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Evaluating the Ecological Impacts of Cultivating Genetically Modified Herbicide-Tolerant (GMHT) Oilseed Rape and Maize

STRIVE
Environmental Protection Agency Programme
2007-2013
Environmental Protection Agency

The Environmental Protection Agency (EPA) is a statutory body responsible for protecting the environment in Ireland. We regulate and police activities that might otherwise cause pollution. We ensure there is solid information on environmental trends so that necessary actions are taken. Our priorities are protecting the Irish environment and ensuring that development is sustainable.

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- Office of Environmental Assessment
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EPA STRIVE Programme 2007–2013

Evaluating the Ecological Impacts of Cultivating Genetically Modified Herbicide-Tolerant (GMHT) Oilseed Rape and Maize

(2007-B-DS-1-S1)

STRIVE Report

Prepared for the Environmental Protection Agency

by

Teagasc

Authors:
Ewen Mullins and Marcus J. Collier
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The EPA STRIVE Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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Details of Project Partners

Dr Ewen Mullins
Teagasc
Crops Research Centre
Oak Park
Carlow
Ireland
Tel.: +353 59 9170298
Email: ewen.mullins@teagasc.ie

Dr Marcus J. Collier
Teagasc
Crops Research Centre
Oak Park
Carlow
Ireland
Tel.: +353 59 9170259
Email: marcus.collier@teagasc.ie
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Executive Summary

With the introduction of genetically modified (GM) crops there has been concern about their potential impact, particularly their possible ecological impact. This report follows from earlier research that examined this issue in detail and investigates the potential biodiversity impact of two GM crops that are most likely to suit Irish agronomic conditions. These are glyphosate or glufosinate herbicide-tolerant (HT) maize and oilseed rape. The research reported here is an extensive analysis of the scientific literature on the subject and was carried out between August 2009 and July 2010. There are several key conclusions from this analysis.

There are many 'wild' species related to oilseed rape in Ireland, none of which are native and many of which are highly unlikely to survive when crossed with the current oilseed rape crop plant (Brassica napus). The only exception is wild turnip (Brassica rapa), which is an earlier oilseed crop now no longer farmed but anecdotally present in marginal habitats. Worldwide, there have been ongoing and intensive surveys of the potential for GMHT B. napus to transfer herbicide tolerance to B. rapa. While it has been shown that this will indeed occur, the primary issue remains the consequence of this gene flow event: that is, what will happen the resulting offspring? Critically, in the absence of a selection pressure (spraying with the herbicide for which they have a tolerance), these GM hybrid individuals have no physical advantage over their non-GM neighbours. As they also contain a significant portion of a crop genome, they will not have the competitive ability that weed populations possess and will therefore not survive over time. In Ireland, marginal habitats are not routinely sprayed so it can be concluded that GM hybrids with a HT trait will not proliferate and spread. Separately, there is no likelihood of maize impacting on wild relatives as none exist in Ireland.

In real-world conditions, there are some scenarios where accidental spraying may occur and where management arrangements may give rise to an opportunity for a GMHT plant to prevail in the landscape. This was examined and presented in a series of five hypothetical scenarios. It was shown that there are no credible scenarios where a GMHT crop can persist or prevail over time any more than a non-GM crop outside of the confines of a managed field environment. Furthermore, it is also shown that it is in the management of the GM or non-GM crop that the potential for biodiversity impact is at its greatest.

Glyphosate and glufosinate toxicity was examined in detail and it was concluded that these two compounds have significantly less toxicity than those compounds currently in use across conventional systems. Using a recently developed index of biodiversity impact (CINMa1), the two GMHT crops were subjected to an analysis of their potential for impact. It was shown that in the management of GMHT maize there is the potential for benefiting landscape biodiversity. The same may be said for oilseed rape management, but there is some likelihood for transfer of genetic material to a wild relative. The potential impact of this is minimal and there is a net beneficial impact as with maize.

1. The index examines four key biodiversity stressors – Chemicals, Introgression, Nutrients, and Management – and grades their potential impact on four agri-environmental zones – the in-field area, nearby semi-natural habitats, the soil column and nearby watercourses.
1 Introduction

1.1 Background

The management of the Irish landscape is in continual flux and future land-use patterns are unknown (Ewert et al., 2005; Rounsevell et al., 2006; Levidow and Boschert, 2008; Angus et al., 2009; Burgess and Morris, 2009). This gives rise to concern over the future impact of agricultural activities on the environment, especially on landscape biodiversity. One issue relates to the potential impact on the Irish agri-environment of cultivating genetically modified (GM) crops suited to the Irish tillage sector. Since the lifting of the European Union (EU) moratorium on growing GM crops, Ireland has not adopted GM cropping regimes. However, as new data and new crops become available and as the technology expands to meet global consumption necessities, it is pragmatic to assume that Irish farmers will soon be afforded the choice of certain GM varieties tailored to Irish agri-environmental conditions (O’Brien and Mullins, 2009).

Worldwide, there are relatively few GM traits currently in crop production and of these insect resistance (Bt) and herbicide tolerance (HT) predominate. Globally, GM crop hectarage has been increasing annually since their first introduction (James, 2008) and while there are several new traits ‘in the pipeline’ (Lheureux et al., 2003; Stein and Rodriguez-Cerezo, 2009) HT is a trait that would be most applicable to Irish tillage systems, with particular relevance to oilseed rape (Brassica napus) and maize (Zea mays) (O’Brien and Mullins, 2009). However, issues have been raised in regards to the potential ecological impact of GMHT crops within the agri-environment and across the wider landscape. This project addresses these issues and follows on from an earlier Environmental Research Technological Development and Innovation (ERTDI) report to the Environmental Protection Agency (EPA) where it was shown that in order to ascertain the potential impact of HT crops on the biodiversity of the Irish landscape it is necessary to examine all aspects of the production of the crop. Alterations and innovations in crop management schemes that are associated with the management of GM crops have the potential for reducing the impact on the wider landscape (Mullins et al., 2009).

Prior to examining this issue further, it is necessary to establish a basis for the research. When looking at the potential impact of GM plants on the Irish flora, for example, it is necessary to outline the current state of knowledge. For many years, multiple surveys of the Irish flora have been carried out (Mitchell, 2000), but a high proportion are localised and place specific, and mainly show presence/absence data only. Many of these data are informed by earlier surveys and these may be subjective as well as unverifiable (Webb, 1986)\(^1\). With the advent of modern concerns for endangered or threatened plants, these early surveys part-led to the creation of the Flora Protection Order in 1987 (SI No. 274), later amended in 1999 (SI No. 94)\(^2\). However, these are not necessarily reliable accounts of species abundance or of the modern distribution of feral crops that would facilitate deductions to be made on the potential national impact of a novel trait or GM crop. Modern surveys of the flora of the Irish landscape are also informed by earlier research and, of the plants that have been identified as noxious weeds or invasive\(^3\), none are feral crops. As the use of the terms ‘invasive’ and ‘weeds’ may give rise to anxiety over biodiversity loss, it is first necessary to clarify the terminology that is used in this report.

1.1.1 Invasiveness

From an ecological point of view, invasive species worldwide are deemed to be a significant threat to biodiversity (CBD, 1992). Indeed, invasive species are considered to be the second greatest threat to biodiversity after habitat destruction (Conference of the Parties: 6, 2002; Anonymous, 2009), but this use of

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3. These include deliberate introductions such as rhododendron (Rhododendron ponticum), giant rhubarb (Gunnera tinctoria) and Japanese knotweed (Fallopia japonica).
‘invasiveness’ and ‘invasive species’ is intended to convey an impact that gives rise to a significant reduction in biodiversity at a variety of scales and time frames. The assignment of biodiversity values (positive or negative), particularly in the case of ‘invasive’ species, is often linked with emotional and subjective appraisals or emotional biogeographies (Ulrich, 1985; Trudgill, 2008) and thus often takes the form of a qualitative argument rather than a quantitative assessment. Invasive species are also deemed to have the potential to disrupt basic ecosystem services and thus may ultimately affect human well-being (van Andel and Aronson, 2006). Any new land usage will give rise, in time, to an alteration in biodiversity. With mainstream agricultural crops it is almost impossible that they themselves will become invasive, as they have been bred to maximise their potential within the confines of a managed field. Yet, commentaries have discussed the potential that they may hybridise with relatives to produce what eventually becomes a novel invasive ecotype. In this report, an invasive species is defined as one that is “dominating and/or negatively impacting on landscape biodiversity, to the detriment of rare, threatened, endangered or protected species and/or habitats”.

1.1.2 Prevalence/Persistence

Used here, ‘prevalence’ describes the spatial distribution of crops in the landscape while ‘persistence’ describes their temporal distribution, i.e. how they can maintain viable populations over time. For example, former crops such as dandelion (Taraxacum officinale) may prevail throughout the Irish landscape and have certainly persisted for many centuries. However, they are not considered to be invasive (i.e. they are not on the list of designated invasive species in Ireland4), though they may be considered to be a ‘weed’ and an indicator of poor land management (Mitich, 1989). Some introduced and now naturalised species may be prevalent and persist in certain landscapes and yet may be deemed non-invasive – that is, not over-dominant or detrimental to habitats or species. For example, buddleia (Buddleia davidii) is prevalent in neglected urban landscapes. However, it is known to benefit urban butterflies, bees and other species even though it is a recent arrival in Ireland (1910). It is mostly found in ruderal, neglected conditions, such as recently disturbed soils, and is vulnerable to competition, especially for light. Feral crop volunteers are similarly challenged by ecological processes outside agronomic conditions, and while some crops prevail for a time, or persist locally, there is no evidence to show that any of the crops grown in Ireland have become invasive in the past or are likely to become so.

While hybridisation is extremely common among closely related plants, genetic introgression is the permanent incorporation of genes from one population into another (Stewart et al., 2003, p. 806). This can only be demonstrated after repeated backcrossing shows the new genome to be stable. So, the persistence and prevalence of a novel trait (such as GMHT) in wild relatives depend on the fitness of the introgressed plant (Meade and Mullins, 2005), that is, its ability to reproduce successfully and thrive. When it comes to crops such as oilseed rape, Norris et al. (1999) demonstrate that the novel traits do not confer any additional ability to improve fitness or to become an ‘invasive’ species. Hybrids arise through natural processes (Ellstrand et al., 1996) and longevity is not necessarily predicated solely on a crop having a GM trait (Wilkinson and Tepfer, 2009). There are no surveys on the prevalence or persistence of feral crops in Ireland (their occurrence, frequency of distribution, etc.) and any evidence is based on observation and not analysis. In addition, where there may be a perceived abundance of feral crops and hybrids, there are no data on whether these pose a threat to habitats and species, or support or harbour benign or detrimental species at higher trophic levels.

1.1.3 Weeds and weediness

Referring to a plant as a ‘weed’ is not scientifically accurate, as it refers to the opinion of the observer relative to the perceived impact that the plant in question may have. Thus, this qualitative assessment is often based on the assumption that a weed is a plant in the wrong place. Weediness, as used in the literature consulted for this report, is a colloquial way of describing the likelihood of a plant to become persistent and/or prevalent. A more accurate and pragmatic definition of a ‘weed’, and which is adopted

in this research, relates to a plant that “interferes with human objectives” (Ellstrand et al., 1999, p. 540) and not the often subjective, aesthetic or emotive opinions of what ‘weediness’ may be.

1.2 Objectives

Some crops can prevail locally after harvesting and persist in the following years; some may persist for a long time in that their finite rate of increase (i.e. the measure of fitness) is amplified year on year (i.e. $\lambda > 1$) (Parker and Kareiva, 1996). While feral maize is far less prevalent (if it is at all), oilseed rape is anecdotally thought to be ubiquitous – that it prevails as feral volunteers. It will be shown that it also may hybridise with some relatives that can be locally prevalent. Therefore, several key questions arise, including:

- Does the prevalence and persistence of a feral crop or crop hybrid constitute a threat to biodiversity in the Irish landscape?
- Is this prevalence and persistence related to the modified traits of the crop?
- Does the presence of hybrid crop/wild relatives constitute an invasive species? and
- Is this a threat to the biodiversity of the Irish landscape?

