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Agronomic and Environmental Aspects of the Cultivation of Transgenic Herbicide Resistant Plants

by

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Agronomic and Environmental Aspects of the Cultivation of Transgenic Herbicide Resistant Plants

Introduction

It it generally accepted in the international field of risk assessment research that the effects of transgenic organisms have to be assessed 'case by case' and 'step by step'. While most physiological effects can be studied in laboratory and greenhouse, ecological, agronomic and economic effects are partly only assessable in field tests or commercial growing and by modelling. Information on these aspects have been retrieved by literature and internet mining and by contacting experts, inter alia, by mailing a questionnaire as included in the appendix of this document. The study is subdivided into the four sections 'Scope and area of application', 'Changes in weed susceptibility', 'Impacts on agricultural practice and agronomy' and 'Impacts on Biodiversity'.

Herbicide resistance in crops can result from two different breeding procedures: traditional and genetic engineering techniques.

Tab 1: Herbicide resistant crops available in North America*

Herbicides	**Crops**
resistance due to traditional breeding	
cyclohexadinones/sethoxydim (SR) (Poast)	corn
imidazolinones (Pursuit)	corn, canola,
sulfonylureas	soybean
triazines	canola
resistance due to genetic engineering	
glufosinate (Liberty, Basta)	canola, corn, soybean
glyphosate (see Tab 2)	soybean, canola, cotton, corn
bromoxynil	cotton, canola

* modified table published by Duke, 1999

Bromoxynil resistant cotton is grown in some parts of the US (Arkansas, Tennessee, Missouri). However, the overall importance of this trait for the OECD member states is low compared to glyphosate and glufosinte. Less than 4% of the cotton growing area in the USA is planted with bromoxynil-resistant varieties (3,7% in 2001, Gianessi et al 2002). Moreover, the acreage has been decreasing. Therefore it was decided not to cover bromoxynil-resistant cotton in this report. The paper focusses on the agronomic end environmental aspects of cultivating genetically engineered HR crops resistant to glyphosate and glufosinate. "HR" refers to these two resistances in the context of this document.

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, Böger 1994).

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 (Böger 1994).

With the termination of the Monsanto patent there are many glyphosate containing products on the market (Tab 2) whereas glufosinate is exclusively marketed (Liberty) by BayerCrop Science:

Tab.2: Companies and examples of glyphosate-type trade marks

Company (former company)	**Examples of glyphosate and glyphosate-type products**
Monsanto	Roundup Ultra, Roundup Ultra MAX, Roundup Ultra DRY, Roundup Custom, Roundup Original, ReadyMaster ATZ, Ranger, Rodeo
Syngenta (Zeneca)	Touchdown
Cheminova	Glyphos
NuFarm	Credit
MicroFlo	Gly-Flo
Dow AgrowSciences	Glyphomax, Glyphomax Plus
BASF	Acquire
BASF (Cyanamid)	Extreme - contains Pursuit, Backdraft - contains Scepter
Honcho, Rascal, Silhouette, Rattler, Buccaneer, Mirage	Several products

Crop Field News 2000

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 EPSP synthase, which confers glyphosate-resistance, partly in combination with the *gox* gene encoding the glyphosate-degrading glyphosate oxidoreductase (GOX). And many crop plants have been transformed with one of the two bacterial genes *pat* or *bar* encoding the enzyme phosphinothricin acetyl transferase (PAT) which detoxifies L-PPT (OECD 1999b) in order to confer glufosinate (L-PPT) resistance.

Glyphosate or glufosinate may be used in HR-crops at other application rates and dosages than comparable conventional herbicides.

The official WSSA (Weed Science Society of America) definitions of "herbicide resistance" and "herbicide tolerance" are used throughout this document:
"*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."

Section I Scope and area of application

I.1 Field trials

The OECD database (OECD 2003, releases until 2000) of field trials lists more than 3000 releases of about 25 transgenic plant species with either glufosinate or glyphosate (incl. stacked traits) resistance. About 150 companies/institutions have received release permits for carrying out field trials with herbicide resistant (HR) plants.
Data obtained from these small-scale trials might be of only limited value in answering questions concerning the environmental and agronomic aspects of growing herbicide resistant plants (HR plants) on a commercial scale. In particular, biodiversity effects and the magnitude of gene flow between neighbouring crops and from transgenic crops to feral populations and wild relatives is recognised to be scale and time dependent (DETR 2000).

More than 200 seed companies sell either glufosinate or glyphosate resistant plants, thereby accepting the conditions of the patent owners (main owners: BayerCrop Science and Monsanto). Since 1996 an immense number of HR plant lines has been generated and the number is still growing.

I.2 Commercial cultivation

Out of the many transgenic glyphosate and glufosinate resistant crop species globally tested in field experiments only four plant species are commercially grown as approved varieties (s. Tab. 3):

Tab.3: Commercially grown HR (glyphosate and glufosinate resistant) crops

crop	herbicide resistance against	country
corn	glyphosate	Argentina, Bulgaria[1], Canada, USA
	glufosinate	Canada, USA
cotton	glyphosate	USA,*
canola (oilseed rape)	glyphosate	Canada, USA
	glufosinate	Canada
soybean	glyphosate	Argentina, Canada, Mexico, Romania, South Africa, Uruguay, USA

http://www.transgen.de, James 2002, [1]Gianessi et al. 2002

* regulatory approval is currently pending for HR (glyphosate) cotton in Australia, Argentina, Mexico and South Africa, the product is under development in Brazil and Turkey

Global HR area

2,6% (48,6 mio ha) of the global crop acreage (1.830,2 mio ha) is currently planted with HR crops (James 2002, FAO 2003). The area will increase if HR wheat and rice varieties are adopted (see below, Tab. 7). The current share of the HR crop areas per global acreage of the four most important crops is shown in Tab. 4.

Tab. 4: HR acreage as % of global crop acreage 2002

crop	global area (mio ha)	HR acreage as % of global acreage* of that crop
soybean	79	46 %
cotton	34	6,5 % (13%)
canola	25	12 %
corn	140	1,6 % (3,1%)

* only HR, in brackets: HR/insect resistant (stacked) included
modified table cited in James 2002 and Crop Biotech Net. 2003 cited in http://www.isaaa.org, global soybean area from http://www.FAO.org

Other crops, which are already approved but not adopted by farmers:

Tab. 5: Approved but not adopted HR crops

crop	**country**
sweetcorn	USA
sugarbeet	USA

Gianessi et al. 2002

Herbicide resistant crops are by far the most planted genetically engineered crops, and HR soybean is by far the most planted HR crop worldwide: 82% of the global (58,7 mio ha) transgenic area were planted with HR crops and 75% of the total HR area was planted with HR soybean in 2002. This was mainly due to the fact, that 79% of the US soybean area and more than 90% of the Argentinian soybean area was planted with HR soybean (James 2002). The increase of the total soybean acreage and HR soybean acreage is illustrated in Fig. 1.
The other HR crops and HR/Insect resistant (stacked) crops are planted at more or less the same acreage and make up about 5% of the global HR area each. Their area has not increased as fast as for HR soybean during the last 3 to 7 years (Tab. 6).

Tab. 6: Global growing areas of herbicide resistant crops from 1996 to 2002 (mio ha)

	1996	1997	1998	1999	2000	2001	2002
HR soybean	0,5	5,5	14,5	21,6	25,8	33,3	36,5
HR canola	0,1	1,2	2,4	3,5	2,8	2,7	3
HR cotton	< 0,1	0,4	--	1,6	2,1	2,5	2,2
HR/BT cotton	0	< 0,1	2,5	0,8	1,7	2,4	2,2
HR corn	0	0,2	1,7	1,5	2,1	2,1	2,5
HR/BT corn	0	0	--	2,1	1,4	1,8	2,2
total	0,6	7,3	21,1	31,1	35,9	44,8	48,6

CropBiotech Net. 2003, cited in http://www.isaaa.org

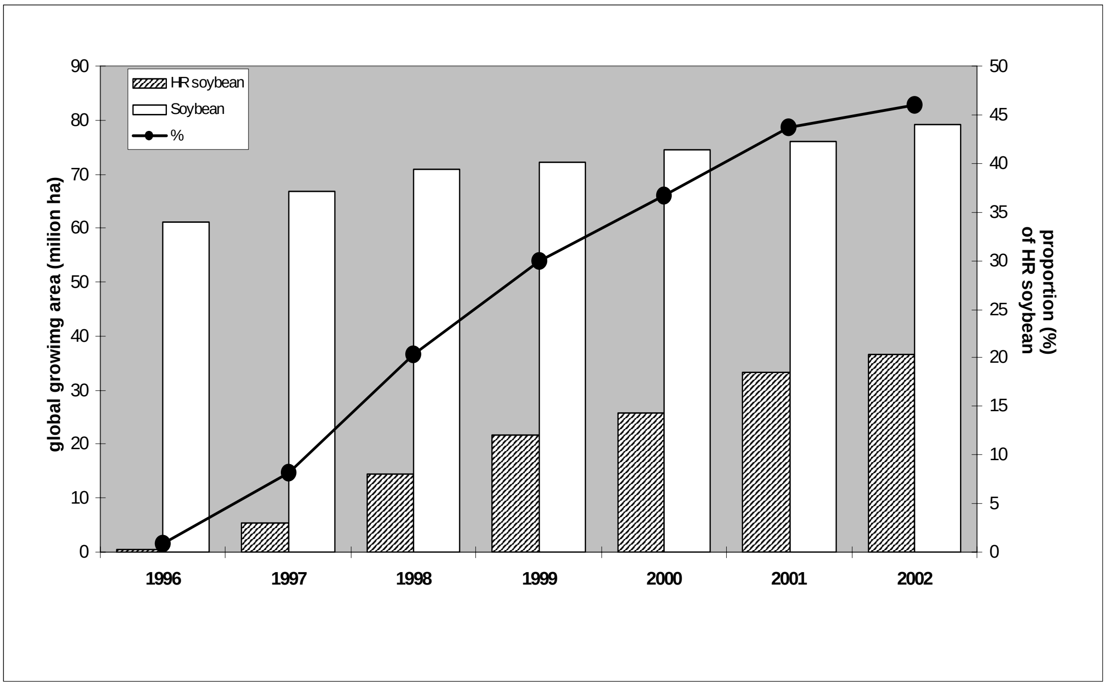

Fig. 1: Global growing area of soybean , HR soybean and proportion of HR soybean

James 2002 (HR area) and http://www.FAO.org (total area)

More HR crops are currently being developed and tested, some of which will probably be adopted in the near future (development of HR crops in the USA: see Tab. 7).

Tab.7: HR crops under development

crop	**potential HR-area in the USA* (mio ha)**
strawberry	0,002.105
lettuce	0,086.639
tomato	0,117.004
sugarcane	0,186.235
potato	0,251.417
rice	0,381.781
alfalfa	0,404.858
wheat	2,388.663
total	**3,818.702**

*according to Gianessi et al., 2002

I.3 Hybrid selection

Transformation with glufosinate or glyphosate resistance genes can be used alone or in conjunction with other genes such as the MS/RF lines (male sterility/fertility restorer) for hybrid selection.

Transgenic male sterility systems are currently being employed for variety development and seed production in chicory, corn, and oilseed rape.

Section II Changes in weed susceptibility

The use of HR crops is connected with several changes in weed control measures and other agricultural activities such as seeding practice, tillage, or land use (see Chapter III). Some of these changes are due to HR, while others are due to government incentives or driven by the world market. The decrease in numbers of herbicides used, and the trend to less tillage or less cultivation is most relevant for HR crops (see Chapter III.1). As a consequence, weeds are now under selection pressure caused by fewer herbicide types (modes of action) than before. Aside from possible gene flow selection pressure contributes to the evolution of new weed biotypes and to shifts in weed communities. Some changes in weed susceptibility to glyphosate have resulted in altered weed control patterns and presently, some farming consultants propose to use additional herbicides in HR crops or to change the pattern of cultivation (see Chapter III.1).

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 weeds. Cold and cloudy weather conditions impair the effectiveness of glufosinate (Ammon et al. 1996, Hommel and Pallutt 2000). The effect of glyphosate on all of three weed species evaluated was negatively affected in evening and night hours (Northworthy et al. 1999). A reduced interception was attributed to the diurnal movement of leafs.

Perennial weeds can easier be controlled than before by glyphosate and HR plants give new options to farmers to control weeds resistant to other herbicides.

Moreover, the maximum weed size for effective control is higher with glyphosate than with other postemergence herbicides (Carpenter and Gianessi, 1999). However, late applications were the pre-eminent reason for yield losses in HR soybean field tests (Hartzler 2003).

There are different sensitivities of target plants to non-selective herbicides (see II.1). There is also considerable intraspecific biotype variability in susceptibility at the whole plant and cellular level (Gressel 1996). Weed biotypes with a higher tolerance or a resistance may contribute to the anticipated shift of the weed flora.

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

- 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 II.1).
- escape and proliferation of the transgenic plants as weedy volunteers (II.2.1),
- hybridization with - and HR-gene introgression into - related weedy species (II.2.2)

II.1 Selection of resistance and weed shifts

Glufosinate and glyphosate are generally considered as low risk herbicides for the evolution of herbicide-resistance in weed populations. Mutations at the substrate binding site of the target enzyme of glufosinate, the glutamine synthetase, are thought to result in low-fitness

biotypes or to be lethal (Böger 2000). The chemical structure, mode of action, and limited metabolism of glyphosate in plants as well as lack of soil persistence, lack of residual activity, limited uptake from the soil (Böger 1994) by plants, and its application pattern are considered as reasons why the evolution of resistance to glyphosate may evolve rather slowly (Heap 2000, Baylis 2000, Nap et al. 1996, Jaseniuk 1995).
Other factors may trigger the selection process of resistant weeds in HR crops: If the non-selective herbicides are used on a larger scale than other conventional herbicides, selection may occur on a broader spectrum of weed flora in cereals and broadleaved crops. The larger the areas planted with crops resistant to the same herbicide will be, these crops being sprayed year after year with likely more than one herbicide application per season, the sooner resistance will evolve (Freudling 1999, Darmency 1996).

As non-selective herbicides can be applied on these HR crops on fall, spring, and summer weed communities, selection can act indifferently, which was previously not the case for most selective herbicides (Darmency 1996). The mechanisms of resistance described for weeds resistant to traditional herbicides include target site insensitivity, target site overproduction, herbicide detoxification, reduced herbicide entry, reduced herbicide translocation, and changes in the intracellular accumulation of herbicides. A total of 249 herbicide-resistant weed biotypes were recorded until January 2001 (http://www.weedresearch.com).

Most of the resistant weed biotypes are resistant to ALS inhibitors (herbicides inhibiting acetolactate synthase) and triazines (atrazine and others). Increasing problems occur in US soybean culture, particularly with acetolactate synthase (ALS) inhibitor-resistant weeds. 30 of the 84 herbicide resistant weed biotypes reported for the USA show resistance to ALS inhibitors, and 11 out of 35 resistant biotypes in Canada respectively (http://www.weedresearch.com).
The fitness of resistant weed biotypes is not always lower than the fitness of susceptible ones. For example, no fitness difference between susceptible and resistant biotypes of *Lolium rigidum* could be detected (Mortimer 1993). Some resistant *L. rigidum* biotypes have been detected in unsprayed areas adjacent to sprayed farmland (Mortimer 1993).
Herbicide-resistance (against any herbicide) does not need to be a consequence of a spread from a few initial sites but can also result from independent evolutionary events (Mortimer 1993).

Cross resistance and multiple resistance

M*ultiple resistance* is defined as the expression of more than one resistance mechanism within individuals or populations. 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, the two cross resistance categories being *target site resistance* and *non-target site resistance* (Powles and Preston 1995).
Multiple resistant weeds have been reported from several regions, including Europe (Niemann 2000). The mechanism of multiple resistance of *Lolium rigidum* seems not to be due to any

barrier to herbicide uptake or translocation, but to the induction of several herbicide degradation enzymes. Resistance to one herbicide may facilitate development of resistance to another one raising implications for weed management in preventing resistance to broad-spectrum herbicides such as glyphosate (Pratley et al. 1999). 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), to ALS-inhibitors, and to glyphosate (http://www.weedresearch.com).

Current weed control limitations of glyphosate and glufosinate

Weed species or populations which are difficult to control are specified in Tab. 8 (glyphosate) and Tab. 9 (glufosinate).

Tab. 8: Reported cases of insufficient weed control by glyphosate

weed	**crop/region**	**indication** tolerance, resistant populations, individuals, degree of control/resistance, management indications	**reference**
Festuca rubra (red fescue)		varying efficiency	Mortimer 1993
Chenopodium album (lambsquarter)		varying control effect	Mortimer 1993
	Argentinia, Minnesota, southern USA	can become tolerant	Anonymous cited in Firbank and Forcella 2000
Aegopodium podagraria		tolerance	Gressel 1996; Koch and Brunotte cited in Umbach et al. 1994
Trifolium repens		tolerance	see above
Sedum (several species)		tolerance	see above
Urtica urens		tolerance	see above
Equisetum (several species)		tolerance	see above
Festuca sp.		tolerance	see above
Euphorbia spp. (spurges)	USA	control not adequate in cotton	Benbrook 2000
Sida spinosa (prickly sida)	USA	control not adequate in cotton	Benbrook 2000
Hemp sesbania (coffeeweed)	USA	control not adequate in cotton	Townsend cited in Deterling 2003
Richardia scabra (florida pusley)	USA, Southeast	control not adequate in cotton	Hayes cited in Manning 3002
Echinochloa crus-galli (barnyard grass)	USA	control not adequate in cotton	Benbrook 2000
Passiflora incarnata (maypop passionflower)	USA	control not adequate in cotton	Benbrook 2000

Cynodon dactylon (bermudagrass)	USA	control not adequate in cotton	Townsend cited in Deterling 2003
Sorghum halepense (johnsongrass)	USA	control not adequate in cotton	Townsend cited in Deterling 2003
Senna obtusifolia (sicklepod)	USA	control not adequate in cotton	Townsend cited in Deterling 2003
late season grasses	USA	control not adequate in irrigated cotton	Townsend cited in Deterling 2003
Ipomoea lacunosa (pitted morningglory)	USA: Cotton Belt	control not adequate in cotton	Hayes cited in Manning 2003
Tradescantia ohiensis (tropical spiderwort, “dayflower“)	USA: Georgia, Florida, Luisiana	peanuts and cotton	Leidner 2003
Amaranthus palmeri (palmer amaranth, pigweeds)	Cotton-Belt	cotton	Hayes cited in Manning 2003
Cyperus ssp. (nutsedges)	Cotton-Belt		Hayes cited in Manning 2003
Oenothera laciniata (cutleaf eveningprimrose)	Cotton-Belt	cotton, favoured by glyphosate, thus tank mix or alternative treatments required	Hayes cited in Benbrook 2000
Polygonum (smartweed)	Cotton-Belt	cotton, favoured by glyphosate, thus tank mix or alternative treatments required	Hayes cited in Benbrook 2000
Salsola iberica (tumbleweed, Russian thistle)	USA: High Plains		Hayes cited in Manning 2003
legumes		hard to control	Gressel 1996
Amaranthus rudis (common waterhemp)	USA Iowa, Missouri Europe	difficult to control, early spraying or higher amounts recommended „could be interpreted as resistant“ biotypes escape due to late germination probable	Hin et al. 2002 Hartzler 2003a Firbank and Forcella 2000
Ambrosia spec. (rag weed)	Midwest USA	decreasing efficiency	Hin et al. 2002
Abutilon theophrasti (velvetleaf)	Midwest USA	decreasing efficiency	Hin et al. 2002 Hartzler 2003a
resistance to glyphosate			
Erigeron (fleabanes)	Cotton-Belt	cotton, favoured by glyphosate, thus tank mix or alternative treatments required	Hayes cited in Benbrook 2000

Conyza canadensis (horseweed)	Delaware, New Jersey, Maryland Tennessee, Indiana, Ohio, East, midwest and southeast US	resistant biotypes, soybean and cotton resistance, difficult to control,	VanGessel 2001, cited in Hin et al. 2002, www.weedscience.org Hayes cited in Deterling 2003, Manning 2003, Hartzler 2003a
Eleusine indica (goosegrass)	Malaysia 1997	in oilpalm after 10 years spraying (8 times/year), population with target site resistance, 4-8fold, only 25% control, 2-5 sites, 101-500 acres	Lee and Ngim 2000, Doll 2000; Heap 2000, http://weedresearch.com
Lolium rigidum (rigid ryegrass)	2x Australia 1996,1997, 1x California 1998 South Africa 2001	populations with resistance. 8-12fold; Australia (NSW):11-50 sites and 1000-10.000 acres (increasing) in apple and wheat after 20 years spraying, (Victoria): 2-5 sites, 11-50 acres 15 years in grain sorghum and wheat South Africa: 11-50 sites and 500-1000 acres (increasing) in vineyards	Heap 2000, Pratley et al. 1999, http://weedresearch.com
Lolium multiflorum (italian ryegrass)	Chile	2 locations, 3 populations 2-6fold resistance in orchards after 8-10 years spraying (3 times/year)	Straszewski 2003, Perez and Kogan 2003

Tab.9: Reported cases of insufficient weed control by glufosinate

weed	**crop/region**	**indication** tolerance, resistant populations, individuals, degree of control/resistance, management indications	**reference**
Ranunculus sp.		not very active	Gressel 1996;
Sedum sp.		tolerance	Heitefuss et al. 1994
Chenopodium album		varying	see above
Equisetum ssp.		tolerance	see above
Viola arvensis	Europe	4.8-8 l not sufficient („satisfying“)	Pallutt and Hommel 1998
Galium aparine	Europe, sugar beet	3x600g ai/ha and more not sufficient	Hommel and Pallutt 2000
Lamium sp.	Europe, sugar beet	conventional herbicides are more effective	Bückmann et al. 2000
Viola arvensis	Europe	not always active	Ammon et al. 1996
Amaranthus lividus	Europe	not always active	Ammon et al. 1996
Amaranthus retroflexus	Europe	not always active	Ammon et al. 1996
Amaranthus rudis	Europe	escape due to late germination probable	Firbank and Forcella 2000
perennials		difficult to control	Hurle 1994
legumes (several species)		hard to control	Gressel 1996

Although the development of resistance to glyphosate was thought to be unlikely (Jasieniuk 1995), three weed species resistant to glyphosate are recorded (http://weedresearch.com): rigid ryegrass (*Lolium rigidum*), italian ryegrass (*Lolium multiflorum*) and goosegrass (*Eleusine indica*).
Horseweed (*Conyza canadensis*) has also acquired resistance according to Hayes (cited in Deterling 2003 and in Manning 2003) as well as fleabanes (*Erigeron*) according to Hayes (cited in Benbrook 2000) and Petersen (pers. communication)(see Tab. 8).
The first glyphosate-resistant rigid ryegrass populations were found in 1996 and 1997 in two distinct populations in Australia, a third biotype was described 1998 in California, and a fourth biotype was reported from South Africa in 2001.

The Australian glyphosate-resistant *L. rigidum* biotype is 9- to 10-fold more tolerant to glyphosate and also aquired a 3-fold higher tolerance 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 times a year), and in Malaysia (*E. indica*) after 10 years (8 applications a year, first report in 1997) (Lee and Ngim 2000, http://weedresearch.com).
The mechanisms of resistance against glyphosate may be cellular or biochemical. They are not fully elucidated (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).

No glufosinate-resistant weed biotype has been recorded so far (see Tab. 9) though weed species with lower sensitivity to glufosinate are known (Nap and Metz 1996, Jansen et al. 2000, Hommel and Pallutt 2000). However, *Populus spec.* (poplars), transformed for elevated level of glutamine synthetase (the target enzyme of glufosinate) to enhance nitrogen utilization, have been found to be more resistant to glufosinate (Gressel 2000).

II.2 HR-gene flow to volunteers or interfertile weeds

Variability of gene flow

In recent years, many data have been collected from field experiments with regard to gene transfer frequencies. A summary of published hybridization distances for crop and wild species was provided by Schütte (1998a). A high variation of results was shown. Results vary so much, that they are of very little prognostic value (Gliddon 1999, Schütte 1998a). The 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 (Schütte 1998a). Different genotypes or varieties sometimes show different frequencies of cross-pollination (Gliddon 1999, Ford-Lloyd 1998, Simpson et al. 1999). Even

self-pollinating plants do cross-pollinate at small or very small levels depending on the genotype. In general, plants are called self-pollinating when the level of cross-pollination does not exceed 10%.
In 6 of 19 studies the rate of cross pollination did not decrease in correlation to distance between referring plants (Schütte 1998a). The (correlation) hypothesis was most often disproved when insect pollination occurred.

Most experiments were done with small pollen sources. 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. Pollen clouds from different fields of a region should be taken into account.

