ASSESSING THE IMPACT OF POLLEN-MEDIATED GENE FLOW FROM GM HERBICIDE TOLERANT *BRASSICA NAPUS* INTO COMMON WILD RELATIVES IN IRELAND

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ABSTRACT

Although now we have had many years of research completed on assessing the potential environmental impact of GM crops, concern remains over their potential impact on biodiversity in the rural landscape. In particular, issues have arisen in regards to the modification of crops with traits that could introgress into sexually compatible wild relatives. In contrast to wheat, barley, potato and maize, *Brassica napus* (oilseed rape) is the only commercial crop grown in Ireland at present with the potential to successfully transfer its DNA, via pollen-mediated gene flow, into inter-related weed species. This review details the species in question and by examining the relevant literature that relates to Irish agronomic conditions, demonstrates that gene flow is likely to occur, especially to an earlier used cultivar, *Brassica rapa*. However, the critical factor remains not that GM traits will flow from the commercial source but what might the consequences of said gene flow events be. This review indicates that the conferred trait in question (in this case, herbicide tolerance) can only impact on weed diversity in the presence of selecting herbicide action. In the absence of the herbicide, the GM traits will be lost from the wild species over time and will not confer any selective advantage that could facilitate population growth.

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INTRODUCTION

The management of agricultural landscapes is in continual flux and future land use dynamics 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 of cultivating GM crops suited to the Irish tillage sector. Although the *de-facto* EU moratorium on the planting of GM crops was lifted in 2004, GM cropping has not been established in Ireland principally due to the lack of varieties suited to the Irish agri-environment. Those traits that would be most suited include disease resistance and herbicide tolerance, with the latter typically conferred through resistance to the broad spectrum herbicide glyphosate. However, 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 conditions (O'Brien and Mullins 2009). Herbicide tolerant (HT) oilseed rape is a crop that would be most applicable to Irish tillage systems; enabling 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 (O'Brien and Mullins 2009). However, while GM HT oilseed rape would increase the flexibility of the cropping regime for the farmer, issues are often raised in regards to the level of risk associated with the escape of a HT trait into wild related species of the crop in question. Although alterations and innovations in GM crop management schemes has the potential to reduce the impact of the crops on the wider landscape (Mullins *et al.* 2009), the potential for genetic introgression of a GMHT trait into Irish wild relatives has yet to be gauged.

When looking at the potential impact of any GM plant in the Irish flora it is necessary to outline the current state of knowledge, but previous studies have demonstrated the lack of research on the impact of crops on the Irish agri-environment (O'Brien *et al.* 2008). Flora surveys are likewise poor. For many years, multiple surveys of the Irish flora have been carried out (Kollmann *et al.* 2009), but a high proportion are localized and place-specific, and mainly show presence/absence data only. 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 (S.I. no. 274), later amended in 1999 (S.I. no. 94) (Government of Ireland 1999). However, these are not necessarily reliable accounts of species abundance or of the modern distribution of fera 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, none are feral crops. This review article is based on research carried out as part of the EPA funded STRIVE programme (Mullins and Collier 2011), tasked specifically to look at the potential impact of GMHT oilseed rape on rural biodiversity in Ireland. It is a critical assessment of the published literature and available data on this subject.

BRASSICA NAPUS IN *IRELAND*

Although *Brassica Napus* a globally important oil crop, it has been predicted that Irish oilseed rape hectarage could increase to 20,000ha by 2018 in order to supply high-value food, feed and industrial ingredients to the domestic market (Fig. 1, Teagasc 2008). Under certain conditions, oilseed rape can prevail locally after harvesting and persist in the following years as volunteers within the confines of a managed cropping system and as feral individuals in semi-wild habitats (e.g. roadways and hedgerows). Studies have shown that it can survive outside agronomic zones for up to five years (Lutman 1993) and possibly up to nine in certain circumstances (Lutman *et al.* 2005). Anecdotally *B. napus* is believed to be ubiquitous in the Irish landscape, prevailing as feral volunteers from agricultural activities. As these populations are coexisting with inter-related weed species, it is important to ask: if GMHT oilseed rape feral/volunteer populations emerge, can they act as a reservoir for the transfer of a HT trait into wild relatives and how long such a modified trait can persist. It is also necessary to examine this under modern agronomic conditions as the management of the crop can have as big an impact on biodiversity as the crop itself (Mullins *et al.* 2009).

