-time farmers who are otherwise employed. 39 % of Illinois farmers consider farming as their secondary job (Hin *et al*., 2001). Simplicity and flexibility may be of high importance for part-time farmers as they have to organise two or more occupations. Fernandez-Cornejo and McBride (2000) found a correlation between the adoption of HR soybean varieties and farm size (particularly for farm sizes of 50 acres to 800 acres; 1 acre = 0,405 ha) and operator’s education.

41. Neither biodiversity nor weed resistance management are significant considerations to the farmer, but aesthetics (better weed suppression, simplicity of control) and production risks (reduced herbicide costs, flexibility in timing) (Owen, 2000; Hin *et al*., 2001; CEC, 2000). Improved weed control was named as the pre-eminent reason for adopting HR in maize (94.3 % of farmers), cotton (76.3 %) soybeans (97.5 %) and canola (50 %). In many parts of Europe, oilseed rape is presently sprayed with herbicides, although it is economically not necessary according to threshold models (Greenadas and Boothsack, 1999).

42. Furthermore, landlords may insist on clean fields (Duffy, 2001). The proportion of rented agricultural lands is high in many regions (e.g. 50 % in Iowa; Owen, 2000).

43. Canadian farmers who were questioned in the province of Manitoba rejected approval of a glyphosate resistant wheat (survey of Mauro and McLachan 2003, see above). 86 % of the responding Manitoba farmers were opposed to this type of variety. Their reasons were as follows (ranked in order): loss of markets, corporate control of food supply, segregation, multiple glyphosate resistance in a rotation, contamination, resistant volunteers and weeds, seed costs and loss of seed saving, loss of livelihood for organic farmers.

II.2 Weed control patterns

II.2.1 Factors influencing the time and the mode of applications

44. In non-HR farming with crop rotation, farmers can choose to apply a sequence of different herbicide modes of action or tank mixtures to control competition of weeds with the crop. Some of these

herbicides can only be applied before crop emergence and are therefore often routinely applied as a precautionary measure. Weeds may survive these control measures because they are non-susceptible to certain herbicides or because they emerge after application of a non-residual herbicide.

45. HR crops allow the postemergence application of a single herbicide with a wide spectrum of activity. Moreover, glufosinate or glyphosate can be used alone, in combination with other herbicides (i.e. preemergence herbicides for programs that provide soil residual control), or with mechanical weeding.

46. The appropriate time span for postemergence control is 3-5 weeks after crop emergence. Variations depend on the herbicide, crop, weed abundance and weather. Glyphosate and glufosinate provide an option to enlarge the time span after crop emergence compared to many other herbicides (Kalaitzandonakes and Suntornpithug, 2001; Pallutt and Hommel, 1998), as late applications of selective herbicides are sometimes not economically sound and are risky (Dewar *et al*., 2000). Selective postemergence herbicides are already available for most crops. Data for oilseed rape indicate that postemergence application practices without HR are common in the UK (about 99 % of acreage) and Germany (90 %) but less in France (44 %) (Amann, 1998).

II.2.2 Herbicide amounts, herbicide application frequencies, and mechanical weeding

47. Changes in overall amounts of herbicides used are difficult to assess because different herbicides are applied at different rates. For example, in Canada glyphosate is applied at rates ranging from 0.6 L/ha in glyphosate resistant canola to 2.5 L/ha in maize to as high as 5L/ha in glyphosate resistant soybeans, whereas atrazine is applied in maize at a rate of 3.2 L/ha, and some AHAS-inhibiting herbicides are commonly applied in many crops at 0.4 L/ha or less (Anonymous, 2008). Additionally, each of these herbicides differ from each other with regards to their environmental behaviour and toxicological profile, meaning that a change in the overall volume 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.

48. Calculating herbicide use is far from simple (USDA/ERS, 2000). USDA/ERS used three different statistical approaches (Hin *et al*., 2001). The USDA/ERS (1999) analyses for 1997 and 1998 ranged from no significant effect to a reduction of 10 % (Hin *et al*., 2001). Duke (2005) stated from his summary of several studies that herbicide amounts (weight per unit area) used in conventional and herbicide resistant varieties did not substantially differ from each other.

49. The results of many different analyses of herbicide use in combination with glyphosate resistant soybeans in the USA ranged from a 7 % increase to a 40 % decrease compared to herbicide use in conventional soybeans in the first years of HR growing. Identifying the reasons for these differing results is hampered by the absence of information regarding the herbicide programs used by soybean growers (Gianessi and Carpenter, 2000).

50. According to Gianessi (2008) the number of active ingredients used on at least 5 % of the USA soybean hectares has declined from 19 in 1996 to only one (glyphosate) in 2005, however there is a significant increase in the amount of glyphosate usage which grew from 1.8 mio kg on US maize, soybeans and cotton hectares in 1990 to 45 mio kg in 2005 which came along with the increase in HR crop hectares.

51. The survey of Klotz-Ingram *et al*. (1999) (covering 12 cotton growing states in the USA) showed that the application frequency of glyphosate (1.3) and the average number of applications of other alternative herbicides were about the same. However, Culpepper and York (1998) concluded from their study, that less application trips and less amounts of herbicides are used in herbicide resistant cotton.

52. Since 1999, a number of weed species have become more troublesome in the US Cotton Belt. The abundance of glyphosate resistant horseweed (Conyza canadensis), which is well-adapted to no-till systems, tremendously increased (Heap, 2008). Tank mixtures (clarity [dicamba] 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). Furthermore, Harvade 5F [dimethipin] mixtures with glyphosate are presently recommended for other troublesome weeds such as teaweed (Sida spinosa), sicklepod (Senna obtusifolia) and morningglory (Ipomoea) (Deterling, 2002).

53. The number of herbicides used in HR (compared to conventional) varieties decreased in canola growing regions of Canada (Canola Council of Canada, 2001) and in soybean growing regions of US (Benbrook, 2001; Hin *et al*., 2001; Duffy, 2001). The amount of herbicide active ingredient applied per hectare of canola declined by 42.8% in Canada (Brimner *et al*., 2005).

1. Amounts of herbicides in the USA used in HR maize, HR soybean and HR cotton were lesser relative to conventional crops in 1996 and 1997 (first years of HR introduction). The trend was reversed afterwards (turning point in 2000) and amounts of herbicides were higher in HR crops than in conventional crops in 2004 (Benbrook, 2003, 2004). The extrapolations of Benbrook are disputed.

54. Herbicide use was generally reduced for several countries between 1996 and 2005 according to Brookes and Barfoot (2005, 2006), although results varied by region and tillage system. Gianessi (2005) calculated a reduction of herbicide use in the USA of 37.5 million lbs since introduction of glyphosate-resistant crops.

55. Herbicide amounts increased in Argentinean reduced tillage systems planted with HR soybeans from the start. The total amount of glyphosate use increased further in 2002/2003 in Argentina (Pengue, 2004).

56. In Australia an overall decrease in herbicide use in cotton since 1994 was due to a reduction of cotton acreage due to drought, the use of Staple (pyrithiobac sodium, a low dose ALS-inhibitor herbicide) and glyphosate resistant varieties (Carpenter and Gianessi, 2000). In a study by Werth *et al.* (2006) the use of glyphosate was higher in HR cotton than in fields planted with conventional cotton. The increase related to a reduction in other herbicides.

57. 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 postemergence in resistant varieties. The preseeding application is currently a two-herbicide mixture (2,4-D or MPCA added) in western Canada according to van Acker *et al.* (2003). Premixed products of glyphosate or glufosinate and other residual herbicides are commercially available. It is not recommended by university weed scientists to solely rely on a postemergence herbicide like glyphosate and glufosinate for maize production (Owen, 2000).

58. HR varieties dominate soybean production (98 % - 99 % of soybean area was HR in 2004) in Argentina. The introduction of RoundupReady soybeans, which are resistant to glyphosate has led to a reduction of 83 % in the use of herbicides with toxicity class II and of 100 % of herbicides with toxicity class III (Qaim and Traxler cited by Trigo & Cap (2003)). Quaim and Traxler (2005) reported for HR soybeans in Argentina that the number of herbicide applications and the amount of herbicides used increased. However, the amount of more hazardous herbicides was reduced significantly with the adoption of HR soybeans.

59. Phipps and Park (2002) extrapolated from field trials that the number and the amounts of herbicides (and of active ingredient) per ha may be reduced using HR oilseed rape, HR maize and HR sugar/fodder beet in standard herbicide programs in parts of Europe. In field experiments the effectiveness of weed control was more than 90 % with 300–500 a.i. glufosinate per ha (Cremer, 1996). Cremer *et al.* (1995) conclude that control of volunteer transgenic crops does not create any new problems and can be controlled by conventional management (mechanical measures, alternative herbicides).

60. In European field trials the number of application trips in glufosinate resistant maize is mostly 2 in HR varieties (with extreme weed infestations 3) instead of 1 in conventional maize (Cremer, 1996; Lechner *et al.*, 1996; Harms *et al.*, 1998). Mechanical weed control is not common.

61. The Farm Scale Evaluation (FSE) demonstrated according to Dewar *et al.* (2005) and Champion *et al.* (2003) 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 were necessary.

62. It is known from several German field tests that the number of applications in HR sugar beet will be 2-3 which is about the same as in conventional varieties (1-3 applications, Hurle (1994)). According to Petersen (pers. communication) the average number of applications in conventional beet in Germany is 3.2. Higher application frequencies are reported for the UK (4-5) in conventional varieties.

63. Mechanical weed control decreased with the introduction of HR varieties in cotton, especially RR flex cotton, US soybeans, Argentinean soybeans (at those locations where it was still employed), and in western Canadian canola fields (Charles and Taylor, 2006; Werth *et al.*, 2006). Mechanical weeding is not commonly employed in European oilseed rape and maize but to a certain extent in sugar beet, which may be omitted in HR varieties.

64. In Canada more mechanical control is employed in conventional than in HR varieties (Canola Council of Canada, 2001). Early annuals/biennials and broadleaved plants are often mechanically controlled.

65. A higher level of weed control in HR crops was reported in several publications (e.g., Westwood, 1997; Read and Bush, 1998; Buckelew *et al.*, 2000; Heard *et al.*, 2003a, 2003b; Harker *et al.*, 2004). Results of a large scale field test conducted in Canada indicate that the level of weed control (diversity and density) increases relative to the frequency of glyphosate resistant crops in rotation.

II.3 Tillage and planting

66. Conservation tillage can help to prevent soil erosion. There are a variety of factors that can influence adoption of low-tillage practices, including government programs, declining costs of pre-emergence herbicides, and improvements in seeding technologies (Zentner, 2002). Reduced tillage can be used with conventional cropping systems (Diercks und Heitefuss, 1990; Kees, 1990), and HR varieties are often adopted in conjunction with low tillage practice, whether as a result or a consequence of reduced tillage.

67. The use of reduced tillage practice on US-soybean acreage increased from 25 % to 48 % before the introduction of HR varieties. The proportion varied between 50 % and 60 % after introduction (Fernandez-Cornejo and McBride, 2002). However, many farmers who reduced tillage subsequently planted HR varieties, and according to the survey of Van der Sluis and Grant (2002), more than 40 % of the soybean and cotton farmers reported that they planted HR varieties in order to reduce tillage. (Fernandez-Cornejo and McBride, 2002; Kalaitzandonakes and Suntornpithug, 2001).

68. Farmer surveys indicate that 2 % of cotton and 3 % of canola farmers planted HR varieties in order to reduce tillage (Ward *et al*., 2002; Klotz-Ingram *et al*., 1999; Canola Council of Canada, 2001; Tab. 4).

69. In Canada, no-till (Low Disturbance Seeding "LDS") practice is the most common in the drier western parts. Low disturbance seeding was practiced on about 40 % of the cropping area in Saskatchewan in 2002 (van Acker *et al*., 2003). In these systems, farmers presently apply glyphosate and sometimes a second herbicide (see Section II.1.2) before seeding in the spring.

70. When a survey of 434 HR canola growers in Canada was taken, 37 %-44 % practiced zero-tillage while 25 % of conventional growers practiced zero-tillage (Canola Council of Canada, 2005).

71. Earlier seeding in Canadian canola is possible when postemergence herbicide application is an option (Canola Council of Canada, 2001). Of 448 respondents who planted transgenic varieties, 44 % said they are seeding earlier in the spring due to transgenics (Canola Council of Canada, 2001).

72. In Argentina, many farmers who adopted HR soybeans also reduced tillage, with 42 % of conventional fields and 80 % of HR fields practicing reduced tillage (Qaim and Traxler, 2002).

73. In Europe HR cropping systems are not widely adopted, therefore a correlation of no-till systems and HR cropping systems is not possible. Furthermore, no-till practice and mulch planting is not so common in many parts of Europe. Cover crops are more frequently adopted by German farmers (Lütke-Entrup, 1995). Cover crops with a high competitive ability (for example, 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 and Hurle, 1998).