II.2.1 Agronomic significance of HR-gene flow to volunteers and in seed production

General relevance

Volunteers are crop plants emerging from buried seeds or plant parts from the previous crop. They can be a problem in agriculture, behaving as a weed in following crops, particularly in those crops with a lower competitive ability. They can also cause problems in following crops of the same species (e.g. different varieties of oilseed rape showing different seed qualities) where they may cause a contamination and/or a reduction in quality. Eliminating volunteers usually involves the application of herbicides, thus increasing herbicide use. Major problems arise when broadleaf volunteers such as oilseed rape show up in sugar beet or other broadleaf crops where they are very competitive and difficult to control with the available herbicides (Madsen et al. 1997). If such volunteers are resistant to the same herbicide as the crop species, the volunteers cannot be controlled by this herbicide, which could result in the use of alternative herbicide mixtures or in an increase of herbicide use (Darmency 1996, Bjerregaard et al. 1997). Double resistance against both herbicides can also occur. The emergence of multiple-resistant oilseed rape volunteers in Canada may serve as an example (Hall et al. 2000, Downey 1999).
Outside the fields volunteers may also play a role in gene transfer from transgenic crops to oilseed rape or wild relatives by serving as stepping stones. In consequence gene flow may be found over larger distances than currently often estimated from common isolation distances and pollen flow models.
While some crops are ready volunteers, 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 Europe oilseed rape volunteers are abundant in cereals, corn, sugar beet, potato, some legumes and linseeds, cereal volunteers occur in sugar beet and cereals and potato in sugar beet. In general, feral populations of crops which are not native to a region have a lower chance of surviving.
Details on the genetics and pollination of corn, oilseed rape, and sugar beet are described in the appendix.

Current relevance of the control of volunteers and of seed impurities

Corn, Zea mays L. ssp. mays

Seed production

Isolation distances in seed production vary between 220 m and 440 m although distances of 500–1000 m have been recommended (Emberlin et al. 1999). The accepted practice for foundation seed production is a distance of 180-200 m - for the production of sweet corn 300m (Neuroth 1997, Niebur 1993). As the accepted level of contamination (2 % in conventional varieties) will be lower for transgenic crops in Europe, isolation distances may become larger.

Unwanted, adventitious herbicide resistance in seed:

Cross pollination in seed production and grain handling, storage and transport are the main sources of contamination. 41% of seed lots imported from North America to France contained low levels (0,2%) of GM seeds (Bock et al. 2002). Another source of seed contamination is the practice of seed exchange between farmers as e.g. done in parts of Mexico (Alvarez-Monzales 2002).

Volunteers

Volunteer corn causes serious weed problems in soybean (Shaner 2000) and sugar beet. Volunteer management is done in the North American Corn Belt (Cremer et al. 1995). Farmers who rotate both glyphosate resistant corn and soybean already use an additional herbicide (e.g. “Select”) to control volunteer corn in soybean (Hartzler 2003a). Corn plants can survive outside the field, e. g. on road sides, in warmer climates, but they show no tendency of invasiveness (de Kathen 1999). Corn seeds have no dormancy and can germinate after harvest, remaining viable for 2 – 10 years (Neuroth 1997).

In Northern and Central Europe corn 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). Despite this fact, adventitious HR corn plants will occur due to seed impurities in Europe and elsewhere:

Conclusion and management recommendations

Seed exchange and cross pollination may be important aspects in Mexico and other centres of diversity of corn (Alvarez-Monzales 2002). Corn volunteers are known in warm regions and additional control methods for them are applied in the US-Corn Belt.

In many colder regions (where corn does not survive low temperatures) the likelihood of growing unwanted HR corn due to impure seed may become relevant.

The probability of growing low levels of unwanted HR (or generally of unwanted GM) corn depends on many aspects in farming, such as field sizes, crop rotations, weather conditions, on the abundance of pollinators and – most important in US and European corn production: seed production management.

Cotton, Gossypium ssp.

Cotton pollen is spiny, comparably heavy and not carried by wind over longer distances. Cross pollination in cotton does only occur at low levels compared to e.g. sugar beet or oilseed rape. A few studies compiled by Schütte (1998a) proved average outcrossing rates of

1% to 2% (5 studies) at a distance of 10 m. The considerable (overall) variation of 0,03-4,7% is likely due to the differences in experimental settings (see above). Higher rates may be found if more settings were studied. The amount of cross-pollination varies with the population of insect pollinators.

Only very small isolation distances are required between different varieties unless there are obvious differences in morphology such as flower colour. In this case about 500 m are required (Jenkins 1993). Contamination of non-GM cotton seed by transgenes has been observed (FOEE 2000).

Commercial cotton varieties do not seem to create severe problems as volunteer plant. Most seeds of modern cultivars do not survive more than one season – in contrast to wild cotton (Jenkins 1993). Nevertheless, the occurrence of volunteer cotton in soybean crops has been reported from the USA (http://www.cropchoice.com/leadstry.asp?recid=87).

Oilseed rape, Brassica napus

Seed production

Isolation distances for breeding range from 100 m to 1000 m (Darmency and Renard 1992, Renard et al. 1993, Gerdemann-Knörck and Tegeder 1997). Isolation distances for non-hybrid seeds are at least 200 m for foundation seed (in Germany: 100 m for certified seed, 200 m for foundation seed). Border areas (8 – 30 m wide) are effective in reducing pollen mediated gene flow (Feldmann 2000, Staniland et al. 2000) more than isolation zones of the same width do, but they cannot completely eliminate gene flow.

The level of HR genes is usually below 0,25% in conventional seeds in Canada (Orson 2002).

In Europe it might be technically possible but economically difficult (see management recommendations below) to maintain a 0,3% seed impurity level and a 1% impurity level in agricultural production when 10% of the rape growing area is transgenic (e.g. herbicide resistant) (Bock et al. 2002). (The assessment of Bock et al. (2000) was based on expert surveys, published information on hybridisation rates and the current agricultural practice.).

Volunteers

Volunteer oilseed rape is creating control problems in many areas in Europe and in Canada. 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).

A substantial amount of oilseed rape seeds is lost at harvest regardless of the harvesting method. Seed loss is estimated to be between 200 to 300 kg/ha on the average, corresponding to 5,000 – 7,000 seeds/m² (Pekrun et al. 1998). The seed loss could not be prevented even in small experimental plots (Darmency and Renard 1992). Seed losses can be partially reduced by avoiding harvest at high temperatures and low air humidity and by avoiding late harvest. Post-harvest cultivation is commonly delayed in Europe in order to control volunteers. Rape seed is also dispersed during transport. Considerable amounts of seed can get lost from harvesters and trucks at field margins and along road sides/rail road tracks (Neemann et al. 1999).

Seeds can germinate in the first year or acquire secondary seed dormancy. Secondary dormancy is favoured by exposing seeds to water stress and darkness (Pekrun et al. 1998, Gerdemann-Knörck and Tegeder 1997, Madsen et al. 1997). Rape seeds can resist cold temperatures (up to -30°C) and remain viable for more than 10 years (Renard et al. 1993), possibly up to 15 years (Gerdemann-Knörck and Tegeder 1997). Oilseed rape, more than many other crops, has the potential to develop large and persistent seed banks from which volunteers can emerge. HR volunteers have been shown to emerge in multiyear field experiments with HR oilseed rape in Germany and France (Ernst et al. 1998, Darmency and Messéan 1999). Multiple-resistant oilseed rape volunteers exhibiting resistance to glyphosate and glufosinate, and/or to imidazolinone have been reported from Canada at all of the 11 locations where implications were measured (Beckie et al. 2001). No compulsory isolation distances are implemented in Canadian oilseed rape production though 175 m isolation have been recommended by Beckie et al. (2001). SCIMAC recommended a 50 m distance between fields for the UK (Orson 2002).
The relative high probability of outcrossing and pollen drift led to the loss of organic canola industry in western Canada (Phillips 2003). Co-existence of farming with and without transgenic plants is endangered in Europe too (see management recommendations).

<u>Feral/volunteer rape as stepping stones</u>
Oilseed rape volunteers may play a role in gene transfer from transgenic crops to wild relatives and possibly serve as “stepping stones”. 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). Feral plants include both volunteers within fields and other populations on field margins, soil dumps and roadsides 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.

<u>Conclusion and management recommendations</u>
Canada
In Canada no management plan has been implemented for canola volunteers so far. Farmers and regulators seem to rely on the options to use alternative herbicides for volunteer control. Several alternative herbicides are available, except for control in legume crops (Beckie et al. 2001). Syngenta has promoted a new product (‘Gramoxone PDQ’) to Canadian farmers for dealing with glyphospate resistant oilseed rape volunteers (<u>http://www.tao.ca/~ban/100MSuk</u> gmtrials.htm).

Europe
Volunteers:
Pekrun et al. (1998) suggested to delay post-harvest cultivation (which is commonly done in Europe) and to repeat shallow stubble tillage in production in order to reduce seed persistence in soil. A complete prevention of volunteer occurrence seems impossible even by a combination of the above post-harvest cultivation and wide rotations according to Dietz-Pfeilstetter et al. (1999). Additional herbicide applications would become necessary due to HR volunteers in other HR crops resistant to the same herbicide (e.g. corn or sugar beet) if they were grown in rotation. However, such a rotation with resistances to the same herbicide

is not very likely due to the expected control problems. Nevertheless, some experts recommended to use tank-mixtures in order to prevent volunteer problems (Stelling et al. 2003), which will have negative consequences on biodiversity (s. IV.3).

Unwanted, adventitious herbicide resistance in seed:
It will be economically difficult to adjust farming practice well enough to meet a 1% impurity level in European oilseed rape production. It may be necessary to minimize overlapping flowering periods between different (HR and conventional) varieties. A regional border management is another option in order to keep impurities below the mentioned level (Bock et al. 2002).

Soybean, *Glycine max* L. Merrill
Seeds are dispersed by pod shattering, particularly if harvest is delayed. Seed survival in soil is poor (Beversdorf 1993).
In Europe, soybean is not weedy (Bjerregaard et al. 1997). In US cotton areas, however, keeping out volunteer soybeans can be a challenge (Hayes, cited in Benbrook 2000). Volunteer management is known to be done (Neuroth 1997, Cremer 1995).

Sugar beet, *Beta vulgaris ssp. vulgaris var. altissima*,

<u>Seed production</u>
Annual forms of wild *Beta vulgaris spp*. grow in Italy and France where European sugar beet seeds are generally produced. Isolation distances in seed production vary from 300 m to 1.000 m (Bjerregaard et al. 1997, Bosemark 1993, Gerdemann-Knörck and Tegeder 1997). A more extended isolation distance of 3.200 m has been recommended (Neemann et al. 1999).
The annual growth habit can cause considerable problems if allowed to contaminate breeding stocks or commercial seed fields. If these are HR beets, herbicide resistant weed beets and volunteer beets may result (Bosemark 1993, Gerdemann-Knörck and Tegeder 1997).

<u>Volunteers</u>
As sugar beet is a biennial species, it is normally harvested before flowering. Some individuals (bolters) may flower in the first year. Bolting can be due to low temperatures (due to early sowing) and to the bolting gene, which is independent of low temperature.
Low temperatures can induce the reproductive state in the first year. The annual growth habit is governed by a dominant gene causing plants to flower and quickly set seeds under long day conditions and reasonably high temperatures. Seeds from such bolters need stratification during winter and can germinate in the following year, again exhibiting an annual growth habit. Seed contamination with annual weed beet can cause serious weed problems in beet crops and reduced sugar yield (Bjerregaard et al. 1997, Madsen et al. 1997, Neemann et al. 1999). Beet seeds can remain viable for 8 – 10 years or more (Bosemark 1993, Gerdemann-Knörck and Tegeder 1997).
Annual weed beets cause serious problems in parts of Europe, including Belgium, Germany, England and northern France. Bolting HR sugar beets can pollinate weed beets , if bolters are not removed before flowering, resulting in HR resistant weed beets. According to Vigouroux et al. (1999) hybridisation between annual weed beets and cultivated HR beet will happen when HR varieties are grown.

In milder climates volunteer plants can also emerge from pieces of beet roots left in the field (Bjerregaard et al. 1997).

Conclusion and management recommendations

Bolters have to be monitored and controlled in root production areas. If the bolting plants and weed beets are not removed immediately, stable weed beet complexes form quickly and are difficult to eradicate (Bartsch et al. 1993, Sukopp and Sukopp 1994, Parker and Bartsch 1996, Hoffmann and Köhler 1999, Neemann et al. 1999).

Moreover, certified seed with low impurity levels should be produced and used. A thorough control of ruderal beets and the implementation of upper isolation distances (see above) in seed production areas will be necessary.

II.2.2 Probability and agronomic relevance of HR-gene flow to weeds

General relevance

There is widespread concern that weeds can not only become resistant to herbicides through selection pressure exerted by the broad use of these herbicides but also by hybridisation with HR crops (either directly or after several backcrosses, depending on the species and event), followed by introgression. Introgression of HR genes due to crossings between (HR-)crops and weedy relatives is a new mechanism for the development of herbicide-resistance in weeds.

If HR crops are cultivated in many countries, the transfer of a given gene will quickly become a worldwide matter. The opportunity for range overlap with compatible relatives may also increase with increasing HR crop areas. Additionally, when bulks of unprocessed wheat or oilseed rape are imported as commodities, effects of introgression from transgenic crops into the weed flora of importing countries should be considered (Gressel 2000).

If a certain proportion of a weed population acquired herbicide-resistance then a certain proportion of the seeds shed will carry herbicide-resistance. These weed 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).

Additional or alternative weed control efforts could be the outcome. The potential for simultaneous occurrence of more than one HR transgene could make management and eradication efforts of volunteers and weeds substantially more difficult (as with multiple resistant volunteers of oilseed rape, see above).

Knowledge on hybridisation frequencies

Cross pollination is a prerequisite for hybridisation. The probability and the limitations for pollen flow and cross pollination are described in chapter II.2. Generally, hybridisation frequencies are lower than cross pollination frequencies between individuals of the same species.

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

Hybridisation frequencies between crop plants and wild species depend on a variety of factors, quite often exerting influence on each other. Potential hybridisation mates have to flower at the same time in a distance allowing pollen transfer. Besides that, many other conditions for successful hybridisation have to be fulfilled (Brown et al. 2000, Feldmann 2000, Chèvre et al. 1999, Kloepffer et al. 1999, Pfeilstetter et al. 1998, Schuette 1998a): Temperature, humidity, time of the day, wind speed and direction, abundance and foraging behaviour of insect pollinators, population size of the pollen donor and the recipient, and the compatibility of crop plants and their wild relative play an important role in hybridisation. Hybridisation rates may also depend on the genotypes of the cross mates. Intra-population genetic variability of wild plants regarding their ability to produce hybrids seems to exist (Darmency 2000). Predictions of what may happen in a given scenario are thus very difficult.

In the UK, some plant families are known to show high numbers of natural hybrids, e.g. *Brassicaceae* and *Poaceae* (Sukopp and Sukopp 1994). Darmency and Renard (1992) assume that spontaneous interspecific crop x weed crosses probably occur more frequently than reported in the literature. Ellstrand (1988) concluded from pollen flow studies between neighbouring plant populations that interpopulation gene flow can proceed over much greater distances and at higher rates than hitherto believed and that escape of engineered genes from crop plants to their wild relatives is not only possible but also likely (see also II.2).

<u>Survival of hybrids</u>

Survival rates and reproductive fitness of resulting hybrids and of progeny of backcrosses are important factors for the establishment of transgenes in plants other than crops.

Once transgenes conferring herbicide-resistance move into weeds, their frequency within local weed populations could increase due to positive selection pressure (when the corresponding herbicide is applied).

According to Colwell (1994) a „rare" hybridisation between crop and weed may be sufficient for the escape of the transgenic trait into the population of a weedy relative. Furthermore, hybrids do not need to be particularly fit in themselves as long as they are able to backcross with the weedy relative, a capacity many interspecific hybrids have.

When the positive selection is missing, a negative selection is probable, 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 trait and could be different in crops and in weeds due to different genetic backgrounds (Snow and Jørgenson 1999, DETR 2000). The fitness of hybrids should be assessed from species to species (see below). But even genotypes with a lower fitness are able to survive when the pollen flow is steady and the source is large. Giddings (pers. communication) estimated from a population model that a steady pollen flow leads to 1% naturalisation of a genotype with 20% lower fitness.

Probability of gene transfer to weedy relatives of current HR crops

Corn

Europe and USA

Since there are no wild relatives in Europe and the USA, gene transfer to wild weedy species in Europe and the USA is highly unlikely (Bjerregaard et al. 1997).

Central America

Corn (2n = 20 chromosomes) is considered to have a multicentered origin. Independent domestications may have occurred in teosinte (*Zea mays ssp. mexicana*) throughout Central America (Niebur 1993). Outcrossing in corn could not be found beyond 200m at a very arid Mexican research location due to the desiccation susceptibility of corn pollen and low wind speeds (Baltazar and Schoper 2002). The potential for transfer of traits from transgenic corn to teosinte is real (Niebur 1993, Colwell 1994). Teosinte, the closest relative of corn, includes three wild subspecies of corn itself and three closely related species of *Zea*, growing as agricultural weeds and wildland species (Colwell 1994). At least in Mexico it is also grown as a forage crop because of its high protein content.

Hybrids of teosinte and corn have been found in the Central Plateau and Valley of Mexico. A study of natural populations in Mexico in different regions showed that hybrids are surviving in nature. Hybrids have been found at five of nine locations. The rate was low (under 1% to 1,5%) at four locations and high (26,9%) at one location (Sánches-Gonzáles, pers. communication). Baltazar and Schoper (2002) concluded from their study that early shedding teosinte plants are able to fertilize late silking corn plants. In contradiction to these findings, Evens and Kermicle (2001) reported that the two forms were physiologically incompatible and their flowering period is known to hardly overlap (Baltazar and Schoper 2002). The closest related genus of *Zea mays* is *Tripsacum* (7 species, all with n = 9 chromosomes). In spite of the different chromosome number, crosses can be achieved with all *Zea* species although under difficulties, resulting in highly sterile hybrids (de Kathen 1999). Although the hybrids are of low fitness, they are nevertheless able to backcross with teosinte.

Cotton

Cotton has been cultivated for about 3000 years. The centres of origin of the predominant cotton for commerce (*Gossypium hirsutum*) are North and Central America and Mexico. The centre of extra long staple cotton (*Gossypium barbadense*) is South America. Both species are allotetraploid (AD1 and AD2 genome respectively). The diploid *G. arboreum* (A1 genome) is predominantly grown in India, small acreage of diploid *G. herbaceum* (A2 genome) are grown in drier regions of Africa and Asia. There are no wild species or relatives of cotton in the US cotton belt that will form fertile hybrids with commercial cotton. However, a wild species (*G. tomentosum*) is cross-fertile with commercial cotton in Hawaii. Here and in other areas of South-East Asia, where wild relatives grow, extra attention should be paid to field isolation of cotton crops to wild and diploid species (Jenkins 1993).

Oilseed rape

Details on the probability of hybridisation of oilseed rape with related species are presented in the appendix.

As direct hybrid formation is very low for *B. napus* and *B. nigra* or *S. arvensis* introgression is not expected in either case (Dietz-Pfeilstetter et al. 1999). Moreover, hybrids exhibit a reduced or no fertility (Dietz-Pfeilstetter et al. 1999). Compatibility with a range of other wild species may exist (Jørgensen 1998).

Introgression of HR into *B. juncea* and *H. incana* is considered as highly unlikely as they are rare in Europe and hybrids occur only with very low probability. Nevertheless, *S. arvensis* may become herbicide resistant by indirect gene flow in regions where *B. rapa* and herbicide resistant *B. napus* are grown in vicinity.

The following weedy plants may raise control problems due to introgression of HR genes from oilseed rape according to Dietz-Pfeilstetter et al. (1999):

- *B. rapa* (which is grown as a crop but also known as a weed) (in Europe and Canada)
- backcrosses of *B. napus/R. raphanistrum* hybrids with the weed parent (in Europe)
- backcrosses *B. napus/Erucastrum gallicum* hybrids with the weed parent (in Canada).

Herbicide resistant weeds are under control, as long as different herbicides are sprayed in cereals (or other rotational crops) (Dietz-Pfeilstetter et al. 1999). Thus, herbicides should be changed from time to time.

<u>Limitations of the above conclusion</u>
Hybridisation with feral oilseed rape may theoretically happen and the feral plants possibly serve as stepping stones. Feral descendants of oilseed rape exist in close proximity with 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). Feral plants include both volunteers within fields and other populations on field margins, soil dumps and roadsides mostly derived from seed spills. Many of these out-of-field populations are not routinely controlled. Feral populations outside fields experience a wide range of selection pressures, leading to diverse forms including individuals that flower when the plant is very small or at various times or late in the season. *B. napus* feral plants have been found together with 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).

Soybean

Cultivated soybean (G*lycine max*) derives from the wild annual legume species *Glycine soja* native to China, Japan, Korea, eastern Russia and Taiwan with which it hybridizes readily resulting in fertile offspring. The genus *Glycine* has been subdivided into the subgenus *Soja* (GG genome) with *G. max* and its ancestor *G. soja* (2n = 40 chromosomes) and the subgenus *Glycine* (40 or 80 chromosomes) with about 16 wild perennial species, most of them growing in Australia, and some of them native to Indonesia, the Philippines and Taiwan. These species are grouped according to their genomes, ability to hybridize and fertility of F1-hybrids. *Glycine* species with similar genomes cross easily and produce fertile offspring, whereas crosses between species with different genomes mostly lead to sterile offspring, if seeds are produced (Zeller 1999). Naturally occurring hybrids between the sub-genera *Soja* and *Glycine* have not been observed (Beversdorf 1993).

Sugar beet

The wild relatives of sugar beet, family *Chenopodiaceae*, genus *Beta*, originated in Asia Minor and in the Mediterranean area. The maritime beet (*Beta vulgaris* L. *ssp. maritima*) growing along European coasts particularly of the Mediterranean Sea is considered to be the ancestor of *Beta*-beets (sugar beet, fodder beet, red beet, and chard). All cultivated beets and a range of wild forms belong to the section *Beta*, all of which are sexually compatible and give fertile offspring with each other. They may thus be considered to be members of the same collective species.

All members of the section *Beta* with exception of *B. macrocarpa* are diploid (2n = 18 chromosomes). *B. macrocarpa*, a variety of the subspecies *B. maritima*, has diploid and tetraploid populations. Tetraploid and triploid sugar beet have been produced too (Bosemark 1993, Gerdemann-Knörck and Tegeder 1997).

The ancestor of sugar beet, wild beet (*Beta vulgaris ssp. maritima*), exhibits a large phenotypic variation and is adapted to a large number of different ecological niches. Populations of wild beets are found on the coasts of Southwest Norway, of the Baltic Sea (Denmark and Germany), and the North Sea, and along the coasts up to the Cape Verde Islands and the Canary Islands, on the coast of the Mediterranean Sea and the west coast of the Indian subcontinent. Populations have also been described in Australia and California and as ruderal inland beets in France (Bartsch et al. 1993, Gerdemann-Knörck and Tegeder 1997, Bartsch and Ellstrand 1999, Desplanque et al. 1999, Driessen et al. 2000).

Hybrids between wild beets and all *Beta vulgaris* cultivars arise wherever the parental plants grow and flower in close vicinity. Weedy sugar beet forms can result which flower the first year and produce only small roots with low sugar content. As wild beet and sugar beet are subspecies of the same species, the relevance of gene exchange is addressed in chapter II.1.

Beet can also hybridise with *B. atriplicifolia* and *B. macrocarpa* both abundant in the Mediterranean area. In California, hybrids between *B. vulgaris* and *B. macrocarpa* are reported to cause weed problems in sugar beet fields (Bjerregaard et al. 1997).

Experimental transfer of herbicide-resistance genes from sugar beet to red beet, chard, and the wild beet as well as from bolters in a field has been demonstrated (Bartsch et al.1999, Vigouroux et al. 1999).

Gene flow to volunteers and interfertile weeds of important HR crops under development

Rice, *Oryza sativa*

According to Gressel (2002) not the rice volunteer plants themselves but the mitigation of introgression of HR genes into weedy relatives will be the main challenge in rice even though rice is predominantly self-pollinated and cleistogamous. Conspecific red rice and weedy relatives such as *O. rufipogon* and *O. nivara* are cross compatible with cultivated rice.

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. Wild conspecific rices occur throughout the tropics of Asia, Africa, Oceania and Latin America (Vaughan 1994 and Bellon et al. 1998 cited in Cohen 1999).

Herbicide resistant hybrids will become an acute problem after 4-7 years when outcrossing exceeds a rate of 1% (this can be concluded from computer modelling according to Gressel 2002). The outcrossing rate may be lower than 1% but will increase where hybrid varieties are grown (see above, chapter II.2:Variability of gen flow). Gressel (2002) discussed diverse containment measures for herbicide resistant rice such as linking the resistance trait with anti shattering -, anti dormancy – or dwarfing genes (these three traits are considered to be detrimental to weedy rice but neutral to cultivars) and male sterility (in order to mitigate outcrossing). Varieties resistant to different herbicides should be used according to Gressel (2002) and a combination of two or more of the above mentioned containment traits. Even the establishment of weed free zones around the HR crops were proposed by Gressel.