PREVALENCE OF WILD BRASSICACEAE IN IRELAND AND POTENTIAL FOR GENE FLOW

The phenomenon of gene flow into wild *Brassiceae* from cultivated *B. napus* has been researched widely, with work dating to long before GM crops were commercialized (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 outside of Ireland 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 three years and concluded that the transfer of herbicide tolerance 'appears to be minimal' (p. 20). Ellstrand *et al.* (1999) state that while hybridization within the *Brassica* family is very common there are no implications for either the 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).

However, it is important to consider the potential for weeds to evolve resistance to commercial herbicides (Waltz 2010) and worldwide a total of 360 'weeds' biotypes (197 species) with an evolved resistance to modern herbicides have been recorded (Heap 2012). Given that resistant weeds are the direct result of herbicide overuse and the lack of an integrated weed management strategy (Egan *et al.* 2011), it is not surprising to see the epidemic of glyphosate-resistant weeds that has resulted from the overuse of glyphosate herbicide in the USA (Wright *et al.* 2010). There are 24 herbicide resistant 'weeds' in the UK and in 1996 one was identified in Ireland, *Stellaria media* (common chickweed). None of these plants are tolerant of, or resistant to, glyphosate and most are resistant to herbicides that are now prohibited (such as simizine) or to acetolactate synthase inhibitor herbicides (such as metsulfuron-methyl to which *S. media* has been found to be resistant). Nonetheless, when considering the potential hybridization between GMHT oilseed rape and its wild relatives it is prudent to bear in mind that the use of a GMHT variety must be implemented in parallel with a robust weed management strategy that incorporates both a diversity of chemical and nonchemical practices to reduce reliance on single herbicide chemistries.

Gene flow between *Brassica rapa*, *Brassica oleracea* and *Brassica nigra* as parents and *B. napus*, *Brassica juncea* and *Brassica carinata* as hybrids is well documented (U. 1935 (U's Triangle); Gill and Vear 1966) and the development and use of plastid (Flannery *et al.* 2006) and nuclear markers has clarified the origin of the amphidiploid *B. napus* (Allender and King 2010) and *Raphanus sativus*, which it is hypothesized was derived from a hybridization event between the *rapa/oleracea* and the *B. nigra* lineages of

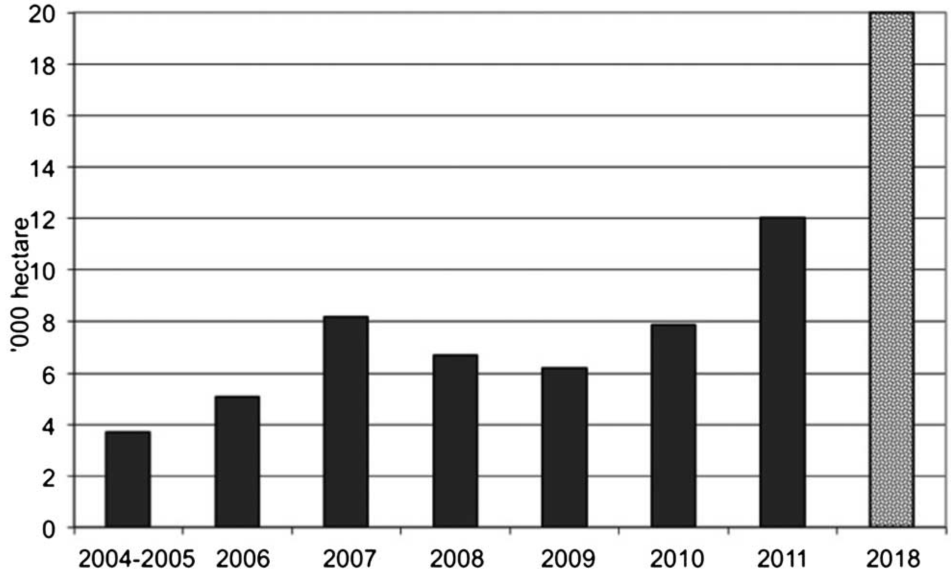

Fig. 1—Current (solid bars) and predicted (shaded bar) status of oilseed rape (in '000ha) (CSO 2010). Projections to 2018 and the projected percentage change from 2004 to 2018 (Teagasc 2008).