II.4 Crop rotation options in HR crops

74. Rotations often lead to reduced input costs because pesticide and artificial nitrogen needs are lesser, the latter 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. Rotations may also help to distribute labour requirements for different crops over time.

75. As glyphosate and glufosinate have a low residual activity, carryover restrictions are low. Thus rotation options are increased in principle (Carpenter and Gianessi, 1999). Most persistent herbicides have been forbidden within the last years in Europe. Thus, carryover restrictions are not likely to be further reduced.

76. In the USA, a waiting period of 40 months is recommended (Rohm and Haas (1998) cited in Carpenter and Gianessi (1999)) before planting canola, sugar beet and many vegetables, when imazethapyr and pendimethalin (pursuit plus) are used in soybean. Also, maize can be damaged by imidazolinones used in previous production of soybean.

77. Some rotational constraints of glufosinate or glyphosate resistant crops are described in the Agronomy Guide (1999/2000). These are, for example:

- glufosinate-treated/resistant soybeans;

4 months for alfalfa, clover, cucumbers, peas, peppers, pumpkins, snap beans, sweet maize, tobacco, tomatoes, white potatoes,

2-3 months for grain sorghum, spring oats, winter barley, winter rye, winter wheat

no restriction: field maize

- glyphosate-treated soybeans (treated with 'Touchdown'):

  1-2 months for alfalfa, clover, cucumbers, grain sorghum, peas, peppers, pumpkins, snap beans, sweet maize, tobacco, tomatoes, white potatoes, spring oats, winter barley, winter rye

  no restriction: field maize, winter wheat

- glyphosate-treated soybeans (treated with 'Round-up'):

  no restriction: alfalfa, clover, cucumbers, field maize, grain sorghum, peas, peppers, pumpkins, snap beans, spring oats, sweet maize, tobacco, tomatoes, white potatoes, winter barley, winter rye, winter wheat.

78. In Argentina HR soybeans displaced about 4,600,000 ha of land that had been planted with cotton, maize, orchards, sunflower, horticulture, as well as fallow and pasture land within 5 years (1999-2004) (Pengue, 2004). Fallow seasons that have been used to grow cattle pasture were replaced for continuous agriculture. They are also being 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). It is not known whether conventional soybeans may have been planted instead of HR varieties, if HR varieties had not been available during this time. A homogenisation of production and landscapes has taken place. The option to use glyphosate in crop may have been one driving factor. The main driving factor was the increasing demand for soybeans on the world market.

79. In future, rotations of rice with soybeans may be replaced with herbicide resistant permanent rice (Annou *et al*., 2001). The authors argue that soybeans are pre-eminently planted in these rotations because of weed control problems in permanent rice.

80. A trend towards extended and diversified cropping systems started in Canada in the early nineties. The changes were mainly driven by price reductions of cereal grains and glyphosate (Miller *et al*., 2001; Orson, 2002). These factors were coupled with changes in government policies, development of new markets and soil management practices to avoid environmental degradation (Lindwall and Larney (1993) and Smith and Young (2000) as cited by Zenter (2002)). Coincident with this trend has been a growing popularity of conservation tillage (reduced or no-till) systems (Zentner *et al*., 2002).

81. Some of the land planted with wheat and summer fallow were instead planted with several crop species in Canada in the more moist soil zones. In the (drier) Brown soil zone mainly pulse crops and canola (oilseed rape) replaced wheat and fallow (Zentner *et al*., 2002), and since 1996 HR canola has allowed for further replacement (van Acker *et al*., 2003) (however a complete elimination of fallow from crop rotation is not likely under present and future economic conditions (Zentner *et al*., 2002). The decline in summer fallow acres resulted from a reduction in tillage reaching approx. 5 million additional cropped acres across western Canada (van Acker *et al*., 2003). 22 % of conventional canola growers adopted summer fallow practice in the late nineties. This portion was lower (13 %) with HR canola growing farmers (Canola Council of Canada, 2001).

82. As volunteer canola cannot be assumed to be susceptible to glyphosate and therefore cannot be assumed to be controlled by preseeding glyphosate sprays, volunteers are likely to cause losses in less competitive crops such as flax, lentils or field beans (see section III.4.3; also Simard and Légère (2003)) unless controlled by some other method. Therefore additional preseeding herbicides are necessary, and it is important for these treatments to be applied at an early stage for optimum control (Légère *et al*. 2006). Care must be taken to avoid using volunteer control methods that jeopardise other crops that could follow in rotation, particularly those herbicides that are persistent in the soil (Harker *et al*., 2004; van Acker *et al*., 2003; Johnson *et al*., 2004; Anonymous 2006-Manitoba Guide to Crop Protection).

83. Van Acker *et al*. (2003) stated that the currently added auxin-type herbicides can seriously injure broadleaf crops such as field peas, field beans, lentil, chickpeas and sunflower and make it difficult to maintain them in the rotation. Tank mix control options are available for control of volunteer HR canola prior to field peas, lentils and chickpeas (Anonymous 2006-Manitoba Guide to Crop Protection). In field peas imazamox/imazethapyr and clethodim can be applied (Harker *et al*., 2004). Johnson *et al*. (2004) compiled information on alternative herbicides for the control of volunteer canola in several crops including peas, lentil, chickpea, soybean and faba bean.

II.5 Yields

84. Data from regions where HR crops are commercially grown can result from surveys or statistical reviews or from field tests. Data from other countries are always gained by field tests. Yield differences in surveys and statistical studies may also be due to other reasons, e.g. site, farm size, soil, climate, tillage system, weed abundance, varieties, crop management practices, weed control practices and the education of the farm operators (Zentner *et al*., 2002; Fulton and Keyowski, 1999). Field tests attempt to deliver results under comparable conditions. Their reliability depends on the time scale and the number of typical locations included. Results can differ from year to year and depend on local factors. Thus results of different field tests can also sometimes differ from each other.

85. According to the 1999 report of the American Cotton Producers Yield Committee ‘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).

86. According to the survey of the Canola Council of Canada (2001) the yield was 10 % higher in HR systems compared with conventional farming. Yields of conventional varieties (Smart Open Pol/herbicide resistant, and Conventional Open Pol) were higher than those of glyphosate resistant varieties, whereas glufosinate resistant varieties yielded the same as shown in the Conventional Open Pol in 1999 (Phillips, 2003). Higher yields in HR compared with conventional canola have been described by Cathcart *et al*. (2006) under high weed densities. A recent survey of 425 Manitoba farmers (Mauro & McLachan, 2003) did not indicate that increased yield was an important reason for adoption of HR crops.

87. Yield losses are reported for TT oilseed rape (Robertson *et al*., 2002). However, 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 herbicide resistant canola varieties (TT oilseed rape). Therefore, comparisons of yields between HR and conventional varieties must also consider the genetic backgrounds of the varieties.

88. A study carried out under the European Commission's FACTT Project (Familiarisation and Acceptance of Crops incorporating Transgenic Technology) examined the agronomic performance of transgenic oilseed rape varieties resistant to glufosinate (Liberty) compared to the yield performance of conventional rape hybrids. The results showed that mean yields from the transgenic varieties were either equivalent (Förster *et al*., 1999) or lower (Greenadas and Boothsack, 1999), HR hybrids showed less grain

mass but a higher seed number/pod (Greenadas and Boothsack, 1999), there are no differences in ramification (branching structure) and pod numbers/plant in HR versus non-HR oilseed rape (Förster *et al.*, 1999), hybrid yields of the transgenic varieties showed a higher degree of variability (Greenadas and Boothsack, 1999).

89. An estimated "small yield increase" for HR soybeans (USDA/ERS, 1999) may have been due to production factors as farm size, planting higher-priced crops on better lands, the experience of farm operators and narrow row production (Carpenter and Gianessi, 1999; USDA/ERS, 1999; Gianessi and Carpenter, 2000; Benbrook, 2001). Many field tests of HR varieties and conventional varieties under comparable conditions resulted in the opposite.

90. Elmore *et al.* (2001) showed that (backcross-derived) non-HR soybean lines outyielded (+5 %) the HR-lines. The yield drag could be due to reduced nitrogen fixation by nodules and a weaker defence response after glyphosate application (Benbrook, 2001; King *et al.*, 2001). Recent reviews indicate that effects of glyphosate applications on microbes in HR crops are transient (Cerdeira and Duke, 2006) and that nodule number and mass (which has been correlated with nitrogen fixation) were not affected by the genetic modification itself (Powell *et al.*, 2007; King and Purcell, 2001; van Berkum *et al.*, 1985).

91. With regard to sugar beet different studies indicate that yield in HR production systems could be increased, depending on the crop management practices used. However, sugar beet yield in (improved) integrated production systems (without HR) was not influenced by 15 % ground coverage of the associated weed flora. The weed flora was managed by one or two herbicide sprays (row spraying) and additional cutting or hoeing of large weeds between the rows. The ground cover can even lead to a 7 % higher yield because of an effective aphid control by natural antagonists (Schäufele, 1991; Häni *et al.*, 1990). In a review, total costs savings of €180×10^6 per year were calculated for the area of 1.7×10^6 ha for glyphosate tolerant (tolerant according to the author's wording) sugar beets in the main EU sugar beet-growing countries (Märländer, 2005).

92. II.6 Conclusions on impacts on agricultural practice and agronomy (Section II)

93. Like any significant change in crop choice, HR crops may have various impacts on the agricultural practice and agronomy. Changes may affect weed control, yields, net income, soil tillage and planting and crop rotations. However, because of the positive correlation of other production factors and the adoption of HR it is nearly impossible to attribute statistically evaluated differences to the adoption of herbicide resistant plants alone. Particularly the results (of different studies) on herbicide amounts and application frequencies, yields and net returns are often not consistent.

94. In HR farming postemergence applications increased but preseeding applications decreased in herbicide resistant cotton in Australia and only regionally in herbicide resistant canola.

95. Preseeding applications in HR farming with no-till systems in canola and soybeans had been omitted in the first years. However, farmers resorted to two glyphosate applications (pre- and postemergence) with occasionally added substances in the USA - predominantly in the Midwest – and in western Canada in early planted soybeans and in no-till systems.

96. Glufosinate and glyphosate should be applied according to the label directions depending on the specific weed species and weed size. As the maximum weed size for effective control is higher with glyphosate than other herbicides, the potential time period for spraying is extended.

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

98. Reported changes in application frequencies for soybean and cotton within the first few years were conflicting. Compared to the first few years of HR growing an increase in herbicides, amounts or applications used was found in some soybean and cotton growing regions in the US, in western Canadian canola and in Argentina.

99. Changes in overall amounts of herbicides used are difficult to assess because different herbicides are applied at different rates. A change in the overall volume of applied herbicides must be considered in terms of the change in the environmental impact of them. A change in amounts does not necessarily imply a change in side-effects or number of applications.

100. Reduced herbicide application frequencies can lower soil compaction and erosion by requiring fewer passes with mechanical equipment. It is difficult to determine the effects of changes in herbicide usage because both the total amount introduced into the environment and the number of applications have environmental consequences.

101. Mechanical weed control decreased with the introduction of HR varieties. Conservation tillage can help to prevent soil erosion. There are a variety of factors that can influence adoption of low-tillage practices. HR varieties are often adopted in conjunction with low tillage practice.

102. The level of weed control increased in HR crops in most cases.

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

104. Rotation options in HR agriculture are increased in principle, because glyphosate and glufosinate have a low residual activity, and therefore carryover restrictions are low. However, when crop rotations were used to control weeds, permanent cultivations with herbicide resistant crops can replace the rotations.

105. “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.

SECTION III 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 has changed and will change weed populations. The planting of HR crops is also connected with several changes in weeed control measures. Where HR crops are planted systematically, weeds may be under selection pressure from fewer herbicides modes of action than before and additionally the trend to less tillage or less soil cultivation is more relevant for HR crops. These effects of agricultural practice will reflect in weed communities and populations. As in non-HR crops, the effectiveness of weed control in HR crops can be undermined by the occurrence of less susceptible or resistant plants. This section highlights the different aspects, that can lead to shifts in weed susceptibility and that have to be considered for risk analyses and environmental assessments.

106. Weeds occur in agricultural production fields and are commonly regarded as pests because they compete with the desired 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 those important stages for the crop.

107. The application of selective and/or non-selective herbicides, possibly in combination with mechanical weed control practices (which include hand-pulling, hoeing, mowing, and tillage), are commonly adopted tools across all crops and by farmers across the world. Generally, farmers have the choice to select a weed control program at their disposition. The actual composition of weed communities or population size shows a wide range of variation, depending on local conditions.

108. Any change of the weed management strategy changes weed populations. This also applies for the use of any herbicide in any cropping system, irrespective of whether technology has been used to generate herbicide resistance in the plants or whether it is naturally present.

