Management of transgenic crops within the cropping system

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ABSTRACT

With the development of Genetically Modified Organisms (GMO) in agriculture, new concerns about crop management have been addressed. Apart from the evaluation carried out with the regulation process before marketing, observations under current agricultural practices are required in order to build suitable agronomic management and design a monitoring system. A multi-year and multi-crop monitoring study has been carrying out in France since 1995 and preliminary results suggest that a more integrated crop management should be required.

INTRODUCTION

After about 15 years of transgenic research carried out by public research teams as well as private companies, the first marketing releases occurred in North America in 1995, while in Europe the first applications are still under discussion. Tobacco resistant to bromoxynil, imports of glyphosate resistant soybean, insect resistant corn through the Bt strategy and some herbicide tolerant rapeseed sum up the current European status for GMO marketing. Several other applications for marketing clearance have been submitted.

Corn, sugar beet and rapeseed are the main crops for which genetic modification has been applied. While several traits have been introduced (oil quality, disease and insect resistance) and are under development, herbicide resistance has been developed extensively and three systems are near marketing : glyphosate and glufosinate resistance for the three crops and bromoxynil resistance for rapeseed.

Development of transgenic plants raises several questions, most of them are not specific to recombinant DNA techniques : ethical concerns, relationship between science, society and organization of collective expertise, marketing of transgenic plants with new rules, protection of biotechnology and patent policy, food and feed safety of these novel plants, environmental and agronomic concerns. With respect to these last concerns, the evaluation has to be performed on a case-by-case basis. The risk assessment of gene flow must take into account the specific trait introduced (e.g. herbicide resistance vs oil quality), the biology of the plant (open vs self pollination, seed dormancy) and the agricultural context (cropping systems, spatial organization of the crops, agricultural practices, ...).

Herbicide resistance is not only one of the first traits for which marketing clearance has been applied but it is also an adequate model to carry out the risk assessment of crop management of transgenic plants. In this paper the main criteria for consideration in herbicide tolerant crops and the effect of their use in cropping systems will be reviewed. Rapeseed and sugar beet provide a good example of the principles involved (Richard-Molard & Gestat de Garambe, 1998).

RISK ASSESSMENT

For several years, the main question with respect to modified rapeseed and sugar beet was : will the transgene be disseminated outside the field and be transferred to other plants and, especially, to weeds ? From many studies carried out by different scientific teams, it can be concluded that transgenes will disseminate and can lead to outcrossing with weeds. Although interspecific crosses between rapeseed and related wild species lead to less fertile plants, they can produce a small quantity of seeds (Kerlan *et al.*, 1992).

As we know that transgenes will disseminate, the question is now : So what ? Could the consequences of such a dissemination be managed ? With respect to long-term effects, no experiments are available for assessing the transgene behaviour. In order to estimate gene flow, simulations using genetic models are performed. These models generally represent the gene transfer from a field towards the wild species located at field edges and take into account various parameters such as the gene migration rate, its dominance level or the competitivity of the hybrid. Long-term behaviour appears to be difficult to predict as the model is highly dependent on specific events. It is thus necessary to take into account the spatial and temporal variability.

On the other hand, we can look for markers already introduced into rapeseed in the past and to survey their behaviour in the non-cultivated areas. Such a survey is being performed in various regions of France, where we are intending to detect the introgression of traits like "low-erucic" in wild species.

Gene flow

In the case of rapeseed, gene flow can occur through two different ways :

* the pollen, either towards rapeseed plants (intraspecific crosses) or towards wild relatives which are quite numerous (interspecific crosses) ;
* the seeds, through volunteers in subsequent crops or seed dissemination during transportation.

The long-term effect of such phenomena on farmers' crop management of transgenic plants and the design of adequate agricultural practices are assessed by carrying out several types of studies :

* Modeling the gene flow. Models of gene flow between two adjacent fields have been designed (Reboud, 1992 ; Lavigne *et al.*, 1994) and are being improved by taking into account crop rotations, spatial patterns of crops and agricultural practices.

* Specific studies about outcrossing have been performed in order to estimate pollination distances and interspecific crosses (Jorgensen and Andersen, 1996 ; Kerlan *et al.*, 1992 ; Eber *et al.*, 1994 ; Baranger *et al.*, 1995). Spontaneous hybridization of rape with wild mustard, wild hoary mustard and wild radish has been demonstrated to occur when using a male-sterile oilseed rape cultivar as the pollen recipient, i.e. without pollen competition (Chèvre *et al.*, 1996). Lefol *et al.* (1996b) have showed that the reciprocal cross can occur in the field with hoary mustard, but it was not been observed with wild mustard to date (Lefol *et al.*, 1996a).