the *Brassica* genus (Yang *et al.* 2002). Though 'naturally occurring' *Brassica* hybrids have not been specifically recorded (Daniels *et al.* 2005, p. 20) it must be recognized that it is relatively difficult to identify hybrid *Brassicas* in a natural landscape. Allainguillaume *et al.* (2006) have examined the fitness of *B. napus* and *B. rapa* hybrids in non-GM crosses and shown that 'substantially fewer' descendants will exhibit any traits that will permit the hybrids to persist. There are numerous studies of *B. napus* gene transfer to wild or feral species in the Netherlands (de Vries *et al.* 1992), Canada (Beckie *et al.* 2006), Australia (Salisbury 2002) and the US (Brown *et al.* 1997), countries where GM crops are currently in cultivation, 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 establish the potential inter-related species of *B. napus* that may be present within the Irish agri-environment, and thus those that may be impacted upon by the flow of genetic material from the future cultivation of GMHT oilseed rape. Hypothetically there are around 100 species capable of hybridizing with *B. napus* (Eastham and Sweet 2002), most of whom have been demonstrated using in-hand or similarly forced experimentation techniques. As these hybridizations do not usually 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. There are sixteen cruciferous species (*Brassicaceae*), which are believed to exist in or near agricultural landscapes in Ireland and are close relatives of *B. napus* (Table 1). The 'rare' and 'occasional' species were mostly identified in urban waste areas and have been assumed to be discarded food plants (Scannell and Synott 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 sixteen species, one is 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 is more likely to have been introduced at some time by accident (Guiry and Guiry 2012). The remaining relatives of *B. napus* are also either deliberately or accidentally introduced (Reynolds 2002; Guiry and Guiry 2012), but these are now classed as 'naturalised' to some degree. Of these listed species, seven have been the subject of the vast majority of research into gene flow in other jurisdictions (Table 2) and have direct relevance to this discussion because the remaining eight have not been researched to any significant extent. However, very few detailed studies have been carried out on gene flow between *B. napus* and either *Brassica alba* or *B. nigra* (FitzJohn *et al.* 2007).

Hybridization between *B. napus* and *B. juncea* under field conditions has been demonstrated (Jørgensen and Andersen 1994). Others show hybrid pollen fertility under laboratory conditions and affirm that the possibility of wild hybridization 'cannot be neglected' (Frello *et al.* 1995, p. 240), though they do not demonstrate this in field trials. Some hybridization events have been recorded in both directions, with female *B. napus* being less successful (Jørgensen *et al.* 1998). Seedling survival is usually low and it has not been shown if any surviving seeds are viable. However, *B. juncea* is

Table 1—List of 16 species found in the 'wild' in Ireland closely related to *Brassica napus*.

BRASSICACEAE (Cruciferae)	*Common name*	*Location*	*Abundance*[a]	*Status*[a]
Brassica 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 juncea[b]	Chinese/brown mustard	Ports, roadsides, (probably introduced with grain feed)	Rare	Introduced
Brassica nigra[b]	Black mustard	Shingle, coastal locations in southern part of Ireland	Rare	Probably introduced
Brassica oleracea[b]	Wild cabbage	Cultivation escape, landfills, waste areas, cliffs (SE UK only)	Rare	Introduced
Brassica rapa (*Brassica campestris*)[b]	Wild turnip	Roadsides, ports, disturbed ground, mostly in southern Ireland esp. near coasts, UK: riverbanks/lakesides	Locally abundant	Probably introduced
Brassica tournefortii	Pale cabbage	Dublin port	Rare	Introduced
Diplotaxis muralis	(Annual) Wall mustard	Railways, dry banks, walls, cliffs, sandbanks	Occasional	Introduced/ invasive
Diplotaxis tenuifolia	(Perennial) Wall rocket	Waste ground, disturbed ground	Rare	Introduced
Eruca vesicaria	Garden rocket	Landfill, waste ground, disturbed ground	Rare	Introduced
Hirschfeldia incana (*Brassica adpressa*)[b]	Hoary mustard	Dublin mainly, disturbed ground, gravel, roadsides, landfills (introduced with grain feed)	Occasional	Introduced/ invasive
Raphanus raphanistrum subsp. *raphanistrum*[b]	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 arvensis[b]	Charlock/white mustard	Cultivated ground, disturbed ground, roadsides, landfills	Occasional	Possibly introduced

[a]Sources: Scannell and Synnott (1972), Scheffler and Dale (1994), Webb, *et al.* (1996), Eastham and Sweet (2002), Reynolds (2002) and Guiry and Guiry (2012). It would, perhaps, be more appropriate to delineate their status as being 'native', 'neophyte', or 'archaeophyte' etc., but the sources for these species listed here do not list them as such, nor are definitions of such status universally agreed upon (Dunn and Heneghan 2011). It is perhaps better to regard the classifications used here in line with Preston *et al.* (2004), who suggest that that their presence is more an hypotheses to be tested in specifically designed studies, something lacking in the Irish landscape.
[b]Species that have been the subject of the majority of published introgression research outside Ireland and thus may be species of concern (Flannery *et al.* 2005).