109. The planting of HR crops is connected with several changes in weed control measures and other agricultural activities such as seeding practice, tillage, or land use. Some of these changes are related to growing HR crops, with the associated herbicide spray regimes, while others are due to government incentives or driven by the world market. The decrease in the numbers of herbicides used, and the trend to less tillage or less cultivation is most relevant for HR crops (see Section II).

110. Where HR crops are planted in sequence, weeds may be under selection pressure from fewer herbicide types (modes of action) than before. In general, selection pressure and gene flow contribute to the evolution of new weed biotypes and to shifts in weed communities. Some changes in weed susceptibility to glyphosate have already resulted in altered weed management techniques and some farming consultants presently propose using additional herbicides in HR crops or to change the pattern of cultivation (see Section II).

111. 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 lower range. As glufosinate is not translocated down into the root system it is not active on perennial structures of weeds. Additionally, weather condition

and diurnal movement of leaves can reduce the control or speed of action (Ammon *et al.*, 1996; Hommel and Pallutt, 2000; Norsworthy *et al.*, 1999; Anonymous, 2009).

112. 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.

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

114. genetic and structural shifts in weed communities and populations as a result of selection pressure exerted by the application of the respective herbicides and the variability in susceptibility of weed species or biotypes (see Section III.1).

115. escape and proliferation of transgenic plants as weedy volunteers (see Section III.2),

116. hybridisation with - and HR-gene introgression into - related weedy species (see Section III.3)

117. All these aspects have to be considered when performing risk/safety analysis or environmental risk assessments for HR crops.

118. III.1 Selection of resistance and weed shifts

119. Glufosinate and glyphosate are generally considered to be low risk herbicides for the evolution of herbicide-resistance in weed populations (Beckie, 2006). The mechanisms of resistance described for weeds resistant to herbicides include target site insensitivity, overproduction of the target protein, herbicide detoxification, reduced herbicide entry, reduced herbicide translocation, and changes in the intracellular accumulation of herbicides. Up until November 2008 a total of 323 herbicide-resistant weed biotypes had been recorded (Heap, 2008).

120. Mutations at the substrate binding site of the target enzyme of glufosinate, the glutamine synthetase, leading to glufosinate resistance via an insensitive target site, are thought to result in low-fitness biotypes or to be lethal (Böger, 2000).

121. Possible reasons for the low resistance risk of glyphosate are suggested to be the timing of application, low occurance 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 lack of soil persistence, lack of residual activity, plants limited uptake from the soil (Böger, 1994), and its application pattern are considered as further reasons as to why the evolution of resistance to glyphosate may evolve rather slowly (Heap, 2008; Baylis, 2000; Nap *et al.*, 1996; Jasieniuk, 1995).

122. The larger the areas planted with crops resistant to the same herbicide, and the more frequent the herbicide applications, the sooner resistance is likely to evolve (Darmency, 1996). The probability of developing resistance to herbicides is comparatively low when the size of the area and/or the weed seed population is small (Diggle *et al.*, 2003).

123. 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). But unless the HR crop is a winter annual, a non-selective herbicide cannot be applied in one particular crop in all three of these seasons.

124. Herbicide resistance is dispersed world-wide, weed species adapted to current agroecosystems are reported, and naturally resistant weed species appear to be increasing. While weed population shifts and the evolution of herbicide resistance are inevitable consequences of the use of herbicide resistant crops and the adapted herbicide(s), the relative economic importance will depend on the specific agroecosystem (Owen and Zelaya, 2005)

125. Hartzler (2003) stated that it makes good sense for farmers to implement a long-term plan to reduce the selection pressure placed on weeds by glyphosate. The simplest way to reduce resistance selection is to avoid using glyphosate as the only weed management tool and to use additional measures to reduce resistance e.g. crop rotation away from continuous glyphosate-tolerant crops, mixtures or sequences with other herbicides (diversified weed management practice, Harker *et al*. (2005)).

126. A combination and rotation of weed management methods is essential to delay resistance evolution in weeds (Ghersa *et al*., 2000; Kropff and Walter, 2000; Bastiaans *et al*., 2000; Heap, 2008; HRAC, 2000; Wolfe, 2000; Ballare and Casal, 2000; Flint *et al*., 2005; Kruger *et al*., 2009). Common weed resistance management practice includes:

- crop rotation, changing the composition of weed populations
- reduced herbicide use and rotation of herbicide mode of action (MOA)
- rotation of cultural practices reducing reliance on herbicides
- alternating sowing times giving crops a competitive advantage over relevant weeds
- "integrated pest management" (IPM) adapted specifically for weed management
- more elaborate scouting, acquiring better knowledge about the types of weeds
- manipulation of light environment during tillage reducing seedling emergence
- additional measures: i.e. cover crops, mixed cropping, manipulation of row width, fallow.

127. In general, fitness penalties can occur in resistant weeds but the fitness of resistant weed biotypes is not always lower than the fitness 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 overexpression (EPSPS overproduction in case of glyphosate) or detoxification is likely to have a significant cost of resistance. These biotypes are likely to disappear when the herbicide is changed to forego selection pressure. The probability, frequency and significance of fitness penalties are not well-understood for all weeds in all cropping systems and experts' estimations are mixed regarding the question. This also regards the frequency of negative cross-resistance to other herbicides as found in triazine-resistant *Echinochloa crus-galli* and *Conyza canadensis* (Gadamski *et al*., 2000).

128. Development of herbicide resistance in different weed populations (against any herbicide) need not necessarily be a consequence of a spread from a few initial sites but may also result from independent evolutionary events (Mortimer, 1993; McNaughton *et al*., 2005).

III.1.1 Cross resistance and multiple resistance

129. *Multiple resistance* is defined as the expression of more than one resistant mechanism within individuals or populations, with the consequence that these plants or populations can exhibit resistance to more than one herbicide mode of action. It 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. *Cross resistance* is defined as the expression of one genetically-endowed mechanism conferring the ability to withstand herbicides from different chemical classes (Powles and Preston, 1995; Debrah *et al*., 1999).

130. Multiple resistant weeds have been reported in several regions, including Europe. The mechanism of multiple resistance of *Lolium rigidum* (rigid ryegrass) seems to be due to the induction of several herbicide degradation enzymes. The evolution of a multiple resistant rigid ryegrass biotype in South Africa may serve as an additional example showing triple resistance to ACCase- inhibitors (herbicides inhibiting acetyl CoA carboxylase), ALS-inhibitors, and glyphosate (Heap, 2008). Multiple resistant *Brassica napus* volunteers in Canada were due to pollen flow between adjacently-planted resistant varieties (Hall *et al*., 2000).

III.1.2 Current weed control limitations of glyphosate and glufosinate

131. Weed species or populations which are difficult to control are specified in www.weedsciences.org (Heap, 2008) and updated regularly.

132. Although the development of resistance to glyphosate was thought to be unlikely (Jasieniuk, 1995), three weed species developed resistance to glyphosate in conventional cropping systems (Heap, 2008): rigid ryegrass (*Lolium rigidum*), Italian ryegrass (*Lolium multiflorum*) and goosegrass (*Eleusine indica*). The first two glyphosate-resistant rigid ryegrass populations were found in Australia in 1996 and 1997, the following in California (1998) and South Africa (2001). Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) was confirmed in 2008 by Norsworthy *et al*., however, the resistant biotype was effectively controlled by most evaluated herbicides.

133. In addition, horseweed (*Conyza canadensis*, also named *Erigeron canadensis*, marestail or fleabane) waterhemp (*Amaranthus rudis*) and buckhorn plantain (*Plantago lanceolata*) were found to be tolerant or resistant to glyphosate. In case of *Conyza canadensis* this is due to a translocation resistance (Feng *et al*., 2004). Further species which are difficult to control have occurred in Argentina and South Africa. The question whether these species developed resistance traits or are dominating the flora because they are "naturally" resistant or insufficiently controlled due to changing agronomic practices is not fully answered. Also weed-to-weed interactions have to be considered. Zelaya *et al*. (2007) confirmed successful introgression of the glyphosate resistance allele of *Conyza canadensis* in hybrids with under greenhouse conditions.

134. The Australian glyphosate-resistant *Lolium rigidum* biotype is 9- to 10-fold more resistant to glyphosate and also acquired a 3-fold higher tolerance[2] to diclofop-methyl relative to susceptible biotypes. The resistance in Australian populations of *L. rigidum* occurred after 15-20 years of glyphosate use (Pratley *et al*., 1999), in Chile (*L. multiflorum*) after 8-10 years (3 applications a year), and in Malaysia (*E. indica*) after 10 years (8 applications a year, first report in 1997). The resistance was obtained in conventional cropping systems (Lee and Ngim, 2000; Heap, 2008).

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[2] The term "tolerance" is used by the cited authors but it may be resistant biotypes.

135. The exclusive reliance on glyphosate as the main tool for weed management results in agroecosystems biologically more prone to glyphosate resistance evolution (Vila-Aiub *et al*., 2008). However, where diversity in weed management systems is maintained, weed control by glyphosate can be sustainable (Powels, 2008).

136. The mechanisms of resistance against glyphosate may be cellular or biochemical. They have not been fully researched (Pratley *et al*., 1999; Feng *et al*., 1999; Lee and Ngim, 2000). Possible mechanisms of resistance may include a different sensitivity of EPSPS to glyphosate and the overexpresion of EPSPS. The reported doubled level of EPSPS could (at least in part) explain glyphosate-resistance in *L. rigidum* biotypes (Gressel, 2000). The glyphosate-resistance of *E. indica* biotypes seems to be due to an altered binding site, a proline to serine substitution of the EPSPS enzyme preventing glyphosate from binding (Doll, 2000), a mechanism that was considered unlikely to confer resistance to glyphosate in weedy plants (Jasieniuk, 1995).

137. No glufosinate-resistant weed biotype has been recorded so far though weed species with lower sensitivity to glufosinate are known (Nap and Metz, 1996a; Jansen *et al*., 2000; Hommel and Pallutt, 2000). Although, *Populus* spec. (poplars), transformed for elevated level of glutamine synthetase (the target enzyme of glufosinate) to enhance nitrogen utilisation, have been found to be more resistant to glufosinate (Gressel, 2000), however this is not unexpected, given that overexpression of the target enzyme is a known resistance mechanisms, and the first glufosinate-resistant plant cell lines were based on this mechanism.

III.2 Seed escape and proliferation of transgenic plants

138. *Volunteers* are crop plants 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 preventative methods (e.g. methods to reduce harvest losses and wide rotations) can be applied (Darmency, 1996; Bjerregaard *et al*., 1997; van Acker, 2003).

139. While some crops are ready volunteers and can easily build up feral populations, e.g. oilseed rape, because of 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 (in the field) and feral populations (in off-field habitats) of crops which are not native to a region tend to have a lower chance of surviving and cause fewer problems.

140. The reproductive rate, growth habit and germination ecology of oilseed rape are similar to typical weed species (Kloepffer *et al*., 1999). 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). 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 seed bank (Pivard *et al*., 2008). Knispel and 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. Volunteer management should therefore be a multi-scale approach.

141. Farmers who rotate both glyphosate resistant maize and soybeans already use an additional herbicide (e.g. “Select” (clethodim)) to control volunteer maize in soybeans (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 have no dormancy and germinate readily under favourable conditions. Volunteer HR soybeans must be controlled with other herbicide mixes at postemergence (Benbrook, 2000).

III.3 HR-gene flow to volunteers or interfertile weeds

III.3.1 Variability of gene flow

142. In recent years, extensive data has been collected from field experiments with regard to gene transfer frequencies. The frequency of outcrossing ranges for oilseed rape from 3.7 %–0.000 3 % on distances from 1m–2500m (Stringham & Downey, 1982; Scheffler *et al*., 1993; Pauk *et al*., 1995; Timmons *et al*., 1995, 1996), for sugar beet from 17.1 %–0.02 % on distances from 0m–200m (Archimowitsch (1949) by Free (1970); Dark, 1971, Jensen & Bogh (1941) by Dark (1971), Champolivier *et al*., 1999; Vigouroux *et al*. (1999) by van de Wiel and Lotz (2006); Bartsch *et al*. (2003) by van de Wiel and Lotz (2006), in Saeglitz *et al*. (2000) up to 83 %), for cotton from 4.7 %–0.0 % on distances from 1m–35m (Green & Jones (1953) by LaSota (1992); Fitt, 1994; Kareiva *et al*., 1994; Llewellyn & Fitt, 1996; Simpson & Duncan (1956) by LaSota (1992); Umbeck *et al*., 1991; Llewellyn *et al*. (2006) up to 30 % only for adjacent fields). For rice Lu and Snow (2005) reviewed data and provide hybridisation rates for crop-to-crop hybridisation with 0.005 to 0.52 %, crop-to-weed with 0.01 to 1 % and crop-to-wild with less than 3 %, maximum distance for outcrossing was 110 m. For soybean Bao-Rong (2005) reported outcrossing rates in field conditions from below 1% to 4.5% and up to 19%.

