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## **TITLE**

Feral genetically modified herbicide tolerant oilseed rape from seed import spills: are concerns scientifically justified?

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## ABSTRACT

One of the concerns surrounding the import (for food and feed uses or processing) of genetically modified herbicide tolerant (GMHT) oilseed rape is that, through seed spillage, the herbicide tolerance (HT) trait will escape into agricultural or semi-natural habitats, causing environmental or economic problems. Based on these concerns, three EU countries have invoked national safeguard clauses to ban the marketing of specific GMHT oilseed rape events on their territory. However, the scientific basis for the environmental and economic concerns posed by feral GMHT oilseed rape resulting from seed import spills is debatable. While oilseed rape has characteristics such as secondary dormancy and small seed size that enable it to persist and be redistributed in the landscape, the presence of ferals is not in itself an environmental or economic problem. Crucially, feral oilseed rape has not become invasive outside cultivated and ruderal habitats, and HT traits are not likely to result in increased invasiveness. Feral GMHT oilseed rape has the potential to introduce HT traits to volunteer weeds in agricultural fields, but would only be amplified if the herbicides to which HT volunteers are tolerant were used routinely in the field. However, this worst-case scenario is most unlikely, as seed import spills are mostly confined to port areas. Economic concerns revolve around the potential for feral GMHT oilseed rape to contribute to GM admixtures in non-GM crops. Since feral plants derived from cultivation (as distinct from import) occur at too low a frequency to affect the coexistence threshold of 0.9% in the EU, it can be concluded that feral GMHT plants resulting from seed import spills will have little relevance as a potential source of pollen or seed for GM admixture. This paper concludes that feral oilseed rape in Europe should not be *routinely* managed, and certainly not in semi-natural habitats, as the benefits of such action would not outweigh the negative effects of management.

## INTRODUCTION

The global area cropped with genetically modified (GM) crops has consistently increased each year since they were first commercially cultivated in 1996 (1.7 million hectares), reaching 148 million hectares in 2010 (James, 2010). The advent of GM crops and their rapid expansion in terms of cropping area evoked intense debate about their safety (Hails, 2000; Devos et al., 2008b; Waltz, 2009; Gaskell et al., 2011). Concerns have been raised that the commercial release of GM crops could result in adverse environmental and economic effects.

Environmental concerns include the potential for altered fitness of the crop itself, and of its wild relatives as a result of gene flow. An increased fitness may enable plants with the GM trait to be more invasive of semi-natural and natural areas with unwanted impacts on valued species and agro-ecosystem integrity, or to be more persistent (weedier) in agricultural habitats, exacerbating a weed problem (EFSA, 2010). Alternatively, and depending on which plant and which transgenes are involved, gene flow to wild relatives may decrease the fitness of hybrid offspring. If rates of gene flow are high, this may cause wild relatives to decline locally, or to become extinct (e.g., swarm effect, outbreeding depression) (Ellstrand, 2003).

The main economic concerns are the impurity GM crops would bring if admixed with non-GM crops, and the costs of the implemented coexistence measures to ensure the side-by-side development of GM and non-GM cropping systems (Demont and Devos, 2008; Devos et al., 2009b). In the European Union (EU), an adventitious or technically unavoidable presence of approved GM material in non-GM food and feed products up to a level of 0.9% is accepted (EC, 2003b). If the content of GM material in a non-GM product exceeds this threshold, the product has to be labelled as containing GM material, which may affect its market acceptability. To preserve particular types of crop production, Member States can adopt tolerance thresholds lower than the previously set threshold of 0.9% (EC, 2010). Since GM crop production is currently considered the 'newcomer' in the European agriculture (EC, 2003a), GM crop adopters are requested by law to put coexistence measures in place to limit unintended GM admixtures, and to bear responsibility for redressing the incurred economic harm caused by GM admixing (Demont and Devos, 2008; Devos et al., 2009b).

The risks of GM oilseed rape (*Brassica napus* L.) have become particularly contentious in the context of the evaluation of market registration applications in the EU (Levidow and Carr, 2007). At present, three GM herbicide tolerant (HT) oilseed rape events (GT73, MS8 x RF3

and T45) are approved for import and processing for food and feed uses in the EU, and can be transported throughout all EU Member States in conformity with any conditions set out in the approval (EU Community Register: [http://ec.europa.eu/food/dyna/gm\\_register/index\\_en.cfm](http://ec.europa.eu/food/dyna/gm_register/index_en.cfm)). GT73 and MS8 x RF3, and T45 are approved for marketing in the EU until 2017 and 2019, respectively. GT73 is tolerant to the herbicidal active substance glyphosate (GLY), and MS8 x RF3 and T45 to glufosinate-ammonium (GLU). In addition to the HT trait, MS8 contains the *barnase* gene that confers male sterility, whereas RF3 contains the *barstar* gene that restores male fertility. None of the above GMHT oilseed rape events can be grown for commercial purposes in the EU, but some have been grown for experimental purposes, mainly in France, UK, Belgium, Germany and Sweden (EU SNIF Database: <http://gmoinfo.jrc.ec.europa.eu/>).