'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. Laboratory hybridization between *B. napus* and *B. oleracea* has been partially successful (Kerlan *et al.* 1992), and though possible in the wild, the likelihood of hybridization in the agricultural landscape is negligible. Where artificial hybridization has been forced under laboratory conditions, the resulting seeds were non-viable and/or malformed. Naturalized

Table 2—List of seven species of Brassicaceae that have a potential for hybridizing with *Brassica napus* in the Irish landscape.

Hybridization potential between B. napus and...	*Research conditions*	*Hybridization frequency*	*Rate of hybrid seedling survival*	*References*
Brassica juncea	Field trials	<3%	N/A	Jørgensen *et al.* (1998)
Brassica oleracea	Laboratory	None	<0.01%	Chiang *et al.* (1977)
Brassica nigra	Laboratory	0.1% (Male *B. napus* and female *B. nigra*)	0	Bing *et al.* (1996)
	Laboratory	0.9–3.4% (Male *B. nigra* and female *B. napus*)	<0.01%	Bing *et al.* (1991)
Hirschfeldia incana (*Brassica adpressa*)	Laboratory	1.3% (Male *B. napus* and female *H. incana*)	N/A	Kerlan *et al.* (1992)
	Laboratory	3.1% (Male *H. incana* and female *B. napus*)	N/A	Kerlan *et al.* (1992)
	Field trials	1.5% (Male *B. napus* and female *H. incana*)/70% (Male *H. incana* and female *B. napus*)	0.1%	Lefol *et al.* (1996b)
	Field trials	0.6%	0	Darmency and Fleury (2000)
	Field trials	0.06%	0.01%	Chadoeuf *et al.* (1998)
Raphanus raphanistrum	Laboratory	0.2% (Male *B. napus* and female *R. raphanistrum*)	<0.01%	Darmency *et al.* (1998)
	Field trials	<0.01% (Male *B. napus* and female *R. raphanistrum*)	0	Chèvre *et al.* (2000)
	Field trials	0.009% (Male *R. raphanistrum* and female *B. napus*)	0	Warwick *et al.* (2003)
Sinapis arvensis	Field trials	0.1% (Male *B. napus* and female *S. arvensis*)/1.2% (Male *S. arvensis* and female *B. napus*)	0	Lefol *et al.* (1996a)
	Field trials	0.0001% (Male *B. napus* and female *S. arvensis*)	0	Daniels *et al.* (2005)
Brassica rapa (*Brassica campestris*)	Field trials	0.4–1.5% (Male *B. napus* and female *B. rapa*)	2%	Scott and Wilkinson (1998)
	Field trials	0.0008	N/A	Daniels *et al.* (2005)
	Wild	7.1% (Male *B. rapa* and female *B. napus*)	N/A	Warwick *et al.* (2003)

populations of *B. oleracea* are mostly located on cliff habitats in the UK, which are poorly suited for hybrid *B. oleracea* × *B. napus* seedling success (Wilkinson *et al.* 2000; Chèvre *et al.* 2004).

Hybrids of *B. napus* and *B. nigra* have been produced using hand pollination, but no hybridization has been recorded under naturally occurring conditions. It has been shown that hybridization between *B. napus* and *Hirschfeldia incana* can occur in field trial conditions (Eber *et al.* 1994), and that there may be some seed dormancy, intermediate between parents (Chadoeuf *et al.* 1998). Backcrossing for five generations produced no viable plants (Darmency and Fleury 2000), thus it can be deduced that there can be little successful gene introgression between *B. napus* and *H. incana*. Hybridization between *B. napus* and *R. raphanistrum* has also occurred under field conditions (Eber *et al.* 1994) but with very low seedling emergence (Guéritaine *et al.* 2003). Integration of *B. napus* genes into *R. raphanistrum* was not observed by the third backcross (Chèvre *et al.* 1998). Similar results to experiments with *H. incana* were shown, after five years of backcross observation (Jørgensen 1999). Warwick *et al.* noted that successful hybridization was 'extremely rare' (2003, p. 536) which mirrors similar conclusions from earlier research by Chèvre *et al.* (1997). Open pollination between *B. napus* and *Sinapis arvensis* did not produce viable hybrids (Bing *et al.* 1991; Lefol *et al.* 1991) and it is concluded that spontaneous hybridization is unlikely to be successful under natural conditions (Bing *et al.* 1996). 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 to be sexually incompatible under natural conditions (Downey 1999; Eastham and Sweet 2002) and there is an 'extremely low probability' of viable hybridization occurring (Moyes *et al.* 2002, p. 103).