To answer these questions it is necessary to examine this under agronomic conditions as the management of the crop can have as big an impact on biodiversity as the crop itself (Mullins et al., 2009).

The principal aim of this project was to examine the impacts on Irish agri-biodiversity of two specific GM crops, GMHT maize ($\textit{Z. mays}$) and GMHT oilseed rape ($\textit{B. napus}$). This was achieved by:

- Quantifying the propensity for pollen-mediated gene flow to wild relatives. Only oilseed rape was considered for this assessment as maize has no wild relatives in Ireland.
- Determining the potential of the HT trait to maintain a viable presence in crop-to-wild hybrids and to persist through successive generations.
- Ascertaining the potential for feral GMHT oilseed rape and GMHT maize populations to establish outside the confines of a managed system.
- Investigating the potential impacts of HT regimes on biodiversity levels both within and outside the confines of the field environment.

This research was carried out by critically assessing the published literature (peer-reviewed papers, conference proceedings, books and edited book chapters) and through interviews with key researchers in the area of GMHT research and development.
2 Potential for Gene Flow into Wild Relatives

2.1 Introduction
The GM trait that is most likely to be used in maize and oilseed rape in Ireland in the near future is that which is coded for tolerance to the herbicides glyphosate or glufosinate. In addition, a non-GMHT oilseed rape variety will soon be available with tolerance to the herbicide imidazolinone (IMI) (Coghlan, 2009). These crop traits will enable farmers to restrict competition by agricultural weeds during the early stages of seedling growth and may also enable farmers to adopt more benign management practices such as no-till (i.e. to eliminate the need for ploughing prior to seeding) or minimum tillage (min-till) management regimes. Each agricultural crop has the potential to spread its genes into the wider landscape through pollen-mediated and/or seed-mediated gene flow, with the possibility of cross-pollination existing for all plants related to the crop in question. This chapter examines the potential for gene flow in Ireland and concerns itself with oilseed rape only because maize, with its origin of Central and South America, is genetically contained due to an absence of wild/near-relatives in Ireland.

2.2 Oilseed Hybridisation
Oilseed rape (B. napus) is a cultivated form of a wild species, which is still present in non-agricultural locations. Hence, there is every likelihood that pollen-mediated gene flow can and will occur with some Brassica relatives. This phenomenon has been researched widely, with work dating to long before GM crops were commercialised (e.g. U, 1935; Davey, 1939; Percival, 1947; Jenkinson and Glynne-Jones, 1953). The issue has come to the fore, however, with the advent of GM crops and there have been multiple field trials completed to establish the propensity for trait transfer from GMHT oilseed rape into near-relatives (Sweet and Shepperson, 1997; Turner, 2004). Daniels et al. (2005) carried out extensive surveys on multiple sites over 3 years and concluded that the transfer of herbicide tolerance "appears to be minimal" (p. 20). Ellstrand et al. (1999) state that while hybridisation with the Brassica family is very common there are no implications for either evolution of problem "weeds" or an extinction risk to "wild" populations (p. 544) and Warwick et al. affirm that there is little evidence to show that the genes that code for herbicide resistance that are found in wild populations are "inherently risky" (2008, p. 7). Nevertheless, hybridisation between oilseed rape and its wild relatives is possible, and in some cases highly likely (Eastham and Sweet, 2002).

With the cultivation of oilseed rape, as with many field crops, it is accepted that some genetic ‘escape’ is unavoidable (Chadoeuf et al., 1998; Chèvre et al., 1998). Gene flow between B. rapa, B. oleracea and B. nigra as parents and B. napus, B. juncea and B. carinata as hybrids is well documented (U, 1935 (U’s Triangle); Gill and Vear, 1966) but no other naturally occurring hybrids have been recorded (Daniels et al., 2005, p. 20). Allainguillaume et al. (2006) have examined the fitness of B. napus and B. rapa hybrids in non-GM crosses and shown that "substantially fewer" descendents will exhibit any traits that will permit the hybrids to persist. There are numerous studies of oilseed rape gene transfer to wild or feral species in the Netherlands (de Vries et al., 1992), Canada (Beckie et al., 2006), Australia (Salisbury, 2002), Austria (Pascher and Gollmann, 1999), the US (Brown et al., 1997), and also from field trials in the UK (Raybould and Gray, 1993). Prior to quantifying the propensity for gene flow to wild relatives from an Irish context, it is first necessary to examine what inter-related species of B. napus may be present within the Irish agri-environment and which hence might be impacted upon by the flow of genetic material from the cultivation of GMHT oilseed rape.

2.3 Crop Biology
Hypothetically, there are ca. 100 species capable of hybridising with B. napus (Eastham and Sweet, 2002), most of which have been demonstrated using in-hand or similarly forced experimentation techniques. As these hybridisations do not occur in real-world situations, it is necessary to first examine only those plants that have the potential to cross with oilseed rape
under typical agricultural conditions. Table 2.1 contains a list of 16 cruciferous species (Brassicaceae) that may be found in or near agricultural landscapes in Ireland and are related to B. napus. The ‘rare’ and ‘occasional’ species were mostly identified in urban waste areas and have been assumed to be discarded food plants (Scannell and Synnott, 1972). Indeed, many of these records date from the early part of the last century and some may have been mistakenly identified (Reynolds, 2002).

Of these 16 species, one was believed to be a native species – Raphanus raphanistrum (Webb et al., 1996). However, it is unlikely that R. raphanistrum is indeed a native species and it is more likely to have been

| Table 2.1. List of 16 species found in the wild in Ireland with genetic similarity to Brassica napus. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| **Brassicaceae (Cruciferae)** | **Common name** | **Location** | **Abundance** | **Status** |
| Brassica alba (Sinapis alba) | White mustard | Cultivated ground, disturbed ground, roadsides, landfills (probably introduced with birdseed) | Rare/Occasional | Introduced |
| Brassica fruticulosa | Twiggy turnip | Dublin port only | Very rare | Introduced |
| Brassica gallica (Erucastrum pollichii) | Hairy rocket | Ports, disturbed ground, roadsides | Occasional | Introduced |
| Brassica juncea1 | Chinese/Brown mustard | Ports, roadsides (probably introduced with grain feed) | Rare | Introduced |
| Brassica nigra1 | Black mustard | Shingle, coastal locations in southern part of Ireland | Rare | Probably introduced |
| Brassica oleracea1 | Wild cabbage | Cultivation escape, landfills, waste areas, cliffs (south-east UK only) | Rare | Introduced |
| Brassica rapa (Brassica campestria)1 | Wild turnip | Roadside, ports, disturbed ground, mostly in southern Ireland especially near coasts; UK: riverbanks/lakesides | Locally abundant | Probably introduced |
| Brassica tournefortii | Pale cabbage | Dublin port | Rare | Introduced |
| Diplotaxis muralis | Wall mustard (annual) | Railways, dry banks, walls, cliffs, sandbanks | Occasional | Introduced/Invasive |
| Diplotaxis tenuifolia | Wall rocket (perennial) | Waste ground, disturbed ground | Rare | Introduced |
| Eruca vesicaria | Garden rocket | Landfill, waste ground, disturbed ground | Rare | Introduced |
| Hirschfeldia incana (Brassica adpressa)1 | Hoary mustard | Dublin mainly, disturbed ground, gravel, roadsides, landfills (introduced with grain feed) | Occasional | Introduced/Invasive |
| Raphanus raphanistrum (subsp. raphanistrum)1 | Wild radish | Cultivated ground, disturbed ground, roadsides | Occasional | Introduced |
| Raphanus raphanistrum (subsp. Maritimus) | Sea radish | Shingle beaches, coastal | Occasional | Introduced |
| Raphanus sativus | Garden radish | Roadsides, disturbed ground, cultivation escape | Rare | Introduced |
| Sinapis arvensis1 | Charlock/White mustard | Cultivated ground, disturbed ground, roadsides, landfills | Occasional | Possibly introduced |


1Indicates species which have been the subject of the majority of published introgression research outside Ireland (Flannery et al., 2005).
introduced at some time by accident (Guiry and Guiry, 2009). The remaining relatives of \textit{B. napus} are also either deliberately or accidentally introduced (Reynolds, 2002; Guiry and Guiry, 2009), but these are now classed as ‘naturalised’ to some degree. Seven species are the subject of the vast majority of research into gene flow in other jurisdictions and have direct relevance to this discussion because the remaining eight have not been researched to any significant extent\(^5\). The seven are:

1. \textit{Brassica juncea};
2. \textit{Brassica nigra};
3. \textit{Brassica oleracea};
4. \textit{Brassica rapa} (\textit{B. campestris});
5. \textit{Hirschfeldia incana} (\textit{B. adpressa});
6. \textit{Raphanus raphanistrum} (subsp. \textit{Raphanistrum}); and
7. \textit{Sinapis arvensis}.

\subsection*{2.3.1 \textit{B. napus} × \textit{B. juncea}}

Hybridisation between \textit{B. napus} and \textit{B. juncea} under field conditions has been demonstrated (Jørgensen and Andersen, 1994). In other studies, hybrid pollen fertility was shown under laboratory conditions and that the possibility of wild hybridisation “cannot be neglected” (Frello et al., 1995, p. 240), though critically they do not demonstrate this in field trials. Some hybridisation events have been recorded in both directions, with female \textit{B. napus} being less successful (Jørgensen et al., 1998) (Table 2.2). Seedling survival is low and it has not been shown if any surviving seeds are viable. However, \textit{B. juncea} is “rarely cultivated in northern Europe” (Jørgensen et al., 2004, p. 259) and to date has not been grown on a large commercial scale in Ireland.

\subsection*{2.3.2 \textit{B. napus} × \textit{B. oleracea}}

Laboratory hybridisation between \textit{B. napus} and \textit{B. oleracea} has been partially successful (Kerlan et al., 1992) and, though possible in the wild, in theory the likelihood of hybridisation in the agricultural landscape is negligible. Where artificial hybridisation has been forced under laboratory conditions, the resulting seeds were non-viable and/or malformed (Table 2.3). Naturalised populations of \textit{B. oleracea} are mostly located on cliff habitats in the UK which are poorly suited for hybrid \textit{B. oleracea} × \textit{B. napus} seedling success (Wilkinson et al., 2000; Chèvre et al., 2004).

\subsection*{2.3.3 \textit{B. napus} × \textit{B. nigra}}

Hybrids of \textit{B. napus} and \textit{B. nigra} have been produced using hand-pollination, but no hybridisation has been recorded under naturally occurring conditions. Experimental crossings resulting in seedling survival were unsuccessful (Bing et al., 1991, 1996) (Table 2.4).

\subsection*{2.3.4 \textit{B. napus} × \textit{H. incana} (\textit{B. adpressa})}

It has been shown that hybridisation between \textit{B. napus} and \textit{H. incana} can occur in field trial conditions (Eber et al., 1994), although backcrossing for five generations produced no viable plants (Darmency and Fleury, 2000), and thus it can be deduced that there can be no successful gene introgression between \textit{B. napus} and \textit{H. incana} (Table 2.5).

\subsection*{2.3.5 \textit{B. napus} × \textit{R. raphanistrum}}

Hybridisation between \textit{B. napus} and \textit{R. raphanistrum} has also occurred under field conditions (Eber et al., 1994) but with very low seedling emergence (Guéritaine et al., 2003). Integration of \textit{B. napus} genes into \textit{R. raphanistrum} was not observed by the third backcross (Chèvre et al., 1998). Research has shown similar results to experiments with \textit{H. incana} after 5 years of backcross observation (Jørgensen, 1999). Warwick et al. noted that successful hybridisation was “extremely rare” (2003, p. 536), which mirrors similar conclusions from earlier research by Chèvre et al. (1997) (Table 2.6).