Recently, and for the first time, the possibility of producing interspecific hybrids in the field between oilseed rape and wild radish as the seed parent have been reported (Darmency *et al.*, 1998). Two hybrids were obtained from 59 wild radish plants grown at low density in the field, but none when wild radish was grown at high density. The germination rate of these hybrids is low and their fitness is reduced.

Other studies have been performed in North America and first large scale releases already took place there. However, climatic and agricultural conditions are quite different in Europe : shorter rotations (every two years in some European regions), winter sown type rather than spring sown types, different kinds of wild relatives. Thus, it appears to be rather difficult to extrapolate data from North America for assessing the gene flow and agronomic impact of herbicide tolerant crops.

Sugar beet is a biannual crop, but some bolter beets can produce seeds from the first year. They are hybrids between crop and wild forms, which have been observing in the seed production area for 25 years (Desplanque *et al.*, 1996). These weed beets are controlled in the current cropping systems by respecting recommendation about lifting bolters. The development of non-selective herbicide tolerant varieties may induce the appearance of a tolerant weed flora, which can develop from bolter beet and volunteer beet.

For the maize, there is no possibility of outcrossing with wild relatives in Europe. But the transgene escape remains possible by pollination between varieties.

A multi-crop and multi-year monitoring study

In order to assess the effect of such outcrossing under agricultural conditions, in 1995 the French technical institutes CETIOM, AGPM, ITB and ITCF, designed and implemented a monitoring study for various transgenic crops on three platforms located in different regions of France : Champagne, Burgundy and Midi-Pyrénées (South-West). Each platform consists of a 6 ha field where transgenic corn, rapeseed and sugar beet are cropped with the usual local cropping system (see Figure 1). The transgenic traits are as follows :

* glufosinate and glyphosate resistance in corn, rapeseed and sugar beet ;

* bromoxynil resistance in rapeseed and corn borer tolerance (using the Bt system) in corn.

A 500 meter area around the field was defined and monitored in order to assess the spatial impact of transgenic crops.

This multi-year experiment aimed mainly at :

* assessing the impact of transgenic crops when cultivated together in the same field area ;

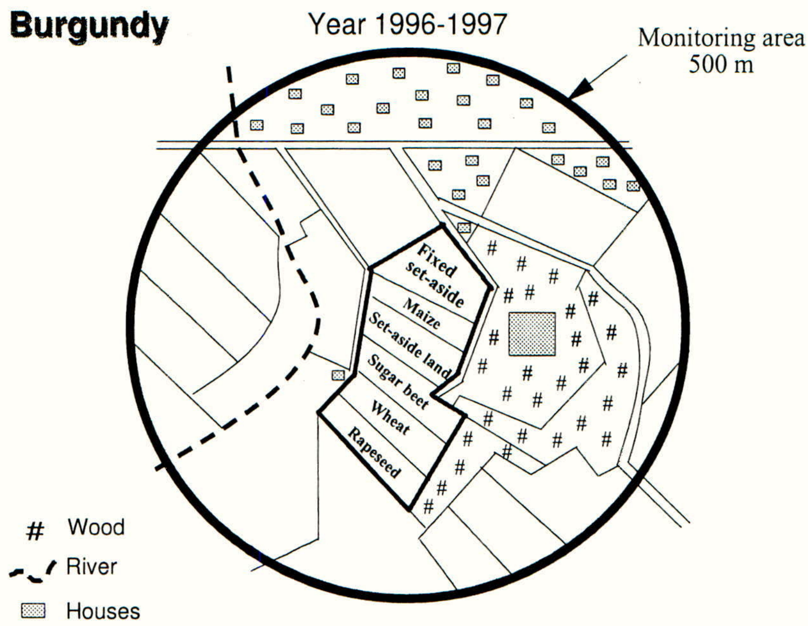
* designing the weed control of volunteers in subsequent crops which are resistant to the same herbicide (e.g. glyphosate-resistant rapeseed volunteers in the subsequent sugar beet resistant to glyphosate) ;

* evaluating the multiple resistance rate when cropping two adjacent rapeseed fields with two different herbicide resistances ;

* estimating the interspecific outcrossing towards the wild relatives under real and local conditions and

* estimating the cost-benefit of herbicide resistance technology with respect to conventional techniques.