143. A high variation of results was shown depending on the distance and local or climatical conditions (Gliddon, 1999). Wind direction and wind speed, climate, variability of the pollination system between varieties of the same species, diversity, abundance and behaviour of pollinators (sometime influenced by land marks) and the size of the pollen donor and acceptor populations are main influencing factors. Different genotypes or varieties sometimes show different frequencies of cross-pollination (Gliddon, 1999; Ford-Lloyd, 1998; Simpson *et al*., 1999; Rieger *et al*., 1999; van Acker *et al*., 2003). Even self-pollinating plants do cross-pollinate at low or very low levels depending on the genotype. In general, plants are called self-pollinating when the level of cross-pollination does not exceed 10 %. Male sterile plants for seed production and hybrid varieties exhibit higher levels of cross pollination due to the reduced pollen competition (Thompson *et al*., 1999; Feldmann, 2000).

144. Pollen flow was observed in distances up to 26 km for oilseed rape (Ramsay *et al*., 2003). Pollen beetles may also contribute to this long distance pollen dispersal. The hypothesis of a negative correlation between distance of referring plants and cross pollination was mainly disproved when insect pollination occurred 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. According to Squire *et al*. (1999) and Timmons *et al*. (1999) gene flow should be considered at the landscape level. Information on intra- and interspecific fertilisation was summarised in the respective OECD Consensus Documents on crop plants.

III.3.2 General relevance of HR-gene flow to volunteers

145. Double or multiple resistance against herbicides can occur in volunteer plants (Hall *et al*., 2000; Downey, 1999). Some of these plants emerged with unwanted herbicide traits due to seed lot impurities or due to former outcrossing events between fields.

146. This mechanism also refers to conventionally generated HR crops. Hall *et al*. (2000) also found resistant volunteers after planting conventional imdazolinon resistant oilseed rape.

III.3.3 General relevance of HR gene flow to interfertile weeds

147. Weeds can also become resistant to herbicides through hybridisation with compatible HR crops (both transgenic and conventional varieties), followed by backcrosses and introgression.

148. As true for any crop, the opportunity for hybridising with compatible relatives may also increase with increasing HR growing areas.

149. Cross pollination is a prerequisite for hybridisation which means the ability to produce viable progeny. Generally, hybridisation frequencies are lower than cross pollination frequencies between individuals of the same species.

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

151. 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.

152. 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 (Feldmann, 2000; Chèvre *et al.*, 1999; Kloepffer *et al.*, 1999; Dietz-Pfeilstetter and Kirchner, 1998; Schütte, 1998; Darmency, 2000)."

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

154. According to Colwell (1994), a "rare" hybridisation 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.

155. 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[3] trait and could be different in crops and in weeds due to different genetic backgrounds (Snow and Jørgensen, 1999; DETR, 2000). The fitness of hybrids should be assessed from species to species (see below). But even genotypes with a lower fitness may survive if the pollen flow is steady and the source is large (Gliddon, 1999).

156. If a certain proportion of a weed population acquired herbicide-resistance then a certain proportion of the shed seeds will carry herbicide-resistance. These seeds will remain in the weed seedbank and some will germinate in years to come, considerably prolonging infestation with herbicide-resistant weeds (Bjerregaard *et al.*, 1997; Darmency and Renard, 1992).

157. Additional or alternative weed control methods for such weeds may have to be employed.

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[3] The term "tolerance" is used by the cited authors but it may be resistant biotypes.

III.4 Aspects of changes in weed and volunteer susceptibility for specific crops

III.4.1 Maize, Zea mays L. ssp. mays

158. The probability of growing low levels of unwanted HR maize depends on many aspects in farming, such as field sizes, crop rotations, weather conditions, on - to a very small extent in maize - the abundance of pollinators. The most important factor in US and European maize production is the seed production management.

159. HR maize volunteers could occur in warm regions. In many colder regions (where maize volunteers do not survive low temperatures) adventitious HR maize plants may only emerge due to impure seed. In Northern and Central Europe maize is unlikely to develop volunteers due to its sensitivity to low temperatures (Bjerregaard *et al*., 1997; Neuroth, 1997; Niebur, 1993) and its inability to shed seeds naturally (Bock *et al*., 2002).

160. The potential for transfer of traits from (transgenic) maize to teosinte is real (Niebur, 1993; Colwell, 1994). Wild forms occur in Mexico and Guatemala (Colwell, 1994; OECD 2003). Hybrids, surviving in nature, have been found (Baltazar and Schoper, 2002).

161. The consequences of seed exchange and (intra- or interspecific) cross pollination are important to consider in Mexico and other centres of diversity of maize (Alvarez-Monzales, 2002).

162. Since there are no wild relatives in Europe and many other countries where maize is grown, gene transfer to wild or weedy species is unlikely (Bjerregaard *et al*., 1997).

III.4.2 Cotton, Gossypium spp.

163. Most seeds of modern cultivars do not survive more than one season – in contrast to wild cotton (Jenkins, 1993). Wild and feral *Gossypium* species can occur (OECD, 2008).

164. In Australia the control of cotton volunteers and ratoon cotton (plants re-grown from left-over rootstock from a previous season) is one of the major crop management issues associated with using the HR technology (Taylor and Charles, 2002). A crop management plan to prevent weed escapes setting seeds should maintain the viability of the HR system in the medium term (Charles and Taylor, 2006, Werth *et al.*, 2008). In Australia the most noticeable shift with the adoption of HR cotton has been to increase the prevalence of two groups of broadleaf plants, *Conyza* sp and *Ipomoea* sp, in the cotton farming system (Werth *et al*., 2006).

165. Cross pollination in cotton rarely occurs compared to e.g. sugar beet or oilseed rape (OECD, 2008).

166. In regions where wild relatives occur, extra attention should be paid to field isolation of diploid cotton crops to wild diploid cotton (Jenkins, 1993). Attention may be paid to regions where the two cultivated tetraploid species are cultivated and are brought within range of tetraploid wild species or primordially wild populations as spontaneous (unaided) hybridisation might occur (OECD, 2008).

III.4.3 Oilseed rape, Brassica napus

167. 28 % of 425 farmers in Manitoba (Canada) had experienced herbicide resistant volunteer oilseed rape. Harvest and transport losses account for many of them. 52 % of those farmers indicated that the volunteers came from outside their operation (Mauro and McLachlan, 2003) and are thus adventitious plants (with resistance to either of the herbicides used in herbicide resistant plants) resulting from

outcrossing or seed impurities. Adventitious plants can also emerge from the soil seedbank depending on the cropping history (e.g. former gene flow from other sites/fields or impurity of former seed - both in combination with harvest losses).

168. Multiple-resistant oilseed rape volunteers exhibiting resistance to glyphosate and glufosinate, and/or to imidazolinone herbicides have been reported from Canada (Hall *et al*., 2000; Beckie *et al*., 2001). Environmental safety/risk assessments must take into account any other traits in the species that have been released into the environment (e.g. trait stacking that occurs as a result of natural processes in the environment).

169. Results of a Canadian study (Beckie *et al*., 2004) suggest that oilseed rape plants with multiple herbicide resistance traits are as sensitive to alternative herbicides for volunteer control as plants without herbicide resistance traits.

170. A substantial amount of oilseed rape seeds is lost at harvest regardless of the harvesting method. Losses of about 6 % (about 120 kg/ha when 2000 kg are harvested) are typical in Canada (Gulden *et al*., 2003). Seed loss is estimated to be between 200 to 300 kg/ha on the average, corresponding to 5,000 – 7,000 seeds/m² in Europe (Pekrun *et al*., 1998). The amounts lost from harvesters and trucks at field margins and along road sides/rail road tracks can be considerable (Neemann *et al*., 1999).

171. Feral descendants of oilseed rape exist in close proximity to rape crop fields throughout the arable land of Central and Western Europe (Squire *et al*., 1999; Menzel and Mathes, 1999; Kloepffer *et al*., 1999; Timmons *et al*., 1996). They are mostly derived from seed spills. Nevertheless, gene flow from fields is expected to be of much greater importance than from a few feral pollen donor plants (van Acker, 2003).

172. Many of these feral populations are not routinely controlled and as detected by Wilkinson *et al.* (2003) the hybridisation frequency can vary between regions. Feral populations outside fields experience a wide range of selection pressures, leading to diverse forms including individuals that flower when the plant is young or at unusual times in the season. *B. napus* feral plants have been found together with sexually compatible wild relatives such as wild radish (*Raphanus raphanistrum*), wild mustard (*Sinapis arvensis)*, and white mustard (*Sinapis alba*), showing overlapping flowering periods (Feldmann, 2000; Menzel and Mathes, 1999).

173. The level of HR genes is usually below 0.25 % in conventional seed in Canada (Orson, 2002). Given current knowledge, it is unlikely that pollen flow could cause contaminations greater than 0.1 % in a single generation of pedigreed seed production. The observed levels greater than 0.25 % were therefore either due to mechanical mixing during harvest or handling or earlier generations of pedigreed seed production. (i.e. Breeder or Foundation Seed; van Acker *et al*., 2003; Downey and Beckie, 2002). Nevertheless, the sowing of a conventional variety will almost certainly result in a population of herbicide resistant plants in a field even when a variety purity standard of 0.25 % is met. This scenario is based on typical rates of harvest losses and plant establishment (Downey and Beckie, 2002). Similar results are stated for Europe (Devos *et al*., 2004; Bock *et al*., 2002).

174. Van Acker (2003) stated that the spread of herbicide resistance traits in western Canada will be greater than measured in outcrossing experiments (Beckie *et al*., 2001) because of the large number of oilseed rape acres (which leads to higher outcrossing rates - Staniland *et al*., 2000), the high rotational frequency, the high abundance of volunteer oilseed rape (Thomas *et al*., 1998a, 1998b; Gulden *et al*., 2003; Leeson *et al*. 2002; Thomas *et al*. (1996) cited in van Acker (2003)), the relatively high level of volunteer oilseed rape survival to flowering (Leeson *et al*., 2002; Thomas *et al*., 1996; Simard *et al*., 2002; Légère *et al*., 2001) and the selection by glyphosate in low disturbance tillage systems. Particularly the latter aspect

led to his conclusion that there is a "transgene bridge" for glyphosate resistance. Nevertheless, the study on gene flow between relatively large adjacent oilseed rape fields (25-100 ha) indicates that the level of outcrossing is much less than 1 % (a maximum of about 0.2 % was observed) (Rieger *et al.*, 1999).

175. To date there is no evidence of selection of herbicide resistant biotypes in unrelated weed species due to herbicides use patterns in HR oilseed rape production in Canada (Warwick *et al.*, 2004). However, Warwick *et al.* (2008) confirmed the persistence of the HR trait over 6 years in Canadian populations of *Brassica rapa* in the absence of herbicide selection pressure.

176. Although GM HT oilseed rape has so far only been grown on a limited scale in Australia since 2006, seedbank analysis of Baker and Preston (2008) indicate a rapid decline of oilseed rape seeds and volunteers in managed cropping systems. Therefore, it is unlikely that HR oilseed rape will become a major weed if volunteers are managed carefully.

III.4.4 Rice, Oryza sativa

177. There are easily shattering forms and those with dormancy characteristics and long-living seeds in both types of *O. sativa*, Japonica and Indica.

178. Intraspecific outcrossing rates are high in wild perennial rices (up to 50 %) and occur up to a rate of 5 % in cultivated rice (i.e. *O. sativa*: Japonica and Indica groups and – in Africa - also *O. glaberrina*) (OECD, 1999d). Hybrids between plants from the Indica and Japanica groups sometimes become vigorous (OECD, 1999d). Gealy (2005) gives in a review article a maximum of 34 % for rice-red rice outcrossing in male-sterile systems and a maximum of 0.7 % for interspecific outcrossing.

179. Wild and weedy conspecific *O. rufipogon*/*O. nivara* and red rices are sexually compatible with cultivated rice. The variation between perennial (*O. rufipogon*) and annual (*O. nivara*) types is nearly continuous. Extensive populations of *O. rufipogon* and/or *O. nivara* occur in India, Sri Lanka, Laos, Indonesia, Thailand, Cambodia and Vietnam. Smaller populations are found in China and other Asian countries (Vaughan (1994) and Bellon *et al.* (1998) cited in Cohen *et al.* (1999)). Wild conspecific rices grow throughout the tropics of Asia, Africa, Oceania and Latin America (OECD, 1999d). Intraspecific hybridisation between cultivated rice and their weedy relatives occurs in many growing areas (OECD, 1999d).