Wheat, *Triticum aestivum*

Wheat volunteer plants are known to occur in sugar beet and oilseed rape fields but hybridisation with wild relatives does not occur in Europe.

In the western United States the weed species *Aegilops cylindrica* (jointed goatgrass) , does form hybrids with wheat. Seefeldt et al. (1999) proposed management strategies to prevent outcrossing of traits into this weed:

- With heavy weed infestation: one preplant burn (or a chisel plough at intervals on highly erodible land) followed by a spring crop or fallow to reduce the jointed goatgrass seedbank
- Herbicide resistant wheat must come from "certified" wheat fields where there is no *A. cylindrica* within half a mile distance to the field
- Wheat varieties should be competitive against *A. cylindrica* and narrow spacing as well as high seeding rates should add to this effect.
- Herbicides should be applied with maximum efficacy to the field and the field borders (even all small infestations of *A. cylindrical* must be killed).
- As much *A. cylindrica* seed as possible should be harvested.
- Seed losses from truck should be minimized and farming machines should be thoroughly cleaned before moving from the field.
- A non-winter crop should be planted in the following year which allows the use of alternative methods to control *A. cylindrica*.
- F1 hybrids, which are easy to detect because of their increased size, should be hand-weeded and destroyed.
- The next winter wheat crop should not be herbicide resistant.

Conclusions on changes in weed susceptibility (chapter II)

The use of HR crops will change weed communities and populations due to the effectiveness and scope of the non-selective herbicides and changes in agricultural practice. The shifts in weed populations will be the greater the higher the changes in weed control effectiveness and scope and agricultural practice are. In general, the more often a specific herbicide is applied on the same field, the more rapidly a weed shift (to less susceptible species) will occur. Nevertheless, the effects from the same transgenic HR-crop can vary greatly from one agricultural ecosystem to another.

The risk of changes in weed susceptibility caused by selection is generally considered to be higher compared to gene flow to weeds in most cropping areas. Regions where highly interfertile weeds are abundant may be excepted in this conclusion. Moreover, gene flow to volunteers is more likely than to weedy relatives.

Conclusion on changes in weed control due to selection and weed shift (II.1)

The data presented make it reasonable to assume that resistance/tolerance to glyphosate will develop if this herbicide is increasingly used in high proportions 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.

USA

In HR crops the decrease in numbers of herbicides used, and the trend to less soil cultivation put a selection pressure on weed communities. Changes of the weed community structure (due to selection of resistance in weeds and volunteers and due to shifts to tolerant species) already resulted in altered weed control patterns (in HR crops) in some regions. Some farming consultants already propose to use additional herbicides in HR crops or to change the pattern of cultivation (see Chapter III.1 and II.1). The percentage of glyphosate resistant soybean fields treated with an additional preemergence herbicide has significantly increased in areas where the HR soybeans have been planted for many years. (Hartzler 2003a). In addition, the glyphosate amounts ("rates") increased. Waterhemp (*Amaranthus rudis*) may be largely responsible for these changes in weed management.

Other new weed control problems and weed shifts have occurred in cotton in the USA (see Tab. 8, Chapter II.1). The most prominent weed problem in HR cotton is caused by horseweed (*Conyza canadensis*) in no-till production. Soybean-corn rotations with both crops being resistant to glyphosate are considered to account for resistance of horseweed. Its seeds are very well dispersed by wind. Many weed scientists recommend to use additional herbicides in glyphosate resistant cotton and multiple applications in soybean resistant to glyphosate (see chapter III.1).

With the current manner in which glyphosate is being used in the Midwest (USA), weed resistance development is inevitable according to Hartzler (2003a). Experts on soybean who responded to a survey (see Chapter III) stated, that the development of resistance is low in US soybean but high in Argentinean soybean. One of 5 canola experts expected resistance selection in Canadian canola to be highly likely too.

In a crop rotation with soybean and corn or soybean and cotton, all crops being glyphosate-tolerant, the selection pressure on weeds is very high and weed shifts are very likely. (Hayes cited in Benbrook 2000). 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.

Europe

In crop rotations with HR-corn and HR-oilseed rape, both crops resistant to the same herbicide, similar weed population shifts may occur since these two crops have many important weed species in common. In fact, German studies showed that 10 of the 20 most important weed species occur in corn, sugar beet, and also oilseed rape (Petersen and Hurle 1998a).

Crop rotations and estimated acreage (ha) in Germany (Petersen and Hurle 1998a)

(cereals), corn	1.084.000
cereals, sugar beet	245.000
cereals, oilseed rape;	314.000
cereals, corn, sugar beet;	150.000
cereals, oilseed rape, sugar beet;	318.000

cereals, oilseed rape, corn;		780.000
cereals, oilseed rape, corn, sugar beet;		150.000
	total	3.032.000 - 12,8% of arable land

These weed species will be put under strong selection pressure. If 50% of the planted corn, oilseed rape, and sugar beet varieties were resistant to glyphosate, 16% of all herbicides used

in Germany would be glyphosate-products - according to Petersen and Hurle (1998a). This is 1,5 times more than the rate of the most widely applied herbicide in Germany, isoproturon (which is not used any more). About 4.600 tons of glyphosate (active ingredient) were used in 2002 (Hommel, pers. comunication), which is about 29% of the herbicides sprayed in German agriculture. Thus the proportion of glyphosate will even be higher than 30% when HR crops are planted.
There are alternative herbicides for all crops available when weed herbicide resistance occurs. But the key question is, whether the new agricultural practices with HR (as already seen in soybean and cotton) or its alternative conventional or integrated practice will be economically and/or ecologically advantageous (see chapter III, IV).

Common methods to delay resistance

Hartzler (2003a) 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 continuously planting glyposate resistant crops. An annual rotation of herbicides should be the foundation of resistance management (Hartzler 2003a).
Resistance in grass species will not evolve as quickly if glyphosate is rotated with other non-selective contact herbicides.

A combination and rotation of weed management methods is essential to delay resistance evolution in weeds (Ghersa et al. 2000, Kropf and Walter 2000, Bastiaans, et al. 2000, Heap 2000, HRAC 2000, Wolfe 2000, Ballare and Casal 2000, Canola Connection 2000, Long 1999):

- 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, getting better knowledge about the kind of weeds
- manipulation of light environment during tillage reducing seedling emergence
- additional measures: i.e. cover crops, mixed cropping, fallow.

Conclusion on changes due to gene flow (II.2)

HR gene flow to volunteers and in seed production (II.2.1)

Volunteers

Oilseed rape-volunteer control may also lead to changes in weed control in European oilseed rape and in Canadian canola. Thus, additional herbicides may be applied in other HR crops rotated with oilseed rape (see chapter III.1).

Bolting sugar beet are considered as a source for cross pollination and HR-introgression into "volunteer -" or weed beet. Thus the control of bolting beet is recommended.
Volunteers of soybean (in cotton) and corn (in soybean) have to be controlled with additional care in some regions of the USA. Glyphosate resistant varieties of all three crops are planted in the USA. Problems in other HR growing regions have not been reported but will be likely to occur in adequate climates.
The relevance of cotton volunteers seems to be low in current HR cotton growing regions.

Unwanted, adventitious herbicide resistance in seed

The prevention of seed contamination has to be addressed in HR plants with a moderate or high chance of cross pollination such as (currently) oilseed rape, sugar beet and - to some extent – corn. The implementation of measures assuring co-existence of farming systems with and without transgenic plants is currently discussed in Europe. Seed production, grain handling, storage and transport are the main sources of contamination.

Tab. 10: General criteria for the relevance of gene flow for weed control

type of problem	region in which the problem may become relevant		
	areas/centres of origin of the crop	wild interfertile relatives abundant	other growing areas
HR in close relatives	x[1,2]		
HR in subspecies	x[1]	x[1]	
HR in volunteers	x[1,2]	x[1,2]	x[1,2]
unwanted HR traits in seed	x[1]	x[1]	x[1]

[1] relevance depends on the frequency and distance of outcrossing events
[2] relevance depends on the competitiveness of volunteers or hybrids

Gene flow into weedy relatives (II.2.2)

The transfer of HR genes will be of importance in areas or centres of origin of the crops and regions where both interfertile and weedy forms of crops occur.
The control of oilseed rape relatives in Europe and the implications of hybridisations between corn and teosinte are addressed in the current biosafety discussion of HR crops. Weed control methods in other crops within crop rotations in Europe have been recommended to control possibly occurring weedy hybrids of oilseed rape and wild species. Mexican researchers are currently investigating and discussing the case of teosinte.
Wheat and rice, two very important crops of which HR varieties are expected to be approved soon, both have weedy relatives in certain anticipated release and growing regions.
Precautious control methods are proposed for the wheat fields in the western USA.
Interfertile weedy relatives of rice are abundant in parts of Asia and red rices (subspecies) are known in many parts of Asia, Oceania, Africa and Latin America. A combination of different modes of containment and genetically introduced containment traits is proposed in order to reduce the likelihood of gene transfer to red rice.

Survey results on volunteers and weedy relatives (II.1 and II.2)

Pollen transfer into weedy relatives or volunteers has to be coped by additional management strategies in sugar beet in the UK and in canola in Canada. No answers were given for corn, US soybean, and European oilseed rape within the survey.

The main concern in canola are oilseed rape volunteers and in eastern Canada also *Brassica rapa*. The problems refer to a few (up to 25% of canola acreage) areas according to 3 experts and to most areas according to 2 experts (sample of 5 experts statements). Strategies to mitigate gene flow are already applied according to 2 and not applied according to 3 of the experts. In addition, 2 experts stated that the current management strategies were sufficient to avoid introgression of HR-genes into weedy relatives and volunteers. The other 3 negated this question.

The UK-expert on sugar beet was concerned about *B. vulgaris* in organic fields and with *B. maritima* by the sea coast. The question "Do farmers adopt particular management strategies in order to avoid introgression of HR-genes into weedy relatives and volunteers?" was answered with "yes" in the case of sugar beet (UK) but the current strategies were estimated to be insufficient.

Section III Impacts on agricultural practice and agronomy

HR crops may have various impacts on the agricultural practice and agronomy. Changes affect weed control, yields, net income, soil tillage and planting as well as 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 amounts and applications frequencies of herbicides, yields and net returns are often not consistent. Field tests and experts views on the other hand may not cover all relevant growing situations.

Thus, published results of field studies and statistical studies are complemented by an expert survey which was conducted for this report and is presented in the following section.

The survey consisted of a questionnaire[1] which was sent to more than 60 institutions and experts in the field of HR crops. The survey aimed to cover the most important HR crops, and areas of commercial HR cropping as well as areas in which intensive research is conducted. The focus was on research and consulting institutions, preferably public, e.g. governmental, not on services with clear connection to the HR promoting industry. As the number of returned questionnaires was rather low (13, 22%), a statistical analyses of the material did not seem appropriate. Nevertheless, the survey gives valuable insights into specific situations. Some general trends as well as a surprising variability of agricultural practices in some areas and also of expert opinions can be seen. Details of individual situations are presented. Some general information (crop, region, typical rotation and portion of HR) about the situations covered by the survey can be found in the appendix.

The results of the survey sometimes allow a more precise picture in combination with the cited published results.

III.1 Weed control patterns

General remarks

In non-HR farming, farmers apply a sequence of different herbicides 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.

[1] See appendix for the questionnaire

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 pre-emergence herbicides for programs that provide soil residual control, or with mechanical weeding.

Glufosinate and glyphosate should be applied as long as weeds are less than 10 cm tall. As the maximum weed size for effective control is higher with glyphosate than with other herbicides, the potential time period for spraying is extended (Kalaitzandonakes and Suntornpithug 2001). From a plant growers view this allows more flexibility

Glufosinate is often applied in combination with a residual herbicide in no-till systems. The performance of some glufosinate-based weed control systems in Iowa State University research was variable depending on the level of weed infestation and environmental conditions (Owen 1998). Glufosinate is not readily translocated within the plant, hence good herbicide coverage is essential for satisfying activity. The average application rate under Central European conditions is 1,2 kg active ingredient/ha (potato, corn, oilseed rape, sugar beet) (Wilke 1994). The glufosinate application rate may vary with weed size, weed species, and herbicide application program. As glyphosate is used in many varieties and regions, its application patterns for different situations are described below.

III.1.1 Factors influencing the time and the mode of applications

Crop injury

Crop injury in the field

Herbicide injury in the crops sprayed is more likely in conventional crop plants than in herbicide resistant plants.

Injury in HR crops may sometimes occur, e.g. when HR varieties are less tolerant to non-selective herbicides than others. Various cases of yellow soybeans after postemergence application of glyphosate have been related, inter alia, to varying tolerance levels of HR varieties (University of Missouri-Columbia 2000).

Herbicide injury may also be due to misapplication. Common mistakes include spraying at the wrong growth stage of the crop, overlapping spray patterns, or spraying directly into the whorl of the corn plant. Proper application can help to prevent herbicide injury (Butzen 1998).

The number of problems associated with leaf cupping has increased with the increase in post-emergence applications in soybeans. Glyphosate resistant soybeans seem to develop this type of response as likely as traditional varieties (Lingenfelter 2000).

Injury in the vicinity of the sprayed field

Herbicide injury through drift in non-HR fields can be serious. Generally speaking, drift problems increase with postemergence applications, daytime applications and increasing numbers of applications. Herbicide resistant crops contribute to these increases according to Owen (1999). Daytime applications are more critical in terms of causing drift than applications in the evening which is due to higher wind speeds (see below).

Daytime and weather

Cold and cloudy weather conditions impair the effectiveness of glufosinate (Hommel and Pallutt 2000), and the time of application can effect the weed control ability of glyphosate.

The effectiveness of glyphosate on all of the three weed species tested in a study conducted by Northworthy et al. (1999) was negatively affected in evening (at 18.30 p.m. a twofold reduction of effectiveness was found) and night hours (fourfold reduction of effectiveness). The interception of the herbicide by the plant surface was reduced in this tests, which was attri-buted to the diurnal movement of leafs. The influence of light and daytime may be compensated by increasing the herbicide rate. However, given a choice between reduced weed control in the evening versus drift (due to higher wind speeds) with day applications, the chances should be taken in the evening. In these situations, the herbicide rate has to be adjusted according to weed species and plant sizes and the potential for a lower level of herbicide activity (Hartzler 2000).

Crop emergence

USA

Many herbicides already allow postemergence applications. The appropriate time span for postemergence control is 3-5 weeks after crop emergence with variations depending on the herbicide and crop. The weather and specific weed populations can influence this scheme (Owen 1999). According to Owen (1999) late applications are not economically sound.

On the other hand, herbicides for soybeans are traditionally incorporated into the soil before sowing, enhancing possibly the soil to dry out. It is possible to directly sow HR soybeans into a relatively undisturbed soil and to apply a postemergence herbicide afterwards. Soil moisture can be conserved in this way (DETR 1999; see also III.4 Tillage).

Europe

Selective postemergence herbicides are already available for most crops. Therefore, herbicide resistance does not generally provide a new option in this sense (Walter 1998). If a farmer wants to spray at postemergence, he will be confined to a very short time period in respect to weed and crop development (Pallutt and Hommel 1998). This can be problematical, if the weather conditions are unfavourable for herbicide applications (see above). 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).

Survey results:

Tab. 11: Survey results with regard to the shift from preemergence to postemergence herbicide application in HR crops (questionnaire: 8.1)

Do farmers shift from preemergence to postemergence application in HR crops?			
Crop	**yes**	**no**	**farmers**
sugar beet; UK	x		most
corn; GE		x	
oilseed rape; GE, F	(no assessment made)		
canola; CA (AB)	x		most
canola; CA (SK)	x		~ half
canola; west. CA	x		most
canola; west. CA	x		most
canola; west. CA	x		most
soybean; AR	x		most
soybean; AR[1]			
soybean; US(IA)	x		~ half
soybean; US (NB)	x		most

Each line represents one expert judgement

[1] Farmers may come back to preemergence application for various reasons, e.g. not all fields can be sprayed in the proper time or climatic condition, or some difficulties with postemergence to control important weeds

The experts were asked whether, and to what extent HR growers shift from preemergence to postemergence applications. About half of the canola growers in Saskatchewan and the US-soybean growers are still applying herbicides before emergence in HR crops according to the survey. Most canola farms shifted to postemergence applications. The expectation on weed control in Europe is, that most farmers will shift to postemergence applications in sugar beet (UK) but not in corn. Postemergence applications are already common in most sugar beet areas in Germany.

Scouting and the use of economic threshold models

As the ease and flexibility of weed control have been major reasons for choosing herbicide resistant plants so far (see below; also Hin et al. 2001, CEC, 2000) the use of economic thresholds would not be done as their first preference. The timing and choice of herbicides is simpler and thus HR serves the desire to simplify weed control (Firbank and Forcella 2000). Not scouting and some agriculturally acceptable occurrence of weeds but ”aesthetic” clean weeding can become a significant consideration to farmers (Owen 2000; see also Table 24, soybeans in Nebraska).

Tab. 12: Results of the survey with regard to the scouting of weeds and the use of economic threshold models (questionnaire: 4.1 and 4.2)

Do farmers scout weeds and use economic threshold models?				
	conventional varieties		**HR varieties**	
crop	**scouting**	**economic threshold models**	**scouting**	**economic threshold models**
sugar beet; UK	>50%	0	>50%	0
corn; GE	>50%	0	>50%	0
oilseed rape; GE	<10%	0	?	?
oilseed rape; F	0	0	0	0
canola; CA (AB)	>50%	14%	>50%	12%
canola; CA (SK)	<10%	<10%	<10%	<10%
canola; west. CA	<10%	<10%	<25%	<25%
canola; west. CA	>50%	>50%	50%	<50%
canola; west. CA	<10%	<10%	<10%	<10%
soybean; AR	0	0	0	0
soybean; AR	0	0	0	0
soybean; US (IA)	<10%	<10%	0	0
soybean; US (NB)	<10%	<10%	<10%	<10%

Each line represents one expert judgement

Scouting is important in corn and sugar beet in Europe according to the expert answers. It is nearly irrelevant in soybean and the estimations for canola vary. The same crop can be handled differently in different regions. There seem to be regional farming traditions in regard to scouting.

While weed scientists in the USA and in Europe recommend control of weeds up to a level that eliminates potential interference with net returns (economic thresholds) growers consider other factors (Owen 2000). Economic threshold models are rarely used in the crops covered by the survey. A small portion of growers (<10%) uses the models in conventional US-soybean but still less in HR soybean. Two of the five experts on canola stated, that the use of economic threshold models will further decrease in HR canola (the portion varying between 12% and >50%) whereas one expert predicted a small increase.

Various databases on integrated weed management and expert systems have been developed to support farmers decisions for weed management in this sense. Official advisory centres on plant protection are offering decision tools to the farmers, but they are not commonly used. Economics determine the use of herbicides and the tolerated weed level, if there is no governmental regulation. “Aesthetics” of fields is a significant consideration of the farmer (Owen 2000; see also Table 24). Weed free fields are then the aim of weed management. This cannot be the desired policy, as biodiversity is a state affair (see also “Overall conclusion” of chapter VI).

The findings above are supported by the adoption reasons stated by farmers and experts (III.6, Tab. 23 and 24; see also III.1.3 chapter on weed control improvement.).

III.1.2 Rates, combinations of herbicides, application frequencies, and mechanical weeding

Reduced herbicide application frequencies can lower soil compaction and erosion. A reduction of amounts does not necessarily mean a reduction of applications. It can result from a diminished dosage, which may vary between different types of herbicides.

Corn

Corn should be free of weeds in the period between the 2-4 leaf stage and the 6-10 leaf stage i.e. until the plant reaches a height of approximately 30 to 40 cm. Corn is very susceptible to early interference with weeds, especially as it is planted early. One herbicide application is usually sufficient in conventional herbicide weed management (see below, survey results). Occasionally, a second application will be required in the presence of persistent weeds.

USA

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 (Owen 2000).

Europe

The number of application trips in HR corn changes to mostly 2 (with extreme weed infestations 3) instead of 1 in conventional corn (see survey results; also: Cremer, 1996, Lechner et al. 1996; Harms et al. 1998). The numbers and amounts of herbicides (formula and a.i.) per ha will be reduced in HR corn in standard herbicide programmes in the EU according to Phipps and Park (2002).

Survey results

Tab. 13: Current weed control in conventional varieties and anticipated measures in HR corn in a typical agricultural area in the State of Brandenburg (Germany)

conventional		**HR**	
100% acreage		0% acreage	
chemical weed control			
gardoprim plus[1]		Glufosinate	
1 spray	May	2 sprays	May
mechanical weed control			
No		no	

Summary based on one expert judgement - [1] terbuthylazin and metolachlor

While an increase in the frequency of herbicide applications to corn (from 1 to 2) is forecasted, the number of active ingredients will be reduced from 2 to 1 in Brandenburg (Germany). There is no mechanical weed control, neither in conventional varieties, and, quite obviously, there will probably be none in HR varieties.

Cotton

Hoe labour and pre-plant weed control is common in conventional cotton (White et al. 2002). Culpepper and York (1998) concluded from their study, that less application trips and less amounts of herbicides are used in herbicide resistant cotton. The typical amount of herbicides used decreased by about 50% in HR cotton according to Carpenter and Gianessi (2000). Application rates in conventional cotton vary between 5,5 and 9 lbs/acre (that are 6,2 and 10,1 kg/ha) and for glyphosate between 2,75 and 4,5 lbs/acre (3.1 and 5 kg/ha) (Carpenter and Gianessi 1999).

Two applications of glyphosate (early post- and mid-emergence) were sufficient in HR cotton infested with broadleaf weeds in the late nineties (Culpepper and York 1998). Some farmers completely rely on glyphosate and others may add diuron at lay-by (Deterling 2002).

The survey of Klotz-Ingram et al. (1999) on the other hand (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. This also referred to the rate of 0,81 lbs/acre (0,91 kg/ha). Nevertheless, the herbicide applied on the largest area (55%) – trifluralin - was applied only 1,1 times at a rate of 0,76 lbs/acre (0,85 kg/ha) on average. The overall decrease in herbicide use in cotton since 1994 is due to a reduction of cotton acreage, the use of staple (a herbicide used at low rates) and glyphosate or bromoxynil resistant varieties (Carpenter and Gianessi 2000).

In the early days of no-till farmers used 2 pounds glyphosate per acre (2,25 kg/ha) in burndown treatments, but the rate was reduced to ½ pound (0,6 kg/ha) later according to Hayes (cited in Deterling 2003). Recently, a number of weed species have become more troublesome in the Cotton Belt. For example, glyphosate resistant horseweed (*Conyza canadensis*), which profits from no-till and reduced till practice, tremendously increased (see also Chapter II) (Haynes cited in Manning 2003). Tank mixtures (clarity and glyphosate), autumn burndown herbicides as valour, or the additional use of peeemergence herbicides (2,4-D; clarity) are recommended against horseweed (Hayes cited in Deterling 2003). Harvade 5F mixtures with glyphosate are presently recommended for other troublesome weeds such as teaweed (*Sida spinosa*), sicklepod (*Senna obtusifolia)* and morningglory (*Ipomoea)* (Deterling 2002). MSMA, dual, gramoxone/boa and basagran are recommended as additional herbicides in case of infestation of glyphosate resistant cotton with tropical spiderwort (*Tradescantia)* (Leidner 2003).

Volunteer HR soybeans resistant to several residual herbicides should be controlled by residual preemergence herbicides cotoran/meturon or caparol/cotton pro. Caparol/cotton pro or karmex/direx both mixed with MSMA can be applicated at postemergence against soybean volunteers (Hayes cited in Benbrook 2000).

A period of reduced applications and rates may be followed by a period of increased herbicide inputs in cotton at least in some areas.

Oilseed rape and canola

Canada

The data generated by a farmer survey in western Canada indicated that the application number slightly increased in HR canola from 1,78 to 2 (Canola Council of Canada 2001) which is not in correspondence with the survey results and with Phillips (2003).

Most conventional farmers use a pre- and a postemergence herbicide resulting in two applications. Farmers using conventional varieties and reduced tillage commonly apply a pree-seed burn-off and fall control with glyphosate added by spot applications for noxious weeds and 2,4-D for volunteers (Canola Council of Canada 2001; see also below: survey results). Syngenta has promoted a new product ('Gramoxone PDQ') to farmers for dealing with glyphosate resistant oilseed rape volunteers (http://www.tao.ca/~ban/100MSukgmtrials.htm).

Europe

Oilseed rape is presently sprayed with herbicides, although it is economically not necessary in most fields in Europe (see below "net income").

In conventional herbicide management practice in oilseed rape, a single herbicide application is made at preemergence. Occasionally, should the first treatment fail, a postemergence herbicide application may be required. The number and the amounts of herbicides (and of a.i.) per ha will be reduced in glufosinate resistant oilseed rape in standard herbicide programs in the EU according to Phipps and Park (2002). A reduction of application trips was supported by the survey results from France but not from Germany (see below).