\subsection*{2.3.6 \textit{B. napus} × \textit{S. arvensis}}

Open pollination between \textit{B. napus} and \textit{S. arvensis} did not produce viable hybrids (Bing et al., 1991; Lefol et al., 1991) and it is concluded that spontaneous hybridisation is unlikely to be successful (Bing et al., 1996) (Table 2.7). If it does occur, it is of such low probability (Warwick et al., 2003) that it is “negligible” (Hails and Morley, 2005). Both plants are considered

---

5. However, very few detailed studies have been carried out on gene flow between \textit{B. napus} and either \textit{B. alba} and \textit{B. nigra} (\textit{Sinapis alba}) (FitzJohn et al., 2007).
Table 2.2. Potential for hybridisation between *Brassica napus* and *Brassica juncea*.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Hybridisation frequency</th>
<th>Seedling survival</th>
<th>Key reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field trials</td>
<td>&lt; 3%</td>
<td>N/A</td>
<td>Jørgensen et al. (1998)</td>
</tr>
</tbody>
</table>

Table 2.3. Potential for hybridisation between *Brassica napus* and *Brassica oleracea*.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Hybridisation frequency</th>
<th>Seedling survival</th>
<th>Key reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td>None</td>
<td>&lt;0.01%</td>
<td>Chiang et al. (1977), reported in Scheffler and Dale (1994)</td>
</tr>
</tbody>
</table>

Table 2.4. Potential for hybridisation between *Brassica napus* and *Brassica nigra*.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Hybridisation frequency</th>
<th>Seedling survival</th>
<th>Key reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td>0.1%¹</td>
<td>0</td>
<td>Bing et al. (1996)</td>
</tr>
<tr>
<td>Laboratory</td>
<td>0.9% to 3.4%²</td>
<td>&lt;0.01%</td>
<td>Bing et al. (1991)</td>
</tr>
</tbody>
</table>

¹Male *B. napus* and female *B. nigra.*
²Male *B. nigra* and female *B. napus.*

Table 2.5. Potential for hybridisation between *Brassica napus* and *Hirschfeldia incana* (*Brassica adpressa)*.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Hybridisation frequency</th>
<th>Seedling survival</th>
<th>Key reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td>1.3%¹</td>
<td>N/A</td>
<td>Kerlan et al. (1992)</td>
</tr>
<tr>
<td>Laboratory</td>
<td>3.1%²</td>
<td>N/A</td>
<td>Kerlan et al. (1992)</td>
</tr>
<tr>
<td>Field trials</td>
<td>1.5%¹/70%²</td>
<td>0.1%</td>
<td>Lefol et al. (1996b)</td>
</tr>
<tr>
<td>Field trials</td>
<td>0.6%</td>
<td>0</td>
<td>Darmency and Fleury (2000)</td>
</tr>
<tr>
<td>Field trials</td>
<td>0.06%</td>
<td>0.01%</td>
<td>Chadoeuf et al. (1998)</td>
</tr>
</tbody>
</table>

¹Male *B. napus* and female *H. incana.*
²Male *H. incana* and female *B. napus.*

Table 2.6. Potential for hybridisation between *Brassica napus* and *Raphanus raphanistrum*.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Hybridisation frequency</th>
<th>Seedling survival</th>
<th>Key reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td>0.2%¹</td>
<td>&lt;0.01%</td>
<td>Darmency et al. (1998)</td>
</tr>
<tr>
<td>Field trials</td>
<td>&lt;0.01%¹</td>
<td>0</td>
<td>Chèvre et al. (2000)</td>
</tr>
<tr>
<td>Field trials</td>
<td>0.009%²</td>
<td>0</td>
<td>Warwick et al. (2003)</td>
</tr>
</tbody>
</table>

¹Male *B. napus* and female *R. raphanistrum.*
²Male *R. raphanistrum* and female *B. napus.*

Table 2.7. Potential for hybridisation between *Brassica napus* and *Sinapis arvensis*.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Hybridisation frequency</th>
<th>Seedling survival</th>
<th>Key reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field trials</td>
<td>0.1%¹/1.2%²</td>
<td>0</td>
<td>Lefol et al. (1996a)</td>
</tr>
<tr>
<td>Field trials</td>
<td>0.0001¹</td>
<td>0</td>
<td>Daniels et al. (2005)</td>
</tr>
</tbody>
</table>

¹Male *B. napus* and female *S. arvensis.*
²Male *S. arvensis* and female *B. napus.*
to be sexually incompatible under natural conditions (Downey, 1999; Eastham and Sweet, 2002) and there is an "extremely low probability" of viable hybridisation occurring (Moyes et al., 2002, p. 103).

2.3.7 \textit{B. napus} \times \textit{B. rapa} (\textit{B. campestris})

Hybridisation between \textit{B. napus} and \textit{B. rapa} is believed to be a common event and most of the research on this subject derives from GM trials (Table 2.8). Sweet et al. (1999) examined in-crop persistence and prevalence of \textit{B. napus} \times \textit{B. rapa} hybrids and deduced that persistence was "not enhanced by specific genetic modification\footnote{Conference proceedings; see: www.regional.org.au/au/gcirc\2/137.htm#P0_0 (accessed: July 2010).}. This was similar to other studies (Crawley et al., 1993). However, introgression in the natural landscape was extensively noted in the UK (Norris et al., 2004), where \textit{B. rapa} is relatively common on riverbanks in some locations. Norris and Sweet (2002) note that while hybrids of \textit{B. napus} and \textit{B. rapa} are fertile, their seed abundance is low. Norris et al. (2004) estimate that non-GM introgression may have occurred over many years.

It is clear that \textit{B. rapa} is the principal relative that hybridises naturally with \textit{B. napus} (Scheffler and Dale, 1994). It is thought to have two ecotypes in the landscape. The first ecotype is a ‘weedy’ remnant of agricultural activity (\textit{B. rapa} subsp. \textit{oleifera}) and may be found within the \textit{B. napus} crop rotation system. Since \textit{B. napus} cultivation is often preceded and/or followed by a cereal crop (such as wheat), \textit{B. rapa} is managed using herbicides targeted for broadleaf weeds. However, when \textit{B. napus} is cultivated, \textit{B. rapa} may reappear within the crop. A second, ‘wilder’ ecotype of \textit{B. rapa} (subsp. \textit{sylvestris}) may be found in semi-natural habitats, mostly riverbanks, canals and lakesides\footnote{A ‘wild’ plant in this case is one that grows and reproduces "without being planted" (Ellstrand et al., 1999) and not necessarily a plant that is native, near-native, naturalised, invasive or introduced.}. Due to its similar morphology to \textit{B. napus}, \textit{B. rapa} in semi-natural landscapes can be difficult to identify accurately. Its exact distribution in the UK is not known (Wilkinson et al., 2003), and no information on its Irish distribution can be located\footnote{It is believed to be absent from Northern Ireland (Wilkinson et al., 2003).}. Indeed, the two ecotypes have not been identified either in the three principal Irish flora surveys – Scannell and Synnott (1972), Webb et al. (1996) and Reynolds (2002) – or in the \textit{New Flora of the British Isles} (2\textsuperscript{nd} Edition) (Stace, 1991).

However, there is some evidence from agri-environmental researchers and farmers that \textit{B. rapa} is locally abundant in marginal landscapes in southern Ireland. Flanagan et al. (unpublished) examined co-synchronous flowering of these \textit{Brassicae} in Leinster, showing a continuous overlap between the flowering periods of two plants and, in outdoor experimental conditions, recorded a hybridisation rate of ca. 6%. Cloney (2003) has also sampled \textit{B. rapa} growing outside cultivated areas in southern Ireland and has shown that these populations may have interbred with early oilseed varieties, but that under ecological conditions this was of no advantage to these wild populations.

2.4 Conclusions

There is no likelihood of gene flow from maize to a wild relative in Ireland as no such relatives exist. Gene flow from oilseed rape to one of its wild relatives (\textit{B. rapa}) can be expected to occur (Lutman et al., 2004; Begg et al., 2006). Gene flow to six near-relatives (\textit{B. juncea}, \textit{B. oleracea}, \textit{B. nigra}, \textit{H. incana}, \textit{R. raphanistrum} and \textit{S. \ldots})

Table 2.8. Potential for hybridisation between \textit{Brassica napus} and \textit{Brassica rapa} (\textit{Brassica campestris}).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Hybridisation frequency</th>
<th>Seedling survival</th>
<th>Key reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field trials</td>
<td>0.4–1.5%\footnote{Male \textit{B. napus} and female \textit{B. rapa}.}</td>
<td>2%</td>
<td>Scott and Wilkinson (1998)</td>
</tr>
<tr>
<td>Field trials</td>
<td>0.0008\footnote{Male \textit{B. rapa} and female \textit{B. napus}.}</td>
<td>N/A</td>
<td>Daniels et al. (2005)</td>
</tr>
<tr>
<td>Wild</td>
<td>7.1%</td>
<td>N/A</td>
<td>Warwick et al. (2003)</td>
</tr>
</tbody>
</table>
arvensis), while technically possible (via artificial hybridisation techniques), will not give rise to a persistent or prevalent population of hybrids in Ireland. Tables 2.2–2.7 illustrate that while there is some potential for gene flow from oilseed rape to its wild progenitors, much of the literature contains examples of forced laboratory experiments, and not conditions that may be found under normal agronomic practices.

While the distribution of B. rapa in Ireland is unknown, it is anecdotally assumed to be widespread. The lack of baseline data impedes functional calculations on rates of hybridisation between B. napus and B. rapa. However, if it is accepted that there is the potential for GMHT gene flow to B. rapa, it is necessary to examine if the resulting hybrids will prevail and persist in the Irish landscape.
3 Potential for Longevity of Genetically Modified Herbicide-Tolerant Traits within ‘Wild’ Hybrids and Volunteers

3.1 Introduction

A novel trait will be judged to have persisted in a wild population via the successful production of seeds, such that these seeds are viable and result in the establishment of a self-sustaining population. In the case of a HT trait, feral and volunteer populations will remain viable if managed with applications of the herbicide that the trait is designed to resist. This surviving population of HT plants would then need to compete successfully with other wild plants in order to prevail in the landscape and persist over time. There are few agricultural crops that can manage this combination, but as oilseed rape plants can be found along roadsides and hedgerows in Ireland (Reynolds, 2002), it is correct to assume that the species has the ability to be feral. This chapter will examine the issue of longevity by taking hypothetical scenarios and examining the potential for this combination of events to take place for both oilseed rape and maize.

3.1.1 Maize

After initial failure in the 1970s, cold-adapted maize was finally introduced to the Irish agri-environment in the 1990s (Crowley, 1998) and has gradually increased in popularity as it is seen to be very profitable and an easy crop to grow (see Table 3.1 for the status of maize). Irish grown maize is not used for human consumption but for biomass or animal fodder, which sees the whole crop being macerated at harvest. It is extremely unlikely therefore that harvested seeds (viable or otherwise) will populate marginal areas. Grain spilt at sowing may give rise to volunteers within and outside the cultivated field (Palaudelmàs et al., 2009), but Irish maize varieties, while cold adapted, are not cold intolerant and hence do not have the capacity to over-winter. There are occasional records that show maize growing outside agronomic conditions but these are very rare (Preston et al., 2002) and are confined to single plants found in two port locations (one each in Limerick and Dublin) (Reynolds, 2002). There is no evidence that these are records of plants derived from agricultural sources and they could, for example, derive from domestic or garden sources (though they are just as likely to be seed spillages at the ports in question). There are no studies that demonstrate that GMHT maize persists, and there is little evidence of any ability to reproduce, outside cultivation. It is not deemed to be invasive (Guiry and Guiry, 2009) and any volunteers are not vigorous and rarely produce seeds (Palaudelmàs et al., 2009). As there are no relatives or near-relatives of maize in Ireland that could act as receptors for transgenic pollen from GMHT maize fields, there is no possibility that crop-to-wild relative hybridisation can occur.