Figure 1. Example of a cropping system in Burgundy (1996-97) using transgenic traits.



Example of cropping system, Burgundy

Year 1995-1996	**Set-aside land**	**Rapeseed**	**Wheat**	**Sugar-beet**	**Maize**	**Fixed set-aside land** (trefoil)
Year 1996-1997	**Rapeseed**	**Wheat**	**Sugar-beet**	**Wheat**	**Maize**	**Fixed set-aside land** (trefoil)
Year 1997-1998	**Set-aside land**	**Sugar-beet**	**Set-aside land**	**Rapeseed**	**Maize**	**Fixed set-aside land** (trefoil)

Table 1. Identification of wild relatives within the monitoring area from 1996 to 1998.

Location		Weed species and number of samples	
		Rapeseed plot	Other crops and survey zone
Midi-Pyrénées		*Sinapis arvensis - 1* *Rapistrum rugosum - 77* *Brassica nigra - 5* *Rapessed volunteers - 13*	*Sinapis arvensis - 6* *Rapistrum rugosum - 1* *Brassica nigra - 77* *Sinapis alba - 51*
	Total	96 samples	135 samples
Burgundy		*Sinapis arvensis - 54*	*Sinapis arvensis - 58* *Rapeseed volunteers - 14* *Arabidopsis thaliana - 1* *Capsella bursa pastoris - 13* *Sisymbrium officinale - 1* *Thlaspi arvense - 1* *Barbarea intermedia - 1* *Alliara petiolata - 18*
	Total	54 samples	107 samples
Champagne-Ardennes		*Sisymbrium officinale - 20* *Capsella bursa pastoris - 15* *Calepina irregularis - 9* *Rapeseed voulunteers - 38* *Sinapis arvensis - 6*	*Sinapis arvensis - 28* *Sinapis alba - 4* *Raphanus raphanistrum - 1* *Capsella bursa pastoris - 13* *Rapeseed volunteers - 22* *Sisymbrium officinale - 2* *Calepina irregularis - 7* *Thlaspi arvense - 4* *Cardamine hirsuta - 4* *Alliara petiolata - 20*
	Total	88 samples	105 samples
Total		238 samples	347 samples

Outcrossing with wild relatives

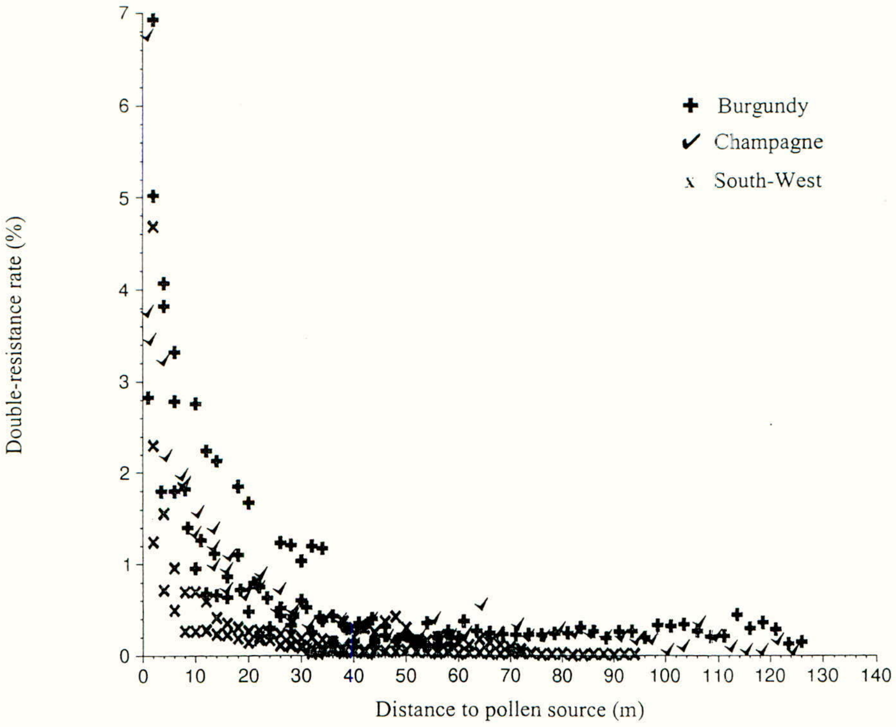
Within the monitoring area, each wild relative plant of rapeseed was located and surveyed until seed maturity. The flowering period was observed and compared with the flowering periods of the transgenic rapeseed crops. Seeds were sampled for assessing the herbicide resistance which was checked by spraying herbicides after re-sowing. Table 1 gives the occurrence of wild relatives observed during three years of the study (1996-1998) : a plot represents one or several plant(-s) located at the same place.