At present, glyphosate is used pre-seeding for volunteer control in 10-25% of oilseed rape fields in the UK (Orson 2002).

The disadvantage of late applications of glyphosate or glufosinate is that the temperature may be too low for successful glufosinate or glyphosate application in winter oilseed rape. In case of infestation with a certain weed species (*Viola arvensis*), two glufosinate applications (4,5 and 8 litre) are recommended in glufosinate resistant oilseed rape (Pallutt and Hommel 1998, Hommel and Pallutt 2000).

Survey results

Several situations for oilseed rape / canola were described by experts, which significantly differed from each other.

Europe

Tab. 14: Current weed control in conventional varieties and anticipated measures in HR oilseed rape in a typical agricultural area in the State of Brandenburg (Germany)

conventional		**HR**	
100% acreage		0% acreage	
chemical weed control			
butisan top[2]; Fusilade ME[3] as needed		glufosinate	
1 –2 sprays	September	2 sprays	September
mechanical weed control			
no		no	

Summary based on one expert judgement.

In Germany, it is anticipated that HR varieties will receive only glufosinate, instead of metazachlor and quinmerac (and – if needed - fluazifop-p-butyl). There is no mechanical weed control (see Tab. 14).

Tab. 15: Current weed control in conventional varieties and anticipated measures in HR oilseed rape in a typical agricultural area in Burgundy (France)

conventional		**HR**	
100% acreage		0% acreage	
chemical weed control			
trifluralin, clomazone,metazachlor		glyphosate or glufosinate	
3 sprays	Sept.	2 sprays	Oct. – March
mechanical weed control			
no		no	

Summary based on one expert judgement

Until now, HR oilseed rape varieties are not commercially planted in France (and in Germany). The number of active herbicide ingredients will decrease from 3 to 1, also the application frequency will decrease according to an expert assessment on changes in case of HR varieties approval.

[2] metazachlor and quinmerac
[3] fluazifop-p-butyl

Canada

Canola cropping in Canada is well covered by several expert assessments.

Tab. 16: Summary of the weed control in canola (AB: Alberta and SK: Saskatchewan, western Canada)

conventional		**HR**	
20 to 28% acreage (2002)		72 to 80% acreage (2002)	
chemical weed control			
ethalfluralin (Edge),	1; Oct.	glyphosate (Round up)	1; Oct and/or June in crop
sethoxydim (Poast Ultra) or quizalofop-p-ethyl (Assure II) and/or clopyralid (Lontrel) or	1; June, in crop.	glufosinate ammonium (Liberty)	1; June, in crop
		imazamox + imazethapyr (Odyssey)	1; June, in crop
ethametsulfuron-methyl (Muster)	1; June, in crop.		
glyphosate (Round up)	1; Oct / April		
mechanical weed control			
	AB: yes, cultivator, before seeding; wild oats, Canada thistle, SK: no		AB: yes, cultivator, before seeding; wild oats, thistle, quack grass SK: no

Based on several expert judgements

In Alberta and Saskatchewan, the chemical weed control is significantly different in conventional versus HR varieties (see Tab. 16). The number of active ingredients as well as the frequencies of application are reduced with all three types of HR varieties. Interestingly, the mechanical weed control is not given up with HR varieties in Alberta.

The weed control patterns from Alberta and Saskatchewan are supported by three more expert assessments from Canada, which had all “western Canada” in focus. While the chemical weed control is very similar, the expert views on mechanical control are obviously different. Taken together, more mechanical control is being applied in conventional than in HR varieties in order to control early annuals/biennials and broadleaved plants.

Soybean

USA

The average number of herbicides (a.i.) was 2,7 in conventional soybean varieties in 1994 and the average number of treatments was 2,6 in 1995 (while 34% of the acreage receives 3 or more applications). In Iowa fields, the average application frequency was 1,55 in HR varieties and 2,45 in non-HR varieties (Duffy 2001).

Farmers who adopted reduced tillage, used a burndown treatment before planting or a soil applied treatment at planting time (Carpenter and Gianessi 1999). A soybean grower survey in

Missouri indicated that of the 36% farmers applying burndown herbicides prior to planting, 91% used glyphosate alone.
About one-third (30%) of soybean fields had about 4,5 kg of ‘Roundup’ applied per hectare for the growing season, and more than 2,25 kg per hectare were applied on 55% of the soybean acreage covered by the study (Smeda et al. 1999).

Changes of amounts and application rates in herbicide resistant soybean relative to conventional varieties are difficult to assess. The many different analyses of herbicide use in combination with glyphosate resistant soybean range from a 7% increase to a 40% decrease compared to herbicide use in conventional soybean production without HR. Identifying the reasons for those differing results is hampered by the absence of information regarding the herbicide programs used by soybean growers (Gianessi and Carpenter 2000). According to Sankula (2000) the amount (applicated per acre) has slightly been increased since the adoption of glyphosate resistant soybean (nine US areas studied).
The data base and the calculations of ISAAA (The International Service for the Acquisition of Agri-biotech Applications) and industry are not described in their reports. Their estimations were done by (not further identified) ”industry” and ”independent researchers”(Hin et al. 2001). Calculating herbicide use is far from simple (USDA/ERS 2000). USDA/ERS alone used three different statistical approaches (Hin et al. 2001). Most glyphosate resistant soybean fields are treated more than once, partly by preemergence herbicides and glyphosate (Benbrook 2001, Hin et al. 2001). The USDA/ERS analyses for 1997 and 1998 ranged from no significant effect to a reduction by 10% (Hin et al. 2001). The difference of calculations between USDA/ERS and Benbrook (see below) partly arises from the fact that USDA/ERS estimated an average use of 1,57 pounds per acre (1,76 kg/ha) whereas Benbrook estimated less than 0,5 pounds per acre (0,6 kg/ha) in conventional systems.

Amounts in no-till systems in soybean
Benbrook (2001) has been the only one who distinguishes between analyses for minimum tillage and for conventional tillage systems and between regions: Soybean fields under no-till production were given 1.7 times more glyphosate than the 30% of soybean fields that required the least amount of herbicides.
In 1998, the total herbicide use in glyphosate resistant soybean was 30% or more higher than in conventional varieties in six states, 10% or more in three states, and modestly lower in five states according to Benbrook (2001). For 2001, Benbrook expected an increase in herbicide use (active ingredient here) in glyphosate resistant soybean compared to conventional varieties.
In Argentina, 80% of the soybean farmers used glyphosate and no-tillage practice, with more than twice the conventional herbicide amounts and higher application frequencies (Qaim and Traxler 2002; see below - survey results).
Agronomists in the USA are recently advising growers of glyphosate resistant soybeans, for example, to use multiple applications of glyphosate on these crops (or to use residual herbicides in addition to glyphosate) in order to achieve intended levels of weed control (Owen 1998). Particularly in the Midwest where early planting is common, single

applications will not provide acceptable weed control according to Owen (2000). As already shown for cotton, herbicide inputs may increase again in HR soybean in the near future.

Survey results

Expert assessments are available from Argentina and from the US (Iowa and Nebraska).

Tab. 17.1: Summary of the weed control in soybean (Argentina)

conventional		**HR**	
2- 5% acreage		95 -98% acreage	
chemical weed control			
metribuzin, imazethapyr, sethoxidim, fenoxaprop-p-ethyl	postemergence	triazolopirimidines imidazolinone glyphosate	
mechanical weed control			
no, yes (opposing expert judgements)		no	

Based on two expert judgements

While HR varieties are by far dominant to conventional soybeans (95 – 98% of all Argentinean soybean is HR) and there are different modes of cropping soybeans, early and late, the table gives a summary of the weed control practice. A variety of active ingredients is applied in conventional systems, but also glyphosate (up to 2.5 times per year). In HR soybeans, not only glyphosate but also triazolopirimidines and imidazolinones are used.

Tab. 17.2: Weed control in HR soybean in the US Corn Belt (Iowa, Nebraska)

conventional		**HR**	
13-20% acreage		80 -87% acreage	
chemical weed control			
pendimethalin fomesafen/ acifluorfen/ lactofen and others	preemergence (April) postemergence (June)	Pendimethalin (in Iowa) glyphosate	preemergence (April) postemergence(June/July)
mechanical weed control			
< 10% of acreage (Iowa) yes (Nebraska): cultivator, 2 times		no (Iowa) Yes (Nebraska): cultivator, 2 times	

Based on two expert judgements

In Iowa, two herbicide treatments (pre- and postemergence) are conducted on both types of varieties, conventional and HR. In Nebraska also a variety of herbicides is used in conventional soybeans, but only one glyphosate application is conducted in HR varieties. The number of active ingredients is lower in HR, for the postemergence treatment is only glyphosate, while different formulations are being used in conventional soybeans. Mechanical weed control is rare in conventional soybean fields, absent in Iowa HR soybeans, but not in Nebraska.

Sugar beet

The critical weed competition phase for sugar beet is between the 4^{th} and 8^{th} week after crop emergence. Weeds emerging within this time frame grow above the crop and shade it from the sun thereby causing yield reductions, whereas late-emerging weeds that grow below the crop canopy have little effect on yield. *Lamium* spec. infestations should be sprayed early (Bückmann et al. 2000). As active ingredients in conventional herbicides are only effective against young weeds, herbicide application must be started at crop emergence and be repeated two to three times. With herbicide resistant sugar beet it is possible to control larger weeds. The numbers of herbicides and amounts of herbicides and a.i. per ha will be reduced in glyphosate resistant sugar/fodder beet in standard herbicide programs in Denmark according to Phipps and Park (2002). 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.

Madsen and Jensen (1995) recommended 2 trips spraying 0,72kg a.i./ha in glyphosate resistant beets. It was more effective than a mixture of phenmedipham and ethofumesate. Petersen and Hurle (1998b) recommended 2 times 0,4 kg/ha (extended to 3 times 0,6kg when *Galium aparine* is abundant) for glufosinate. Phipps and Park (2002) calculated a standard use of 1,08kg a.i./ha (glyphosate, 2 times). The same rate was used by Dewar et al. (2000).

A low rate (row spraying) in combination with economic threshold evaluation and postemergence application can be used in sugar beet without economic losses (Dewar A. M., personal communication; Coghlan 2003). A similar approach (a 50% smaller herbicide rate was used here) has successfully been tested in fodder beet (Elmegard and Pederson 2001).

Survey results

The following changes in weed control are anticipated for sugar beet in the UK (Tab. 18):

Tab. 18: Current weed control in conventional varieties and
anticipated measures in HR sugar beet varieties in the UK

conventional		**HR**	
100% acreage		0% acreage (100% if accepted)	
chemical weed control			
various herbicides		glyphosate	
4 –5 sprays (approx. 6 active ingredients)	March - June	2 sprays	May (June)
mechanical weed control			
no 70%	yes 30%	no	

Based on one expert judgement

A significant reduction of the frequency and number of active ingredients, and a stop of mechanical weed control is forecasted.

III.1.3 Weed suppression

A higher weed suppression in HR crops was reported in several publications (e.g., Westwood 1997, Read and Bush 1998, Buckelew et al. 2000).

Survey results

Tab. 19: Survey results on the effectiveness of weed control (questionnaire: 6.1)

Is weed control improved in HR crops ?			
crop	**yes**	**no**	**if "yes": reasons (1, 2, 3); and area** 1: due to substitution of less effective herbicides 2: due to substitution of mechanical weeding 3: (any additional)
sugar beet; UK	x		1; 2; 3: less effect of weather; in most cropping areas; particular with perennial weeds (volunteer potatoes, wild beet)
corn; GE		x	
oilseed rape; GE	x		1; in about half of the cropping areas
oilseed rape; F	x		1
canola; CA (AB)	x		1; in most cropping areas
canola; CA (SK)	x		1; 2; 3: no incorporation required
canola; west. CA	x		1; in most cropping areas
canola; west. CA	x		1; 2; 3: better timing of weed control
canola; west. CA	x		1; HR canola (esp. RR) may even be used as clean up crop
soybean; AR	x		1; 2; 3: suppression of difficult weeds; in most areas
soybean; AR		x	improved weed control only in few areas
soybean; US (IA)	x		3; areas with perennial weed problems
soybean; US (NB)	x		1; most cropping areas

Each line represents one expert judgement

Survey results on the improvement of weed control with HR crops show a clear effect of HR. Weed control is improved in HR crops except in corn (Germany) according to one expert statement.

Weed control in Argentinean soybean is improved according to one expert, whereas the other expert stated an improvement only for a limited area (see Tab. 19). 81% of HR farmers reported an improved weed control in Alberta. In western Canada (glyphosate resistant) canola fields the weed control is so effective, that this may also serve as clean up crop.

It was due to a substitution of less effective herbicides and the substitution of mechanical weeding in western Canada, Saskatchewan, and Argentina (mid and north states).

The above findings are supported by the fact, that a superior weed control is overall the most frequently stated reason and the reason of highest importance for the adoption of HR by farmers (see chapter III.6).

Summary on weed control patterns

No overall picture about changes in weed control patterns can be drawn. Regional differences and changes in time can be seen.

Postemergence applications increased in herbicide resistant soybean and canola. Approximately half of the soybean farmers and more than half of the canola farmers shifted to postemergence applications - the proportion of cotton farmers is not known. Furthermore, postemergence applications are expected to increase in herbicide resistant sugar beet (UK survey) but not in corn. Information on oilseed rape is missing.

Changes in overall rates of herbicides used are more difficult to assess because different herbicides are applied at different particular, but still varying, (see III.1) rates. Results on changes in soybean vary. A slight overall reduction, but also increased amounts in reduced or no till systems (at least in Argentina) have been reported. Furthermore, numbers, rates, and amounts are currently increasing again in some areas where HR soybean have been planted for many years. The latter also refers to some cotton regions. A decreased herbicide use in cotton was attributed to several reasons, one of which was the adoption of glyphosate resistant varieties.

The survey indicates that canola farmers may spare one application in HR varieties in Canada, but this is not supported by publications. A decrease in application frequency is expected for oilseed rape in France (-1) but an increase in Germany is expected at least with glufosinate resistant cultivars. No changes in application frequencies, or differing results have been reported for soybean and cotton.

The number of herbicides used in HR varieties (compared to conventional ones) decreased in Canadian canola, in US and Argentinean soybean and probably in cotton areas. They are expected to decrease in European sugar beet and oilseed rape. Information on corn is missing.

Mechanical weed control decreased with introduction of HR varieties in cotton, in US soybean (from < 10% to 0 in Iowa), in Argentinean soybean (at those locations where it was still done), and may have decreased in Canadian canola (see Tab.19, but not according to Tab. 16). It is expected to decrease in sugar beet (30% to 0% of the acreage in the UK). Mechanical weeding is not common in European oilseed rape and corn according to the findings presented here.

Weed control is improved in most cases. It is expected to be improved with HR sugar beet and HR oilseed rape, but not with HR corn, in Europe.

III.2 Yields

Generally, data of independent research institutions on yield differences between conventional varieties and HR varieties are scarce. Farm surveys may not meet scientific requirements to clarify this question, for yield differences may also be due to other reasons, e.g. site (farm size, soil, climate) and the education of the farm operators (see below). Results of field tests can differ from year to year and depend on local factors.

Thus results of different field tests and of farm surveys and expert surveys can sometimes be contradictive.

Survey results

Tab.20: Survey results on yields in HR and conventional varieties (questionnaire: 10)

	Are there yield gains in HR varieties?			
crop	**yes**	**no**	**farmers**	**comments**
sugar beet; UK	x		most	5% in normal situations, 15% if weather is hot or frosty during spraying
corn; GE		x		
oilseed rape; GE	?	?		
oilseed rape; F		x		the varieties used so far are old, ‚classical' varieties, but GMO
canola; CA (AB)	x		most	
canola; CA (SK)	x		~ half	better cultivars, better weed control, earlier seeding
canola; west. CA	x		rarely	better weed control (esp. *Galium aparine*), moisture conservation, better varieties
canola; west. CA	x		~ half	better varieties and earlier weed control
canola; west. CA		x		
soybean; AR		x		
soybean; AR		x		
soybean; US (IA)		x		
soybean; US (NB)		x		yield losses with weed free situations

Each line represents one expert judgement

Published findings and survey results

Corn

USA

Corn yields were similar for both glyphosate resistant hybrids and non-resistant isolines that were evaluated by Roth (2000). HR corn showed significant increases in yield (5-30%) in all but one region in resistant over non-resistant (USDA/ERS 2000a, 2000b).

Europe

In Germany, yields of herbicide resistant corn did not significantly differ from conventional varieties (Hommel and Pallutt 2000). Petersen et al. (2002) found higher yields in glufosinate-resistant corn than conventional corn when both were sown into cover crops.

Cotton

USA

Yields of herbicide resistant cotton did not increase relative to conventional cotton in several states (OECD 2000, Klotz-Ingram et al. 1999, Culpepper and York 1998, Carpenter and Gianessi 2000). HR cotton (glyphosate-resistant) was the only engineered crop which showed no significant increase in yield in either region where it was surveyed (USDA/ERS 2000a, 2000b). In one case- HR cotton in one region - a significant reduction in yield (12%) relative to nonengineered varieties was found. 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).

Actually, yield losses were reported in some U.S. states. Some entire glyphosate resistant cotton fields shed their bolls or developed small, malformed bolls with reduced fibre length (Edminsten 1998a, 1998b, 1998c, 2000; McCarty 1998, Myerson 1997).

In contradiction to the above findings are White et al. (2002). They compared the production of irrigated conventional and glyphosate resistant cotton varieties in the Texas High Plains in 1998. Six producers were investigated that all produced both types of varieties. The yields were higher in HR cotton (555 lbs/acre i.e. 623 kg/ha) than conventional (461 lbs/acre i.e. 517 kg/ha). Furthermore, Ward (2002) measured an increase in yields for HR cotton, but only when strip tillage was done.

Oilseed rape

Canada/Canola

In western Canada herbicide resistant canola yields were increased on black but not on brown soils[4]. Yields clearly depend on the location and farm management skills (Fulton and Keyowski, 1999).

The results of a survey of 650 Canadian farmers (Canola Council of Canada 2001) regarding yields and net income in HR versus conventional varieties are of limited statistical value because of the presumable positive correlation of production factors and the adoption of HR varieties. Relevant production factors in this sense are, for example, farm size, planting higher-priced crops on better land, education, and the experience of farm operators. According to the mentioned survey yields of farmers growing herbicide resistant canola were on average 10% higher whereas the maximum yield was 24% higher with conventional farming (72bu/acre to 55 bu/acre, i.e. 4.800 to 3.700 kg/ha). Yields of conventional varieties (Smart Open Pol, Coventional Open Pol) were higher than of glyphosate resistant varieties, whereas glufosinate resistant varieties yielded the same as Conventional Open Pol in 1999 (Phillips 2003).

The survey conducted for this study indicated, that an unclear portion of farmers growing HR varieties increased their yields.

Europe

In Germany, yields of herbicide resistant oilseed rape did not significantly differ from conventional varieties (Hommel and Pallutt 2000, see also survey Tab. 20). Yield gains are not expected for HR oilseed rape in France (according to the survey) either.

A study carried out under the European Commission's FACTT Project (Familiarisation and Acceptance of Crops incorporating Transgenic Technology) examining the agronomic

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[4] Canadian soil zones: http://interactive.usask.ca/ski/agriculture/soils/soilform/soilform_zone.html

performance of transgenic oilseed rape varieties resistant to glufosinate (Liberty) compared to the yield performance of conventional rape hybrids 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.
- there are no differences in ramification 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,
- the glufosinate resistant varieties usually produced reduced financial returns when treated with herbicides compared to the situation when receiving no herbicide treatment at all. Any small increases in yield derived from weed control were usually insufficient to cover the extra cost of the herbicide, including glufosinate-ammonium (Greenadas and Boothsack 1999).

Soybean

USA

It is nearly impossible to attribute statistically derived yield differences to the adoption of herbicide resistant soybean because of the positive correlation of production factors and the adoption of these varieties. Relevant production factors in this sense are, as mentioned above, farm size, planting higher-priced crops on better land, education, and the experience of farm operators and narrow spaced rows in HR crops (Carpenter and Gianessi 1999, USDA/ERS 1999, Gianessi and Carpenter 2000, Benbrook 2001).

Nevertheless, USDA/ERSS (1999) estimated a ”very small yield increase” by herbicide resistant soybean. Hin et al. (2001) deduced an insignificant to small yield increase from a compilation of various assessments. In addition, Fernandez-Cornejo and McBride (2000) found a correlation of the adoption of HR soybean varieties and farm size (particularly for farm sizes of 50 acres to 800 acres) and operators education.

Estimations given in the survey and drawn from field to field comparisons are in contradiction to the conclusions cited above:

No yield increase has been seen in HR soybean by survey respondents (see. Tab. 20), in the contrary, one expert suggested yield losses.

Carpenter and Gianessi (1999) concluded from their review, that yields of glyphosate resistant varieties should be “about the same or less” as with conventional varieties. Hartzler (2003a) reviewed 24 experiments in Iowa and Illinois (1997-1999) without finding yield differences overall. In cases where yields were reduced, late herbicide applications were the main reason. Researchers in Minnesota concluded that yields in a glyphosate system compared to conventional herbicide systems were equal (Breitenbach and Hoverstad 1998). Duffy (2001) found that conventional soybean outyielded the HR counterpart. His data set contained observations for 172 fields in Iowa in 1998 and 2000.

Findings from 3000 yield trials resulted in an overall small yield drag of 4% for HR (Oplinger et al. 1999, cited in CEC 2000; OECD 2000). The yield drag may be due to the use of minor elite varieties in these trials as stated by industry (Hin et al. 2001). But Elmore et al. (2001) showed that (backcross-derived) non-HR lines outyielded (+5 %) the HR-lines. Hence, there must be other reasons for yield differences and yield variability.

It is conceivable that the positive correlation of production factors (narrow row production, field size, education - see above) with the adoption of HR in commercial cultivation (which makes statistical analyses of farm surveys uncertain, but not the field trials) compensate a yield drag. Herbicide resistant soybean can be planted in narrow rows (7,5 inches). The narrow rows may account for the increased yields in commercial farming, which could not be confirmed in field trials with wide rows (Carpenter and Gianessi 1999). The yield drag could be due to reduced nitrogen fixation and a weaker defense response (Benbrook 2001). Some nitrogen fixing bacteria are susceptible to glyphosate under dry conditions and the herbicide reduces the level of aromatic acids (responsible for defense response) in plants under stress (King et al. 2001, several references cited in Benbrook, 2001). In addition, some glyphosate resistant soybean varieties have been reported to crack up in hot weather (Coghlan 1999).

Argentina

Yields in HR soybean were lower than in conventional soybean in Argentina (Qaim and Traxler 2002). Yields were equal according to the experts and to Penna and Lema (2003).

Sugar beet

Europe

In some tests in Germany and the UK, yields of herbicide resistant sugar beet did not significantly differ from conventional varieties (Bückmann et al. 2000, little but not significant increase, Dewar et al. 2000). Yields of HR sugar beet increased in other field tests in the Netherlands and the UK (Wevers 1998, May 2003). The UK-expert expects higher yields for HR sugar beet (see Tab. 20). Conventional herbicides combined with planted cover crops resulted in less yield than postemergence-glyphosate applications (Petersen et al. 2002).

Sugar beet yields in improved integrated production systems (without HR) were not influenced by 15% ground coverage of the associated weed flora. The ground cover can even lead to a 7% higher yields because of an effective aphid control by natural antagonists. The aphid predators were attracted by the associated flora (Schäufele 1991, Häni et al. 1990).

III.3 Net income

In general, the economic threshold of chemical weed control is not reached on large portions of the arable land. This overall portion is about 50% in Germany, as representative studies show (Lettner et al. 2001). It is much smaller in oilseed rape (about 30%) and grassy weeds are often abundant on only 10% of cereal fields (Gerhards et al., 1998). Despite of this fact, herbicides are generally used on all parts of the fields and in all crops covered by this document.

Survey results

Tab. 21: Survey results on farmers economic returns (questionnaire: 11)

Does HR (positively) alter farmers economic return?				
crop	**yes**	**no**	**farmers**	**comments**
sugar beet; UK	x		most	on all commercial farms due to lower production costs (e.g. herbicides, machinery, consultation) and increased yield (e.g. less weed competition)
corn; GE	x		rarely	lower production costs
oilseed rape; GE		x		no changes in economic return
oilseed rape; F				(no estimation)
canola; CA (AB)	x		most	average + $10.62/acre; higher yields (due to less weed competition) and lower dockage
canola; CA (SK)	x		~ half	better cultivars, better weed control, earlier seeding
canola; west. CA	x		rarely	higher yields, less dockage
canola; west. CA	x		~ half	lower production costs, higher yields
canola; west. CA		x	~ half	actually, these are economic losses. Low-disturbance direct seeding farmers have higher costs for pre-seeding herbicides. Glyphosate cannot be used because of RR volunteer canola. Additionally, RR trait of canola in western Canada are not restricted to adopters, also non-adopters have added costs to control Roundup resistant volunteers
soybean; AR	x		most	large and mid sized farms gain, small (<~200 ha) suffer negative impact, because of difficulties to finance the modern machinery/technology
soybean; AR	x		most	low herbicide costs; partly low seed costs through (illegal) use of self harvested soybean
soybean; US (IA)		x		
soybean; US (NB)	x		most	reduced costs

Each line represents one expert judgement

Published findings and survey results

Cotton

In HR cotton (on strip tillage systems) net return increased by savings in variable costs (Ward et al. 2002). Farmers save time or labour (Deterling 2002) in reduced-till and no-till cotton with glyphosate resistance. White et al. (2002, see above, 6 producers study HR cotton in Texas) also reported increased net returns in HR cotton. The increase was mainly due to reduced herbicide costs and to less tillage costs. As discussed later (III.4, III.6) reduced tillage is not necessarily connected with HR crops.