3.1.2 Oilseed rape

Oilseed rape is also a recent arrival into the Irish agri-environment (see Table 3.2 for the status of oilseed

<table>
<thead>
<tr>
<th>Crop</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2009</th>
<th>2015</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>16</td>
<td>21</td>
<td>23.8</td>
<td>23.4</td>
<td>40</td>
<td>122</td>
</tr>
</tbody>
</table>

Table 3.1. Current and predicted status of maize (in ‘000 ha), and the projected percentage change from 2005 to 2015 (Teagasc, 2008, 2010).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Oilseed rape</td>
<td>3.7</td>
<td>5.1</td>
<td>8.2</td>
<td>6.7</td>
<td>20</td>
<td>441</td>
</tr>
</tbody>
</table>

Table 3.2. Current and predicted status of oilseed rape (in ‘000 ha) (CSO, 2009). Projections to 2015 and the projected percentage change from 2004 to 2015 (Teagasc, 2008).
However, in contrast to maize, oilseed rape can survive outside agronomic zones for up to 5 years (Lutman, 1993) and possibly up to 9 in certain cases (Lutman et al., 2005). Case studies of now obsolete varieties of oilseed rape have shown them to persist as feral populations (Wilkinson et al., 1995), which supports observational records that discuss feral *Brassicae* along roadsides, hedges, gardens and railway embankments in the Irish countryside (Aalen et al., 1997; Preston et al., 2002). As was shown earlier, *B. napus* can successfully hybridise with *B. rapa* and hybrid fitness studies are numerous. Allainguillaume et al. (2006) show that the fitness of F1 hybrids is low, and when added to data from multiple sampling surveys in the UK there is the likelihood of a decline in "transgene abundance" within the F1 population (p. 1,182). The ability of transgenic oilseed rape to remain viable over time is unknown (Senior and Dale, 2002), though D'Herterfeldt et al. (2008) recorded GMHT traits in a small number of volunteers in a field that held a GMHT crop 10 years previously. However, this study did not account for any nearby GMHT oilseed rape crops that were grown in the meantime and thus it is unclear if the volunteers in question relate to the original GMHT crop. Indeed, Lutman et al. (2005) concluded that feral GMHT oilseed rape plants were not more likely to become persistent than non-GM oilseed plants, though they stressed that extended timescales and after-harvest management can be limiting factors and thus need to be carefully monitored. Others have shown that feral survival is generally low but that some GMHT volunteers do persist after 2 (and possibly 3) years (Daniels et al., 2005). In an extensive review with Warwick et al. (2009), it was shown that there are few data available with which to establish the pervasiveness of these populations.

Herbicide-tolerant varieties of oilseed rape generated through mutagenic treatment, as opposed to GM, also exist and it is predicted that one such crop, IMI-tolerant oilseed rape will be commercialised for use from 2013 (Coghlan, 2009) and may be grown in Ireland soon after that. Derived using non-GM technology, the issues that were discussed in Chapter 2 regarding gene transfer, pollen flow, introgression to wild relatives and persistence remain and apply to this new variety of oilseed rape as much as they do with a GM-derived HT oilseed rape variety. Yet, as IMI oilseed rape was not developed using GM technology, it is exempt from the EU regulations that govern the release of GM crops. Also, IMI oilseed rape is not covered by EU-wide coexistence strategies for GM crops, implying that the management regime for this crop is likely to be similar to that of crops currently on the market in Ireland. As this oilseed rape variety has the same management advantage as the GMHT oilseed rape, it is likely to be popular with Irish farmers for the same reason that GMHT varieties will be. However, the herbicide IMI has a higher toxicity than either glyphosate and glufosinate (Coghlan, 2009).

### 3.2 Worst-Case Scenario Case Studies

To examine the implications of gene flow from a HT crop variety in the Irish landscape, it is necessary to hypothesise scenarios in the form of notional case studies, which serve to illustrate how these crops are grown and what the avenues for, and consequences of, gene flow are. The following five case studies examine hypothetical (yet realistic) ‘worse-case scenarios’ and the potential impact of a GMHT crop ‘escaping’ into the Irish landscape. Because there are multiple variables that need to be considered in any agri-environmental scenario, it is necessary to establish some parameters from the outset. Therefore, the following scenarios cover a 10-year time frame. This is in line with the maximum time frame used in the research findings that were drawn upon for this study. These scenarios are also based on current management regimes and available technology though this would be altered with the advent of novel-trait crops. Figure 3.1 is an aerial photograph of a notional location where the following scenarios take place.

#### 3.2.1 Inefficient seed control (maize)

**Scenario**

In this scenario Farmer A uses an inefficient/unsealed seed spreader to sow maize in the spring, and this has resulted in GMHT maize seeds being deposited in the crop field margin, the hedgerows, farm storage areas and along some local roadsides. Derived using non-GM technology, the issues that were discussed in Chapter 2 regarding gene transfer, pollen flow, introgression to wild relatives and persistence remain and apply to this new variety of oilseed rape as much as they do with a GM-derived HT oilseed rape variety. Yet, as IMI oilseed rape was not developed using GM technology, it is exempt from the EU regulations that govern the release of GM crops. Also, IMI oilseed rape is not covered by EU-wide coexistence strategies for GM crops, implying that the management regime for this crop is likely to be similar to that of crops currently on the market in Ireland. As this oilseed rape variety has the same management advantage as the GMHT oilseed rape, it is likely to be popular with Irish farmers for the same reason that GMHT varieties will be. However, the herbicide IMI has a higher toxicity than either glyphosate and glufosinate (Coghlan, 2009).
glyphosate, so in the rare circumstance that any maize seedlings did manage to survive the Irish winter these would have a selective advantage over the background flora only if their location is sprayed with glyphosate herbicide. If the spraying time coincides with competition with the surrounding flora, such as late spring/early summer, then the feral maize could possibly survive to maturity.

**Issue/Concern**
The main concern with this scenario is that there may be some GMHT maize ‘escapes’ into the wider landscape and that this may be accentuated by the use of herbicides in the management of the agri-environment.

**Outcome**
Under current management practices, Irish farmers and local authorities do not use herbicides in field margins or roadsides; they are usually trimmed (if at all) with a flail or bar cutters. Therefore, any escaped maize will not be subjected to selection pressure and thus will not thrive, due to a lack of competitive advantage. If any seeds do survive through the winter, it is very unlikely that they could produce a second generation ($F_1$) due to their inability to produce viable seeds in any quantity and to their sensitivity to low temperatures. Evidence for this can be seen in the lack of anecdotal evidence supporting the existence of feral maize populations. It is safe to conclude therefore that

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Figure 3.1. A notional aerial photograph where each scenario (Scenarios 3.2.1, 3.2.2, 3.2.3, 3.2.4 and 3.2.5) takes place.
under current climatic conditions and in the absence of selection pressure there is no likelihood of GMHT maize persisting over adjacent flora and hence there would be no detrimental impact on the Irish landscape should GMHT maize seed be lost pre-sowing.

### 3.2.2 Inefficient seed control post-harvest (oilseed rape)

**Scenario**

Oilseed rape is a prolific seed producer, with varieties generating up to 130,000 seeds/m$^2$ (Fray et al., 1996). In this scenario, Farmer B is transporting GMHT seed to a nearby silo after the harvesting of a GMHT oilseed rape crop. Because the trailer was not adequately sealed prior to leaving the GMHT field, seed has spilt out of the trailer within the field, and scattered along hedgerows and the open road. In addition to this, upon arriving at the silo the GMHT seed is sent by rail/road to the processing plant, with similar consequences due to inadequately covered trailers. As it can be expected that a percentage of lost seed will germinate and survive through to the following season, the issue in this scenario is that the GMHT oilseed rape seeds will populate and thrive in the semi-natural zones and even act as secondary sources of GM traits for neighbouring non-GM oilseed rape crops, i.e. acting as a ‘genetic bridge’ (Flannery et al., 2005).

**Issue/Concern**

The concern here is that residual GMHT oilseed rape seeds ‘escape’ into semi-natural habitats where they thrive and maybe even act as secondary sources of GM traits.

**Outcome**

**Field volunteers.** Several management techniques will minimise the impact of seed loss within the field environment. These include delaying ploughing for several weeks after harvest (Devos et al., 2004) to induce germination and decrease the potential for secondary dormancy induction (Lutman et al., 2004). Applying a herbicide 4 weeks post-harvest followed by an additional time lag of 4 weeks will ensure that lost seed has the maximum chance to germinate, upon which the second volunteer flush can be destroyed through the ploughing of the field (see Scenario 3.2.3).

**Roadside.** Oilseed ferals will populate roadsides (Norris and Sweet, 2002) but these individuals are susceptible to competition from grasses and other perennial plants. On recently completed roads, or roadsides where works were carried out, ‘escaped’ oilseed rape may grow and thrive due to the short-term lack of competition, but in time successional colonisation will result in these plants being out-competed by the more persistent species (Crawley and Brown, 1995). In the absence of management by herbicide, ‘escaped’ GMHT oilseed rape will have no enhanced ecological advantages and unless the roadside is continually disturbed feral populations will not prevail (Devos et al., 2004).

**Hedgerows.** Field margins are rarely managed with herbicides as they can be prohibitively expensive for the size of land and such a practise will damage the entire hedgerow system and the ecological services they provide. The high level of shading and vegetation competition in a hedgerow will not permit any ‘escapes’ to prevail, especially in the absence of a herbicide for which the GM trait was originally designed to resist (Crawley et al., 1993). As with

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**Railway lines.** Canadian studies have shown that transgenic *B. napus* may be found along rail lines (Yoshimura et al., 2006). However, feral *B. napus* is unlikely to thrive as it would be growing in a harsher medium (i.e. diverse substrates and/or gravel) than that for which it is designed (i.e. richer soils), though some *Brassicae* wild relatives and/or feral volunteers have been noted in these locations (see Table 2.1). However, railway management is carried out regularly and herbicides that are not glyphosate or glufosinate based are regularly used. This management regime will prevent the GMHT trait from gaining any selective advantage and ensure that feral oilseed rape populations do not expand in population size.

**Canadian railway lines** are significantly different to Irish railways in that they traverse wide, open prairies where light is available, unlike Irish railways which often are shaded by embankments and vegetation. In addition, Canada has an extremely large area of landscape under oilseed rape cultivation and trains pass through many kilometres of oilseed rape fields. Thus, it would not be appropriate to extrapolate this to Irish conditions.

9. For discussions on these services, see Collier and Feehan (2003), Feehan and Keena (2001), Reif and Schmutz (2001).
maize, any volunteer populations of GMHT oilseed rape will only have an advantage if in the management of the landscape the farmer uses any of the herbicides to which the plant is resistant. So without any advantage being conferred, the volunteer GMHT oilseed rape plants will be subject to the dynamics of normal competition and thus may be no more likely to persist than ‘escaped’ non-GM cultivars which are currently found in the landscape (Norris et al., 1999).

### 3.2.3 Pollen-mediated gene flow from GMHT oilseed rape to B. rapa

#### Scenario

In this scenario it is assumed that successful pollen flow has occurred from a GMHT oilseed rape crop to some wild B. rapa growing within the crop field and/or in a nearby marginal habitat (such as in a hedgerow or along a roadside). The following planting season Farmer C decides to spray field weeds as part of the preparation for the next crop in the rotation, which in this scenario is wheat. During this spraying, there is some accidental drift into a nearby hedgerow. At the same time, the local authority in the area also decides to spray along the roadside. Both sprays are glyphosate based, which is the subject of the trait that has been introgressed into the wild B. rapa and as a result small populations of hybrid B. rapa exist with physiological resistance to glyphosate.

#### Issue/Concern

The concern is that pollen from GMHT oilseed rape will give rise to successful hybrid populations and thus confer a selective advantage on these populations in the landscape.

#### Outcome

**Field.** Growing within the wheat, Farmer C notices both volunteer oilseed rape from the previous year and what appears to be some B. rapa. As the wheat is not herbicide tolerant, Farmer C now uses a different herbicide (e.g. metsulfuron) to remove the Brassica weeds. Using this management approach, all GMHT crop volunteers and any hybrids within the field will be removed. Thus, the in-field population of hybrid B. rapa and any GMHT B. napus volunteers would be destroyed.