RESULTS

Preliminary results indicated that no herbicide resistance with wild mustard and other mustard species occurred during the first two years. Unfortunately, wild radish was not present in our situations and a specific location site has been implemented in 1998 in the South-West region. frequency of outcrossing will continue to be studied in the subsequent years and will allow us to increase the precision of this frequency.

There was some weed beets in our situations. The frequency of outcrossing with the sugar beet varieties can be estimated from 0.07 to 0.2%. The distribution of resistant seeds let believe that insects are involved in the pollination. This hypothesis will be tested in the further years.

Figure 2. Average dispersal curves for the three location sites



Multiple resistance and dispersal of pollen

The three herbicide resistant rapeseed varieties were cropped in adjacent fields and double resistant plants were detected in two different ways :

* by applying the herbicides on volunteers whose emergence occurred after harvesting ;
* by sampling seeds and re-sowing using a specific design of experiments and direct application.

Both methods gave similar results with respect to the rate of double resistance. Although the results were dependent upon the variety, the average rate of double resistance can be estimated under our specific conditions : about 2 % at a one meter distance, 0.2 % at 20 meters and less than 0.01 % at 65 meters. (Figure 2)

The pollen dispersal of sugar beet outside the field was assayed by introducing male-sterile plants at various distances from the field, to biologically trap pollen. At maturity, seed production was recorded, seeds collected and tested for resistance by screening a portion of the sampled seed with herbicide. The analysis of seed production of the male-sterile plants showed a rapid decrease of the pollen flow with the distance (- 85% for 30 meters).

Asynchrony of flowering between the two varieties of maize limited the study of gene flow. The first results showed that the seed production on emasculated plants at 150 m from the field dropped to about 10%.

CONCLUSIONS

Although further data are still required, these results seem to indicate that multiple resistance should probably be the major concern for farmers rather than interspecific crosses. Suitable agronomic practices should be proposed in order to provide a sustainable use for transgenic crops such as herbicide tolerant oilseed rape.

Preliminary results obtained during the first years of our project confirmed what was expected from previous studies. Thus results have been obtained under current farmer practices and provided data which will be used to fit simulation models for gene flow. While further data will be obtained for three more years, adequate crop management practices can already be discussed and recommendations provided for public decision-making.

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Assessing the impact and consequences of the release and commercialisation of genetically modified crops

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ABSTRACT

Genetically modified (GM) crops will have impacts on agriculture and both the agricultural and natural environment. Analysing the consequences for the agricultural environment requires study of the characteristics of the GM crop and its hybridising relatives, and study of the management systems involved in growing the GM crop and other crops grown in rotation with it. GM crops may also have impacts on uncultivated land and natural environments. Thus risk assessments are concentrating on whether the genetically modified characteristics of a GM crop are likely to change the behaviour of the plants in their environments to the extent that ecological balances are altered. This paper discusses approaches to risk assesments and reviews results of risk assessments of GM Brassica crops.

INTRODUCION

Genetically modified (GM) crops have potential benefits for growers, processors and consumers. However many will have impacts on agriculture and both the agricultural and natural environment while others will have food quality and safety implications.

Pest, disease and herbicide resistant crops will require different (often reduced) pesticide and herbicide inputs in order to exploit these novel traits. These modified management systems will themselves have an impact on agricultural environments. Thus analysing the consequences for the agricultural environment requires study of the characteristics of the GM crop and its hybridising relatives, and study of the management systems involved in growing the GM crop and other crops grown in rotation with it. For example GM herbicide tolerant (HT) crops will be treated with different herbicides, with different activity spectra, at different crop development growth stages. Thus the effects on botanical diversity in the GMHT crop and in subsequent crops will be a product of the GM crop and the herbicide treatment.