Oilseed rape

Canada

The overall net return in conventional versus herbicide resistant canola is as difficult to assess as in soybean (see below; CEC 2000, Fulton and Keyowski 1999).

The study of Fulton and Keyowski (1999) indicates lower yields and lower economic returns for HR varieties ($242/acre compared to $238/acre with glufosinate resistance and $225/acre for glyphosate resistance). Fulton and Keyowski (1999) discuss this finding on the background of dramatically increasing HR canola acreage in Canada. They emphasize the heterogeneity of the farms, which explains this alleged contradiction. For example, those farms which have machinery for reduced tillage may have advantages planting HR canola, those which don't, are probably better off with conventional varieties.

Net income was higher for growers who used herbicide resistant varieties in 3 of 4 years according to the above mentioned survey done by the Canola Council of Canada (2001). The increase in net return was mainly due to reduced herbicide costs and to less tillage costs. However, the strong correlation between the use of HR varieties and the practice of reduced tillage is doubted (see III.4 and III.6). The limitations of this sort of study are - as mentioned before - the possible positive correlation of production factors and adoption of GM crops. For example, small sized (<32ha = 80 acres) farms were not covered by the statistical analysis. The difference in net returns was smaller in the case studies than in the statistical study presented by the Canola Council of Canada (2001).

A two year study of Lethbridge Research Centre, Canada, revealed that in some regions conventional oilseed rape varieties gained comparable yields to HR varieties. But this resulted in a higher net income of farmers because of reduced production costs, e.g. seed and herbicide costs in HR rapeseed (Lethbridge Research Centre Report 13.01.2000). Phillips (2003) stated, that a sometimes lower yield in HR fields is compensated by better income due to lower dockage and that yields can be higher due to earlier seeding in HR canola.

The expert survey reflects a mostly positive development for about half of the HR canola growers. This finding is more or less in accordance with the published results and discussions. The heterogeneity of farms (tillage, yield, farm size, soil type, seeding, dockage) seem to account for mixed differences in results. Nevertheless, more than 50% of Canadian canola farmers use HR varieties (James 2002).

The problem of rising costs through added efforts in order to control HR volunteers even in areas where no HR is grown is raised by one expert.

Europe

As winter oilseed rape is a quite competitive crop, weed control is not economically justified on 23-74% of the investigated oilseed rape field area in Germany. In the UK, it was uneconomic at any of 4 sites (Werner and Garbe 1998, Greenadas and Boothsac 1999). The study, carried out under the European Commission's FACTT Project (Familiarisation and Acceptance of Crops incorporating Transgenic Technology; see also III.2) evaluated the agronomic performance of transgenic rape varieties resistant to glufosinate (Liberty) compared to conventional rape hybrids. The herbicide trials on the transgenic varieties included:

- Conventional herbicides, either soil residual (metazachlor) or contact (benzolin/clopyralid/cycloxydim) herbicides
- A herbicide program (unspecified) based on glufosinate-ammonium
- Untreated controls

Averaged across variety and site, glufosinate gave a negative margin over herbicide response of minus £16/ha for 1997 and minus £13/ha for 1998. Other herbicide treatments applied to UK winter oilseed rape gave negative margins over herbicide ranging from minus £13/ha to minus £99/ha, "showing that the benefits of weed control in oilseed rape are not consistent" (Greenadas and Boothsack 1999).
The respondents of the survey did not expect gains either for HR oilseed rape in Europe.

Soybean

Due to seed and technology fees[5], the costs of glyphosate resistant soybean technology is marginally higher than that of conventional soybean. Net returns to HR systems were shown to lag behind returns of conventional soybean varieties, regardless of irrigation treatments (Oriade and Popp 1999).

Duffy (2001) found that conventional soybean outyielded the HR counterpart. His data set contained observations for 172 fields in Iowa in 1998 and 2000. The overall production costs in resistant and conventional varieties (including land charge and insurance) were equal. Duffy concluded from his study that lower herbicide and management costs equalize lower yields and higher seed costs. There was essentially no difference in total returns between the two types of systems in 1998 and 2000.
Two of three US studies on income effects cited in Qaim and Traxler (2002) show an advantage for soybean (HR). According to CEC (2000) and Mara (2002) reduced herbicide and labour costs may outweigh yield losses and higher seed prices in soybean. According to the U.S. National Centre for Food and Agricultural Policy, genetically altered glyphosate resistant soybeans produce about the same yield (see III.2 for yields) and require the same overall volume of chemicals to kill weeds as traditional varieties but save farmers about $ 220 million annually through cheaper chemicals (Gianessi and Carpenter 2000). As discussed in chapter III.1, herbicide inputs increase again in some HR soybean growing areas due to problems in weed control.
The expert on soybean production in Iowa did not recognize increased net returns in HR soybean, whereas the Nebraska expert suggested economic gains through reduced costs.

Argentina

In Argentina, net income increased by savings in production costs in herbicide resistant soybean even though yields slightly decreased (Qaim and Traxler 2002). Reduced herbicide costs and less tillage (machinery costs / fuel) were calculated to mainly account for the better net return by Qaim and Traxler (2002). When tillage is done in the same way, herbicide costs accounted for an overall 5-7 % increase in returns in HR soybean according to Penna and Lema (2003).

[5] It seems to be widespread, though illegal, that farmers do not buy seed but instead use own soybeans that were harvested in previous season as seed (see survey results)

Sugar beet

A low herbicide rate (row spraying) in combination with economic threshold evaluation and postemergence application can be used in HR sugar beet without economic losses (Dewar, A. M., IACR Broom´s Barn, Higham, Bury St. Edmunds, Suffolk IP28 6NP, UK, personal communication; Coghlan, 2003). Integrated production systems without HR (as described in chapter III.2) with high weed coverage can also positively influence farmers income .
Positive income effects are expected for HR sugar beet in the UK (see Tab. 21). Herbicide costs are significantly lower in European HR-sugar beet.

Conclusion on yields and net returns

The yields did not clearly increase due to the adoption of several HR varieties in many regions. Results on the effects of HR cotton on yields are very mixed. A certain portion of canola farmers (approximately 50%) seemed to improve production. The farm size, tillage system, soil, weed abundance and operators education are influencing yield and net income results.

Higher net returns were achieved by some (approximately 50%) canola farmers. The outcome probably depended on the type of farm. No clear-cut increase in net returns can be stated for Iowa (HR) soybean but for Nebraska and Argentinean (HR) soybean. When the net income increased in a HR crop, the better profits were mostly attributed to lower herbicide costs and less tillage (which implies less labour and fuel costs) often summarised as production costs. The correlation between less tillage and HR may not commonly be given (see III.4 and III.6), which implies that cost reductions are smaller and mainly due to reduced herbicide costs. Highly suppressive herbicides seem to be of importance only in the first one or two years of tillage reduction (see IV.1, Belde et al. 2000).

In the UK one expert predicted higher yields and net returns in HR sugar beet which is confirmed by some field tests. Only very little or no changes are expected for German growing sites in corn, sugar beet and oilseed rape.

III.4 Tillage and planting

Tillage

Cover crops and conservation tillage can help to prevent soil erosion. As HR varieties often allow low till management, the introduction of HR has been supported by this argument. Also, the promise of higher biodiversity of soil organisms is given with the assumption that no-till or reduced-till agriculture will increasingly be adopted with HR (Monsanto 1998, Duke 1999).
Approximately 54% of US-soybeans were planted under conservation tillage conditions in 1998, up from 30% in 1989 (Conservation Tillage Information Center 1999). A recent study on US cotton indicated that 59% of the overall cotton acreage (Manning 2003) and about 50% of the HR cotton acreage (Kalaitzandonakes and Suntornpithug 2001) are grown under no-till or reduced till practices.
It should be noted that the development and introduction of reduced till or no till agriculture does not depend on herbicide resistance of the crops. Tillage has been reduced since many

years in the USA due to a variety of reasons, e.g. government programs, in order to reduce erosion and organic matter loss, and because of compliance with regulations for water quality. In addition, the precision of machinery for direct drilling has been improved. Reduced tillage in Canada had also started long before the introduction of HR canola (see survey results). Moreover, cover crops with a high competitive ability like, 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 are planted and exhibit a high competitive ability (Petersen and Hurle 1998b). However, HR facilitates reduced-tillage, minimizing the risk of high weed pressures when for example low temperatures disturb the competing ability of cover crops. Currently, huge amounts of glyphosate (about 4.600 tons in 2002/Germany) are sprayed pre-seeding in reduced-till systems and on fallow land in Europe. Some of the early glyphosate applications in reduced-till systems may be done after sowing and emergence in herbicide resistant crops.
Generally, findings are mixed referring to the question of tillage, because it is difficult to decide whether planting HR crops is a side effect of reduced tillage or the availability of these crops leads to the adoption of reduce tillage practice (Ward 2002). Reduced tillage practice and the planting of HR cotton are both increasing and encouraging each other (Kalaitzandonakes and Suntornpithug 2001).
Surveys indicate that only 2% of cotton and 3% of canola farmers but 42% of corn and 46% of soybean farmers planted HR varieties in order to reduce tillage (Ward et al. 2002, Klotz-Ingram et al. 1999, Canola Council of Canada 2001, Van der Sluis and Grant 2002) (Tab. 23).
86% of conventional canola farmers made 2,63 tillage passes compared to 76% of transgenic HR canola growers who conduct 1,79 passes on average (survey of 650 farmers in western Canada, Canola Council of Canada 2001). However, the ploughed area in Alberta is 100% with HR and only 40% in conventional canola (survey result not shown in a table). Experts judgements on canola within the survey carried out for this study are somehow in contradiction to the above findings. Tillage reduction is seen as an adoption reason of HR varieties of very high importance for canola farmers. Experts estimated that about half of the HR canola farmers and the US (HR) soybean farmers shifted to reduced or no tillage (see Tab. 22). One explanation of these contradictions may be, that farmers who had already shifted to reduced or no tillage planted HR varieties afterwards.
Most of the Argentinean (HR) soybean farmers shifted to reduced or no-tillage according to the expert statements. An increase in minimum soil tillage acreage is expected for sugar beet in the UK (from 10 to 25% of the sugar beet acreage, see also Tab. 22).

The experts expected an expansion of reduced tillage practice for the next 5 years. Sugar beet was the crop with the least low till acreage expected (in the UK). Glufosinate-resistant oilseed rape varieties are not expected to be managed with reduced tillage.

Tab. 22: Survey results on tillage in HR crops (questionnaire: 9.1/2)

Do farmers shift from 'normal' tillage to reduced or no tillage in HR crops?						
	current situation			**expectation (next 5 years)**		
crop	**yes**	**no**	**farmers**	**yes**	**no**	**farmers**
sugar beet; UK	(no commercial growing to date)			x		few (20%)
corn; GE	(no commercial growing to date)			x (RR)		~ half
oilseed rape; GE	(no commercial growing to date)			x (RR)	x (LL)	most
oilseed rape; F	(no commercial growing to date)			x		~ half
canola; CA (AB)	x		~ half	x		most
canola; CA (SK)	x		few	x		most
canola; west. CA	x		most	x		most
canola; west. CA	x		~ half	x		(unsure)
canola; west. CA		x[1]		x		~ half[2]
soybean; AR	x		most	x		most
soybean; AR	x		most	x		most
soybean; US (IA)	x		~ half	x		few
soybean; US (NB)				x		most

[1] this movement started long before the introduction of HR canola in western Canada
[2] not necessarily because of HR crop
Each line represents one expert judgement

Planting

Herbicide resistant soybean and cotton can be planted in ultra narrow rows (7,5 inches distance for soybean) (Carpenter and Gianessi 1999, Kalaitzandonakes and Suntornpithug 2001). The narrow rows may account for the increased yields in commercial farming, which could not be confirmed in field trials with wide rows. Earlier seeding may be possible in canola in Canada when postemergence application is an option (Canola Council of Canada 2001).

III.5 Crop rotation

Crop rotations can help to control pests, diseases and weeds, and thus save pesticides. Input costs are often reduced in rotations because of the need for less nitrogen when legumes are planted. Crop rotation can also facilitate no-till production, as shown in corn-soybean systems: Soybean stubble and fall-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 corn.

Theoretically new crop rotation options in HR crops:

As glyphosate and glufosinate have very 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 will not likely be further reduced in relation to the current situation in Europe.
In the USA, a waiting period of 40 month is recommended (Rohm and Haas 1998 cited in Carpenter and Gianessi 1999) before planting canola, sugarbeets and many vegetables, when imazethapyr and pendimethalin (pursuit plus) are used in soybean. Also, corn can be damaged by imidazolinones used in previous soybean.

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

- glufosinate-treated/resistant soybeans:
  4 months for alfalfa, clover, cucumbers, peas, peppers, pumpkins, snap beans, sweet corn, tobacco, tomatoes, white potatoes,
  2-3 months for grain sorghum, spring oats, winter barley, winter rye, winter wheat
  no restriction: field corn
- glyphosate-treated soybeans (treated with ’Touchdown’):
  1-2 months: alfalfa, clover, cucumbers, grain sorghum, peas, peppers, pumpkins, snap beans, sweet corn, tobacco, tomatoes, white potatoes, spring oats, winter barley, winter rye
  no restriction: field corn, winter wheat
- glyphosate-treated soybeans (treated with ‘Round-up’):
  no restriction: alfalfa, clover, cucumbers, field corn, grain sorghum, peas, peppers, pumpkins, snap beans, spring oats, sweet corn, tobacco, tomatoes, white potatoes, winter barley, winter rye, winter wheat.

Expectations and evidence

Experts were asked about their prediction if HR-crops and other new transgenic varieties are likely to change crop rotations or management methods in the long run (in about 5-10 years) due to new options for farmers. Some experts predicted the integration of more crop species and some of less crop species in rotations with canola or soybean. One expert expected a wider rotation for sugar beet locations.
In the case of herbicide resistant rice rotations with soybean there may be a change into growing rice permanently (Annou et al. 2001). It was argued that soybean is pre-eminently planted because of weed control problems in permanent rice in these rotations.
The use of glyphosate allowed the increased planting of “weed-dirty” crops such as peas and lentils into the rotation at the expense of summer fallow in Canada (Orson 2002; see also survey results). 22 % of conventional canola growers adopt summer fallow practice. This portion is lower (13%) with HR canola growing farmers (Canola Council of Canada 1999). (See also III.1, survey results: in one situation, canola in Canada, RR varieties may be used as “clean up crops”).

In Nebraska less crops are expected in the future in soybean rotations. Especially grain sorghum and winter wheat will be decreasing, because of the ease of the glyphosate resistant soybean system and the lack of HR varieties in grain sorghum and winter wheat.
A general conclusion on the question whether HR and other transgenic varieties will lead to more or less crops in rotations cannot be drawn. Yet evident is the fact that summer fallow acreage was reduced due to the introduction of HR varieties in Canada and in Argentina (Questionaire 15, appendix).

III.6 Reasons to adopt HR crops

The acreage of HR crops has significantly increased worldwide during the last years. A further increase can be expected. From the farmers perspective, what are, or what could be the reasons to grow HR varieties instead of conventional crops? Answers to this question may point to general rationals and problems, and also to specific situations, current as well as anticipated. Results of the survey carried out for this report as well as published surveys and expert statements are presented below.

Tab. 23: Published surveys on adoption reasons of HR crops

adoption reasons for HR	percent of the respondents who stated the reason **crops**			
	canola	**corn**	**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!

Tab. 24: Expert survey on important reasons to adopt HR varieties (Questionnaire: 5)

crop and region	adoption reason	
	very high importance	**high importance**
sugar beet (UK)	reduced herbicide costs, convenient timing of weed control, reduced application frequency, simplicity of control, farmers profit wish to reduce tillage better consulting	better weed control, higher yields
corn (Brandenburg, GE)	-	convenient timing of weed control, wish to reduce tillage (with glyphosate resistance)
canola (western Canada)	better weed control, wish to reduce tillage, simplicity of control	reduced herbicide costs, convenient timing of weed control, reduced application frequency, simplicity of control, higher yields
oilseed rape (Burgundy, F)	better weed control	reduced herbicide costs, reduced labour costs
oilseed rape (Brandenburg GE)		reduced application frequency
soybean (AR)	better weed control, reduced herbicide costs, reduced labour costs, simplicity of control	reduced application frequency, convenient timing of weed control, wish to reduce tillage, simplicity of control, no farming consultation needed
soybean (USA)	better weed control, reduced herbicide costs, avoidance of crop injury concerns, simpler herbicide system	convenient timing of weed control, simplicity of control, (clean) appearance

The experts were given several potential reasons why a farmer would adopt HR varieties. The possible reasons were listed and could be ranked in relation to the "importance" in five classes: no; low; medium; high; very high (Tab. 24., see questionnaire in the appendix, part 5). A summary for reasons or possible reasons, which were ranked "very high" or "high" by the experts is given in Table 24.

The simplicity of weed control was ranked as the most important reason (4 x very highly important, 2 x highly important) (Tab. 24). This statement may rather be due to the effectiveness than to the timing of applications, as the timing is quite crucial (at postemergence) according to Hommel (pers. communication) and Owen (1999).

Better weed control was the second most important adoption reason in the survey conducted for this study (4 x very highly important, 1 x highly important) (Tab. 24). In addition, it was the most often stated reason in the surveys presented in Tab. 23.

The reduction of herbicide costs was the third adoption reason in terms of importance (3 x very highly important and 2 x highly important).

Neither higher yields nor higher returns turn out to be under the first important reasons overall. This partly reflects the findings on yields and net income (see III.2 and III.3). Nevertheless, cost reductions and labour reductions were often stated as important reasons in the reviewed surveys (Tab. 23).

The option to reduce tillage (2 x highly important, 2 x important) was ranked below the reduction of herbicide costs. The convenience in timing of weed control and the reduced herbicide application frequency are further important reasons (1 x highly important, 3 x important).

The picture drawn from these survey leads to the conclusion that the desire to reduce production risks is very strong.

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 according to them. Interestingly, the adoption rate of glyphosate resistant cotton was highest in South Carolina due to an improved control of palmer amaranth (*Amaranthus palmeri*) and sicklepod (*Senna obtusifolia*) (Carpenter and Gianessi 1999). Firbank and Forcella (2000) also underline the flexibility in timing and the simplicity as important reasons. 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).

The option to save labour, the simplicity and the flexibility of weed control is of particular interest for farmers who hold other jobs apart from their farm. 39% of Illinois farmers consider farming as their secondary job (Hin et al. 2001).

Section IV Impacts on biodiversity

One of the prevailing political aims in regions, where most of the land is under cultivation, is to stop and to reverse the decrease of biodiversity in agriculture. In Germany, for example, agricultural and forested land make up 84% of the total area and additional 11% are sealed by streets, buildings and so forth. In the UK over 70% of the land is farmed. For this reason biodiversity conservation has to be integrated in agriculture. The decrease in farmland biodiversity indicated by the decrease of farmland birds is also an important issue in the USA and in Canada too (see below, IV.3).

Herbicide resistance does not increase the fitness and invasiveness of plants in semi-natural or natural habitats. The possible direct and indirect impacts of HR crops on biodiversity are thus related to farming. Changes in farming practices due to the cultivation of HR crops may include crop rotations, planting and spacing of the crops, soil tillage, pesticide application, use of fertilizers and so forth (see Section III).

IV.1 Effects of changes in agricultural practice

Crop rotation

Although in theory, the options for crop rotations with HR varieties seem to be more numerous there is no evidence of a trend to widen rotations yet. On the contrary, summer fallow acreage decreased in Argentina (Questionaire 15, appendix) and in Canada in connection with the use HR varieties. HR canola allows the increased adoption of “weed dirty” crops such as peas and lentils into the rotation at the expense of summer fallow (Orson 2002).

Planting

Herbicide resistant soybean and cotton can be planted in ultra narrow rows, because no mechanical weeding is necessary (Carpenter and Gianessi 1999, Kalaitzandonakes and Suntornpithug 2001). The competative 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. Amounts of fungicides and insecticides used in these production systems are likely to increase. The crop architecture and the loss of forage plants restrain the habitat of some wildlife and beneficial species, the altered microclimate favours fungal diseases. Narrow row production can be seen as an element of further intensification in agriculture.

The practice of direct seeding is predicted to increase in rice production when HR varieties are available (Gressel 2002). The increase may come true at the expense of paddy (wetland) rice production. Wetland habitats are essential for wintering waterbirds such as waterfowls (Ducks Unlimited 2003).

Tillage

Cover crops and conservation tillage can help to prevent soil erosion. It has been assumed that no-till or reduced-till agriculture will increasingly be adopted with HR (Monsanto 1998, Duke 1999). Generally, findings from the USA are mixed referring to the question of tillage, because it is difficult to decide whether farmers reduce tillage because of planting herbicide resistant crops or whether the wish to reduce tillage has led to the adoption of HR varieties. Conventional tillage is commonly becoming less popular because of necessary compliance with federal regulations for water quality and governmental programs on the one hand. Reduced tillage systems often account for decreased production costs (fuel, labour and machinery). Surveys in North America indicate that only 1,8% of cotton and 3% of canola farmers but 42% of corn and 46% of soybean farmers planted HR varieties in order to reduce tillage (see III.6, Ward et al. 2002, Klotz-Ingram et al. 1999, van der Sluis and Grant 2002). The survey conducted for this study indicates that the wish to reduce tillage is an adoption reason for planting HR crops beside others in soybean and corn. It may become a reason in sugar beet and in corn in Europe (see above).

No-tillage and reduced tillage agriculture does not depend on herbicide resistance. Cover crops with a high competitive ability like legumes or mustard can help to suppress weeds. Traditional herbicides can be used (Kees 1990, Heitefuss et al. 1994, Auerswald et al. 2000) but they are not always necessary (Petersen and Hurle 1998b). However, HR does facilitate reduced-tillage, e.g. by minimizing the risk of high weed pressures when cold temperatures restrain the competitiveness of cover crops. Currently, huge amounts of glyphosate are sprayed in reduced till systems before sowing in Europe.

Effects of reduced tillage and reduced mechanical weeding on the soil fauna

The adoption of reduced-tillage in agriculture may improve conditions for several soil dwelling species. Particularly, the abundance of the important group of earthworms (one very important effect of earthworm abundance and diversity is the reduction of erosion) is increased. However, large populations of earthworms and of other soil organisms are only found especially in soils in which easily decomposable litter and/or organic fertilizer are available (Mackeschin 1997). A more effective way than conservation tillage to increase their abundance is to plant clover-grass-mixtures (Krück et al. 1997). Populations of other beneficial organisms (except spiders to some extent) will not significantly increase in fields with conservation tillage unless a plant coverage mitigates cold temperature in winter (Bürki and Hausammann 1993, Stippich and Krooß 1997).

Mechanical weeding had no negative effect on important predatory organisms (ground beetles, staphilinids, spiders) (Lorenz 1995). It can have an impact on small arthropods, but presumingly does not influence the density of epgeal predators (Basedow et al. 1991).

Reduced tillage and associated flora (weeds)

The associated flora of crops is the most important group of organisms as it provides food sources and habitats for most other (biodiversity) indicator groups (see also Werner et al. 2000). As pointed out in the section below, it is of great importance to stop and reverse the loss of the agricultural flora and of its seed banks. Belde et al. (2000) studied the long-term impact (4-25 years) of reduced-tillage systems with traditional selective herbicides on the flora. The review of nine studies showed, that the abundance of broad-leaved weeds was reduced in four studies and maintained stable in another four cases. In one case, the plant abundance was increased whereas the seedbank abundance decreased. Not only tillage, but also herbicide use was reduced in five studies, which mitigated herbicide impacts. The use of herbicides with a higher and broader effectiveness (such as glufosinate and glyphosate) than alternative ones is thus predicted to result in a decrease of vegetation biodiversity (with consequences on biodiversity in general, see below).