**Semi-natural habitats.** The issue is different in the semi-natural areas. After being treated with glyphosate by the local authority, the bulk of the roadside vegetation dies with the exception of the B. rapa × GMHT B. napus hybrids. The Brassica hybrids are now free to grow to seed and to self-perpetuate with little competition. Similarly, the glyphosate that Farmer C was using has accidentally drifted into the hedgerow base and the vegetation is desiccated, with the exception of the hybrid and feral GMHT Brassicae, both of which are able to mature without competition. In such a situation, the question arises: what is the likelihood of these hybrid populations persisting? While the probability of interspecific gene flow is quite low, it is not zero (Raybould and Gray, 1993; Ellstrand et al., 1999). Using B. napus and B. rapa, Warwick et al. (2003) were the first to demonstrate the movement of modified genes from a crop to a near-relative in natural, non-confined conditions, but it was not shown if these genes were fully introgressed. Later, Warwick et al. (2008) demonstrated that GMHT oilseed rape genes can persist for up to 6 years or more in B. rapa, but in the process there was a gradual retreat to the original B. rapa genes and a gradual loss of the genes of B. napus (Fig. 3.2).

Glyphosate and glufosinate are systemic, decay rapidly and become inert upon contact with the soil. Thus, in all sprayed locations in this scenario there will be a definite regrowth of ‘natural’ vegetation within a few months of the initial spray treatment. Depending on the time of initial herbicide application, this vegetation may either compete with the established feral HT population or its seeds the following season. There is also evidence that B. rapa would need to be in high abundance in the local landscape for introgression to occur to a significant level (Johannessen et al., 2006) and, as discussed earlier, the exact distribution of B. rapa is not quantified in Ireland. Therefore, Farmer C has no need to be concerned because if there is no selection pressure then any GMHT hybrids/ferals in the marginal habitats will have no competitive advantage over adjacent flora populations (Simpson et al., 1999; Beckie et al., 2001)

11. This can be one of the negative agricultural consequences of using these 'milder' forms of herbicide, in that vegetation often returns more rapidly.
Norris and Sweet, 2002; Norris et al., 1999, 2004). On the contrary, if selection pressure is applied via glyphosate applications then there is an increased likelihood of hybrid and/or feral plants. Critically, the fitness of these populations will diminish outside agronomic management regimes as the new hybrid and/or feral plants will also possess genes bred into oilseed rape to ensure maximal performance under the managed environment of the field but which will actually reduce plant fitness outside the field (Warwick et al., 2008).

3.2.4 Increased use of GMHT oilseed rape in the Irish landscape

Scenario
In this scenario, a GM clustering arrangement is in place in a particular county in the south-east of Ireland, of which Farmer D is a participant (for a detailed explanation of GM clustering see Mullins et al. (2009), Chapter 3). All oilseed rape in this cluster is GMHT (e.g. for glyphosate) and the cluster has been producing for 10 years. The management regimes that are used in this system are now more dependent upon the use of glyphosate than in other areas. The concern here is that the concentration of GMHT oilseed rape will provide a continual supply of HT pollen, which will ensure some HT *B. rapa* populations, as well as that volunteer GMHT oilseed rape populations are maintained through continuous selection. As with Scenario 3.3.2, there is also an elevated level of seed scatter, resulting in increased feral populations of GMHT oilseed rape in marginal habitats. This may result in high levels of sub-populations of feral *B. napus* as well as hybrid *B. napus* × *B. rapa*. If more than one GMHT variety is prevalent (such as glyphosate and glufosinate), this may give rise to populations with stacked genes (i.e. with a resistance to both herbicides). In combination, these populations may act as ‘genetic bridges’ and gradually prevail in marginal habitats outside the cluster zone. A second concern may be that the increased presence of glyphosate and glufosinate would force other species to evolve resistance (known as ‘weed shifts’ – emerging

![Figure 3.2](http://www.aiast.com.au/upload_docs/Suzanne%20Warwick%20Presentation.pdf) (accessed: July, 2010).
unrelated plant species with a tolerance to those herbicides).

Issues/Concerns
The first concern is that a combination of feral crop and hybrid *Brassicae* may now act as ‘genetic bridges’ and spread along marginal corridor habitats outside the cluster zone. A second concern is that the increased presence of glyphosate and glufosinate would force other species to adapt to a HT phenotype.

Outcome
Guidelines have been established for the management of GM crops in the Irish landscape (McGill et al., 2005). One recommendation includes the establishment of clusters of GM-licensed farmers within a region or agricultural zone to simplify the key EU requirement of monitoring GM cropping sites post-cultivation (European Commission, 2001). This monitoring is composed of a general surveillance phase and a more focussed case-specific analysis. The general survey is the responsibility of the network of GM farmers within the cluster along with the GM crop company representatives. The case-specific survey offers the opportunity to monitor the persistence of feral populations and test for phenomena such as ‘gene stacking’. As with the earlier scenarios, the response may entail an application of different herbicides or to manage these habitats by non-chemical means. Furthermore, as with earlier scenarios, ecological processes will apply in these semi-natural habitats and vegetation competition (which not all crops tolerate) will mitigate population expansion. Yet, in a clustering situation, monitoring will only take place in semi-natural habitats and fields in the vicinity and, as there is the possibility for ‘genetic bridging’, semi-natural areas that are beyond the GM cluster zone are unlikely to be monitored on a regular basis. Conversely, such areas outside the GM cluster are also unlikely to be managed using these herbicides, and thus the absence of selection pressure will also impede feral/hybrid persistence over time. Separately, the case-specific monitoring must survey for the propensity for non-Brassicaceae weeds to spontaneously mutate and develop herbicide tolerance. These ‘weed shifts’ are suspected to have occurred already, though weed species diversity is not known to have declined as a consequence (Beckie et al., 2006). Beckie et al. also suspect that increased reliance on herbicides will lead to an increased potential for selection pressure to occur, but this is based on a 10-year assessment of Canadian farms where the entire landscape was subjected to herbicide applications.

3.2.5 Increased use of IMI oilseed rape in the Irish landscape

Scenario
It is 10 years since the uptake of IMI oilseed rape and many farmers nationwide, including Farmer E, now grow this variety in the absence of any regulation as it was not developed through GM technology. There is no restriction on its use and no assessments of introgression into wild relatives in any of the marginal semi-natural habitats as was the case in the above scenarios. The key concern is that un-monitored and un-clustered farming of IMI oilseed rape may give rise to large populations of IMI-resistant volunteers and hybrid *Brassicae* in semi-natural habitats. In this scenario, a hypothetical survey of ruderal habitats within oilseed rape farmland landscapes is carried out as part of a student research project. This survey reveals multiple populations of feral *B. napus* and hybrid *B. napus × B. rapa*, all with IMI tolerance.

Issues/Concerns
The concerns here are the same as for Scenarios 3.2.3 and 3.2.4, but with a non-GM variety of oilseed rape. A further concern is that IMI tolerance will increase the levels of toxic exposure in the landscape.

Outcome
The outcome of this scenario would be similar to those of Scenarios 3.2.3 and 3.2.4, discussed above. As IMI is not used to manage semi-natural habitats there will therefore not be any selection pressure applied and thus the situation in marginal habitats will be the same as the scenarios above. If there are any accidental applications of IMI, the same outcomes as in Scenario 3.2.3 would arise. If all oilseed farmers are using the IMI variety and there are the same number of oilseed farmers as today, hybrid and volunteer *Brassicae* ought to be the same as current levels in the absence of any advantage being conferred to these populations. However, the research student has hypothesised that populations of hybrids or volunteers in semi-natural habitats may be increasing. Having
discovered this, the farmer can manage the ‘problem’ using alternative herbicides or using non-chemical management. Finally, as farmers become reliant on regularly using this herbicide (IMI), levels will build up in the landscape and, as with Scenario 3.2.4, there may also be weed shifts. None of these issues point towards IMI oilseed rape being different from GMHT oilseed rape in that regard.

However, as there are no regulatory mechanisms for the (mandatory) monitoring of IMI crops it is highly unlikely that any surveys will take place, and thus issues regarding persistence will go unnoticed but, critically, in the absence of selection pressure they will have the same lack of advantage as current *Brassicae* feral and/or hybrid populations. If, on the other hand, IMI oilseed rape is grown in proximity to GMHT oilseed rape, then there is the possibility of stacked tolerant genes being located in feral or hybrid *Brassicae*. There are some reports of multiple-trait oilseed rape in Canada (Downey, 1999; Hall et al., 2000; Beckie et al., 2003), in France (Mésean, 1997; Champolivier et al., 1999) and in the UK (Simpson et al., 1999) and, while these populations can be controlled through alternative management regimes, their prevalence erodes existing strategies and increases the risk of these gene-stacked populations persisting and expanding.

The concern that IMI is significantly more toxic than either glyphosate or glufosinate, will be examined in Chapter 4.

### 3.3 Conclusions

The five case studies were specifically designed to hypothesise the worst-case scenarios that could occur with the cultivation of GMHT maize and oilseed rape crops. There are no realistic scenarios that can give rise to maize persisting for any appreciable time in the landscape, GM or non-GM. In contrast, scenarios exist whereby the GMHT/IMI traits could persist in feral/volunteer populations of *B. napus* and/or be successfully introgressed into wild populations of *B. rapa*. While hybridisation is not the exception, it is the rule in natural systems. It is the consequence of the gene flow events that is critical for, without selection pressure through the application of herbicides, these plants are no more likely to persist than current conventional oilseed-rape-derived populations. 

Furthermore, if HT *B. napus* × *B. rapa* hybrids or HT *B. napus* volunteers are identified, they can be eradicated using alternative agricultural herbicides. If populations of GMHT/IMI *B. rapa* do arise and are subjected to selection pressure (spraying), they will have an opportunity to increase their numbers in the short term before the rest of the vegetation returns. In the long term, typical ecological processes will prevail (e.g. successional growth), as will competition (e.g. for light, nutrients, water, etc.), and will impact on the HT populations as with all plants. Thus, it is shown that the limiting factor in all scenarios is the management regimes of the crop or of the semi-natural areas or of both. The requisite monitoring of GMHT oilseed rape and maize will reveal any persistence issues in the short term. This monitoring or control is not required for IMI crops and thus any issues regarding persistence may not be noticed until some time has passed. By following current best practices in farm management, coupled with the newly established guidelines for the growing of GM crops in Ireland, the risk of either GMHT crop becoming any more prominent in the landscape than existing non-GM crops is remote.

Yet, given the principle of precaution in these matters, might there be the potential for the management of *Brassicae* (GM and non-GM) to impact detrimentally on farm landscape biodiversity? Even under the rare likelihood of a population of HT hybrids or crop volunteers persisting locally, there is no evidence that these may become invasive or in any way impact detrimentally on Irish species and/or habitats. There are no data to show that *Brassicae* have invaded or are otherwise occupying an ecological niche of a native species. The prevalence and persistence of escaped *Brassicae* have not been shown to have impacted Irish flora in a negative manner that would pose a problem for landscape biodiversity. In addition, these flowering plants may provide additional support for nectar feeders in the rural landscape. As keystone species such as bees are in decline in Ireland, a counter-hypothesis is possible – that the presence of *Brassicae* may be more supportive than detrimental. However, in the absence of any data to the contrary, this is the subject of speculation and, perhaps, further investigation.
4 Potential Impacts of Herbicide-Tolerant Management Regimes on the Biodiversity of Non-Target Organisms

4.1 Introduction

It is widely accepted that the management of agricultural landscapes has been detrimental to biodiversity (Chamberlain et al., 2000; Robinson and Sutherland, 2002; Stoate et al., 2009), and that land management regimes, such as the use of pesticides, have further impacted on non-target organisms (NTOs) (e.g. Carson, 1965; Birch et al., 2004; Devine and Furlong, 2007). These impacts can be either direct, by being toxic to some NTOs, or indirect, by impacting what sustains them. Changes in management brought about by the introduction of a novel-trait crop offer an opportunity to examine these impacts. In respect to the first generation of GM crops, these were designed to address targeted inputs to crop management in order to make the crop in question more economical to the farmer. While less management may imply less impact on landscape biodiversity (as was shown in an earlier report: Mullins et al., 2009), there is still a great deal more to learn on the impacts of GMHT management regimes on specific NTOs and habitats. Much of the research into the impact of HT crops has examined gene flow in the landscape (as reported earlier), but significantly less research has been carried out on the effects of management change on NTOs.