GM crops may also have impacts on uncultivated land and natural environments. These environments may be affected by characteristics of crop and wild species induced by novel genetic constructs and their products. Thus risk assessments concentrate on whether the genetically modified characteristics of a GM crop, and of similarly modified hybridising relatives, are likely to change the behaviour of the plants in their environments to the extent that ecological balances are altered. Risk assessments study both the severity and extent of the hazard or damage as well as the likelihood and frequency at which the damage will occur. Thus the definition:

Risk (Impact) = Frequency x Hazard

GENE FLOW MEASUREMENTS

The impact of transgenes on particular wild species will depend on whether genes will introgress into wild populations and the rate of that introgression. Introgression of a transgene is a product of cross pollination with transgenic relatives, survival of the hybrid and its hybridisation with the wild population to the extent that the gene becomes established in a proportion of the population. If there is sexual incompatability between species the assumption is that no gene transfer will take place and thus the risk is zero. However, in experiments, certain wild Brassica and other crucifer species previously considered incompatible with *B. napus* have shown some ability to hybridise (Scheffler and Dale, 1994). Thus there is the possibility that hybridisation can occur, albeit at very low frequencies, and that transgenes have a route to introgression. Studies to determine the frequency of these interspecific hybrisations are described in other papers in this conference report. However information is also required on whether the hybrids can survive and back cross with their wild parental species so that the gene can become established in the wild population. Studies by Scott and Wilkinson (1998) of *B. rapa* (*B. campestris*) populations growing outside fields of *B. napus* in England indicated that cross pollination frequencies were low (0.4-1.5%) in 7% of *B.rapa* populations surveyed. The remaining 93% of populations contained no hybrid seed. In addition they found that on average less than 2% of all seedlings survive, so that unless the trangene conferred higher survival characteristics, establishment of GM *rapa* x *napus* hybrids would be very poor and introgression of the gene into rapa populations would be very slow. Hybridisation frequencies appear to be much higher where *B. rapa* occurs as a weed in *B. napus* crops (Jorgensen *et al* 1998 and Sweet and Norris, unpublished data). However in the UK there is no indication that *B. rapa* has modified its behaviour or is spreading as a weed in *B. napus* crops as a consequence of hybridisation. The relationship between weedy and wild *B. rapa* is yet to be determined but may be a significant route for introgression of a transgene from oilseed rape.

Chevre *et al* (1998) have shown that hybridisation and backcrossing with *Raphanus* and *Sinapis* can occur at low frequencies under particular conditions. Studies are underway in the UK to determine whether oilseed rape genes can be detected in *Sinapis* spp. (Dale and Moyes, pers comm.) However little is known of the ability and frequency of hybridisation of weedy crucifers eg. *Sinapis* and *Raphanus* with *B. napus* under natural conditions and the survival and reproductive characteristics of the interspecific hybrids. Thus we appear to be far from determining whether natural hybridisation frequencies are zero or close to zero for many related cruciferous spp.

Hybridisation between weedy crucifers and oilseed rape would tend to lead to introgression of advantageous adaptive characters into the weedy populations such as increased waxyness and reduced leaf hairs associated with tolerance to the herbicides currently used on oilseed rape. There are indications that this may have occured in weedy *B. rapa* populations occurring in oilseed rape crops in Denmark (Jorgensen *et al* 1998) and in England (Sweet and Norris, unpublished data). However no morphological modifications associated with enhanced herbicide tolerance has been reported in populations of other weedy crucifers. Some *Sinapis* and *Raphanus* populations appear to have partial tolerance to some oilseed rape herbicides, though whether this is due to gene exhange, evolution or selection from an inherent natural variability is unknown.

There is also a need to study indirect gene flow through intermediary species which are compatible with both oilseed rape and with other wild crucifers. Thus we need to determine whether transgenes are able to introgress from *B. napus* into *B. rapa* and in hence to *Sinapis* or *Raphanus* spp.

There have been and continue to be numerous studies of transgene flow through seed dispersal, pollination and hybridisation within the same species. These are gradually being scaled up to levels that reflect field scale releases and the results confirm those found with non-GM pollen. There are less reports of studies of the persistance and survival of introgressed genes in volunteers. Sweet *et al* (1998), and Simpson *et al*, in this report, studied volunteer populations of winter and spring oilseed rape occuring at release sites in subsequent crops. There are also reports on volunteer GM rape from France (Messean, 1997) but none of the studies have reported increased numbers or fitness of herbicide tolerant volunteers.

Impact of plant species

Many studies have concentrated on measuring frequency phenomena such as gene flow and inter-specific hybridisation without necessarily considering the impact of the transgene when it has dispersed or introgressed into other populations or species. Frequency and hazard are dependant on the characteristics of both the crop that is modified and of the GM trait and so risk assessments require measurement and study of the hazard or impact of both the crop/plant and the trait. In addition the impact of the release of the GM plant will depend on the type and location of the environment into which it is being released. This means that risk assessments are not necessarily transferable from one site, area, region or country to another.