III.4.5 Soybeans, Glycine max L. Merrill

180. In continuously grown glyphosate resistant soybean, resistance in *Conyza canadensis* (horseweed) was detected within 3 years. Glyphosate was the only herbicide used in this cropping system (van Gessel, 2001). It has to be taken into account that glyphosate was used several years before the introduction of HR crops and the selection of resistant weeds might have started much earlier (Cerdiera and Duke, 2006). The percentage of glyphosate resistant soybean fields treated with an additional pre-emergence herbicide has significantly increased in areas where the HR soybeans have been planted for many years (Hartzler, 2003). In addition, glyphosate is sprayed at higher rates. Waterhemp (*Amaranthus rudis*) may be largely responsible for these changes in weed management.

181. In Europe, soybeans are not weedy (Bjerregaard *et al.*, 1997). Seeds are dispersed by pod shattering, particularly if harvest is delayed. Seed dormancy is rarely displayed in cultivated soybeans (OECD, 2000).

182. In colder growing regions, volunteers are usually not a concern, because most seeds germinate within a few weeks after harvest and die during the winter. Soybean volunteers are sometimes present in US cotton and must be controlled (Hayes, cited in Benbrook (2000)). HR resistant soybean volunteers

occurred in Argentina and Uruguay (Iglesias, 2003). Survival of soybean volunteers is only likely in the warmer, northern parts of these countries.

183. Volunteer HR soybean is a management concern in cotton crops (Canola Council of Canada, 2005) and should be controlled by residual pre-emergence herbicides cotoran/meturon or caparol/cotton pro. Caparol or cotton pro [prometryn] or karmex/direx [diuron] both mixed with MSMA must be applicated at postemergence against soybean volunteers (Benbrook, 2000). Broadcast postemergent applications will damage the cotton crop (Griffin, 2004).

184. Naturally occurring hybrids between the sub-genera *Soja* and *Glycine* have not been observed (Beversdorf, 1993; OECD, 2000), and attempts to hybridise between species of the subgenera were unsuccessful (Bao-Rong, 2005).

185. Of the same sub-genus (i.e. *Soja*), cultivated soybeans (*Glycine max*) can easily be crossed with the two other species of the same subgenus *G. soja* and *G. gracilis*. Both species are wild or semi-wild. *G. gracilis* is a weed in northeast China. *Glycine soja* is native to China, Japan, Korea, eastern Russia and Taiwan. The subgenus *Glycine* comprises of at least 16 wild perennial species, growing in Australia, Asia and the southern Pacific islands (Bao-Rong, 2005).

186. Outcrossing rates of soybean in field conditions is usually less than 1 %. But also outcrossing rates of as high as 4.5 % were observed. Gene flow to wild and weedy soybeans was estimated by multilocus analysis to range from 9.3 % to 19 %. In field tests a maximum of 5.8 % natural hybridisation between cultivated and wild soybeans was reported. Gene transfer by cross-pollination from cultivated soybeans to weedy relatives could therefore become a concern in those regions, where *G. soya* and *G. gracilis* occur (Bao-Rong, 2005).

III.4.6 Sugar beet, Beta vulgaris ssp. vulgaris var. altissima,

187. Sugar beets are biennial and are harvested in the first year of growth, before the second year when they are able to set seed. Sugar beet volunteer plants are therefore rare (Bjerregaard *et al*., 1997). However, bolters (individuals which flower in the first year), which are usually far below 1 % in the crop, can produce flowers which can result in gene flow and/or volunteers.

188. If HR volunteers are not removed before flowering, they can pollinate wild or non-resistant beets, thereby resulting in HR weed beets. It is difficult to distinguish between cultivated beets and weed beets, which are sometimes found in spring cereals (seldom in winter cereals). They are common and difficult to control in potato and peas due to similar cultivation techniques and a limited selection of herbicides (OECD, 2001). According to Vigouroux *et al*. (1999) hybridisation between annual weed beets and cultivated HR beet will happen when HR varieties are grown. Hybrids are formed in the wild and in seed-production fields (Bartsch *et al*. (1999) cited in OECD (2001)). One of four fields in England will have viable beet seed in the topsoil (Hojland and Pederson (1994) cited in OECD (2001)). Annual forms of *Beta vulgaris spp*. occur in Italy and France where European sugar beet seeds are generally produced.

189. The maritime (wild) beet (*Beta vulgaris* L. *ssp. maritima*) is considered to be the ancestor of *Beta*-beets (sugar beet, fodder beet, red beet, and chard) and occurs in coastal regions of Europe, in India, Canary Islands, Maderia, Central and South America, California and Australia (OECD, 2001; Tab. 3; Bartsch *et al*., 1993; Gerdemann-Knörck and Tegeder, 1997; Bartsch and Ellstrand, 1999; Desplanque *et al*., 1999; Driessen *et al*., 2001).

190. There is extensive evidence of hybridisation between cultivated and wild beet (Bartsch *et al*., 1993; OECD, 2001). Hybrids are often vigorous.

191. Cultivated beets as well as a range of wild forms belong to the section *Beta*, all of which are sexually compatible and generate fertile offspring (OECD, 2001). They may thus be considered to be members of the same collective species. In California, hybrids between *B. vulgaris* and *B. macrocarpa* are reported to cause weed problems in sugar beet fields (Biancardi *et al*., 2005; Bjerregaard *et al*., 1997).

III.4.7 Wheat, Triticum aestivum

192. Cereal volunteers occur for example in sugar beet, cereals and oilseed rape fields. Volunteer wheat can persist at least 5 years in 5 % to 10 % of fields (Thomas and Leeson (2000) cited in van Acker *et al*. (2003)). It is difficult to distinguish between new and old seeds because the seed longevity is unsure (Harker *et al*., 2005).

193. Wheat is a basically autogamous plant (1 % allogamy that varies according to variety and is higher in hybrids). Outcrossing can be induced by applying water stress (Briggs *et al*., 1999). Outcrossing occurs at rates of 1 % to 2 % and below increasing up to 3.7 % to 9.7 % in warm dry weather (OECD, 1999a). Depending on cultivar and distance Waines and Hedge (2003) reported estimated outcrossing rates up to 6.7 % and for male-sterile systems of up to 90 %. The cross-pollination rate of wheat is about 10 % according to the CFIA 1999. Therefore HR traits could be transferred to non-resistant plants, and additional management is required to minimise pollen flow, especially in rotations where wheat is grown frequently (Anderson and Soper, 2003). However, a tolerance level of 0% transgenic wheat in non-transgenic wheat grain is unrealistic.

194. Pollination within 200m distance remains below 0.5% (Matuz-Cadiz *et al*., 2004) and declines with distance from the source (Matuz-Cadiz *et al*., 2004; Stoskopf and Rai, 1972).

195. Interspecific hybridisation is possible (Claesson *et al*., 1990). Hybridisation with wild relatives does occur in the Mediterranean. Potential interfertile species are listed within the Consensus Document on *Triticum aestivum* (OECD (1999a), natural wild crosses with members of the genus *Aegilops*: van Slagern (1994) cited in OECD (1999a), pp. 21). In the western United States the weed species *Aegilops cylindrica* (jointed goatgrass) can form hybrids with wheat (Seefeldt *et al*., 1999).

196. Volunteer persistence and density of wheat can be similar to oilseed rape in individual cases (van Acker *et al*., 2003). Therefore concerns and recommendations for oilseed rape are probably relevant for wheat too: Glyphosate resistant wheat could contribute to a gene bridge. Still, the cross-pollination rate of wheat (10 %, CFIA (1999)) is lower than that of oilseed rape (20-30 %, CFIA (1994)). Seed persistence of wheat is clearly lower compared with oilseed rape, and there is evidence that the introduction of glyphosate resistant spring wheat will not increase the short-term agronomic or environmental risk related to volunteer wheat (Harker *et al*., 2005).

III.5 Conclusions on changes in weed and volunteer susceptibility (Section III)

197. The effects of changing weed control (and agricultural practice) on HR crops will result in changes in weed communities and populations. In general, the more often a specific herbicide is applied on the same field, the more probable a weed spectrum shift (to less susceptible species) will be. Effects of the same transgenic HR crop can vary depending on the agricultural ecosystem.

198. In HR crops the potential decrease in numbers of used herbicides (active substances) (discussed in Section II.2.2) and the trend to less soil cultivation can put a different 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 some regions.

199. The data presented makes it reasonable to assume that resistance/ tolerance to glyphosate will develop if this herbicide is used frequently enough in high numbers of crop fields. Resistance may evolve not earlier than after 10 to 20 years of glyphosate use, as glyphosate is not sprayed more than 1 to 3 times in currently planted HR crops.

200. Several weed species have caused control problems within the last years in connection with the frequent use of glyphosate in non-HR and HR varieties and with changed agricultural practice in parts of the USA and Argentina. Weed shifts are more likely than resistant weed biotypes to result in further changes to weed management practices as they have been reported since roughly 5 years after the first approval of HR crops.

201. In the last few years many weed scientists have recommended using additional herbicide modes of action to control HR ratton cotton and multiple applications/additional substances to control resistant weeds in HR soybean.

202. In a crop rotation with soybeans and maize or cotton, all crops being glyphosate-resistant, the selection pressure on weeds is very high and weed shifts are most likely. The continuous application of glyphosate is also contraindicated wherever a weed species (depending on its germination pattern) is abundant in large quantities at both preplant and postemergence.

203. A combination and rotation of weed management methods is essential to delay resistance evolution in weeds.

204. Crops with characteristics such as easy shattering and persistence of seed are likely to emerge as volunteer plants. Problems with HR volunteers will be more common with crops which easily occur as volunteers and exhibit high outcrossing rates. However, volunteer management is not a new problem which is specific for HR crops. There are several alternative herbicides that are used for volunteer control.

205. The risk of changes in weed susceptibility caused by selection and gene flow to volunteers is considered to be higher compared to changes due to gene flow to weeds in most cropping areas. However, there are regions where highly interfertile weeds are abundant. In that case management proirities may be different.

206. The transfer of HR genes to relatives should be taken into account in centres of crop origin and regions where interfertile and weedy hybrids occur.

SECTION IV IMPACTS ON BIODIVERSITY

The relevance of conservation and protection or even restoration of native biodiversity is acknowledged by all countries. In general, direct and indirect impacts of HR crops on biodiversity are related to agricultural practices like herbicide use, cropping system, etc. The goal of this section is to review the available information of possible impacts of HR cropping systems on biodiversity and to give an overview on aspects that have to be considered, like baseline comparators, direct effects of the toxicity of the used herbicides and effects that can be attributed to the growing of HR crops and further aspects of sustainable agriculture and possible mitigation of environmental effects. This section also refers to the fact that different countries may have different management goals to protect biodiversity.

207. The relevance of biodiversity itself and the implementation of modes to preserve it as well as the sustainable use of biodiversity is a widely shared goal. Its importance is underscored by the fact that the political intention to achieve this is laid down within the Convention on Biological Diversity (CBD, www.cbd.int).

208. One of the prevailing management goals in regions where most of the land is under cultivation is to stop or to reverse the decrease of biodiversity in agriculture. In Germany, for example, agricultural and forested lands make up 84 % of the total area and an additional 11 % are sealed by streets, buildings, etc. In the UK over 70 % - 75 % of the land is farmed (Hails, 2002; Robinson and Sutherland, 2002). The decrease in farmland biodiversity indicated by the decrease of farmland birds is also an important issue in the USA and Canada. The significance and relevance of biodiversity to agriculture have widely been assessed (El Titi and Landes, 1990; van Emden 1990; Wijnands and Kroonen-Backbier, 1993; Lewis *et al*., 1997). Many potential pests are still controlled by natural antagonists, and decreasing the latter could lead to higher pesticide inputs substituting 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 *et al*. (1997)).

209. However, in some countries with a high level of exotic species in the agricultural environment, the management goals for conserving native biodiversity might be different (e.g. Australia).

210. Herbicide resistance does not increase the fitness and invasiveness of plants in semi-natural or natural habitats (Dale *et al*., 2002; Crawley *et al*., 2001). Therefore, direct and indirect impacts of HR crops on biodiversity are related to farming practices in general. Changes in farming practices due to the cultivation of HR crops may include changes in crop rotations, planting and spacing of the crops, soil tillage, pesticide applications, use of fertilisers and so forth. In addition, the assessment of species diversity within an agricultural field is dependent upon the type and choice of species used as baseline comparators.

211. Off-field habitats can be affected indirectly when herbicide resistant weeds evolve or invade. Additional herbicides may be used in such a case and the species composition may change.