4.1.1 Chemical toxicity

Numerous herbicide products are cleared by the Department of Agriculture, Fisheries and Food for use on forage maize and oilseed rape. Weed control in oilseed production generally involves one pre-emergence application per year for spring oilseed rape and a further post-emergence application for winter oilseed rape. A further application may be used to desiccate the crop prior to harvest, especially in winter crops. For forage maize production there are generally two applications (one pre-emergence and one post-emergence). Herbicide application is highly dependent upon the extent of ‘weed’ coverage in the field, the species of ‘weed’ present and prior usage of the field. The approved Irish list of active herbicidal ingredients for both crops is shown in Table 4.1. Farmers will select the active ingredient depending on the management required, the pest species in question, and so on. The aim of introducing a HT trait into these crops is to replace these chemicals with a move to glyphosate-based herbicides.

Glyphosate was first produced by Monsanto in May 1970, claiming that it had “unique biological properties” (Franz et al., 1997, p. 5) that would enable it to be used to control ‘weeds’ with negligible impact on non-target species. Glyphosate claims to be:

- A broad-spectrum, non-selective, post-emergence herbicide;
- Virtually non-toxic to animals and does not bioaccumulate;
- Systemic (translocated from leaves to roots rapidly) in its action;
- Static in the environment in that it binds tightly to soil particles and thus does not leach into groundwater;
- Metabolised by soil micro-organisms; and
- Unable to penetrate tree stems (Franz et al., 1997, p. 7–11).

The United States Environmental Protection Agency (USEPA) classifies glyphosate as a Category IV environmental chemical (i.e. it is the least toxic herbicide) and a Category D pesticide (i.e. “not classifiable as to human carcinogenicity evidence”) based on lack of evidence, though some research has speculated towards a possible link to cancer and reproductive disruption in humans which may need to

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be further examined (Pieniazek et al., 2003; Gasnier et al., 2009). However, glyphosate is currently considered to be a low toxicity herbicide from an environmental standpoint (Cerdeira and Duke, 2006). Glufosinate is also considered to have low toxicity in the environment and to human health. In addition, it has been observed that with the introduction of glyphosate- and glufosinate-resistant crops there has been a move away from herbicides that are significantly more toxic (Gianessi and Carpenter, 2000; Heimlich et al., 2000; Trewavas and Leaver, 2001).

A comprehensive series of risk assessments of herbicides was taken by Peterson and Hulting (2004) using USEPA data in a wheat growing system. They demonstrated that glyphosate can be very slightly toxic to some organisms (e.g. earthworms and rainbow trout) but that no herbicide is totally non-toxic (i.e. to NTOs). However, they show that bromoxynil, 2,4-D and trifluralin were significantly more toxic to a range of mammals, amphibians, fish and invertebrates (Peterson and Hulting, 2004). The most extensive data

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Table 4.1. Active ingredients in herbicides approved for use in maize and oilseed rape production (PCS, 2010). The potential levels of environmental toxicity are classified according to the Pesticide Action Network of North America.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Active ingredient</th>
<th>Acute toxicity</th>
<th>Human toxicity</th>
<th>Water toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oilseed rape</td>
<td>Carbetamide</td>
<td>Low</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Cycloxydim</td>
<td>Low</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Diquat (dibromide) (desiccant)</td>
<td>High</td>
<td>Potential</td>
<td>Potential</td>
</tr>
<tr>
<td></td>
<td>Fluazifop-P (-butyl)</td>
<td>Low</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Glyphosate</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>Metazachlor</td>
<td>Low</td>
<td>Low</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Propaquizafop</td>
<td>Low</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Quinmerac</td>
<td>Low</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Quizalofop-P-ethyl</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Tepraloxydim</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Trifluralin/Treflan(^2)</td>
<td>Low</td>
<td>Potential</td>
<td>Potential</td>
</tr>
<tr>
<td>Maize</td>
<td>2,4-D</td>
<td>Moderate</td>
<td>Potential</td>
<td>Potential</td>
</tr>
<tr>
<td></td>
<td>Bromoxynil</td>
<td>Low</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Fluafenacet</td>
<td>Low</td>
<td>Low</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Fluroxypyr</td>
<td>Low</td>
<td>Low</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Isoxaflutole</td>
<td>Moderate</td>
<td>High</td>
<td>Potential</td>
</tr>
<tr>
<td></td>
<td>Mesotrione</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Nicosulfuron</td>
<td>Low</td>
<td>Unknown</td>
<td>Potential</td>
</tr>
<tr>
<td></td>
<td>Pendimethalin</td>
<td>Moderate</td>
<td>Potential</td>
<td>Potential</td>
</tr>
<tr>
<td></td>
<td>Rimsulfuron</td>
<td>Low</td>
<td>Potential</td>
<td>Potential</td>
</tr>
<tr>
<td></td>
<td>Terbuthylazine</td>
<td>Low</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

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\(^2\)Used in the management of oilseed rape in Ireland, but not listed on the Pesticide Control Service database.
analysis of pesticides in the EU, the EU eco-footprint database\textsuperscript{16}, lists the ecotoxicity of glyphosate as being between zero and moderate and glufosinate as being between zero and low. It ought to be noted that while the US and EU databases contain a wide variety of research data on herbicides, they are by no means complete. Because of poor evidence of any significant toxicity of these substances, wider and continual research has not been deemed necessary, with most of the research being carried out on glyphosate.

Chassy et al. (2003) have commented that Directive 91/414/EEC (EEC, 1991) on the use of crop management chemicals does not include an assessment of risk to in-crop biodiversity, which is now a requirement of Directive 2001/12/EC (European Commission, 2001), and thus herbicide impact is assessed differently for non-GMHT (e.g. IMI-tolerant crops) and GMHT crops. While a review by Kleter et al. (2008) shows that the use of glyphosate has a better environmental impact quotient (EIQ) than earlier, more toxic herbicides used on non-GM crops, it should be noted also that EIqs do not necessarily examine the effects on biodiversity, but rather assess the impact on the broader environment. Therefore, this chapter will examine the impact that a GMHT regime might have on NTOs. The issue of impact has two aspects. The first is the type of herbicide (its relative toxicity) and the second is the level or rate of herbicide usage in the agri-environment. Significantly more research has been carried out on the former, with the latter highly dependent on the agronomic systems of the country where the crop is grown, the landscape characteristics and farmer choice.

4.2 Herbicide Usage

The introduction of HT crops has brought about a reduction in the rates of herbicide usage, especially in the US (Gianessi et al., 2002; Kleter and Kuiper, 2003) and Canada (Brimner et al., 2005). As there are no GMHT crops commercially grown in the EU (James, 2009), there are no data available of similar reductions. As their management is derived from the use of a less toxic chemical, it ought to follow that GMHT crops may have a lower environmental footprint. However, impacts on the environment are difficult to measure because it is necessary to select the parameters for measurement, the weighting of each component or organism being impacted upon, how reliable the data are, and how to evaluate the combination of issues such as economic and human health impacts (Levitan et al., 1995). There is one comprehensive examination of this issue with relevance to Ireland – the UK Farm-Scale Evaluations (Firbank, 2003; Squire et al., 2003). These were a study of a microcosm of agronomic conditions and herbicide applications in management.

While herbicide usage varied significantly between conventional and GM crops (Champion et al., 2003), the overall study did not show any cumulatively negative impact on biodiversity in maize systems (Heard et al., 2006). There were some declines in ‘weed’ feeding species in spring oilseed rape systems within the managed environment of the field (Andow, 2003). Declines in dicot ‘weeds’ were observed in winter oilseed rape systems, which might affect species at higher trophic levels, but declines in species diversity were not recorded (Bohan et al., 2005). The overall conclusion was that the introduction of GMHT crops would possibly have some detrimental impacts on some species due to changes in ‘weed’ diversity and distribution, but that much would be determined by the management regimes that would be brought about by their introduction in to the agri-environment (Hawes et al., 2003).

4.2.1 Soils

Glyphosate is non-volatile and becomes inert when it comes into contact with the soil regardless of pH or organic matter content (Franz et al., 1997). When used correctly, it is non-contaminating and there is little evidence from long-term studies of glyphosate residues persisting over time (Cerdeira and Duke, 2006). Much of the research on the effects of glyphosate in the soil has been carried out, by necessity, using lysimeters, i.e. in experimental conditions and not in field conditions. Though field studies are few, in an extensive review of their literature Motavalli et al. deduce that “despite widespread public concern, no conclusive evidence has yet been presented that currently released transgenic crops, including both herbicide and pest resistant crops, are causing significant direct effects on

\textsuperscript{16} See: \url{http://sitem.herts.ac.uk/aeru/footprint} (accessed: July 2010).
stimulating or suppressing soil nutrient transformations in field environments” (2004, p. 186). Under GMHT management systems, Irish farmers would move to a minimum tillage (min-till) regime. The effects of this type of land-use change should be beneficial to soil ecology due to less compaction, disturbance and soil erosion, improved soil composition and structure, and less disturbance of soil infiltration capacity (Holland, 2004). It has also been discussed that while some minor effects on soil bacteria were noted under glyphosate regimes, conservation tillage regimes may enhance soil organic carbon and increase vegetation residues and thus aid in buffering the potential effects of glyphosate (Locke et al., 2008).

Some elements of the soil microbial community may be impacted upon by glyphosate. For example, Zablotowicz and Reddy (2004), following earlier data from Carlisle and Trevors (1986), show that mycorrhizal fungi necessary for nitrogen fixing may be temporarily affected, thus necessitating additional nutrient inputs. However, most soil biota also appear not to be adversely affected in the majority of the studies on this topic. There is also some evidence of differences in rhizosphere microbial activity under GMHT and non-GMHT cropping systems (Siciliano et al., 1998; Dunfield and Germida, 2004). However, crop cultivation has the potential for altering soil quality via altered root exudes and root architecture and, in an extensive review in the UK, some negative effects of GM cropping on soils were noted, though most were transient (Cartwright and Lilley, 2004). Research into this area is now becoming more commonplace but as the soil is such a complex ecosystem, and its biodiversity so necessary for many ecosystem functions, there is still quite a long way to go in understanding the impacts that human activities may be having (Lilley et al., 2006), regardless of the origin of the crop ecotype. Still, soil microbial tolerance under GMHT and non-GM maize cultivation shows little difference under glufosinate management regimes in detailed glasshouse research (Griffiths et al., 2008) and under normal agricultural conditions (Griffiths et al., 2007). Liphadzi et al. (2005) found no differences between conventional and GM cropping maize systems and Cerdeira and Duke conclude that “no agriculturally significant effect of glyphosate on soil micro-organisms has been documented” (2006, p. 1,638). The effects of management on soil communities may have a more significant impact over time (Birch et al., 2007; Bohanec et al., 2007).

4.2.2 Air

The adoption of min-till or no-till management regimes in GMHT agriculture will result in lower emissions due to less vehicular activity and less spraying (and hence potential spray drift). Few studies have examined this specifically, but from a human health point of view Bennett et al. (2004) use a model-based approach to show that a GMHT crop system (sugar beet, in this case) has less harmful emissions than a conventional system. Glyphosate use has been shown not to be an atmospheric contaminant (Cerdeira and Duke, 2006).

4.2.3 Water

Though soluble in water, glyphosate and glufosinate are readily adsorbed to particles of soil and thus leaching to watercourses does not occur (Franz et al., 1997), nor can it be traced in groundwater (Miller et al., 1995). Indeed, some have proposed that moving from less toxic herbicides to the more benign glyphosate may assist in improving drinking water quality in intensive agricultural landscapes (Shipitalo et al., 2008). Much of the research into the effects of glyphosate on waterbodies has been carried out by forestry researchers. Here, they have shown that there may be some negative aquatic effects from aerial spraying and the bioaccumulation of herbicides (Solomon and Thompson, 2003), such as atrazine (Gunkel and Streit, 1980) or 2,4-D (Wang et al., 1994), for example. However, such usage in the Irish landscape does not normally occur. In all, no significantly adverse effects on aquatic life have been shown to date, though there is much comment on the paucity of research into this area (Cerdeira and Duke, 2006).