In contrast, hybridization between *B. napus* and *B. rapa* is believed to be a common event and most of the research on this subject derives from GM trials. Sweet *et al.* (1999) examined in-crop persistence and prevalence of *B. napus* × *B. rapa* hybrids and deduced that persistence was 'not enhanced by specific genetic modification' (Sweet *et al.* 1999 online). This was similar to other studies (Crawley). However, introgression in the natural landscape was extensively noted in the UK (Norris *et al.* 2004), where *B. rapa* is relatively common on riverbanks in some locations. Norris and Sweet (2002) note that while hybrids of *B. napus* and *B. rapa* are fertile, their seed abundance is relatively low. Norris *et al.* (2004) estimate that non-GM introgression may have occurred over many years.

It is clear that *B. rapa* is the principal 'wild' relative that can hybridize with *B. napus* (Scheffler and Dale 1994). It is believed that there are two subspecies in the Irish landscape. The first subspecies is a 'weedy' remnant of agricultural activity, *B. rapa* subsp. *oleifera*, and this may be found within the *B. napus* crop rotation system. Since *B. napus* cultivation is often preceded and/or followed by a cereal crop (such as wheat) *B. rapa* is managed using herbicides targeted for broadleaf weeds. However, when *B. napus* is cultivated *B. rapa* may reappear within the crop. A second, 'wilder' subspecies of *B. rapa* (subsp. *sylvestris*) may be found in semi-natural habitats, mostly riverbanks, canals and lakesides (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, naturalized, invasive or introduced.). Due to its similar morphology to *B. napus*, *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 (though it is believed to be absent from Northern Ireland (Wilkinson *et al.* 2003)). Indeed, the two subspecies 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 *New Flora of the British Isles* (2nd edn) (Stace 1991).

However, there is some evidence from agri-environmental researchers and farmers that *B. rapa* is locally abundant in marginal landscapes in southern Ireland. The flowering time of *B. napus* (both Winter and Spring varieties) has been shown to overlap with the flowering time of some of its wild relatives, especially *B. rapa* (Colbach *et al.* 2008). Flanagan (2010)) examined co-synchronous flowering of these *Brassicae* in Leinster, also showing a continuous overlap between the flowering periods of two plants and in outdoor experimental conditions recorded a hybridization rate of *c.* 6%. Cloney (2003) has also sampled *B. rapa* growing outside cultivated areas in Southern Ireland and shows that these populations may have interbred with early oilseed varieties, but that under ecological conditions this was of no advantage to these wild populations.

Thus, gene flow from *B. napus* to *B. rapa* can be expected (Lutman *et al.* 2004; Begg *et al.* 2006), but is there likelihood that such a crop-to-wild relative hybrid population will prevail? Traits such as herbicide tolerance (from GMHT *B. napus*) have been shown to gradually diminish over time within hybrid populations (Warwick *et al.* 2008). Gene flow to the other six near-relatives (*B. juncea*, *B. oleracea*, *B. nigra*, *H. incana*, *R. raphanistrum* and *S. arvensis*), while technically possible (via artificial hybridization techniques), is unlikely to give rise to a persistent or prevalent population of hybrids in Ireland. Table 2 illustrates the principal trials in this area and shows 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.

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 in the coming years will soon be afforded a choice as to whether they wish to avail of specific GM varieties. Herbicide tolerance will be one of the first traits available and with its uptake, the management practices of Irish farmers will change, and despite commonly held beliefs, such land management changes may be of a more long-term benefit to overall landscape biodiversity (Mullins *et al*. 2009; Collier and Mullins 2010; Mullins and Collier 2011).

Whereas the distribution of *B. rapa* in Ireland is unknown it is anecdotally assumed to be widespread, but the lack of baseline data impedes functional calculations on rates of hybridization between *B. napus* and *B. rapa*. Furthermore, while gene flow from GMHT *B. napus* gene flow into *B. rapa* will occur, the HT trait will not persist unless selected for by the use of the relevant herbicide (Wilkinson *et al*. 2003). Although, there may be many relatives of *B. napus* in the Irish agri-environmental landscape, and these are likely to be neophytes or even archaeophytes (and there is now a requirement for this to be explored in detail), the potential for successful gene flow from *B. napus* to these relatives is negligible. Finally, it should be borne in mind that there are now a plethora of specifically designed crops that are *not* derived using GM technology but will express a novel phenotype (e.g. herbicide tolerance). Due to the current regime for authorizing crops within the EU, this material does not require a license, although it will be exhibiting the same trait as per a GM-derived variety. In light of this scenario, it is therefore critical that an evaluation and sufficiently robust monitoring programme for all crops and their associated management regimes be established.

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