Populations of problematical weed types like grasses and perennials often increase in reduced tillage systems (Swanton et al. 1993, Tab. 2), whereas broadleaved annual plants, which provide nectar and pollen for important aphid predators, may decrease in some reduced tillage systems (Knab and Hurle 1986, Thomas and Frick 1993, Sievert 2000, Belde et al. 2000). Belde et al. (2000) concluded from their study, that wild plant abundance increases in the first years of reduced tillage but their abundance and diversity will decrease on 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 contradictive (Zwerger 2002, Swanton et al. 1993). The increase in species dispersed by wind and grasses seems to be an unquestioned finding.

The seedbank dynamics and biodiversity in reduced tillage systems with HR plants has not been investigated yet.

Reduced tillage/mechnical weeding and vertebrates

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 stragies 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).

Conclusion on agricultural practice and biodiversity

Neither the effects of using increased amounts of broad-spectrum herbicides in minimum-tillage on wild plants nor the effect of HR and conventional tillage on erosion have been studied in the field.

The long-term experiences with reduced-tillage indicate that diversity and abundance of broad leaf plants will further be decreased 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 rescheduled and adopted to wildlife (vertebrates), and when wild plant abundance is not further decreased by highly effective (broad spectrum) weed control (plants provide habitat and food and influence the microclimate for vertebrates and invertebrates).

Changes in weed control are discussed below (see IV.3 Plants).

The trend to narrow row production in soybean and cotton and the loss of summer fallow acreage indirectly induced by HR in Canadian canola and in Argentina influence biodiversity because of the loss of undisturbed habitats.

IV.2 Toxicological attributes of glyphosate and glufosinate

Summary of published knowledge on direct toxicity to animals and water organisms

Glyphosate

Glyphosate is classified as toxic to fish and aquatic invertebrates (Ohnesorge 1994). It is also known to harm ground beetles of the genus *Bembidion* (Diercks and Heitefuss 1990). Slightly harmful effects on beneficial insects, predators and parasitoids, were detected in 4 of 17 species (Hassan et al. 1988). Glyphosate reduced the growth rate of the earthworm *Aporrectodea caliginosa* at all rates of application (Springett and Gray 1992). The risk to different arthropods tested (mainly predators) varies between high risk, medium risk, and slight risk and harmful effects cannot be excluded (European Commission, 1999). Glyphosate (as formulated product) has very high to very low toxicity to algae, water plants, and fish. The chronic toxicity to fish and crustaceans is moderate (CTB 2000, Cox 2000). The formulated products are also toxic to predatory mites and moderately toxic to some beneficial spiders and (parasitic) wasps. Low toxicity to earthworms and low acute toxicity to birds were found (CTB 2000).

Glufosinate

Glufosinate was classified as toxic for the aquatic fauna and for fish (Ohnesorge 1994). 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 formulated products (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). The highest concentration expected after applications in agriculture is 0,25 mg/l in small lakes (formulated product).

General ecotoxicological profile (land plants excepted, see below)

According to Sandermann (1994) and Ohnesorge (1994) the knowledge about the toxicity of herbicides is not sufficient for a scientifically based comparison or judgement. The relevant data and original reports are still considered confidential and have not been published (Landsmann et al. 1998). Nevertheless, the opinion based on the few pieces of information published is that the toxicity of both broad spectrum herbicides (glyphosate, glufosinate) for mammals is a little lower relative to other herbicides.

In Germany and in other countries officially requested ecotoxicological tests cover only a few beneficial species. The effect on other non-target species is not known (Forster 1995). In addition, the biological significance of many tests is limited due to highly artificial exposure conditions, which may not relate well to natural exposure conditions (Giesy et al. 2000).

Hommel and Pallutt (2000) referred to an assessment of the cumulative effect of active ingredients of pesticides (Gutsche and Rossberg 1997) even though the toxicity of herbicide products to water organisms is higher compared to the active ingredients (see below). Neither results nor indications about the methodology of the toxicity-tests with daphnia, fish, earthworms, and algae on which the assessment is based were presented (Gutsche and Rossberg 1997). Hommel and Pallutt (2000) stated that glufosinate is less toxic to three of the tested groups (all but the earthworms) compared to the reference herbicide Butisan Top®. Qaim and Traxler (2002) used the WHO toxicity classification for assessing environmental effects, but this classification is also based on the type of tests used by Gutsche and Roßberg (1997). In addition, the WHO is committed to human health and not to environmental issues. Both working groups concluded that glufosinate with respect to glyphosate causes a ”lower potential biological risk”. These statements are based on tests which do not cover effects on insects and spiders (see above), which make up most of the animals in the fields. Arthropod populations were reduced in field studies with HR crops (see below). This effect was explained by the damage and elimination of the flora (due to herbicide use) (Giesy et al. 2000).

However, these indirect effects have deliberately to be focused on because the dimension of impacts on biodiversity induced by the destruction of habitats and the elimination of food sources is greater than of the herbicides toxic (non-target) effects (Körner 1990, DETR 2000). The tendency to exclude indirect effects when assessing environmental effects of herbicides (Fernandez-Cornejo et al. 2003, Kalaitzandonakes 2003, Council Directive 91/414/EEC), or moreover to take mammalian toxicity as an indicator for effects on the environment (Nelson and Bullock 2003, Qaim and Traxler 2002) is quite common. But as shown below, results of these restricted assessments are misleading regarding biodiversity.

IV.3 Effects on the food chain

According to an European assessment of the impact of HR on biodiversity, the impact of HR regimes is slightly more negative than the impact of conventional or integrated farming without HR. The assessment was based on the comparison of the impact of the whole agricultural production systems, which was evaluated step by step in detail. Biodiversity was measured by main indicator groups such as three beneficial predatory arthropods, three farmland vertebrates and the associated flora. The model indicated that the unsatisfying situation will even become a little worse with HR (Werner et al. 2000). The results were confirmed by an expert survey. The possibly increased effect of herbicides was seen as decreasing the (ecological) quality of agricultural ecosystems (Werner et al. 2000).

Plants

The effects of selective herbicides and mechanical weeding are described in order to give a reference system for the new weed control practice within the covered HR plants.

<u>The effects of selective herbicides and mechanical weeding (as a reference systems to non-selective herbicides used in HR crops) to biodiversity</u>

Selective herbicides and mechanical weeding

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 1000-2500 seeds/m^2 within the last decades (Pallutt and Haass 1992). 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 (inhabiting the soil surface) arthropod fauna species diversity by 45-85% (Heydemann 1983). Their biomass decreased even further (Koch and Kunisch 1998). Adults of many beneficial organisms lose valuable pollen and nectar sources if weeds are reduced (Schütte 1998). 12 years of herbicide use in wheat led to a decline of the soil seedbank by 35-60% (Pallutt and Burth 1994). In Denmark the abundance of the associated flora in agriculture was reduced by 60% in connection with the increasing use of herbicides (1970-1990) (Madsen pers. communication). Similar declines of farmland species were observed in the UK (Johnson 1999). 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 has been reported from most agricultural regions including Canada and the USA (McLaughlin and Minneau 1995, Ducks Unlimited 2003).

Mechanical weeding does not reduce the density and diversity of the weed flora and associated flora as much as herbicides. In Germany, their abundance was - on an average of 12
studies - 3 times higher (range: 0,3-10 times) (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). The diversity (medians of species numbers) was on average doubled in mechanically weeded fields. The overall means of species numbers varied from 2 (Oesau 1998) with chemical and 43 (Becker and Hurle 1998) with mechanical control per test site. 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 has been reduced since decades of herbicide use. This important component of biodiversity can only be regenerated when the rare associated floral species can disperse their seeds in these fields. As several seed dispersal mechanisms do not work any more in modern agriculture, it could only be intentionally re-established with high efforts (Mayer and Albrecht 1998, Poschlod and Schuhmacher 1998, Auerswald et al. 2000). In Switzerland, seeding of rare and beneficial wild plants is done for conservation reasons and financed by public incentives.

Conclusion: Herbicides and other elements of modern agriculture have caused a systematic depletion of seed banks and difficulties to reverse this tendency do exist. The aim of weed control has often been to eliminate, not to manage, weed populations. The use of threshold models, which tolerate a certain level of weediness, is limited (see below). 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 herbicides play a prevailing role in negatively affecting biodiversity. In addition, the herbicides are even becoming more effective, especially with HR cropping. This was stated by most experts and proved in sugar beet and oilseed rape. Effects in corn may be different (see above):

The probability of using the new and more effective control options is very high, as farming history (see above) and farmer surveys indicate (see above).

Economic thresholds and improved weed control with non-selective herbicides in HR crops

Weed scientists in the USA and in Europe recommend control of weeds 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 et al. 1997, Werner and Garbe 1998). However, the databases on integrated weed management and the expert systems are rarely used in practice (see chapter III.1.1). Growers consider other factors (Owen 2000). Neither biodiversity nor weed resistance management are significant considerations of the farmer, but aesthetics (better weed suppression, simplicity of control, “clean” fields; see Table

23, 24), production risks (reduced herbicide costs, flexibility in timing) (Owen 2000, see adoption reasons - chapter III.6). Improved weed control was named as the pre-eminent reason for adopting HR in corn (94,3% of farmers), cotton (76,3%) soybean (97,5%) and canola (50%). It is the main reason in any surveyed region (9 US states, Alberta, western Canada, Saskatchewan, France [Burgundy], Argentina [north and mid states]) except some parts of Argentina (questionnaire on adoption reasons). Furthermore, landlords may insist on clear fields (Duffy 2001). For example, 50% of the agricultural land is rented in Iowa (Owen 2000). In many parts of Europe, for example, oilseed rape is presently sprayed with herbicides, although it is economically not necessary (Grenadas and Boothsack 1999).

The use of economic thresholds and mechanical weeding, both measures would favour the associated flora in fields, further declined with the introduction of HR varieties (chapter III.1.1 and III.1.2). According to Hommel (pers. communication) the use of economic thresholds in oilseed rape can become easier with HR-crops in Europe, but it is questioned whether they will be used in agricultural practice.

Weed suppression and effectiveness

Glyphosate and glufosinate are more effective on a broader range of species than currently used conventional herbicides (Westwood 1997). Weed suppression is clearly improved in most crops and regions where HR crops are planted due to the substitution of less effective herbicides and sometimes mechanical weeding (see chapter III.1.3). Buckelew et al. (2000) also found negative effects on arthropod abundance due to high weed suppression in HR sugar beet.

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 (Firbank et al. 2003, Squire et al. 2003). In HR sugar beet, HR fodder beet and HR oilseed rape the density, biomass and seed rain were between one-third and one–sixth lower (relative 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 of 6 species in oilseed rape. Emergence increased in only one 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). Less field flora resulted in decreasing forage and consequently less arthropods (see below).

Findings in HR corn (glufosinate-resistant) were different. Nevertheless, some reservations have to be discussed: The conventional fields have been spayed with atrazine which is highly effective on a broad range of plants. It is forbidden in Germany and other countries because of its long persistence. Effects of managing HR-corn should be compared to conventional management without atrazine. A comparison of this sort has been done in Germany (Brandenburg). In these trials, Hommel and Pallutt (2002) found a higher seed rain in one species (*Chenopodium album*), but the authors state, that this result has to be confirmed in further tests. Additionally, the variability of results (4 fields, 3 plots with different herbicide applications: HR1, HR2, conventional) was high. Moreover, these tests are not representative for most cropping areas in Germany. The rotation (winter oilseed rape, winter rye, corn, winter wheat) was not representative and the management was not fully representative. No herbicides were used in rye in any plot, and in some plots (HR2-plots in field 1 and 3) no herbicides were used in HR oilseed rape either (while herbicides were used in conventional oilseed rape).

A high abundance of *Chenopodium album* was detected in a plot without herbicides in rye, oilseed rape and corn (HR2-plots, field 1). This plot was not sprayed in 3 out of four crops/seasons. The other of the two HR-plots (out of 6 or 8 HR-plots, results for 2 plots not shown) with a high abundance was on field 3 (HR1). In addition, not the diversity of the field flora has been focussed, but the abundance of only 10 out of 33 species. Effects of glufosinate-resistant corn has to be studied further with a representative methodology. Glyphosate-resistant corn should be studied too, as glyphosate is effective on more species and farmers will prefer it to glufosinate:

Interestingly, weed control is improved in most HR varieties, even though yields are often not clearly increased (particularly not in soybean, the most abundant HR crop). This also refers to the net income in US soybean. Net income is increased in some situations in canola, but mostly due to reduced production costs or sometimes less dockage (chapter III.1.3, III.2, and III.3). A few highly damaging weed species are the target of “improved” weed control, but many harmless and benign wild plants are killed by the non-selective herbicides too. In this sense, weed suppression has been overdone in many regions and is even further “improved” in HR crops. As shown by the above findings, less amounts or less applications of highly effective herbicides do not cause less damage to biodiversity.

Impacts at field margins

In addition; field margins may increasingly be sprayed with herbicides in oilseed rape and canola: This is because volunteers and weedy relatives of oilseed rape have to be controlled at field margins when HR oilseed rape is planted. Field margin management has not been changed in Canada yet, but volunteer control is becoming important (Orson 2002, experts survey III.3: Tab. 21). As the agricultural systems in Europe are different, additional spraying of fallow land, which is an important refuge for wildlife in agriculture, may quite often be done in order to reduce the risk of gene flow to weeds and volunteers. Spraying field margins is currently prohibited in Germany. Drift of non-selective herbicides to field margins is another important issue of concern to nature conservation and biodiversity of 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 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-corn. As discussed above, findings in corn may be due to the use of atrazine in conventional plots and should be confirmed with other conventional herbicides.

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).

Probability of using additional selective herbicides in HR crops or rotations

Oilseed rape volunteers and at least two interfertile weedy relatives may have to be controlled in the fields respectively in the subsequent crop, because they can become herbicide-resistant (chapter II). The need to control them may undermine efforts to enhance biodiversity by reducing cereals in rotations to 50% in Europe. Weed control can be omitted in cereals in such rotations (Pallutt and Haass 1992)[6.] Pre-seeding and pre-harvest use of glyphosate (the extent of this practice is not known) in the UK at present may be substituted by paraquat+/-diquat. Paraquat+/-diquat can have a negative impact on hares (Orson 2002).

In future, the use of even more effective herbicide mixtures with glyphosate and glufosinate provides the option to further improve weed control (with further effects on biodiversity). Examples are 2,4 D and several other herbicides in soybean and cotton see chapter III.1 and above; atrazine or dicamba (Bradley et al. 2000, Hamill et al. 2000, Owen 2000; or mixtures in oilseed rape against volunteers and weeds: Stelling et al. 2003)

Conclusion: There is much evidence that the seedbank, wild flora and whole food webs in agricultural fields will further be reduced, if herbicide resistant beet and oilseed rape are planted and sprayed with broad-spectrum herbicides. The positive effects of HR corn should be confirmed relative to conventional corn without atrazine applications.

Microbes

Several herbicides have a negative impact on microbial biodiversity. Microbes are of significant ecological and agronomic importance e.g. as symbiotic partners, antagonists to pathogens, and food source for the micro-fauna. Glyphosate and glufosinate suppress soil microorganisms. The suppression can last 60 days and more at temperatures far below 20°C. At temperatures of about 20°C, regeneration can be observed within a week. A reduction of bacteria and fungi of approximately 40% (measured by several tests and indicators) or more (sometimes less, several references in Schütte 2000) lasting a few to 8 weeks will suppress the microfauna feeding on bacteria and fungi and thereby negatively influence the whole food web. The relevant growing and reproduction season for many invertebrates does not last more than 18 to 25 weeks in countries with temperate climate. This has to be evaluated, taking into account that mostly two and sometimes three herbicide applications are recommended by farming consultants (see chapter III.1).

Beneficial microorganisms like *Rhizobium leguminosarium* and *Trichoderma* species (mycoparasitc) are negatively affected by just one application of glufosinate, unlike some plant pathogens (Broer 1995, Ahmad and Malloch 1995, Kremer et al. 2000). According to Ahmad and Malloch (1995), the dominance structure of the soil biocoenosis is changed by the

[6] A maximum of 50% cereals is advocated by the German Umweltbundesamt and most non-governmental organisations (Gemeinsame Plattform von Verbänden 2001). Less cereals in the crop rotation would stop the ongoing selection of a small number of typical cereal weeds that are difficult to control.

herbicide glufosinate. Some mycorrhiza species were sensitive to multiple dosages of glyphosate (Chakravarty and Chatarpaul 1990).

Invertebrate Fauna

Volkmar et al. (1998 and 2000) compared the density activity of staphylinids, carabids, and spiders on test plots of 0.5 ha size using pitfall traps. They compared farming systems with standard herbicide application, with HR, and without herbicide use over a period of three years. The activity density of staphylinids and carabids was higher in HR-plots with less weed ground cover (Volkmar et al. 1998) and activity density of spiders was less in HR-plots (Volkmar et al. 2000). However, the biological significance of these activity density results is poor. Firstly, the ground activity of these beetles (of which many are able to fly) but not their real abundance (numbers of individuals per area) often increases when the soil cover of field flora is low, because of low levels of prey and less obstruction allowing increased activity (Hassall et al. 1992). Secondly, even test plots of 20 ha are too small to exactly measure their real density by activity and lead to an underestimation of negative effects because of the compensation from the vicinity (Booij and Noorlander 1992) (further methodical discussion: Möwes et al. 1997, Welling 1990, Chiverton and Sotherton 1991). Insects and spiders can move from one small plot to the other. Theoretically, the optimal plot size (no compensations of losses by colonizers from the vicinity of the plot) for studying effects on population densities of flying insects would be the centre of a 1000-ha area (van Emden 1990). Thirdly, the biomass of predatory arthropods (not only the density) should be measured and compared, because the same numbers of very small individuals (with an overall low biomass, which often dominate in modern conventional agriculture) do not control pests as effectively as larger ones.

Moreover, arthropod biomass is the primary criteria for the evaluation of positive impacts on other animals, such as birds and small mammals feeding on them. Large scale investigations are necessary to detect the effects of changing herbicide application patterns discussed above. As an example, Schütte (1990) found an up to 2,6 times higher arthropod biomass in integrated farming systems (less herbicides used) compared to conventional farming (5 years, whole farms of more than 100 hectares compared - not small parts of a field, use of pitfall traps like Volkmar et al. [1998]).

Several arthropod sampling methods were used in the large-scale trials in Britain (scale and plot size: see above) in order to compare the abundance of different arthropod groups (Firbank et al. 2003). 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). Effects in HR corn were reverse, but the findings may be due to the atrazine use in conventional plots as discussed above. The indirect effects of plant suppression and habitat destruction (see above “Plants” and conclusion - IV.3) are the key to invertebrate (and vertebrate) biodiversity.

Vertebrates

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, DETR 1999, Werner et al. 2000, SRU 1996). The causes of these declines are a combination of factors. For farmland birds it is widely accepted that changes in agricultural management practices are responsible for these developments. Birds are both major targets and important indicators of agricultural change (Ormerod and Watkinson 2000) and recently adopted as a key measure of agricultural sustainability in the UK (Johnson 1999). 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. The decline in farmland birds has been at least partially attributed to the use of herbicides and broad spectrum insecticides and the increased efficiency of their application (DETR 2000, Chamberlain et al 2000, see above). Simulations from the UK show that one consequence of planting herbicide resistant crops will be a major loss of food sources for seed consuming farmland birds, if the adoption of the new system ”co-varies with current weed levels” (Watkinson et al. 2000, and see above IV.3 Food chain: Plants).

The effects of high amounts of a specific herbicide (sprayed on most fields of the area) in the surface water due to the runoff after spraying in coincidence with erosive events should not be ignored according to Auerswald (pers. communication)[7]. This is of relevance as glyphosate and glufosinate both influence the aquatic ecosystems (see above, toxicological attributes).

Overall conclusion on biodiversity

Reduced amounts of herbicides, considered to have less toxic effects to vertebrates than several other herbicides, have to be balanced against negative effects of a stronger weed and wild plant suppression (and its effects on the food web including vertebrates), loss of fallow land, drift effects on margins and uncropped land, additional volunteer control effects in oilseed rape/canola, increased narrow row production, and probable additional volunteer control effects in oilseed rape/canola – depending on the production systems.

As shown by the above findings, less amounts or less applications of highly effective herbicides in HR crops do not cause less damage to biodiversity but result in the opposite.

Watkinson et al. (2000) and Firbank and Forcella (2000) suggest that the regional-scale consequences of farm-level decisions might be the key to predicting the impacts of such herbicide-resistant crops on biodiversity. The decrease in biodiversity compounded over time (Heard 2003b) and large areas (Haughton 2003) will be much greater than detected in the UK-trails. Wolfenbarger and McCarty (2003) are investigating consequences of HR on farmland

[7] Silty soils and clay soils are not suited for no-tillage agriculture. HR and spraying non-selective herbicides on soils in tillage systems can result in more erosion than with traditional herbicides in parts of Europe. The recommended timing of glufosinate or glyphosate applications in corn and sugar beet coincides with periods of high precipitation, at least in western Europe. If the field flora was not eliminated by these herbicides, it could – to a certain density-dependent extent - prevent erosion caused by precipitation. An assessment of this aspect has only been done for the unrealistic one-application scenario (Auerswald 1994).

birds in Nebraska for three years. However, the duration of this project is too short to find a response in bird populations. In general, such significant effects occur with a time lag of about 6 years (see above).

Nevertheless, HR systems might be modified to favour biodiversity (Firbank and Forcella 2000).
Field tests with a 50% dosage in fodder beet (Elmegard and Pederson 2001), and band spraying in combination with economic threshold evaluation and postemergence application in sugar beet (Dewar et al. 2003, Dewar et al. 2000, Coghlan 2003) resulted in a higher wild plant abundance followed by a higher abundance of beneficial predator arthropods on sites with a rich seedbank reservoir. Changes in diversity of field plants and in the seedbank should be investigated in such innovative field tests, in order to predict long-term changes. Unsprayed patches and patchy (precision) fertilisation would positively contribute to these effects (Dzinaj et al. 1998, Gerhards et al. 1998, Lettner et al. 2001). As seedbank losses have already been quite dramatic in agriculture, it would be important to conserve locations with a still diverse seedbank (Otte et al.1988, Jüttersonke and Arlt 1998) (But particularly the use of HR in high biodiversity fields that is predicted by experts [Firbank and Forcella 2000]).

The propagation and implementation of the above concepts as well as adoptions in the timing of agricultural operations (see IV.1) or the re-establishment of seedbanks will need high initial efforts. The use of non-selective herbicides and HR crops are less damaging, if integrated management systems, particularly economic threshold models and patchy weed control, were developed and applied. However, patchy weed control of difficult weeds with selective herbicides and ecological farming are more favourable than non-selective herbicides. For this reason, McLaughlin and Mineau (1995) addressed and explained the need for selective products in agriculture. It should additionally be noted, that the positive effect of economic threshold models on biodiversity declines relative to declining pesticide costs. Low herbicide costs is one of the three pre-eminent adoption reasons for HR crops. Thus, economic threshold models should be complemented by additional measures.
Moreover price reductions for agricultural products account for a strong trend to save tillage runs by applications of non-selective herbicides. Some experts consider soil conservation as more important than biodiversity. However, both resources are highly important. The challenge is to conserve both resources. Biodiversity is a national and international affair and cannot solely be shouldered by farmers in the context of low and falling product prices.
As long as the use of economic thresholds and additional measures such as e.g. selective and precision control are not common practice, a negative overall effect of non-selective herbicides and HR is predicted because of the simplicity, and the desire to eliminate wild plants irrespectively of whether they are harmful or benign. The need for a regulatory system which encourages agricultural methods favourable to biodiversity is evident. In addition, many monitoring concepts for transgenic plants do not even include the monitoring of field flora and seed rain/seedbank dynamics, although these are the key indicators for biodiversity under different herbicide regimes.

Summary

Introduction

The data and information for this document were collected through literature review, internet search, and expert surveys. Genetically modified bromoxynil resistant crops were not covered because of the low relative relevance compared to glyphosate and glufosinate resistant crops.

Section I Scope of Application

While herbicide resistance can also result from selection, the focus is on resistance due to genetic engineering. This is because the latter crops are planted at huge cropping areas at the moment (especially soybeans). It can be expected that this trend will continue, because other important crops with the HR-trait, like sweet corn, sugar beet, rice and wheat are already approved or under development. The most important traits in this sense are crops resistant to one of the two herbicides glufosinate and glyphosate.

Commercial cultivation of glyphosate and glufosinate resistant transgenic crops:

crop	global cropping area (mio ha)	% HR of global area	herbicide resistance against	country
corn	140	1.6 (3.1**)	glyphosate	Argentina, Bulgaria[1], Canada, USA
			glufosinate	Canada, USA
cotton	34	6.5 (13**)	glyphosate	USA, *
canola (oilseed rape)	25	12	glyphosate	Canada, USA
			glufosinate	Canada
soybean	79	46	glyphosate	Argentina, Canada, Mexico, Romania, South Africa, Uruguay, USA

http://www.transgen.de, James 2002, [1]Gianessi et al. 2002

** in brackets: HR/insect resistance (stacked)

* regulatory approval is currently pending for HR (glyphosate) cotton in Australia, Argentina, Mexico and South Africa, the product is under development in Brazil and Turkey

Section II Changes in weed susceptibility and weed population shifts

Generally, the selection pressure of a particular herbicide is enhanced, if it is more often applied than others and if the herbicide is highly suppressive. Glyphosate and glyfosinate are non selective herbicides. They are effective to a very large range of weed species. And they are applied in a still increasing number of different HR-crop species accompanied by changes in agricultural practice.