4.2.4 Non-target organisms

Dinehart et al. (2009) show that glyphosate usage in intensive agronomic conditions has no discernible effects on reptile and amphibian indicator species in North American farmscapes, though Mann and Bidwell (1999) noted that in Australian farmland there may be some negative effects on tadpole growth. These experiments were not carried out under agricultural conditions or in landscapes or soils found in Ireland, so
it would be conjecture to extrapolate to Irish conditions. Gardner and Nelson (2008) demonstrate that combined with min-till or no-till management in intensively farmed landscapes the introduction of HT crops has had a positive benefit on mammal populations. This was reflected in studies of managed forest landscapes (McComb et al., 2008). Some impact has been noted at very high levels of application. Haughton et al. (1999) recorded negative impacts on field spiders in a barley crop managed using glyphosate. After reviewing the canon of available and peer-reviewed data, Cerdeira and Duke (2006) conclude that glyphosate has not been shown to have any direct effect on invertebrates, but that the reduction in vegetation upon which many feed may reduce populations in intensively managed landscapes. Direct ingestion of GMHT crops by animals has been shown to have no discernible effect (Cerdeira and Duke, 2006). ‘Weed’ species in agricultural landscapes are often also linked to invertebrate and other species diversity. The use of herbicides as a management tool, therefore, would imply a greater impact on ‘weed’ availability and thus a lowering of biodiversity at other trophic levels.

The most comprehensive source of information on the potential impact on the biodiversity of NTOs, similar to those found in the Irish agri-environmental landscape, is the recent UK farm-scale evaluations (FSEs)\(^\text{17}\). These trials were the largest of their kind in the world and GMHT maize and GMHT spring and winter oilseed rape were tested for their potential impact; GMHT maize fields contained higher levels of ‘weeds’, non-crop seeds, butterflies, springtails and bees than non-GM fields. ‘Weed’ levels in GMHT winter oilseed rape fields were no different to non-GMHT winter oilseed fields and butterfly and bee numbers were slightly lower, with springtail numbers slightly higher. ‘Weed’ levels in GMHT spring oilseed rape fields were lower than non-GMHT oilseed rape fields; springtails and bees were higher and butterflies were slightly lower (with seasonal differences). The overall conclusions from this extensive survey were that there was little difference, if any, between GMHT crops and non-GMHT crops and that any difference had nothing to do with the GM aspect of the crops. Differences resulted largely due to changes in management (DEFRA, 2005).

4.2.5 Evolved resistance

Benbrook (2001, 2004) shows that there has been a net increase in the use of glyphosate since its introduction, especially as its price has fallen and as it is ‘bundled’ with other Monsanto seed and land management products. Heimlich et al. (2000) illustrate that, while it may not be the case with GM crops in the US, there is still some concern of an increase in non-crop resistance to this and other herbicides, and it has been shown that there has been an increase in herbicide-resistant non-crop plants since the 1990s (see Fig. 4.1). There are 24 herbicide-resistant ‘weeds’ in the UK and in 1996 one was identified in Ireland, Stellaria media (common chickweed) (Heap, 2009). None of these plants are tolerant of, or resistant to, glyphosate and most are resistant to herbicides that are now prohibited (such as simazine) or to acetolactate synthase (ALS) inhibitor herbicides (such as metsulfuron-methyl).

Worldwide, a total of 347 ‘weeds’ have been recorded with an evolved resistance to certain herbicides. For example, of the chemicals listed in Table 4.1, there are 28 plants reported with resistance to synthetic auxins (2,4-D) and three with resistance to bromoxynil (Heap, 2009)\(^\text{18}\). Eighteen plants have an evolved resistance to glyphosate. Despite the affirmations of Franz et al. (1997), there has been some evolution of glyphosate resistance in an Australian ryegrass, which was the first to be documented, 20 years after the market release of glyphosate (Powles et al., 1998; Pratley et al., 1999). This was later noted in a South American related species (Perez and Kogan, 2003). While there is no evidence of natural dicot resistance to glyphosate (Duke and Powles, 2008b), some plants may tolerate it, being able to recover after an application of the herbicide. Such a plant is the field bindweed/morning glory (Convolvulus arvensis) (Bradshaw et al., 1997).


With the increased global use of HT crops, attention is now being paid to glyphosate resistance in non-crop plants. In Europe, there is one report of evolved resistance in a *Lolium* species in Spain and one report in a *Conyza* species in France (Powles, 2008). After an extensive review of the subject, Duke and Powles (2008b) and Powles (2008) conclude that diverse ‘weed’ management regimes, and not solely glyphosate-dependent ones, should prevent this issue becoming more serious. Such regimes may include the alteration of GM crops and non-GM crops in the cropping rotation (Heap, 1997), for example. Genetically modified HT crops may bring about resistant plants in a number of ways in addition to introgression to wild relatives and feral volunteers. First, plants that are naturally resistant to glyphosate may occupy the niche vacated by ‘weeds’ that are vulnerable to glyphosate, and in so doing may become new pest species. Second, glyphosate use may select for ‘weeds’ with a natural tolerance (evolved resistance). However, these issues are not new to land managers and are the same as those currently experienced in other herbicide regimes.

### 4.3 CINMa Assessment of GMHT Maize and Oilseed Rape

In a previous report on this topic (see: Mullins et al., 2009, Chapter 4), a new index of biodiversity stress and management impact assessment was designed to quantify the potential impact of GM crops (Collier and Mullins, 2011). This index, called CINMa, establishes both the potential impact of the management of GM crops in the landscape and is a general monitoring tool to meet the assessment demands of the EU regulations on the release of GM crops (European Commission, 2001). The index examines four key biodiversity stressors – Chemicals, Introgression, Nutrients, and Management – and grades their potential impact on four agri-environmental zones – the in-field area, nearby semi-natural habitats, the soil column and nearby watercourses. The index is calculated using the available data from similar landscapes and non-industrial sources of information. Tables 4.2 and 4.3 show the index as calculated for GMHT (winter) oilseed rape and GMHT maize, respectively. The values identified in Tables 4.2 and 4.3 reflect a quantitative assessment of the available peer-reviewed data for GMHT oilseed rape and GMHT maize, respectively.

Table 4.4 shows the range of scores in relation to the potential stress upon landscape biodiversity from the management of both GM crops. As can be seen, GMHT maize may have a net positive effect and GMHT oilseed rape may have a wider impact range. With this crop, as discussed earlier, there is a likelihood of introgression to wild relatives. However,
Ecological impacts of cultivating GMHT oilseed rape and maize

The negative score may be balanced by the positive impact of the management of this crop, as GM crops are designed for use with systemic and low-impact herbicides (e.g. glyphosate).

Table 4.5 shows the range of scores that illustrate the potential impact on the landscape zones. As can be seen, both crops are shown to have a potentially beneficial impact in these areas.

4.4 Pipeline Crops

Finally, it is worth noting that this report covers just a single aspect of a whole range of modifications that are in the development pipeline (Table 4.6). While most of the maize and oilseed rape crops in the development pipeline continue to be modified for insect resistance, the move is also towards drought resistance, enhanced nutritional content, nitrogen-use efficiency and/or stacking for herbicide and insect resistance. The list of pipeline crops is in constant flux as new products arrive and older ones are removed. The general move worldwide in GM crops is towards stacked events and while the current crops are mostly designed to be pest or herbicide resistant, future crops will also have enhanced composition, enhanced yield, resistance to abiotic stress (drought) and modified protein output (Lheureux et al., 2003). In Ireland, the crops that are likely to be attractive will be those which reduce nutrient and other chemical inputs, enhance...
Table 4.4. CINMa\(^1\) – biodiversity stressor mean score range.

<table>
<thead>
<tr>
<th>CINMa score</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize – HT</td>
<td>1 0.75</td>
<td>0.5 0.25</td>
</tr>
<tr>
<td>Oilseed rape – HT</td>
<td>0</td>
<td>0.25 0.5 0.75 1</td>
</tr>
</tbody>
</table>

\(^1\)Chemicals (C), Introgression (I), Nutrients (N), and Management (Ma).

The shaded cells represent the range of scores taken from the ‘Biodiversity Stressor Total’ rows in Tables 4.2 and 4.3.

Table 4.5. CINMa\(^1\) – zone mean score range.

<table>
<thead>
<tr>
<th>CINMa score</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize – HT</td>
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</tr>
<tr>
<td>Oilseed rape – HT</td>
<td>0</td>
<td>0.25 0.5 0.75 1</td>
</tr>
</tbody>
</table>

\(^1\)Chemicals (C), Introgression (I), Nutrients (N), and Management (Ma).

The shaded cells represent the range of scores taken from the ‘Zone total’ columns in Tables 4.2 and 4.3.

Table 4.6. Crops grown in Ireland or suitable to future Irish agronomic conditions and are currently in the development pipeline (due between 2010 and 2015) (adapted from Stein and Rodriguez-Cerezo, 2009).

<table>
<thead>
<tr>
<th>Crop</th>
<th>Developer/Country</th>
<th>Product name</th>
<th>Event namegenes</th>
<th>Trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>Monsanto</td>
<td>n/a</td>
<td>LY038(^1)</td>
<td>Crop composition – high lysine content</td>
</tr>
<tr>
<td></td>
<td>Syngenta</td>
<td>n/a</td>
<td>3272(^1)</td>
<td>Crop composition – amylase content</td>
</tr>
<tr>
<td></td>
<td>Pioneer Hi-Bred</td>
<td>Optimum GAT</td>
<td>90140</td>
<td>Herbicide tolerance – ALS inhibitors and glyphosate</td>
</tr>
<tr>
<td></td>
<td>China</td>
<td>n/a</td>
<td>n/a</td>
<td>Crop composition – high lysine content</td>
</tr>
<tr>
<td></td>
<td>China</td>
<td>n/a</td>
<td>n/a</td>
<td>Crop composition – phytase enzyme</td>
</tr>
<tr>
<td></td>
<td>Monsanto</td>
<td>n/a</td>
<td>MON87754</td>
<td>Crop composition – high oleic content</td>
</tr>
<tr>
<td></td>
<td>Dow Agro Sciences</td>
<td>DHT</td>
<td>n/a</td>
<td>Herbicide tolerance</td>
</tr>
<tr>
<td></td>
<td>BASF Plant Science</td>
<td>NutriDense</td>
<td>n/a</td>
<td>Crop composition – protein, amino acid and phytase content</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oilseed rape</td>
<td>Bayer Crop Science</td>
<td>n/a</td>
<td>n/a</td>
<td>Herbicide tolerance</td>
</tr>
<tr>
<td></td>
<td>Bayer Crop Science</td>
<td>n/a</td>
<td>n/a</td>
<td>Crop composition – oil content</td>
</tr>
<tr>
<td></td>
<td>BASF Plant Science</td>
<td>n/a</td>
<td>n/a</td>
<td>Crop composition – fatty acid content</td>
</tr>
<tr>
<td></td>
<td>BASF Plant Science</td>
<td>n/a</td>
<td>n/a</td>
<td>Crop composition – oil content</td>
</tr>
<tr>
<td>Potato</td>
<td>BASF Plant Science</td>
<td>Amflora</td>
<td>EH92-527-1</td>
<td>Crop composition – amylopectin content</td>
</tr>
<tr>
<td></td>
<td>Tecnoplant</td>
<td>n/a</td>
<td>SY230(^1)</td>
<td>Virus resistance – to potato virus Y</td>
</tr>
<tr>
<td></td>
<td>Tecnoplant</td>
<td>n/a</td>
<td>SY233(^1)</td>
<td>Virus resistance – to potato virus Y</td>
</tr>
<tr>
<td>India</td>
<td>n/a</td>
<td>RB</td>
<td></td>
<td>Disease resistance – to late blight</td>
</tr>
<tr>
<td>India</td>
<td>n/a</td>
<td>Nt-Inhh, iIRINV</td>
<td></td>
<td>Reduction in cold-induced sweetening</td>
</tr>
<tr>
<td>India</td>
<td>n/a</td>
<td>A20 oxidase</td>
<td></td>
<td>Dwarfness</td>
</tr>
<tr>
<td>AVEBE</td>
<td>Cisgenic</td>
<td>n/a</td>
<td></td>
<td>Crop composition – starch content</td>
</tr>
</tbody>
</table>

\(^1\)Authorised for use but not commercialised (2009).