Plants vary in the degree to which they dominate or are invasive of certain environments and in their ability to disperse genes to different populations and species. Thus they will have different environmental impacts when genetically modified and, for any particular country or region, plants can be classified as being high, medium and low impact.

High Impact plants

Plants in this group are hardy, perennial, competitive, open pollinating and prolific, have a wide range of relatives with which they hybridise and an ability to colonise a range of natural and semi natural habitats. Examples of such plants are perennial grasses (eg *Lolium perenne* - perennial ryegrass) and certain indigenous and introduced trees and shrubs which form a significant proportion of forests and woodlands (eg poplar -*Populus* spp.) Modifications of these plants which affect their competitiveness and behaviour could have significant impacts on the ecology of a range of environments.

Medium Impact plants

Plants in this group are open pollinating, hybridise with some wild relatives, prolific and colonise a limited range of habitats. Examples of such plants are oilseed rape, oats, sugar beet and rice all of which have closely related wild relatives with which they hybridise and an ability to colonise disturbed ground. These plants and their close relatives rarely form climax populations except in particular environments such as coastal areas or in disturbed ground.

Low Impact plants

These are usually annual or biennial species, largely self pollinating with few hybridising relatives that are poorly adapted to the area in which they are cultivated. In the UK examples are maize and sunflower.

It is important to appreciate that the impact of plant species will depend upon the environment into which they are being released. Maize and potato are considered low impact plants in England. However in countries of Central and South America where their centres of genetic diversity occur, along with many wild relatives, their impact would be considered very high.

Impact of Transgenes

Transgenes, operating through their expression in plants, will have different impacts on environments. Since genes often operate uniquely it is not easy to classify transgenes as having high or low impact. In addition their impact is also dependant upon the nature of the receiving environment.

High Impact Transgenes

Generally genetic modifications which improve the fitness of GM plants by increasing their reproduction, competitiveness, invasiveness and/or persistance will have the greatest environmental impact. Thus transformations which significantly increase plant productivity and overcome constraints and stresses such as pests, diseases, drought etc will have the highest impact. Thus very high yielding and vigorous GM plants with enhanced and broad spectrum pest, disease and stress tolerance will have the greatest impact.

Many pest and disease resistance genes will have effects on non-target species either directly through gene products which destroy or debilitate non-targets or indirectly by altering relationships between pests and beneficials. It is important that these non-target effects are thoroughly understood before commercialisation progresses.

Low Impact Transgenes

These are genes that do not noticeably enhance the fitness of the modified plant or of other organisms so that they have minimum ecological impact. Examples would be herbicide tolerance and genes that modify seed composition eg high lauric acid genes in oilseed rape, high starch genes in potato. However it is important to verify that these genes do not significantly increase seed tuber overwinter survival through enhanced frost resistance, or dormancy characteristics of oilseed rape seed so that it has enhanced soil survival characteristics.

AGRICULTURAL IMPACT

Genetic modification can have a range of impacts on agricultural systems and hence will require specific management. Genetic modification can alter the nature of crop volunteers in subsequent crops and the GM trait can disperse to other crops and weeds through cross pollination and seed dispersal. Low impact genes such as herbicide tolerance, which have little impact on natural environments, become highly significant in the agricultural environment because of the changes in herbicides required for their management. These herbicides will differ in the effect they have on plant and other species diversity in cropped fields. These aspects are now the subject of several research projects at NIAB and other European institutes.

Deployment of high impact genes such as pest and disease resistance will result in reductions and changes in pesticide usage and thus offer opportunities to enhance diversity in cropped fields, especially if the trangene products are very specific to selected pest species. However it is important that the selection pressures they impose on pests and diseases do not encourage the development of virulent races of pests and pathogens which are resistant to the genes and require additional pesticide treatments..

Impacts, Consequences and Monitoring

There are considerable concerns in the UK and Europe about the environmental effects of releases and the indirect effects through agriculture of the commercialisation of GM crops. Risk assessments conducted for regulatory purposes tend to concentrate on the direct effects of the GM crop and its relatives on the natural environment. However it is now becoming apparent that the agricultural consequences of the deployment and management of GM crops could also have significant impacts on the environment in regions where a very high proportion of the total land area is managed by man. Plans are being developed for monitoring the early years of the commercialisation of each GM crop so that its impact on both agriculture and the environment can be evaluated in farm scale releases.

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