While weed control in HR crops is currently more simple and effective in many cases, this can be undermined in the long run by:

- 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.
- escape and proliferation of the transgenic plants as weedy volunteers,
- hybridisation with - and HR-gene introgression into - related weedy species.

II.1 Selection pressure

Estimations based on plant physiology generally led to the conclusion that glyphosate and glyfosinate are low risk herbicides with respect to the evolution of herbicide-resistance in weed populations. On the other hand, the application patterns (large scale, dominating herbicides, large time window) may contribute to the selection processes.
Some weeds are difficult to control with glyphosate and glufosinate and some already developed resistance against glyphosate such as (officially recorded): rigid ryegrass (*Lolium rigidum*), italian ryegrass (*Lolium multiflorum*) and goosegrass (*Eleusine indica*). Some experts additionally identified marestail (*Hippuris vulgaris*) and fleabanes (*Erigeon*) as resistant. The mechanisms of resistance against glyphosate are partly elucidated. No glufosinate-resistant weed biotype has been recorded so far.
It is reasonable to assume that more resistant species and biotypes will develop if glyphosate is regularly used in a considerable proportion of crop fields. Judging from the experience with the above species, resistance may evolve after 10 to 20 years, if it is used 1-3 times a year. Many weed scientists recommend to use additional herbicides in glyphosate resistant cotton and multiple applications of glyphosate or residual herbicides and glyphosate in soybean, particularly in regions where glyphosate has been used for a long time period now. The implementation of a long-term plan to reduce the selection pressure on weeds by glyphosate is also recommended by some experts. It should be avoided, for example, to plant glyphosate resistant crops continuously.

II.2 Herbicide resistant volunteers resulting from intraspecific and interspecific gene flow

Gene transfer frequencies are highly variable. Influencing factors aside from species specific ones include wind direction and wind speed, climate, variability of the pollination system between varieties of the same species, abundance, diversity, and behaviour of pollinators (sometimes influenced by land marks) and the size of the pollen donor population. Also, different genotypes or varieties sometimes show different frequencies of cross-pollination. Most experiments were done with small pollen sources. Large pollen sources, such as crop fields make gene flow more likely.

Intraspecific gene flow

Intraspecific gene flow generating herbicide resistant offspring has two aspects, the generation of weedy volunteers and seed impurities.
As crop plants can be volunteers in subsequent crops they also may have to be controlled by herbicides or other means.
Oilseed rape (canola), cereals, and potato are examples of crops that often have to be controlled in other crops. Volunteer control is of high importance in oilseed rape. Bolting sugar beet are considered as a source for cross pollination and HR-introgression into “volunteer -“ or weed beet. Volunteers of soybean (in cotton) and corn (in soybean [and sugar beet]) are known from parts of the USA, where glyphosate resistant varieties of three of these crops are grown. If the volunteer crop is resistant against the herbicide used in the subsequent crop, major problems may arise.
Seed impurities can lead to financial losses when plants are sprayed with a herbicide against which they are not resistant. It is also of importance that many consumers want to choose between genetically modified food and organic or conventional food. The latter aspect is important for all transgenic traits, not only HR. The prevention of seed contamination has to be addressed in HR plants with a moderate or high chance of cross pollination such as (regarding the currently grown HR crop species and cropping regions) oilseed rape, sugar beet and - to some extent – corn. Seed production, grain handling, storage and transport are the main sources of contamination.

Details for relevant HR-crop species:
Corn: Gene flow though cross pollination and seed exchange by farmers may be important aspects in Mexico and other centres of diversity of corn. Corn volunteers are known in warm regions and additional control methods for them are applied in the US-Corn Belt. Problems are recorded from soybean and sugar beet. In many colder regions (where corn does not survive low temperatures) the likelihood of growing unwanted HR corn due to impure seed may become relevant.
The probability of growing low levels of unwanted HR (or generally of unwanted GM) corn depends on many aspects in farming, such as field sizes, crop rotations, weather conditions, on the abundance of pollinators and – most important in US and European corn production: seed production management.

Cotton: Commercial cotton varieties do not seem to create severe problems as volunteer plant. Most seeds of modern cultivars do not survive more than one season – in contrast to wild cotton. Nevertheless, the occurrence of volunteer cotton in soybean crops has been reported from the USA.

Oilseed rape: Volunteer oilseed rape is creating control problems in many areas and crops in Europe and in Canada. Oilseed rape volunteers and feral plants may play a significant role in gene transfer from transgenic crops to wild relatives and possibly serve as stepping stones. Feral plants include populations at field margins, soil dumps and roadsides mostly derived from seed spills.
In Canada no management plan has been implemented for canola volunteers so far. Farmers and regulators seem to rely on the options to use alternative herbicides for volunteer control but this practice is not considered as sufficient by some experts. The level of HR genes is usually below 0,25% in conventional seeds in Canada. Organic canola industry has stopped because consumers are not willing to buy contaminated products.
In European agriculture it might be technically possible but economically difficult (see management recommendations below) to maintain a 0,3% seed impurity level and a 1% impurity level in agricultural production when 10% of the rape growing area is transgenic (e.g. herbicide resistant). It was suggested to delay post-harvest cultivation and to repeat shallow stubble tillage in production in order to reduce seed persistence in soil. It may be necessary to minimize overlapping flowering periods between different (HR and conventional) varieties. A regional border management and the use of additional herbicides are other options to keep impurities below the mentioned level. A complete prevention of volunteer occurrence seems impossible even by a combination of the above post-harvest cultivation and wide rotations. The use of additional herbicides against volunteer oilseed rape is proposed by some experts.

Sugar beet: Cross pollinating bolters and annual weed beet as well as the contamination of organic seed and *B. maritima* at the sea coast are of concern in the scientific discussion on gene flow in sugar beet. Annual weed beets cause serious problems in parts of Europe, including Belgium, Germany, England and northern France. The control of bolting beet is recommended in order to prevent outcrossing of HR into weedy forms. Bolting HR sugar beets can pollinate weed beets resulting in HR resistant weed beets. The hybridisation between annual weed beets and cultivated HR beet is likely to happen when HR varieties are grown. Bolters have to be monitored and controlled in seed production areas. If the bolting plants and weed beets are not removed immediately, stable weed beet complexes form quickly and are difficult to eradicate. Moreover, certified seed with low impurity levels should be produced and used. A thorough control of ruderal beets will be necessary and the implementation of upper isolation distances (1000 m and more) in seed production areas may be necessary.

Soybean: In Europe, soybean is not weedy. In US cotton and corn areas, keeping out volunteer soybeans can be a challenge. Glyphosate resistant varieties of all three crops are planted in the USA.

Interspecific gene flow

The relevance of *interspecific* gene flow of a herbicide resistant plant to weeds highly depends on the cropping region and the abundance of interfertile relatives of a crop. In the current biosafety discussion of HR crops the control of oilseed rape relatives in Europe and the implications of hybridisations between corn and teosinte in Mexico are addressed. Weed control methods in other crops within crop rotations in Europe have been recommended to control possibly occurring weedy hybrids of oilseed rape and wild species.

The following weedy plants may raise control problems due to introgression of HR genes from oilseed rape:

- *B. rapa* (which is grown as a crop but also known as a weed) (in Europe and Canada)
- backcrosses of *B. napus/R. raphanistrum* hybrids with the weed parent (in Europe)
- backcrosses *B. napus/Erucastrum gallicum* hybrids with the weed parent (in Canada).

Herbicide resistant weeds are under control, as long as different herbicides are sprayed in cereals (or other rotational crops). Thus, herbicide use in cereals may become an obligation although it could be omitted in particular integrated farming systems.

Some Canadian experts stated that the current management strategies were not sufficient to avoid introgression of HR-genes into weedy relatives and volunteers in Canada.

Mexican researchers are currently investigating and discussing the case of teosinte.

Wheat and rice, two very important crops of which HR varieties are expected to be approved soon, both have weedy relatives in certain anticipated release and growing regions. Precautious control methods are proposed for the wheat fields in the western USA.

Interfertile weedy relatives of rice are abundant in parts of Asia and red rices (subspecies) are known in many parts of Asia, Oceania, Africa and Latin America. A combination of different modes of containment and genetically introduced containment traits is proposed in order to reduce the likelihood of gene transfer to red rice.

Section III Impacts on agricultural practice and agronomy

HR cropping induces changes in agricultural practices and agronomy, e.g. altered weed control, yields, net income, soil tillage, planting and crop rotation.

III.1 Weed control patterns

In non-HR farming, farmers apply a sequence of different herbicides 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.

HR crops allow the post emergence application of a single herbicide with a wide spectrum of activity.

Spraying at postemergence can imply a restriction to a very short time period in respect to weed development. This can be problematical, if the weather conditions are unfavourable for herbicide applications.

Glufosinate or glyphosate can be used alone, in combination with preemergence herbicides for programs that provide soil residual control, or with mechanical weeding. As the maximum weed size for effective control is higher with glyphosate than with other herbicides, the potential time period for spraying is extended. This allows more flexibility.

No overall picture about changes in weed control patterns can be drawn:

Crop injury within the sprayed field is expected to be lower in HR crops but injury caused by drift is expected to be higher. More postemergence applications as well as daytime applications of the highly supressive herbicides contribute to drift problems. The effect of glyphosate and glufosinate is higher at daytime and wind speeds are higher too.

Postemergence applications increased in HR resistant soybean and canola, postemergence applications are expected to increase in herbicide resistant sugar beet (UK) but not in corn. Information on possible changes in oilseed rape in Europe is missing.
According to the experts statements, the adoption rate of *economic threshold models* is low in any crop covered by the study. It will further decrease in canola and probably in soybean.

Changes in overall *amounts of herbicides* used are more difficult to assess because different herbicides are applied at different particular, and varying rates. In soybean, a slight overall reduction, but also increased amounts in reduced or no till systems (at least in Argentina) have been reported. Recently, increasing herbicide use is observed in some areas where HR soybean has been planted for many years because of evolving resistant or tolerant weeds (see above) and is recommended for some HR cotton areas (additional herbicide types recommended here which results in higher amounts too). One reason of several others for an overall decrease in herbicide use in cotton was the adoption of glyphosate resistant varieties. Amounts used in European corn or sugar beet field tests have been less in HR plots. Oilseed rape should not be sprayed with herbicides as it is mostly not economically sound in Germany and the UK.
A reduction of amounts does not necessarily mean a reduction of effectiveness (see below) or of application numbers.

Reduced herbicide *application frequencies* can lower soil compaction and erosion. The survey indicates that canola farmers may spare one application in HR varieties in Canada, but this is not supported by publications. Application frequencies in soybean also decreased. A decrease in application frequency is expected for European sugar beet and oilseed rape in France. An increase in German oilseed rape is expected with glufosinate resistant cultivars. An additional application is predicted for HR corn in Germany too. No changes in application frequencies, or differing results have been reported for soybean and cotton.

The *number of herbicides (types)* used in HR varieties (compared to conventional ones) decreased in Canadian canola, in US and Argentinean soybean and probably in cotton areas. Nevertheless numbers of herbicide types are probably increasing according to experts recommendations in some cotton areas. In HR soybeans, not only glyphosate but also triazolopirimidines and imidazolinones are used. Herbicide numbers are expected to decrease in European sugar beet and oilseed rape and probably in corn.

Mechanical weed control decreased with introduction of HR varieties in cotton, in US soybean (from < 10% to 0 in Iowa), in Argentinean soybean (at those locations where it was still done), and may have decreased in Canadian canola. It is expected to decrease in European sugar beet (30% to 0% of the acreage).

Weed suppression is improved in nearly all HR crops and regions. It is expected to be improved with HR sugar beet and HR oilseed rape, but not with HR corn, in Europe.

III.2 Yields

Reliable data of independent research institutions on yield differences between conventional varieties and HR varieties are scarce. Varying results make general statements impossible.
One major problem is the correlation with co-variables, e.g. farm size, education and skills of the farmers.

Corn: Mixed results on yield differences (no differences and increased yields in HR varieties) in the USA are recorded. No significant differences have been found in German test fields.

Cotton: Varying results make general statements impossible.

Oilseed rape: Yields in Canadian canola are higher on average with HR in about half of the growing conditions according to the expert survey. Maximum yields were gained by a non-HR variety. No differences were found in European field tests.

Soybean: Mixed results were published for US soybean. In summary and on average yields of HR varieties were about the same or less. Argentinean HR soybean varieties yielded less than their conventional counterparts.

Sugar beet: Yields of HR varieties increased but results were not statistically significant in Germany. Yields increased in other european field tests too. Yield gains are expected by UK experts.

III.3 Net income

Corn: Gains were only rarely found in the German HR varieties (compared to non-HR).

Cotton: Net returns increased due to reduced herbicide costs in the USA.

Oilseed rape: About half of the HR growing farmers in Canada had higher returns according to the experts. The outcome was accounted to a lower dockage, earlier planting and reduced herbicide costs. Some published results indicate lower yields and lower economic returns for HR canola, probably depending on the farm and soil type. No gains and sometimes losses were found in European field tests with HR oilseed rape. Weed control is often not economically justified (Germany, UK).

Soybean: Savings through cheaper herbicides often equalized or outweighed higher seed costs and sometimes lower yields in the USA. No clear-cut increase in net returns can be stated for Iowa (HR) soybean but for Nebraska and Argentinean (HR) soybean.

Sugar beet: Higher net returns are expected for HR sugar beet compared to conventional varieties by UK experts due to higher yields and lower herbicide costs.

When the net income increased in a HR crop, the better profits were mostly attributed to lower herbicide costs and less tillage (which implies less labour and fuel costs) often summarised as production costs. The correlation between less tillage and HR may not commonly be given, which implies that cost reductions due to HR are mainly due to reduced herbicide costs. Highly suppressive herbicides seem to be of importance in the first one or two years of tillage reduction.

III.4 Tillage

The adoption of conservation tillage has widely been enforced and propagated since many years. It does not depend on herbicide resistant crops. Surveys indicate that 1,8% of cotton and 3% of canola farmers but 46% of soybean farmers planted HR varieties in order to reduce tillage. Findings on the significance of HR for the adoption of reduced tillage practice in cotton and Canadian canola were mixed.

Soybean farmers who used no-till had a higher probability of adopting HR, but the use of HR did not affect no-till adoption in the late nineties. Nowadays, reduced tillage practice and the planting of HR cotton are both increasing and seem to encourage each other. Experts predict an increase in reduced tillage when HR varieties are planted in Europe.

Most of the Argentinean (HR) soybean farmers shifted to reduced or no-tillage.

However, price reductions for agricultural products account for a strong trend to save tillage runs by applications of non-selective herbicides – pre-seeding or in HR crops, worldwide.

III.5 Crop rotations

In Canada and Argentina some loss of fallow land, which was planted to HR crops, has been recorded.

III.6 Reasons to adopt HR crops

Several reasons may theoretically account for the adoption of HR-crops by farmers, e.g. improved weed control, cost reduction and yield increase. For most cases, a combination of reasons can be assumed, and different priorities for different crops and growing situations are given. Simplicity, high effectiveness, and low herbicide costs in HR crops are the most mentioned and most highly ranked reasons in published results as well as in the expert survey. The option to reduce tillage, the convenience in timing of weed control and the reduced herbicide application frequency are further important reasons. In general, farmers are adopting HR because they want to reduce production risks.

Section IV Impacts on biodiversity

Agricultural biodiversity is of very high concern. Where agricultural land covers a large proportion of the land, many conservation strategies have to include agricultural practices.

Reduced amounts of herbicides, considered to have less toxic effects to vertebrates than several other herbicides, have to be balanced against negative effects of a stronger weed and wild plant suppression (and its effects on the food web including vertebrates), loss of fallow land, drift effects on margins and uncropped land, increased narrow row production, and additional volunteer control effects in oilseed rape/canola – depending on the production systems.

In general, herbicides are known to have more indirect effects on biodiversity through plant suppression (with consequences for the food chain) than direct toxic effects.

European large scale tests with sugar beet and oilseed rape showed, that less amounts or less applications of highly effective herbicides in HR crops do not cause less damage to biodiversity but the opposite. Diversity and abundance of the field flora and most arthropdods (including important pollinators and benefial pest predators) declined.

The results indicate, that compounded data on direct toxic effects to a restricted number of tested animals are an insufficient indicator for environmental effects of herbicides. The indirect effects (highly efficient and non-selective weed control) accounted for the outcome.

The effectiveness of weed control in commercial HR crops in Canada, the USA and Argentina is also higher than in conventional systems.

The decrease in biodiversity compounded over time and large areas would be much greater than detected in the UK-trials.

Findings in HR corn were different. Biodiversity was higher in glufosinate resistant corn than in conventional corn where atrazine was used in the large-scale trials mentioned above. A comparison without atrazine (which is forbidden in some countries) is missing. As an overall result, the strong relation between field flora and arthropods was obvious.

Some HR systems can be modified to favour wild plant abundance, but it is questioned whether it will be done without further encouragement. Field tests with a 50% dosage in fodder beet, and band spraying in combination with economic threshold evaluation and postemergence application in sugar beet have shown to result in a higher wild plant abundance followed by a higher abundance of beneficial predator arthropods on sites with a rich seedbank reservoir.

Unsprayed patches and patchy (precision) fertilisation would also positively contribute to these effects. The development and propagation of patchy weed control and its devices may encourage this new practice of weed control. Nevertheless, patchy weed control of difficult weeds with selective herbicides and ecological farming are likely to be more favourable, particularly to field plant (species) diversity. As seedbank losses are already quite dramatic, it would be important to conserve areas with a still diverse seedbank through adapted agricultural practices. However, use of HR in high biodiversity fields is predicted by experts. The propagation and implementation of the above biodiversity favouring concepts as well as adoptions in the timing of agricultural operations and the reestablishment of seedbanks would make some of these options realistic.

Price reductions for agricultural products account for a strong trend to save tillage runs by applications of non-selective herbicides. Some experts consider soil conservation as more important than biodiversity. However, both resources are highly important. The challenge is to conserve both by an integrated concept.

In addition, many monitoring concepts for environmental effects of transgenic plants do not even include the monitoring of field flora and seed rain/seedbank dynamics, although these are the key indicators for biodiversity under different herbicide regimes.

The need for a regulatory system which encourages agricultural methods favourable to biodiversity is evident.

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Appendix

Questionnaire on Herbicide Resistant Crops

(for: OECD Consensus Paper)

The following answers refer to the Crop : ……………………………………………

If you can cover a second HR crop, please fill out another copy of this questionnaire[8].
Please also use two (or more) copies of this questionnaire, if two (or more) agricultural situations should be discriminated, in which the crop is cultivated, e. g. soil types, farm types, landscapes. In this case, you may not need to answer all questions, but please answer all questions which are specific to the second HR crop respectively the second agricultural situation.

GENERAL questions

1. Agricultural reference system

Your answers in this questionnaire refer to the

1. agricultural region of________________________________
   (name of geographical region)

2. typical agricultural system/crop rotation of

   ____

2. Personal Expertise

Your answers in this questionnaire are given on the background of:
(multiple answers possible)

Short term field tests	9
Long term field tests including at least a whole crop rotation	9
Experience with commercial use	9
Statistical analysis of commercial use	9
Desk studies	9
Experience as farming consultant	9

3. Which crops are grown in the specified region and how high do you estimate the proportion of HR varieties of these crops?

Crop	area planted with HR-varieties (% of total area of this crop)
……………………	.. ………………………………………………………………..
……………………	.. ………………………………………………………………..

[8] e.g. request by e-mail to ustachow@zalf.de; or simply make a Xerox-copy of the blank form

4. Agricultural details **in conventional crops (cont. [3]1)**	**Please describe the current agricultural practice in conventional varieties** **4.1 Conventional Varieties** • **Application frequency, timing and types of herbicides** Type of herbicide (e.g. brand name, active ingredient) | Frequency | Timing of application(s) (month) ………...
	• **Application timing and type of fertilizer (Legume cover crop, mineral fertilizer, organic (solid or liquid) fertilizer)** **Type of fertilizer** | **Frequency** | **Timing of application(s) (month)** …………………………………………………………………………………………
	• **Is mechanical weed control done in general?** Yes…9 No … 9 If yes, please describe timing and devices: device | timing | Weed problem **... .**
	• **Do farmers scout weeds and use economic threshold models in their conventional crops?** No ………………………………….. .9 Very few farmers …………………… 9 A few farmers…………………………9 More or less one half………………… .9 Most farmers………………………….. 9 I have no estimation…………………. 9
	• **Which is the approx. proportion of the area in which economic threshold models are used?** 0– 10 %………………………………… 10 – 25 %…………………… ……... 25 – 50 % ………………………….. more than 50 %…………….………. I have no estimation………………… .
	• **Is weed control carried out by contractors?** No ………………………………… On very few farms……………………… On a few farms…………………………. More or less half of the farms………….. On most farms………………………….. I have no estimation……………………..

4. Agricultural details **in conventional crops** **(cont. [3]2)**	• **On which approx. proportion is weed control carried out by contractors?** 0– 10 %…………………………………. 10 – 25 %……………………………….. 25 – 50 %……………………………….. more than 50 %…………………………. I have no estimation……………………..
	• **Please describe typical soil tillage systems and devices.** Soil tillage system 1: device timing ………………………….. . ……………………………………… Additional remarks to system 1: ▪ Approximate proportion of area, where system 1 is applied (% of total area of the crop species): Soil tillage system 2: device timing ………………………….. . ……………………………………… Additional remarks to system 2: ▪ Approximate proportion of area, where system 2 is applied (% of total area of the crop species): Soil tillage system 3: device timing ………………………….. . ……………………………………… Additional remarks to system 3: ▪ Approximate proportion of area, where system 3 is applied (% of total area of the crop species):
	• **Typical/average size of fields planted with conventional varieties** (in acre or hectare; please indicate) ………………………………………………………………………………………………….

4. Agricultural details **in conventional crops** **(cont. [3]3)**	• **How many farmers, who grow conventional varieties hold secondary jobs of their farms?** 0-10 % …………………………………. 10-25 % ………………………………… 25-50 %…………………………………. 50-75 %…………………………………. 75-100%…………………………………. No estimation……………………………..

4. Agricultural details **in HR Varieties** **(cont. [3]1)**	**Please describe the current agricultural practice in HR varieties**

4.2 HR Varieties

- **Application frequency, timing and types of herbicides**

Type of herbicide (e.g. brand name, active ingredient)	Frequency	Timing of application(s) (month)
………………………………	………………………	………………………………………

- **Application timing and type of fertilizer (Legume cover crop, mineral fertilizer, organic (solid or liquid) fertilizer)**

Type of fertilizer	Frequency	Timing of application(s) (month)
………………………………………………………………………………………		

- **Is mechanical weed control done in general?** Yes…9 No … 9

If yes, please describe timing and devices:

device	timing	Weed problem
………………………………………………………………… .		

- **Do farmers scout weeds and use economic threshold models in HR-crop?**

No …………………………………… .9
Very few farmers …………………… 9
A few farmers………………………….9
More or less one half………………… 9
Most farmers………………………….. 9

I have no estimation…………………. 9

- **Which is the approx. proportion of the area in which economic threshold models are used?**

0– 10 %…………………………………
10 – 25 %…………………… ……...
25 – 50 % …………………………..
more than 50 %…………….……….

I have no estimation………………….

- **Is weed control carried out by contractors?**

No …………………………………
On very few farms……………………
On a few farms………………………….
More or less half of the farms…………..
On most farms…………………………..

I have no estimation…………………….

4. Agricultural details **in HR Varieties** **(cont. [3]2)**	• **On which approx. proportion is weed control carried out by contractors?** 0– 10 %…………………………………. 10 – 25 %……………………………….. 25 – 50 %……………………………….. more than 50 %…………………………. I have no estimation……………………..
	• **Please describe typical soil tillage systems and devices (reduced – no-tillage?)** Soil tillage system 1: device timing ………………………….. . ……………………………………… Additional remarks to system 1: ▪ Approximate proportion of area, where system 1 is applied (% of total area of the crop species): Soil tillage system 2: device timing ………………………….. . ……………………………………… Additional remarks to system 2: ▪ Approximate proportion of area, where system 2 is applied (% of total area of the crop species): Soil tillage system 3: device timing ………………………….. . ……………………………………… Additional remarks to system 3: ▪ Approximate proportion of area, where system 3 is applied (% of total area of the crop species):
	• **typical/average size of fields planted with HR varieties** (in acre or hectare; please indicate) ……………………………………………………………………………………………… …….

4. Agricultural details **in** **HR Varieties** **(cont. ³3)**	• **How many farmers who grow HR-varieties hold secondary jobs of their farms?:** 0-10% …………………………………. 10-25 % …………………………………. 25-50 %…………………………………. 50-75 %…………………………………. 75-100%…………………………………. No estimation…………………………….