ALS, acetolactate synthase.
crop quality and protein content, and resistance to abiotic stresses (O’Brien and Mullins, 2009).

4.5 Conclusions

Farming systems are dynamic in nature – they are constantly being changed and updated as new technologies become available and with movements in the market and regulation regimes. Glyphosate is not the sole purview of agricultural usage, though agricultural production may be the majority user. Glyphosate is freely available without regulation to the general public for domestic usage and to other land managers such as local authorities, rail companies and so on. ‘Weed’ resistance or tolerance may be more noticed in farmed landscapes but there is no concern in the literature of evolved resistance in domestic or parkland sites, though continual usage in urban and peri-urban landscapes is just as likely to stimulate evolved resistance as it is in agricultural landscapes. This inconsistency in scientific concern may have more to do with production economics than environmental realities.

This chapter has examined the potential impact of the modified management systems on NTOs and on elements of biodiversity in the landscape. Available data have shown that the chemical toxicity of glyphosate is comparably low under agronomic conditions and that the use of glyphosate in agri-environmental management regimes has little or no impact on the biodiversity of soils and water. The recorded effects of glyphosate on NTOs is similarly low and though there is the possibility of an evolved ‘weed’ resistance to glyphosate this may be managed using traditional farm management techniques. The CINMa index shows that for GMHT oilseed rape, as discussed earlier, there may be a slightly negative impact on biodiversity and this may be offset by other, more positive effects of long-term management regime change. For GMHT maize the effects of management may also have a long-term positive effect.
5 Conclusions

Changes and intensification of agricultural activity over the decades have brought about significant alterations in land management practices and landscape change. This has had a largely negative effect on species and habitats, but in Ireland it has also created the landscapes that are familiar today. With the global area of GM cropping increasing and with the ever-growing demand for food from an expanding population, it is inevitable that farmers will soon be afforded the choice as to whether or not they wish to avail of GM seeds in the coming years. Herbicide tolerance will be one of the first traits available and, with its uptake, the management practices of Irish farmers will change. These changes will include herbicide application timing and application rates (frequency of spraying and herbicide combination), rotational management and land-cover management. This report indicates that such land management changes may be of a more long-term benefit to overall landscape biodiversity.

From an initial exploration of the potential for genetic transfer to wild relatives, it was shown that there is no likelihood of this being an issue for maize, as it has no wild relatives in Ireland. In contrast, while there are many relatives of *B. napus* in Ireland (none of whom are considered to be native), gene flow from *B. napus* (oilseed rape) to *B. rapa* will occur. So, while gene flow from *B. napus* to other closely related species is unlikely, with the introduction of GMHT oilseed rape, there may be some genetic transfer to *B. rapa* populations. However, it is unknown if these will persist or prevail in the Irish landscape to the extent that they may present a threat to biodiversity. However, the management of non-crop areas in Ireland does not entail the use of glyphosate, and therefore this will negate any potential selection of those plants with a GMHT trait.

In order to envisage how the agri-environmental landscape may change with the introduction of GMHT oilseed rape and maize, it was necessary to investigate the potential for longevity of the enhanced traits within non-crop populations. Using a series of five hypothetical (‘worst-case’) scenarios, it was demonstrated that if GMHT crops were introduced, and if the correct management regimes were adopted to farm them, there would be no greater likelihood of enhanced traits persisting in the landscape than may be occurring under current management practices. In any event, there is no evidence of any potential for invasiveness and it was shown that repeated backcrossing (of *B. napus*) can result in the GM trait becoming diluted in wild populations. There is an obvious presence of feral *Brassicae* in marginal habitats (roadsides, rail embankments, etc.) today, but this has not been deemed to be of concern from an invasive species or biodiversity threat point of view and thus there are no proscribed actions for this.

Currently, available data do not show that the introduction of GMHT crops may have any increased detrimental effect on biodiversity in Ireland. Glyphosate has been shown to be a low toxic threat and extensive research has failed to demonstrate that it has a long-term negative impact on soil and water biodiversity. In contrast, by using the newly created CINMa index, it is shown that such an introduction may have a net benign effect with the reduction of farm management intensity and chemical toxicity over time. This assumption requires field validation before any conclusive deductions can be made. This will entail a series of field trials.

It should also be noted that with the introduction of oilseed rape varieties that are resistant to IMI (a herbicide that is slightly more toxic than glyphosate and glufosinate), as is currently planned, there will be the same perceived threats. However, because this crop was not derived through recombinant technology (necessitating the regulation requirements of GM crops in Europe), it will therefore not be subject to any introgression and selection trials, any case-specific impact monitoring or any general surveillance post-commercialisation as is the case for GM crops. This policy deficit needs to be addressed as, while this is only one case, it should be borne in mind that a plethora of new and specifically designed crops (not derived using GM technology) will become available in
the near future. With commitments to the Convention on Biological Diversity (CBD, 1992) and the continuing concerns over invasiveness, it is critical that an evaluation and monitoring programme for these crops and their associated management regimes be established prior to their release.
References


Brown, J., Brown, A.P., Davis, J.B. and Erickson, D.,


Crawley, M.J. and Brown, S.L., 1995. Seed limitation and the dynamics of feral oilseed rape on the M25


Ecological impacts of cultivating GMHT oilseed rape and maize

Teamann: The Irish Journal of Agri-Environmental Research 1: 79–94.


## Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS</td>
<td>Acetolactate synthase</td>
</tr>
<tr>
<td>Bt</td>
<td>Insect resistance</td>
</tr>
<tr>
<td>CBD</td>
<td>Convention on Biological Diversity</td>
</tr>
<tr>
<td>CINMa</td>
<td>The CINMa index examines four key biodiversity stressors – Chemicals, Introgression, Nutrients, and Management – and grades their potential impact on four agri-environmental zones – the in-field area, nearby semi-natural habitats, the soil column and nearby watercourses.</td>
</tr>
<tr>
<td>EIQ</td>
<td>Environmental Impact Quotient</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental protection Agency</td>
</tr>
<tr>
<td>ERTDI</td>
<td>Environmental Research Technological Development and Innovation</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>FSE</td>
<td>Farm-scale evaluation</td>
</tr>
<tr>
<td>GM</td>
<td>Genetically modified</td>
</tr>
<tr>
<td>HT</td>
<td>Herbicide tolerant</td>
</tr>
<tr>
<td>IMI</td>
<td>Imidazolinone</td>
</tr>
<tr>
<td>NTO</td>
<td>Non-target organism</td>
</tr>
<tr>
<td>USEPA</td>
<td>United States Environmental Protection Agency</td>
</tr>
</tbody>
</table>
An Ghníomhaireacht um Chaomhnhú Comhshaol

Is í an Gníomhaireacht um Chaomhnhú Comhshaol (EPA) comhlachtá reachtúil a chosnaionn an comhshaoil do mhuintir na tire go léir. Rialaímid agus déanaimid maoiriú ar ghníomhachtaí a d’fhéadfadh truailláit a chruthú murach sin. Cinnímid go bhfuil eolas cruinn ann ar threochtait a comhshaoil ionsa go nglactar aon chéim is gá. Is iad na príomh-nítithe a bhfuilnúimíodhdh ghníomhach leo ná comhshaoil na hÉireann a chosaint agus cinntiú go bhfuil forbairt inbhunaithe.

Is comhlacht poiblí neamhspleách í an Ghníomhaireacht um Chaomhnhú Comhshaol (EPA) a bunadhodh i mí Iúil 1993 faoin Acht fán nGníomhaireacht um Chaomhnhú Comhshaol 1992. Ó thaobh an Rialtais, is í an Roinn Comhshaol agus Rialtais Áitiúil a dhéanann ciúnacht uirthi.

RIALÚ ASTUITHE GÁIS CEAPTHA TEASA NA HÉIREANN
- Cúimniúchótt astuithe gáis ceaptha teasa na hÉireann ná ghearrthóirí a chéile in gcomhthéacs ár dtiomantas Kyoto.
- Cuireadh an bpriomhacht na Treorach um Thrádáil Astuithe, a bhfuil baint aige le híos cionn 100 cuideachta atá ina mór-ghinearáidí dé-oiseáid charbhóin in Éirinn.

TAIGHDE AGUS FORBAIRT COMHSHAOL
- Taighde ar shaolcheisteanna comhshaoil a chomhshaoil (cosúil le caighdeán aerí agus uisce, athrú aeráide, bithéagsúlacht, teicneolaíocht comhshaoil).

MEASÚNÚ STRAITÉISEACH COMHSHAOL
- Ag déanamh measúnú ar thionchar phleananna agus chláracha ar comhshaoil na hÉireann (cosúil le plé a phleanann bainistíochta drámaíola agus forbartha).

PLEANÁIL, OIDEACHAS AGUS TREGAIR COMHSHAOL
- Treoir a thabhairt don phobal agus do thionscail ar cheisteanna comhshaoil a éagúsula (m.sh., iarratais a cheadhúnais, seachaint drámaíola agus rialacháin comhshaoil).  
- Eolas níos fearr ar an gcomhshaoil a scaipeadh (trí cláracha teifísithe comhshaoil agus pacáistí acmhainne do hbrayscleanna agus do mheánscoileanna).

BAINISTÍOCHT DRAMHAÍOLA FHOIRGHníOMHACH
- Cur chuimhneachaint agus laghdú drámaíola trí chomhshaoil An Chláir Náisiúnta um Chosc Dramhaíola, lena n-áirítear cur i bhfeidhm na dTionscnamh Feachtachta Taighdeoirí.
- Cuir i bhfeidhm Rialachán ar nós na treoracha maidir le Trealmh Leictreach agus Leictreoneach Caite agus le Srianadh Substaintí Guaiseacha agus substaintí a dhéanann idió ar an gcroíos ósóin.
- Plean Náisiúnta Bainistíochta um Dramháil Ghuaiseach le hFeachtas a thabhairt gach mheánscoile as a sheachaint agus a bhainistíú.

STRUCTÚR ÁR NA GNÓNIOMHAIREACHT
Bunaodh an Ghníomhaireacht i 1993 chun comhshaoil na hÉireann a chosaint. Tá an eagraíocht a bhall an sruth agus ceartach na sruth a bhall i bhfeidhm na scothríu amach a bhfuil scéal f LEVEL 6.
Science, Technology, Research and Innovation for the Environment (STRIVE) 2007-2013

The Science, Technology, Research and Innovation for the Environment (STRIVE) programme covers the period 2007 to 2013.

The programme comprises three key measures: Sustainable Development, Cleaner Production and Environmental Technologies, and A Healthy Environment; together with two supporting measures: EPA Environmental Research Centre (ERC) and Capacity & Capability Building. The seven principal thematic areas for the programme are Climate Change; Waste, Resource Management and Chemicals; Water Quality and the Aquatic Environment; Air Quality, Atmospheric Deposition and Noise; Impacts on Biodiversity; Soils and Land-use; and Socio-economic Considerations. In addition, other emerging issues will be addressed as the need arises.

The funding for the programme (approximately €100 million) comes from the Environmental Research Sub-Programme of the National Development Plan (NDP), the Inter-Departmental Committee for the Strategy for Science, Technology and Innovation (IDC-SSTI); and EPA core funding and co-funding by economic sectors.

The EPA has a statutory role to co-ordinate environmental research in Ireland and is organising and administering the STRIVE programme on behalf of the Department of the Environment, Heritage and Local Government.