212. In general, when performing risk/safety analysis or environmental risk assessments for HR crops the following aspects have to be taken into consideration:

- the chosen baseline comparators and already existing effects of conventional agriculture (Sec. IV.1)
- indirect effects of changes in agricultural practice (Sec. IV.2)
- direct effects of the toxicity of the used herbicides glyphosate and glufosinate (Sec. IV.3)
- direct and indirect effects that can be attributed to the growing of HR crops (Sec. IV.4)
- further aspects of sustainable agriculture and possible mitigation of environmental effects (Sec. IV.5)

213. Potential environmental impacts of growing HR crops can be assessed by using techniques like life-cycle assessment (Bennet *et al.*, 2006), bow-tie risk management (Pidgeon *et al.*, 2007) or other conventional system safety techniques (fault tree analysis, casual factor charting, and event tree analysis). All these techniques involve a certain subjectivity that can never be eliminated. Efforts have to be made to make the assumptions and decisions transparent.

IV.1 Effects of conventional agriculture

214. The effects of agronomic management practices in conventional cropping systems are described in order to give a reference system for the new weed control practice within the covered HR plants.

215. Over the period of increasing herbicide use (1950-1985), species diversity (measured as number of species) of the associated agricultural flora was reduced by 30 % - 70 % in Germany (Hanf, 1985). The reservoir of seeds in soil has been reduced from 30,000-300,000 seeds/m^2 to 1,000 - 2,500 seeds/m^2 within the last decades (Koch and Kunisch, 1998).

216. Many insect species depend on a specific plant species during early larval stages, which makes each plant species essential for an average of 10-12 insect species in northern Europe (Heydemann, 1983). In Germany, this dependency and the decrease of floral diversity partly led to the decline of epigeal (soil dwelling) arthropod fauna species diversity by 45 % - 85 % (Heydemann, 1983). Their biomass decreased even further (Heydemann, 1983). Adults of beneficial organisms may lose pollen and nectar sources if weeds are reduced.

217. 12 years of herbicide use in a long-term trial in wheat led to a decline of the soil seedbank by 35 %-60 % (Pallutt and Haass, 1992). Similar declines of farmland species as for Germany were observed in the UK (Johnson, 1999). Arthropod numbers (abundance) have decreased by 60 %-80 % in Sussex (UK) from 1970 to 1989 (Aebischer, 1991). The whole food chain including hares and farmland birds has been affected by these reductions in associated flora (and arthropod) abundance and diversity. A decrease of farmland birds and other taxa has been reported from most agricultural regions including Canada and the USA (McLaughlin and Minneau, 1995; Krebs *et al.*, 1999; Robinson and Sutherland, 2002).

218. Mechanical weeding does not reduce the density and diversity of the weed flora and associated flora as much as herbicides. However, it is much harder on soil sustainability and more labour intensive for farmers. In Germany, the abundance of arable weeds was - on an average of 12 studies - 3 times higher (range: 0.3-10 times) in mechanically weeded fields compared to fields where herbicides were employed (Meisel, 1979; Callauch 1981, Frieben, 1990; Anger and Kühbauch, 1993; Pallutt and Burth, 1994; Albrecht and Mattheis, 1996; Korr *et al.*, 1996; Köpke, 1997; Pallutt, 1997; Becker and Hurle, 1998; Dubois *et al.*, 1998; Oesau, 1998; Richter *et al.*, 1999; Hülsbergen, 2000). All compared fields were ploughed.

219. Plant diversity differences in conventional and organic farming varied from a very small gain to a ten times higher diversity in organic farming. These differences in results were due to the different "intensity" and duration of herbicide use at the test sites before the beginning of the comparative studies (Albrecht and Mattheis, 1996; Köpke, 1997; Dubios *et al*., 1998). The seedbank reservoir as component of biodiversity has been reduced through decades of herbicide use. Regeneration is often only possible with high efforts (Mayer and Albrecht, 1998; Poschlod and Schuhmacher, 1998; Auerswald *et al*., 2000). In Switzerland e.g., seeding of rare and beneficial wild plants is done for conservation reasons and financed by public incentives (Herzog *et al*., 2001).

220. Herbicides and other elements of modern agriculture have caused a systematic depletion of seed banks and made it difficult to reverse this tendency. The aim of weed control has often been to eliminate, not to manage weed populations. Threshold models are rarely used. The loss in biodiversity is also due to the reduced number of crop 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 role in negatively affecting biodiversity within agricultural ecosystems. Promotion of weed species diversity and reduction of weed seed banks can be achieved by conservation tillage and crop rotation (Murphy *et al*., 2006).

IV.2 Effects of changes in agricultural practice

221. A decline of abundance and diversity of birds over the last 20 – 30 years has been observed in many countries. Many species are endangered (Chamberlain *et al*., 2000; SRU, 1996). For farmland birds it is widely accepted that changes in agricultural management practices are responsible for these developments. They are major targets and important indicators of agricultural change (Ormerod and Watkinson, 2000) and adopted as a key measure of agricultural sustainability in the UK (Johnson, 1999).

222. Based on the analysis of multi-year data (1962 – 1995), Chamberlain *et al*. (2000) found a strong correlation between agricultural change and the onset of farmland bird population decline. The observed delayed response (time lag of about 6 years) of bird populations to agricultural intensification implies that effects of change in habitat quality may not become apparent for several years.

223. The decline of birds is best monitored and analysed in UK. The farmland bird indicator decreased by 44 % between 1970 and 2004 in the UK. Individual species populations within the index may be increasing or decreasing, irrespective of the overall indicator trends. Comparing numbers of birds from a wide range of habitats the most obvious declines are shown by birds of farmland (Furness and Greenwood, 1993). Several reasons for this decline have been identified (Furness and Greenwood, 1993; Evans, 1997; Chamberlain *et al*., 2000; DETR, 2000; Leech, 2002):

- Increased use of herbicides and their increased efficacy has led to a decrease in the abundance of weeds growing on arable land and therefore to a decrease in both the availability of seeds to foraging birds and the abundance of invertebrate prey.

- The use of broad spectrum insecticides has led to a reduction in insect numbers important as food for chicks.

- Destruction of hedgerows and field margins has reduced the area available for colonisation by seed-producing weed species and invertebrates.

- The amount of grain spillage has decreased ten-fold due to an increased efficiency of harvesting.

- 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 are able to forage during the winter

224. Most adult birds feed on weed seeds in winter (Evans, 1997) whereas many chicks feed on insects which make both of them biological control agents. In warmer climates as for example in Florida, 190 of 200 bird species are potentially beneficial (USDA/CREES, 2004). At present, little direct evidence exists to link variation in the survival rates of declining farmland birds with specific changes in agricultural practices. This is partly due to the fact that data concerning such changes (e.g. variation in seed abundance) are not available at a sufficient temporal and spatial resolution to permit reliable analyses of relationships with long-term variation in survival rates (Leech, 2002). Also changes in agriculture have been closely interlinked. However, the results of several studies suggest that food supply is an important factor (Evans, 1997; Siriwardena *et al*., 1999; Peach *et al*., 2001). Agricultural intensification during the post-war period has greatly reduced the amount of food available to foraging birds (Robinson and Sutherland, 2002). The seed density in farmland soils fell from averagely 1750/m^2 in 1900 to about 125/m^2 in 2000 (Leech, 2002).

IV.2.1 Crop rotation

225. Fallow land, sensitive areas and several crop species (planted in rotation) have been replaced by HR soybeans in Argentina. This is one factor why a homogenisation of landscapes and production has taken place (Pengue 2004).

226. Control levels of glyphosate allow the increased adoption of “weed dirty” crops such as peas and lentils into the rotation (in combination with conservation tillage systems) at the expense of summer fallow in Canada (Orson, 2002). The crop diversification and the reduction in fallow started long before the introduction of HR varieties and went on after their introduction. An intensification of the cropping frequency has taken place.

227. The options for crop rotations with HR varieties appear theoretically to be numerous because of the lower residual activity of glyphosate and glufosinate, although there is no clear evidence of a trend to widen rotations yet. There is the possibility that if GM crops provide a new dimension of control over pests, diseases and weeds in a poorly targeted way, they will drive agriculture farther toward monoculture and the excessive control of the agricultural environment (Dale *et al*., 2002).

IV.2.2 Planting

228. Similar to all cropping systems where herbicides are used, also herbicide resistant soybeans and cotton can be planted in ultra narrow rows, because no mechanical weeding is necessary (Carpenter and Gianessi, 1999; Kalaitzandonakes and Suntornpithug, 2001). The competitive ability of crop plants is sometimes higher in narrow rows and thus herbicide applications may sometimes be reduced. Nevertheless, the abundance and diversity of the associated weed flora is likely to decrease in narrow row production due to stronger competition of the crop.

229. The trend to narrow row production in soybeans and cotton and the displacement of fallow land or diversified cropping systems negatively influence biodiversity (Duelli, 1997; Krebs *et al*., 1999; Robinson and Sutherland, 2002; Wijnands and Kroonen-Backbier, 1993; EISA, 2004).

230. 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 (wetland) rice production. Wetland habitats are essential for wintering waterbirds such as waterfowls (Ducks Unlimited, 2003).

IV.2.3 Tillage

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

232. Effects of reduced tillage are mixed in the case of ground beetles (Kromp, 1999; Stinner and 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 and Hausammann, 1993; Stippich and Krooß, 1997).

233. Mechanical weeding had no negative effect on important predatory organisms (ground beetles, staphilinids, 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).

234. However, Bitzer *et al*. (2002) showed for HR soybeans that soil disturbance can negatively affect abundance of Collembola more than use of herbicides.

235. The associated flora of crops is an important group of organisms as it provides food sources and habitats for most other (biodiversity) indicator groups.

236. Belde *et al*. (2000) studied the long-term impact (4-25 years) of reduced-tillage systems with traditional selective herbicides on flora. Nine case studies were followed after conventional management was changed to integrated or organic management. The abundance of broad-leaved weeds was reduced in four studies and remained stable in another four cases. In one case, the plant abundance was increased whereas the seedbank abundance decreased. But not only tillage, also herbicide use was reduced in five of the studies, which mitigated herbicide impacts.

237. Populations of problematical 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 and Hurle, 1986; Belde *et al*., 2000). Belde *et al*. (2000) concluded from their study, that wild plants increase in the first years of reduced tillage but their abundance and diversity 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 with no 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, 2002; Swanton *et al*., 1993), and the studies reviewed here have been conducted using selective herbicides in the crop. The relative increase in species dispersed by wind and in monocots is a less questioned finding.

238. 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 positive 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, the life cycles and the adaptation of farming practices to life cycles, e.g. timing of planting, plant protection, and harvesting operations (McLaughlin and Mineau, 1995; Meyer-Aurich *et al*., 1998).

IV.2.4 Additional herbicides

239. Atrazine, acetoclor or dicamba (Bradley *et al*., 2000; Hamill *et al*., 2000; Owen, 2000; Shaner, 2000; or mixtures in oilseed rape against weeds: Stelling *et al*., 2000) have been recommended for use in tank mixtures with glufosinate or glyphosate. The addition of 2,4-D and/or other herbicides to glyphosate or glufosinate-based weed control programs, or multiple glyphosate applications for control of oilseed rape volunteers or resistant weeds has become necessary in parts of the oilseed rape, soybean and cotton growing areas. The ‘double knockdown’ approach is also being more frequently advocated as a tool to address resistance development (Weersink *et al*., 2005).

240. Effects on biodiversity with regards to changes in herbicide applications need to be considered in comparison to effects that would be caused by herbicides that are already in use in the cropping system being replaced.

IV.3 Effects of ecotoxicological attributes of glyphosate and glufosinate

241. Specific legal frameworks regulating the approval procedures and assessment criteria for herbicides are established in different countries (e.g. in the EU Directive 91/414/EC, subsequently Regulation (EC) No 1107/2009).

IV.3.1 Glyphosate

242. The WHO classified technical grade glyphosate (without surfactants) as slightly to very slightly toxic to aquatic invertebrates and moderately to very slightly toxic to fish in 1994 (www.who.int). For aquatic invertebrates, reported LC50 values for glyphosate (tested as the acid or the isopropylamine (IPA) salt) range from 55 mg a.i./L for *Chironomus plumosus* ($LC50_{48\text{-}h}$) to 5600 mg a.i./L for *Chironimus riparius* ($LC50_{48\text{-}h}$) (Giesy, 2000). The toxicity of glyphosate (tested as the acid or the IPA salt) to aquatic vertebrates ranges from 10 mg a.i./L for chum salmon (*Oncorhynchus keta*, $LC50_{96\text{-}h}$) under certain test conditions to >1000 mg a.i./L for sheepshead minnow (*Cyprinodon variegatus,* $LC50_{96\text{-}h}$) (WHO (1994): www.who.int). Glyphosate (acid) EC50 values (96 to 168 hr exposure) for different algal species range from 0.64 mg/L to 590 mg a.i./L, and $EC50_{14\text{-}d}$ values for aquatic macrophytes range from 1.6 to 25.5 mg a.i./L (Giesy, 2000). The direct risk of technical grade glyphosate is also low for honey-bees, birds and mammals according to the classification of the WHO in 1994 (www.who.int).