5. Main reasons to decide for growing HR **- general -**	**What are the main reasons for adoption of HR varieties?** **Please mark reasons in regard to their importance (no importance to very high importance) in relation to the cropping of conventional varieties**

Crop: ___________________		Importance				
		no	low	medium	high	very high
A	Better weed control					
B	Higher crop yields					
C	Reduced herbicide costs					
D	Reduced labour costs					
E	Convenient timing of weed control					
F	Reduced herbicide application frequency					
G	Simpler herbicide system[1]					
H	Wish to reduce tillage[2]					
I	Better consulting service					
j	(?)[3]					

[1] simpler weed management because of low number of herbicides
[2] this answer implies that farmers would not reduce tillage without HR
[3] fill in additional reason

<table>
<tr><td rowspan="3">6.
Main reasons to decide for growing HR

- specific -</td><td>6.1 Is weed control improved in herbicide resistant crops?</td></tr>
<tr><td>Yes No
If yes:
Due to substitution of less effective herbicides
Due to substitution of mechanical weeding
Due to____________________

In most cropping areas
In about one half of them
In a few areas
In particular situations such as…………………………………………………………………..
…………………………………………………………………………………………..
…………………………………………………………………………………………..
…………………………………………………………………………………………..

I have no estimation</td></tr>
<tr><td>6.2 Crop rotation: Is weed control altered within the crop rotation with HR-crop, other than within the HR-crop itself?
Crop | Weed problem | Weed management
…………………|……………………………………|…………………………………
…………………|……………………………………|…………………………………
…………………|……………………………………|…………………………………
Crop | Weed problem | Weed management
…………………|……………………………………|…………………………………
…………………|……………………………………|…………………………………
…………………|……………………………………|…………………………………
Crop | Weed problem | Weed management
…………………|……………………………………|…………………………………
…………………|……………………………………|…………………………………
…………………|……………………………………|…………………………………</td></tr>
<tr><td>7. HR

Susceptibility to glyphosate or glufosinate</td><td>Are there weeds, which are less susceptible to glyphosate or glufosinate, and if yes, which ones?

…………………………………………………………………………………………
…………………………………………………………………………………………</td></tr>
</table>

<table>
<tr><td>8. HR

farmers shift in:
herbicide use</td><td>8.1 Do farmers shift from pre-emergence to post-emergence application in HR crops?
……………………………………………………………………………………………
at present:
Yes.........................……. . No..............................….. .
If yes:
A few farmers …………
More or less one half……
Most farmers……………. I have no estimation…….</td></tr>
<tr><td></td><td></td></tr>
<tr><td></td><td>8.2 What is your expectation for the future (next 5 years):
Will farmers shift from pre-emergence to post-emergence application in HR crops?

Yes.........................…. No................................
If yes:
A few farmers …………
More or less one half…..
Most farmers…………...
I have no estimation……</td></tr>
<tr><td>9. HR

farmers shift in:
tillage</td><td>9.1 Did farmers shift from ‘normal’ tillage to reduced or no-tillage in HR crops?
at present:
Yes.........................…. No................................
If yes:
A few farmers …………
More or less one half…..
Most farmers…………...
I have no estimation……</td></tr>
<tr><td></td><td>9.2 What is your expectation for the future (next 5 years): Will farmers shift from ‘normal’ tillage to reduced or no-tillage in HR crops?

Yes.........................…. No................................
If yes:
A few farmers …………
More or less one half…...
Most farmers…………...
I have no estimation……</td></tr>
</table>

10. HR **Impact of HR on:** ***yield***	**Do you observe yield gains in HR varieties?** Yes...................... no changes……… Yield losses…… Rarely......................…. About 50% .... Mostly......................…. I have no estimation…… **With particular situations/locations/farms such as ……………………………………** **……………………………………………………………………………………………..** **The gains or losses are presumably due to …………………………………………….** **……………………………………………………………………………………………..**
11. HR **Impact of HR on:** ***farmers economic returns***	**Does HR alters farmers economic returns?** Yes...................... no changes……… Economic losses…… Rarely......................…. About 50%..................... Mostly......................…. With particular farms such as…………………………………………………………….. ……………………………………………………………………………………………. Gains or losses are presumably due to……………………………………………………. …………………………………………………………………………………………….. I have no estimation……
12. HR **Gene flow**	**Gene flow to weedy relatives and volunteers** 12.1 Is pollen transfer into weedy relatives or volunteers a problem which has to be coped by additional management strategies in the referring region? Yes..................…………… No...................... In a few areas………………. In about half of the areas ….. In most cropping areas……… In particular situations/areas such as……………………………………………………. ……………………………………………………………………………………………. Too difficult to estimate……… Due to which weedy relative? …………………………………………………………… …………………………………………………………………………………………….

12. HR **Gene flow**	**12.2 Do farmers adopt particular management strategies in order to avoid introgression of HR-genes into weedy relatives and volunteers?** Yes...... No...... I have no estimation...... Which weedy relative or volunteer is their concern? ……………………………………. ……………………………………………………………………………………………..
	12.3 Are the current management strategies sufficient to avoid introgression of HR-genes into weedy relatives and volunteers? Yes...... No...... I have no estimation...... Due to which weedy relative or volunteer? …………………………………………….. …………………………………………………………………………………………….. ……………………………………………………………………………………………..
13. HR **Resistance selection**	**How high do you estimate the risk of a selection of new resistances against particular herbicides (due to an overuse in agriculture)?** Glyphosate Glufosinate Imidazolinone High ………… ……………………....... .... Low ………… ……………………....... .... Difficult to estimate………… ……… .... Problems with resistance selection will occur, because …………………………………. ……………………………………………………………………………………………. …………………………………………………………………………………………….

14. HR **Crop rotation**	Will HR-crops and other new transgenic varieties change rotations or management methods in the long run (in about 5-10 years) due to new options for farmers?) Yes......................……………. No........................ *If yes:* What changes do you expect? New crop species in rotations……….. Less crops in crop rotation…………... No changes... Too difficult to estimate…………..… *If yes:* Which crops and which new traits will become important in this respect?……………… …………………………………………………………………………………………… …………………………………………………………………………………………… Why do you think so?…………………………………………………………………….. ……………………………………………………………………………………………. ……………………………………………………………………………………………..
15. HR **Crop acreage**	**Are HR varieties grown in areas, where this crop has not been grown before?** Yes......................……… No....................... Which crop(s) has/have been replaced / reduced in acreage? a)………………………………………………………………………………………… b) ………………………………………………………………………………………… c) ……………………………………………………………………………………………

Background of expert judgements (respondents to the survey)

Crop	Region	Typical rotation	Commercial HR cropping?
Sugar beet	United Kingdom	Sugar beet / winter wheat	Not allowed to date
Corn	Germany, State of Brandenburg	Oilseed rape / winter rye / corn / winter wheat	Not allowed to date
Oilseed rape	Germany, State of Brandenburg	Oilseed rape / winter rye / corn / winter wheat	Not allowed to date
Oilseed rape	France; Burgundy	Oilseed rape / wheat / barley	Not allowed to date
Canola	Canada, Alberta (AB)	Canola / wheat / barley	On 72% of 0.7 Mio ha
Canola	Canada, Saskatchewan (SK)	Canola / cereal / annual legume / cereal	80% of all canola
Canola	Canada, western	Canola / cereal / annual legume / cereal	83% of all canola
Canola	Canada, western	Canola / cereal / annual legume / cereal	80% of all canola
Canola	Canada, western	Canola / cereal / annual legume / cereal	80% of all canola
Soybean	Argentina; Rolling Pampas, Mid and North States	Wheat / soybean / Corn / soybean	90 – 95% is HR
Soybean	Argentina; South Santa Fe, North Buenos Aires, East Cordoba	Wheat / soybean / Corn / soybean	98% is HR
Soybean	USA, Iowa (IA)	Corn / soybean	80% is HR
Soybean	USA, Nebraska (NB)	Corn / soybean	87% is HR

Results of the survey not shown in Detail

Question No: 12.1

Is pollen transfer into weedy relatives or volunteers a problem which has to be coped by additional management strategies in the referring region?				
crop	**yes**	**no**	**area**	**remarks**
sugar beet; GE	x		most	b. vulgaris in organic fields; b. maritima by sea coast
corn; GE		x		
oilseed rape; GE		x		
oilseed rape; F				volunteers are a problem
canola, CA(AB)	x		few	about 23% of farms reported hr volunteers are a problem
canola, CA(SK)	x		most	volunteers, esp. in no-tillage, alternative herbicide needed
canola, CA	x		few	volunteers
canola, CA	x		few	volunteers
canola, CA	x		most	volunteers; esp. low-disturbance direct seeders
soybean, AR		x		no weedy relatives are known in argentina
soybean, AR				(no estimation)
soybean, IA (USA)		x		
soybean, NB (USA)	x			In few areas, where HR soybean follows HR corn

Question No: 12.2

Do farmers adopt particular management strategies in order to avoid introgression of HR-genes into weedy relatives and volunteers?			
crop	**yes**	**no**	**which species? additional remarks**
sugar beet; GE	x[1]		b. vulgaris, b. maritima
corn; GE		x	
oilseed rape; GE			no estimation
oilseed rape; f			no estimation
canola, CA (AB)	x		concern is introgression into conventional canola
canola, CA (SK)		x	volunteers (glyphosate, imidazolinone), eastern canada: b. rapa
canola, CA		x	
canola, CA	x		volunteer canola (rr)
canola, CA		x	volunteer canola
soybean, AR		x	no evaluation of studies about this issues has been done; no management strategy is promoted
soybean, AR		x	
soybean, IA (USA)		x	
soybean, NB (USA)		x	

[1] estimate of what they will be asked to do

Question No: 12.3

Are the current management strategies sufficient to avoid introgression of HR-genes into weedy relatives and volunteers?			
crop	**yes**	**no**	**which species? additional remarks**
sugar beet; GE		x	
corn; GE		x	
oilseed rape; GE			(no estimation)
oilseed rape; F			(no estimation)
canola, CA (AB)	x		hr volunteers in conventional canola fields
canola, CA(SK)		x	volunteers (glyphosate, imidazolinone), eastern canada: b. rapa
canola, CA		x	non hr canola, and volunteers
canola, CA	x		
canola, CA		x	volunteer canola
soybean, AR			(no estimation)
soybean, AR		x	(difficult to estimate)
soybean, IA (USA)		x	
soybean, NB (USA)			(no estimation)

Question No: 13

How high do you estimate the risk of a selection of new resistances against particular herbicides (due to an overuse in agriculture)?				
crop	**glyph.**	**gluf.**	**imid.**	**remarks**
sugar beet; GE	low	low	high	unlikely to occur, beet is grown mostly in 1 of > 3 years
corn; GE	low	low		only if herbicide is overused
oilseed rape; GE	low	low		only if herbicide is overused
oilseed rape; F				difficult to estimate
canola, CA (AB)	low	low	high	problems will occur because of increased use of these hebicides on greater acreage
canola, CA (SK)	low	low	high	
canola, CA	low	low	high	
canola, CA	low	low	high	
canola, CA	high[9]	low	high	
soybean, AR	high	high	unclear	
soybean, AR	high	high		
soybean, IA (USA)	high	low	low	
soybean, NB (USA)				(no estimation)

[9] RR canola is treated in-crop, hence farmers spray large populations of summer annual weeds many of which have populations with high genetic diversity. This change in agriculture practice increases the risk in selecting for glyphosate resistant weed biotypes.

Question No.: 14

Will HR-crops and other new transgenic varieties change rotations or management methods in the long run (in about 5-10 years) due to new options for farmers?			
crop	**yes**	**no**	**details**
sugar beet; GE	x		additional crops in rotation, e.g. potatoes or others where weed control is difficult
corn; GE	x		many changes possible due to many possible new traits
oilseed rape; GE	x		many changes possible due to many possible new traits
oilseed rape; F	x		less crops (only those with good economic return)
canola, CA (AB)	x		additional crops in rotation, e.g. field peas and other pulse crops
canola, CA(SK)	x		(desease resistance; stress tolerance in cereals and oil seeds)
canola, CA		x	
canola, CA	x		
canola, CA	x		less crops in rotation; HR services the trend to simplify
soybean, AR	x		less crops in rotation
soybean, AR	x		new varieties in rotations (e.g. RR wheat; RR corn; IMI sunflower)
soybean, IA (USA)		x	
soybean, NB (USA)	x		Decrease in grain sorghum and winter wheat

Question No.: 15

Are HR varieties grown in areas, where this crop has not been grown before?			
Crop	**yes**	**no**	**details**
sugar beet; GE		x	might be if reduced costs allow for bioethanol production
corn; GE		x	
oilseed rape; GE		x	
oilseed rape; F			
canola, CA (AB)	x		cereals have been replaced/reduced
canola, CA (SK)	x		fallow acreage has been replaced/reduced by HR canola
canola, CA		x	
canola, CA	x		summer fallow and cereals have been replaced/reduced
canola, CA		x	
soybean, AR	x		hr soybean has replaced/reduced cotton, corn, sunflower, ochards, horticulture, cattle; also areas not farmed before
soybean, AR		x	
soybean, IA (USA)		x	
soybean, NB (USA)		x	

Genetics and pollination of corn, oilseed rape, and sugar beet

Corn

Corn is protandrous with pollen being shed before the silks of the female ear are receptive, but as there is some overlap, up to 5 % self-pollination can occur.

Even though most of the corn pollen (98%) remains within a 25m-50m radius of most of the corn fields according to Sears et al. (2002) there is a probability of cross pollination over longer distances.

Firstly, corn is primarily wind - but also insect pollinated. Studies by Emberlin et al. (1999) showed, that the majority of corn pollen transported by bees was carried as far as 2,4 km , some of it even up to 14,5 km. With most honeybee colonies regularly foraging up to 2000 m from the hive some pollen transfer and fertilisation up to 4000 m must be expected (Ramsay et al. 1999). Outcrossing could be detected up to 800m (Salamov 1940, cited in Bock et al. 2002).

Secondly, a field of corn may release enormous quantities of pollen (approximately 70 kg/acre or 175 kg/ha) over a period of up to 13 days. Moreover, Emberlin et al. (1999) concluded from their studies that wind (at speeds of about 2m/s) and convection can lead to transport distances of 1 km (in 4 minutes) up to 172 km (in one day). A wind speed of 10 m/s and turbulent conditions in the boundary layer could lead to 36 km (in 1 hour) or 864 km (in a day). Pollen can remain "viable" from 3 hours up to several days, cold temperature and high relative humidity extending the life span. Corn pollen remains capable of fertilization for 24 hours in most weather conditions prevailing in the UK.

Oilseed rape

Oilseed rape (*Brassica napus*), also called canola, is a member of the genus *Brassica*, family of *Brassicaceae*. Brassica is well adapted to cool and moist growing conditions. Oilseed rape is an annual or winter biennial species with considerable morphological variability. *Brassica napus* (2n = 38 chromosomes, genome AACC) is amphidiploid, probably resulting from spontaneous cross-hybridisation between field mustard (*Brassica rapa/B. campestris*), with chromosome number 2n = 20 and genome AA, and cabbage (*Brassica oleracea*) with 2n = 18 chromosomes and genome CC. At least four independent hybridisation events have been determined (Renard et al. 1993, Gerdemann-Knörck and Tegeder 1997, OECD 1997).

Although B. rapa and B. oleracea, the presumed parent species, are cross-pollinating, B. napus can be both self-pollinated and cross-pollinated. The average level of outcrossing in western Canada is about 20 % (Downey 1992, Hall et al. 2000). An average of 61% of flowers on male-sterile bait plants were pollinated at 100 m from the genetically modified pollen source (resulting in about 50% transgenic seed set) (Thompson et al. 1999).

Oilseed rape fields flower during a period of about a 3 – 4 weeks, which can be prolonged by low temperatures and rain.

Oilseed rape can be regarded as the current "worst case" transgenic crop plant (wooden plants can cross-pollinate at much larger distances) in respect to the question of cross pollination at long distances. It produces a huge quantity of small sized pollen and long range pollen transport and pollination can occur by wind and by insects.

Contamination of non-GM rape seed by transgenic varieties grown about 4 km apart has been reported (FOE 2000). As bees and bumble bees are important pollinators, distances of cross pollination have to be taken into account as described for corn (see above).

In Canada, where HR oilseed rape has been grown for many years now, hybridisation rates between fields are about 1% at field edges and 0,1-0,2% in 50m-400m within the crop (Beckie et al. 2001). Multiple HR resistant volunteers are common in HR production areas in Canada (see above). Timmons et al. (1996) found a rate of 1,2% at a distance of 1500m in hybrid varieties.

Bees from a hive placed 800 m from a transgenic oilseed rape field carried GM pollen in their largely non-GM *Brassica* pollen load.

Sugar beet

Sugar beet is wind (predominantly) and insect pollinated and is most often self-incompatible. Wind borne beet pollen can disperse over distances of up to 5 km, possibly even 8 km. Field experiments showed, that 0,8% of the hybrid progeny of weed beets in vicinity to a HR beet area were HR resistant (Vigouroux et al. 1999). They found a level of 10% hybrids in 3m distance and 1% in 15m distance from each other. Hybridisation between these forms was not at random.

Hybridisation of oilseed rape with related secies

Hybridisation with closely related species

B. napus can cross with a variety of *Brassica* species and wild relatives, interspecific crosses are more successful if an allopolyploid species is used as the female parent and if there is one genome in common with the male parent. Controlled and spontaneous reciprocal hybridisations of *B. napus*, genome AACC, with field mustard (*Brassica* rapa), genome AA, and brown mustard (*Brassica juncea*), genome AABB, are easily possible and result in up to 3,3 % hybrid plants (Renard et al. 1993, Gerdemann-Knörck and Tegeder 1997). Data and summaries of hybridisations (and techniques used) between *Brassica napus* and wild relatives can be found in OECD (1997), Gerdemann-Knörck and Tegeder (1997), Scheffler and Dale (1994), Renard et al. (1993) and at: http://www.environment.detr.gov.uk/acre/pgs/index.htm.

Brassica rapa/B. campestris

Considerable attention has been given to introgression of genes from transgenic *B. napus* to field mustard, bird rape, or wild turnip (*Brassica rapa/B. campestris*) since hybrids (also named *B. x harmsiana*) with this obligate outcrossing parental species have been found in natural populations and field mustard/turnip mustard is sometimes grown as a crop but also behaves like a weed. Various morphotypes are economically important weeds in many countries and are often seen in oilseed rape fields.

Frequencies of hybridisations between *B. napus* and *B. rapa* have been reported from field experiments and survey of natural populations of the wild species, ranging from 0 – 69 % of the seeds. In general, *B. rapa* produces more hybrids than oilseed rape (Jørgensen 1999). Both types of hybrids between oilseed rape and field rape show hardly any dormancy. However, dormancy can be restored in seeds from the first backcross with the weedy *B. rapa*, which, just like the other weedy species known to hybridise with oilseed rape, expresses the dormancy trait. In agro-ecosystems with efficient weed control, seed dormancy allows the seed to ensure optimum germination conditions and hence will be selected as a beneficial trait with a positive effect on survival.

Hybrids between *B. napus* and B. *rapa* seem to be less fit under conditions that are similar to cultivation. They produce fewer seeds than *B. napus*. Combining fitness components such as survival and seed production, hybrids are intermediate to *B.rapa* and *B. napus.* Offspring from backcrosses and F2 matings had a reduced fitness relative to offspring from matings of the pure species (Jørgensen 1999, Hauser et al. 1998a +b). Seedling vigour and fitness can be regained in the following generations by backcrossing to either parental species (Hauser et al. 1998a + b). Since there is a large variation between lines (Darmency 2000), some individual hybrid plants can be as fit as parental lines. Therefore, low fitness in F1 and possibly subsequent generations will not completely prevent introgression from *B. napus* to *B. rapa.* There seem to be no general fitness costs associated with transgenic glufosinate-tolerance when introgressed from *B. napus* into *B. rapa* (Snow and Jørgensen 1999).

In field experiments, spontaneously produced interspecific transgenic hybrids gave rise to fertile offspring if backcrossed with *B. rapa* (Mikkelsen et al. 1996). Such transgenic, glufosinate-resistant weed-like plants with crop-mustard morphology and chromosome number are produced in the first backcross generation when transgenic, herbicide-resistant interspecific hybrids are grown in the vicinity of mustard.

The likelihood of introgression of transgenes might be dependent on whether the gene is integrated into the A or the C genome of *B. napus* (Metz et al. 1997). It should be possible to find out whether the transgene is in the A or C genome with high resolution linkage maps of oil seed rape (Parkin et al. 1995). But even if inserted in the C genome, a herbicide-resistance transgene may still escape from oilseed rape (Jørgensen 1999). Using a population genetic model, Tomuik et al. (2000) questioned that integration of a transgene into the C genome of *Brassica napus* would reduce introgression into *B. rapa* genomes because experimental results did not indicate any specific chromosomes to be safer candidates for an integration of transgenes.

Brassica juncea

Hybridzation between oilseed rape and brown mustard or Chinese mustard (*Brassica juncea*) has also been reported both under co-cultivation in the field (Downey 1992) and spontaneously. Chinese mustard is a rarely grown crop plant. *B. juncea* female plants produce more hybrids than *B. napus* plants. Depending on proportions of the parental species up to 3 % of offspring harvested on *B. juncea* plants were hybrids, but their pollen fertility was mostly rather low (Jørgensen et al. 1998, Jørgensen 1999).

Hybridisation with weedy relatives

Raphanus raphanistrum (wild radish)

Interspecific hybrids between glufosinate-resistant *B. napus* plants cross-pollinated by *Raphanus raphanistrum* (2n = 18, *genome RrRr*) can be found under optimal conditions (male-sterile oilseed rape and the same ratio of crop and weed). Much lower frequencies are found under agronomic conditions primarily depending on the female cultivar (Chèvre et al. 1997, 1999, 2000, Rieger et al 1999). Interspecific hybrids exhibit very poor female fertility. Nevertheless, after successive backcross generations the fertility can increase to almost the level found in wild radish. Under agricultural conditions, when wild radish is the female parent, the rapid transfer of herbicide-resistance genes into this wild species may be a rare event but cannot be ruled out, particularly if bridging is involved and should be taken seriously (Dietz-Pfeilstetter et al. 1999).Gene flow between oilseed rape and wild radish occurs in both directions under field conditions, as simulated by Rieger et al. (1999).

Hirschfeldia incana, syn. Brassica adpressa

Hoary mustard (*Hirschfeldia incana*) is a rare weed in Europe. Spontaneous hybrids can be formed between oilseed rape and hoary mustard, however, this will rarely occur due to the different flowering periods (Dietz-Pfeilstetter et al. 1999). Interspecific hybrids show low reproductive fitness and intermediate seed dormancy, but they are more competitive than their weed parent. Their morphology is very close to oilseed rape. This could explain why hybrids were seldomly identified in the past (Chèvre et al. 1999, Darmency and Fleury 2000). Survival of buried hybrid seeds was lower than survival of *H. incana* seed but higher than that of *B. napus* seed (Darmency and Renard 1992).

Sinapis arvensis

Believed to be native to the Old World, wild mustard (*Sinapis arvensis*) is now widely introduced and naturalized in temperate regions around the world. It is an important and common weed in Europe and also in North America (Moyes et al. 2000, Warwick et al. 2000). Hybridisation between oilseed rape and wild mustard was shown to occur at very low rates only under experimental conditions (summary of data in Warwick et al. 2000). Brown et al. (2000) and Downey (1992) obtained no hybrids repectiveley no fertile hybrids from the crosses between *B. napus* and wild mustard.

Since reciprocal crosses between *B. rapa* (2n = 20, genome AA) and *S. arvensis* (2n = 18, genome SS) produced mature seeds, such offspring, most likely being an allotetraploid with 2n = 38 chromosomes and genome AASS, might act as a bridging species for gene transfer. Possessing the same chromosome number as *B. napus* (2n = 38, genome AACC) and the common genome A, such a hybrid could act as a bridging species with oilseed rape. It could thus add to the risk of gene flow of herbicide-resistance transgenes into weedy species (Brown et al. 2000). Bridging may also occur via an intermediate male sterile hybrid that is cross-pollinated.

Brassica nigra

Hybridisation between *B. napus* and black mustard (*Brassica nigra*) seems possible, but hybrids produced only a few seeds on backcrossing with *B. napus* (Downey 1992). Brown et al. (2000) could not detect hybrids from crosses between oilseed rape and black mustard.

Erucastrum gallicum

In western Canada, the *Brassica* weed dog mustard (*Erucastrum gallicum*), is abundant. Experiments (pollination by hand) indicate that gene flow from oilseed rape and field mustard (cultivars of which are grown in some areas of Canada) into dog mustard may be possible (Downey 1999). Backcrosses of these hybrids with dog mustard should be taken seriously (Dietz-Pfeilstetter et al. 1999).