243. A surfactant is generally added to glyphosate in the formulation to facilitate the penetration of the active substance through the waxy surfaces of plants. The toxicity of glyphosate-containing formulations to aquatic vertebrates and invertebrates as well as to algae can vary considerably depending on the surfactant used in the formulation. For example for common carp (*Cyprinus carpio*), $LC50_{96\text{-}h}$ values for glypohsate-containing formulations have been reported ranging from 2.4 mg/L to >895 mg/L (WHO (1994): www.who.int; Durkin, 2003). $LC50_{48\text{-}h}$ values for the aquatic invertebrate *Daphnia magna* have been reported to range from 3 mg/L to 676 mg/L for different glyphosate formulations (USEPA, 1993; Durkin, 2003). Green algae (*Selenastrum capricornutum*) E_bC50 values for concentrated glyphosate formulations have been reported ranging from 2.1 mg/L to 150 mg/L. (Durkin, 2003). Based on toxicity, some glyphosate-containing products are labelled as toxic to fish and aquatic invertebrates, and restrictions may be placed on these products. For example, it is forbidden to spray such products close to or on wetlands in Germany (BVL, 2010; Ohnesorge, 1994). Also, these products should not be allowed to contaminate fresh water and should not be sprayed when there is a risk that rainfall could wash the product away (BVL, 2010).

244. Many studies suggest that the surfactants have a significantly higher toxicity than the active ingredients. A frequently used surfactant in glyphosate formulations is POEA (polyethoxylated tallowamine). The $LC50_{96\text{-h}}$ values for amphibians for Roundup formulated with POEA ranged from 0.88 mg a.i./L (2.8 mg formulation/L) for African clawed frog larvae (*Xenopus laevis*, Gosner stage 25) exposed to the formulation at pH 7.5, to 15.6 mg a.i./L (50 mg formulation/L) for *X. laevis* embryos exposed at pH 6.0 (Edginton *et al*., 2004). The $LC50_{96\text{-h}}$ value for larvae is in good agreement with $LC50_{16\text{-d}}$ reported by Relyea (2005). Mann and Bidwell (1999) found that $LC50_{48h}$ values in the laboratory ranged from 8.1 to 32.2 mg formulation/L (2.5 to 9.9 mg a.i./L[*]) in four Australian tadpole species. Testing without the surfactant (glyphosate isopropylamine salt) led to much higher values ranging from >343 mg ai/L to >466 mg ae/lL (Mann and Bidwell, 1999). In another study, half of the tadpoles (*Pseudacris triseriata*) studied by Smith (2001) died at 0.75 mg glyphosate IPA salt/L when the formulated product was tested.

245. Temporary wetlands are important sites for tadpole reproduction. Thompson *et al*. (2004) demonstrated that even though laboratory studies indicated formulations containing POEA exhibited significant toxicity to amphibians, native amphibian species in shallow natural wetlands were not affected by an overspray application of a glyphosate formulation containing POEA at the application rate of 2 kg/ha, the maximum rate used in Canada for forestry applications. It has been demonstrated that the dissipation of POEA is rapid in a water / sediment system (Wang *et al*., 2005) which may explain the difference in results between the lab, where testing is done without sediment and the field.

246. 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 have been done with 11 arthropod species such as: predatory species of the following taxa: Beetles (5n - Staphilinidae and Carabidae), flies (2n – Chrysopidae and Tachinidae), mites (1n - *Typhlodromus pyri* [Phytoseiidea]), bugs (1n – *Orius insidosius* [Anthocoridae]), spiders (1n - *Pardosa spec.* [Lycosidae]), and aphid parasitoids (1n – *Aphidius* rhopalosiphi [Braconidae]). The mortality of 8 species was determined using substrates sprayed with glyphosate formulations and of 6 species was determined using substrates sprayed with a glyphosate trimesium formulation or the salt itself. Mortality of 53-100 % was found for half of these species when tested on an inert substrate with a simulated application rate range of 0.7 kg a.i./ha to 7.7 kg a.i./ha (EC 2002). When the most sensitive of these species were tested on natural substrates with a simulated rate of 3.6 – 3.7 kg a.i./ha, however, the mortality was reduced to ≤30 % (EC 2002) indicating low risk under more realistic conditions.

247. The CTB (2000) (Board for the Authorisation of Pesticides, Netherlands) found that formulated products containing glyphosate are toxic to predatory mites and moderately toxic to some beneficial spiders and (parasitic) wasps. A glyphosate formulation was reported to be harmful to ground beetles of the genus *Bembidion* according to Diercks and Heitefuss (1990) but not according to a semifield test reviewed by the EC (2002). The effects of glyphosate on hoverflies (Syrphidae) which provide a high level of aphid control (Krüssel *et al*., 1997) have not been described in the published literature.

248. The CTB (Board for the Authorisation of Pesticides, Netherlands) also determined that glyphosate formulation products were of low toxicity to earthworms (CTB, 2000). 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 which is more than three times the maximum single use application rate.

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[*] In the Mann and Bidwell paper, it was assumed that the formulation indicated as 360 g a.i./L was 36% glyphosate acid by weight. In fact, because the density of the product is greater than 1, the formulation is actually 30.7% glyphosate acid by weight. Glyphosate acid values have been derived from the formulation values based on 30.7% glyphosate acid in the formulation.

Formulated glyphosate (Roundup Original and Roundup Transorb) is practically non-toxic to honey-bees (LD 50: >250µg/bee, contact 48 hours) and earthworms (LC50:> 10,000 mg/kg dry soil, 14 days) (Monsanto Canada, 2002).

249. Glyphosate has been shown to suppress some soil microorganisms. Microbes are of significant ecological and agronomic importance e.g. as symbiotic partners, antagonists to pathogens, and food source for the micro-fauna. The suppression can last more than 60 days at temperatures far below 20°C (Chakravarty and Chatarpaul, 1990). At temperatures of about 20°C, regeneration can be observed within a week. Other studies basing on standardised tests indicate that there were no long-term effects on microorganisms in soil also at rates that exceed maximum use rates (Sullivan & Sullivan, 2000). Some laboratory tests have shown effects on nitrogen-fixing bacteria (Moorman *et al*., 1992; Santos and Flores, 1995) and soil fungi (Estok *et al*., 1989; Busse *et al*., 2001). Studies of Means *et al*. (2007) and Zablotowicz *et al*. (2004) indicate the potential for reduced nitrogen fixation in the HR soybean system; however, yield reductions due to this reduced N_2 fixation in early stages of growth have not been demonstrated. A recent review indicates that effects of glyphosate applications on microbes in HR crops are transient (Cerdeira and Duke, 2006) and there are also soil organisms that degrade glyphosate (Araújo *et al*., 2003). For any conclusion it is important to distinguish between results of artificial laboratory studies and field studies in a normal environment and seasonality (Hart *et al*. 2009) and with normal application rates.

250. Toxicity assessments with mammals indicate that the toxicity of glyphosate to mammals is lower relative to other herbicides. An LD_{50} based indicator simulation indicated that HR soybean technology is more environmentally friendly in terms of acute toxicity to mammals than conventional systems (Nelson and Bullock, 2003). Formulated Glyphosate (Roundup Original and Roundup Transorb) is practically non-toxic to rats via acute oral, dermal and exhalation exposure and to Mallard Duck (*Anas platyryhnchos*) (Monsanto Canada 2002).

251. The metabolite AMPA (aminomethylphosphonic acid) degrades much slower than glyphosate (see EC (2002)). AMPA has been shown to be of low toxicity to birds and aquatic organisms (Giesy, 2000).

IV.3.2 Glufosinate

252. In gentically modified glyphosate-resistant plants, the L-isomer of glufosinate is metabolized into the non-phytotoxic stable compound Acetyl-phosphinothricin (OECD, 2002).

253. 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).

254. 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 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 (Bock (1991) cited in Dorn *et al*. (1992)). Hommel and Pallutt (2000) referred to an assessment of the cumulative effect of active ingredients of pesticides (Gutsche and Rossberg, 1997). Hommel and 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.

255. Glufosinate has also been shown to suppress some soil microorganisms (Ahmad and Malloch, 1995; Ismail, 1995).

IV.4 Effects of HR agriculture

IV.4.1 Effects on flora and seed bank

256. Glyphosate and glufosinate are more effective on a broader range of species than currently used conventional 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.

257. The effects of the HR cropping-technique on abundance and species-diversity were investigated in a large-scale trial (60-75 fields, 3 years, size of plots: half fields) 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, 2003b; Firbank *et al*., 2003b). In sugar beet and fodder beet (glyphosate-resistant) and 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 HR crops mentioned above (Heard *et al*., 2003a, 2003b). The emergence of 8 species was lower in HR beet and in 6 species in oilseed rape. Emergence increased in one weed species in HR oilseed rape. 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.

258. Findings in glufosinate-resistant maize of the FSE were different (glyphosate-resistant maize has not been tested), and showed a higher biodiversity for weed species compared to the 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 since 2003 no longer approved in the EU. Effects of managing HR maize should be compared to the weed management practices that they are likely to displace. A ban on triazine is likely to reduce but not negate relative benefits of glufosinate resistant maize according to the assessment of Perry *et al*. (2004).

259. A transfer of the results of the British FSE is not applicable throughout the world. Nevertheless, the study shows the high complexity of farm-environment interactions.

260. Other studies have shown that with a changed herbicide management strategy the use of transgenic plants can result in higher weed diversity compared to conventional management (Dewar *et al*., 2003; Strandberg & Pedersen, 2002) or like in the BRIGHT study, no significant decrease in botanical (species) diversity were observed (Sweet *et al*., 2004).

261. In Canada, several effects of different rotations with high frequencies of crops resistant to non-selective herbicides were studied for three years (Harker *et al*., 2004). Five rotations with one, two, or even three glyphosate or glufosinate resistant HR crops were planted at six locations. Different seeding dates and types of tillage were tested too. The overall species diversity of arable weeds declined by 26 %, and their density by 66 %. When only no-till systems were compared to plots that were conventionally tilled, the density of weeds was on average 23 % lower. These reductions were explained by a very dry year at most sites and by the treatments. A high frequency of glyphosate resistant crops led to increasing levels of weed control according to the authors.

262. Any decrease of the weed seed bank has to be assessed in the context of the receiving environment. This may include consideration of the importance of 'in-crop' weeds for broad biodiversity conservation goals. In contrast, in some countries the majority of agricultural weeds are exotic species which represent a threat of invasion into native vegetation and/or 'in crop' weeds do not represent a significant repository for native biodiverstiy. This is the case in Australia, where there is a focus on reducing weed seed banks in agricultural areas through integrated weed management.

263. 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 and van der Poll, 1999). Field margins often harbour rare plant species. The impact of non-selective herbicides on these plant populations (and on the fauna depending on them) is of particular significance (Mahn, 1994). The scorching of vegetation was more than doubled in HR crops (1.6 % to 3.6 %) in the large-scale field tests in Britain 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 beet, flowering and seeding were 34 % and 39 % lower. Cover (+28 %) and flowering (+67 %) in margins was higher in HR maize.

264. 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).

265. In Australia, resistance management and spray drift is a concern for these sites too. Extreme care should be used to prevent injury to native desirable vegetation, open bodies of water and waterways.

266. IV.4.2 Effects on fauna

267. In the USA, Buckelew *et al.* (2000) found less canopy arthropods and Jasinski *et al*. (2004) found significantly less spiders and green lacewings in HR soybeans than in conventional cultivars. However, studies of Jackson and Pitre (2004) and Morjan and Pedigo (2002) revealed no significant differences between HR soybean fields and conventional soybean for pest and beneficial insects. In a study of Goldstein (2003) over three generations of Collembola no effects of RR soybeans or RR maize as food source were found.

268. Several arthropod sampling methods were used in the large-scale trials in Britain in order to compare the abundance of different arthropod groups (Firbank *et al*., 2003a).

269. Results for 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). Population densities will be reduced, when forage is reduced over large HR-crop areas (Haughton *et al*., 2003). Herbivores, pollinators (e.g. bees, butterflies) and beneficial natural enemies of pests were reduced (Hawes *et al*., 2003). The effects were dependent on the relative efficiency of comparable conventional herbicide regimes. They changed in the same direction as their resources (Hawes *et al*., 2003). The importance of the correct timing of the application was shown by Strandberg *et al*. (2005). In summer arthropod fauna was even higher in HR fodder beets than in conventional beets if the glyphosate application followed the recommendation, but weed diversity and biomass was lower. Extremely low weed diversity density was observed when glyphosate was applied earlier than recommended.

270. Results for maize: 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 indirect effects of plant suppression and habitat destruction are the key to invertebrate (and vertebrate) biodiversity.

271. Models from the UK simulating the planting of herbicide resistant plants on a larger scale show that one consequence will be a major loss of food sources for seed consuming farmland birds (Watkinson

et al., 2000). A model using data from the FSE also predicts a distribution of more fields with lower weed densities, which could affect animal populations on farmland, if HT 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 affect taxa at higher trophic levels such as some birds dependent on them as a seed food source.

272. Potential effects after replacing equivalent conventional crops with HR crops on farmland birds were also estimated by modelling from Butler *et al.* (2007). In this model only limited effects after nationwide introduction of HR crops in the UK were predicted, and only one bird species may change to a less favourable conservation status. Those species which 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.

273. The replacement of atrazine and alachlor in maize by glufosinate or glyphosate reduces runoff loads in watersheds (Wauchope *et al.*, 2002). Glyphosate and glufosinate are less toxic to mammals than atrazine and alachlor but on the other hand a common glyphosate formulation is toxic to amphibians. Farmland mammals such as hares may benefit from glyphosate and glufosinate being less toxic to them than other herbicides.

274. The importance of the correct timing of the application was shown by Strandberg *et al.* (2005). In summer arthropod fauna was even higher in HT fodder beets than in conventional beets if the glyphosate application followed the recommendation, but weed diversity and biomass was lower. Extremely low weed diversity and weed density was observed when glyphosate was applied earlier than recommended.

IV.5 Further aspects of sustainable agriculture

275. Measures to mitigate environmental effects of herbicides in conventional systems have been developed in some countries, such as controlling the time and the place where herbicides can be used on farmland. The introduction of unsprayed field margins and unsprayed areas in field controls on timing of applications and maximum doses has allowed weeds and associated biota to develop in restricted areas of fields, in areas where these endpoints are management goals.

276. Similar measures have been proposed for HR sugar beet (Pidgeon *et al.* 2001, 2007; Beckie *et al.*, 2006) which restores biodiversity while having little impact on overall crop productivity per hectare because of the increases in yields associated with HT sugar beet. To mitigate decreases in weed seed production and weed biomass production associated with management practices of HR sugar beet, 2% to 4% unsprayed areas in a field are required. Tilled margin effects could be mitigated by increasing the margin from 0.5 m to 1.5m.

277. For decades weed scientists in the USA and in Europe have been recommending weed control up to a level that eliminates potential interference with net returns (economic thresholds). A clean field or a 95 % control is not necessary for the exclusion of competitive effects of weeds and non-target or beneficial wild plants to crops (Korr *et al.*, 1996; Pallutt, 1997, Werner and Garbe, 1998). The use of economic thresholds and mechanical weeding would favour the associated flora in fields in areas where this is needed to support conservation goals. However, while the databases on integrated weed management and the expert systems are rarely used in practice, growers consider other factors (Owen, 2000) and largely attribute the introduction and movement of weeds to factors outside their control (Wilson *et al.*, 2008). In addition, the recommendations for managing resistance development in HR crops must be considered.

278. Within one season, production systems with HR fodder or sugar beet could be modified in order to favour biodiversity (Dewar *et al.*, 2000; Coghlan, 2003). However, delayed spraying had only transient positive effects on herbicide resistant beet, and only on sites with a rich soil seed bank (Dewar *et al.*, 2000;

Elmegard and Pederson, 2001; Strandberg and Pedersen, 2002). The soil seed bank is reduced in the long term even when applications are delayed according to the comprehensive assessment of Freckleton *et al.* (2004). An early band spraying for herbicide resistant beet (May *et al*., 2005) would have positive overall effects and would be in accordance with possible IPM (Integrated Pest Management).

279. Moreover, band spraying or patchy weed control (Dzinaj *et al*., 1998; Gerhards *et al*., 1998; Lettner *et al*., 2001) with conventional herbicides is better for biodiversity than with non-selective ones. However, any modification in this sense is only effective when the seedbank is not already depleted.

280. In HR sugar beet low-dose (row spraying) postemergence application is practicable in connection with an economic threshold evaluation and does not lead to economic losses (Dewar *et al*., 2002; Coghlan, 2003; Elmegard and Pederson, 2001).

IV.6 Conclusions on impacts on biodiversity (Section IV)

281. Maintaining farmland biodiversity is an important issue of public concern, particularly in regions with a high percentage of farmland and high land use by residential cover or traffic areas.

282. When the cultivation of an HR crop yields high economic returns or is otherwise considered to be superior to non-HR varieties by farmers, the proportion of this crop may increase in the crop rotation and replace other crops or fallow. High weed control levels in HR cropping systems would allow the adoption of other crops with high weed infestation, but there is at present no clear evidence of a trend to widen crop rotations by growing HR crops. Given theses trends the adoption of HR crops may be accompanied by some loss in agrobiodiversity.

283. The environmental consequences of each herbicide tolerant crop will depend on the cultivation of the crop, the herbicide, the dose of the herbicide, the time and frequency of applications of the specific and other herbicides, other management features of the HR crop and of other crops in rotation with the HR crop. 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 earning potential associated with commodities futures.

284. Similarly, environmental impacts of the conventional herbicides applied to non-GM comparator crops vary because of these same factors, so that it is very difficult to establish detailed baselines in a very dynamic situation for comparison of GM-HR systems with other systems. In addition, life cycle factors such as impacts of herbicide production on non-sustainable energy usage, atmospheric and water pollution are also factors that need to be considered.

285. For areas where biodiversity within agricultural fields is less of a concern, the indirect impacts associated with the cultivation of HR crops may be different and more difficult to assess. The use of HR crops in environmentally favourable low and no-till systems, the reduction in use of more toxic herbicides and the decreases in fuel use associated with fewer tractor-passes for tillage and herbicide applications that are sometimes associated with HR crops may have a positive effect on biodiversity.

286. Where weeds are exotic species, fields with a low number of weeds are desirable to prevent invasions into native populations and alterations of the population structure of insects relative to natural ecosystems.

287. HR crops can be planted in narrow rows as mechanical weeding is not needed. Narrow rows additionally suppress the establishment of weeds in the crop due to competition. A high crop density is generally a common measure in conventional agriculture for preventive, non-chemical weed regulation to save herbicides and money.

288. Reduced/zero 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. Growing HR crops makes the management of reduced/zero tillage systems easier and consequently may support their expansion.

289. Long-term experiences with reduced-tillage indicate that the diversity and abundance of broad leaf plants may further decrease in reduced-tillage with HR. Reduced tillage could clearly be favourable to biodiversity when combined with cover crops and mulching (for soil invertebrates), when farm operations are re-scheduled and adopted to wildlife (vertebrates), and when wild plant abundance is not further decreased by highly effective (broad spectrum) weed control (plants providing habitat and food and influencing the microclimate for vertebrates and invertebrates).

290. There is much evidence that the seedbank, wild flora and whole food webs in agricultural fields will further be reduced, if HR beet and HR oilseed rape are planted and sprayed with broad-spectrum herbicides. For HR maize the use of less toxic herbicides to replace e.g. Atrazin can be benefical for biodiversity. The risk to the environment posed by these observations is dependent on the receiving environment and appropriate consideration needs to be given to this issue.

291. The weed population shifts to perennial and grass weed species in systems with reduced or zero tillage in combination with application of non-selective herbicides. This trend will probably continue in systems with HR techniques.

292. The soil seed bank is mostly higher in reduced tillage systems than in intensive tillage systems since seed germination is reduced in the former. This effect can be reversed in the long run if the input of new weed seeds is reduced by highly effective weed management that prevents seed depostion.

293. This effect is not contradictory to the aims of many conventional farmers but may be contradictory to the overall aim of reduction of biodiversity loss.

294. It has to be assessed on a case-by-case basis, using current weed control practices in conventional crops as the baseline, if the adoption of a specific HR crop in a country or region will lead to a reduction of weeds as forage for invertebrates and if this may lead to a further decrease of the field fauna, e.g. birds.

295. Impacts on nesting vertebrates are mostly minimised in zero tillage systems. Invertebrates do as well, e.g. earthworms profit from non-inversion tillage because their habitat is undisturbed and the amount of organic residues on the soil surface is high. Living or dead mulch is often more influential on arthropods abundance than the reduction in soil disturbance.

296. According to the WHO glyphosate is classified as unlikely to present hazard in normal use. Glufosinate is classified as slightly hazardous (www.who.int). Glyphosate and glufosinate, particular their formulated products, affect some individual species of the field fauna. In general, toxicity is often lower compared to other herbicides. Microbial activity can be suppressed by glufosinate and glyphosate.

297. There is at present no evidence that the introduction of HR plants increases fitness and invasiveness of plants in natural and semi-natural habitats as there is no selection by herbicides on those areas.

298. Economic threshold models are rarely used in conventional or HR cropping systems. While weed scientists in the USA and in Europe recommend weed control up to a level that eliminates potential interference with net returns (economic thresholds) growers consider other factors. A small portion of conventional soybean growers uses the models.

SECTION V OVERALL SUMMARY AND CONCLUSION

299. Agriculture has the task to produce food and feed in a sustainable way. Herbicide use is common for the production of food and feed in most regions throughout the world.

300. Using HR techniques means using effective non-selective herbicides post-emergence. The application of herbicides has wanted and unwanted implications in terms of, direct and indirect impacts on crops, weeds, agricultural practice, biodiversity and environment. For sustainable agriculture longer time spans have to be considered.

301. Crop injury by herbicides is less likely in HR crops.

302. Crops with characteristics such as shattering and seed persistence are likely to emerge as volunteers. Thus the HR trait can be distributed in an area by gene flow in time. Specific agronomic measures (soil tillage, crop rotation) have to be considered for reducing volunteers in both HR and conventional cropping systems. The transfer of HR genes to wild relatives should be taken into account in centres of crop origin and regions where interfertile and weedy hybrids occur.

303. Weed biotypes with tolerance, resistance or even multiple resistance and cross-resistance to glyphosate have been selected in the field unintentionally in conventional and HR cropping systems. Crop volunteers with resistance to glyphosate and glufosinate have been detected in fields even when HR crops have not been planted there previously. It has to be expected that more weed species become resistant to herbicides when the use of some specific herbicides expands, and if outcrossing occurs from HR plants into the same or related species.

304. There is at present no evidence that the introduction of HR plants increases fitness and invasiveness of plants in natural and semi-natural habitats as there is no selection by herbicides on those areas.

305. Besides the direct impact of herbicides on weed species, shifts in weed composition are often attributed to changes in the whole cropping systems, for instance to the conversion from inversion tillage by a plough to conservation tillage (reduced or zero tillage). There is an interaction of using HR crops and changing the cropping system. Therefore, the growing of HR plants, may result in shifts in weed population as a results of direct and indirect effects.

306. Using HR crops leads to a simplification of weed control, i.e. the choice of the product is easier, the effectiveness of the herbicide is high, the flexibility in timing is high (extended time window for spraying), a wide spectrum of weed species is target of the herbicide. HR crops allow post-emergence application instead of precautionary and routinely pre-emergence herbicide application in conventional crops.

307. Reasons for farmers to adopt HR systems are the reduction of production risk (extended time window for spraying), currently low herbicide prices, lower costs in HR systems (particular in combination with conservation tillage), for other production factors (labour, fuel), higher effectiveness in weed control. For farms with low labour input (e.g. part-time farmers, large farms) HR systems simplify the management.

308. There are no exact conclusions on the development of yields, because several factors are involved. Overall, the final economic success (costs vs. returns) and not the crop yield is decisive for adoption of a new system.

309. HR crops can be well adapted to conservation tillage systems, where mechanical weed control is reduced or omitted. Reduction of soil erosion, saving costs and energy are benefits of these systems. Conservation tillage may expand if more HR crops are provided.

310. Crop rotations may change due to volunteer problems (e.g. wider crop rotations with non-GM oilseed rape after GM oilseed rape). HR volunteers which survive preseeding herbicides can cause undesirable effects in less competitive crops. Other herbicides may have to be used in these cases. It can be difficult to control e.g. HR oilseed rape volunteers in other broadleaved crops (peas, lentils, flax, beet).

311. If a HR crop is widely adopted, shorter crop rotations and reduced crop diversification are likely. There is the tendency towards monoculture of HR crops in some countries, which can lead to higher pressure of diseases and pests.

312. Growing HR crops is associated with the use of comparatively less hazardous herbicides. Nevertheless, there are toxic effects on some aquatic species.

313. If weed reduction in HR systems is clearly higher compared to conventional farming, flora and fauna biodiversity and abundance could be reduced. This includes direct effects (depletion of the weed seed bank, low weed density) and indirect effects (loss of animals feeding from weeds, or predators of these animals). However, this is only a consideration in countries where the conservation of on farm biodiversity is a critical part of the overall nature conservation strategy.

314. Sustainable use of HR crops needs appropriate management strategies to suppress weed resistance development and unintended effects on biodiversity.

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