EU Member States are concerned about the spillage of seed during the import, transportation, storage, handling and processing of GMHT oilseed rape commodities. While most (GMHT) oilseed rape seed is imported by boat and crushed in or near the ports of entry in the EU, a fraction of it can be transported inland to small independent crushing facilities by boat, truck or rail. Some EU Member States contend that imported GMHT oilseed rape will escape and persist outside agricultural fields as feral plants and thereby mediate transgene movement among sexually compatible plants in the landscape (e.g., Levidow and Carr, 2007). The particular concerns related to feral GMHT oilseed rape fall within the range of general concerns stated above. They may cause a change in fitness, leading to invasion of semi-natural habitats, or to a colonisation of agricultural fields, where additional herbicide applications for weed control may be required due to the unintended stacking of HT traits. Feral GMHT oilseed rape plants may extend the potential for gene flow by acting as stepping stones and by forming populations that accumulate transgenes, thereby contributing to admixtures with commercially grown oilseed rape varieties. Based on such arguments, three EU Member States invoked national safeguard clause measures to provisionally ban the marketing of specific oilseed rape events on their territory [i.e., GT73 (Verordnung, 2006); MS8 x RF3 (Verordnung, 2008); MS1 x RF1 and Topas 19/2, for which the EU market approval period ended in 2007 (reviewed by Bartsch, 2008; Sabalza et al., 2011)].

The EU authority responsible for providing advice on the safety of GM plants (European Food Safety Authority, EFSA) considered the national ban on the marketing of GT73 and MS8 x RF3, and concluded that, in terms of risk to the environment, no new scientific

evidence had been presented (EFSA2009a,b) that would invalidate the previous risk evaluations of GMHT oilseed rape (EFSA, 2004a, 2005, 2008). EFSA reiterated its opinion that unintended environmental effects due to the accidental spillage of GMHT oilseed rape seed will be no different from that of conventional oilseed rape (see also EFSA, 2004b, 2006). These scientific opinions have been passed to the European Commission, and it now lies within the EU comitology decision-making process to decide whether the national ban should be lifted (Christiansen and Polak, 2009).

This paper explores whether the concerns about feral GMHT oilseed rape, potentially originating from seed spills during imports, are scientifically justified. Available scientific evidence on feral oilseed rape is reviewed in order to assess the possible environmental and economic impacts of feral GMHT oilseed rape resulting from seed import spills. Since the risk assessment strategy for GM plants usually seeks to compare the GM plant with its conventional counterpart (EFSA, 2010), non-GM oilseed rape is taken as a comparator. More specifically, this paper examines: (1) the biology and population demography of feral oilseed rape; (2) the ability of feral oilseed rape to act as a significant genetic bridge between different commercially grown oilseed rape varieties and therefore accumulate and pass on transgenes; (3) whether feral GMHT oilseed rape is more persistent or invasive than its conventional counterpart in the environment; (4) the extent to which feral GMHT oilseed rape might contribute to GM admixtures in non-GM crops; and (5) whether the risks are great enough that feral oilseed rape needs to be managed. Relevant data for feral plants derived from cultivation (as distinct from import) will be considered as a worst case, representing conditions where exposure and potential impact are expected to be the highest. These data will be used to assess the role of feral GMHT oilseed rape resulting from seed import spills. This paper focuses on the import of commodities from EU approved GM oilseed rape events only, as the EU operates a 'zero-tolerance' policy towards unapproved genetically modified organisms (GMOs) (so-called low-level presence), which may be approved in other countries (Stein and Rodríguez-Cerezo, 2010).

## **GENERAL CHARACTERISTICS OF FERAL OILSEED RAPE**

### **Definition and sources**

Feral oilseed rape plants are defined as crop-derived plants occurring *outside* agricultural fields, often in ruderal – non-cropped disturbed – habitats (see Occurrence and population

characteristics), where they can survive and reproduce successfully without management (Gressel, 2005; Bagavathiannan and Van Acker, 2008). In contrast, volunteers are plants living *within* agricultural fields as a result of previous cropping.

Feral oilseed rape is part of a complicated, and variously connected, metapopulation of plants in which the most numerous are crop plants and volunteers (Simard et al., 2005; Gruber and Claupein, 2007; Messéan et al., 2009; Middelhoff et al., 2011; Squire et al. 2011). Feral oilseed rape typically originates either from the spillage of seed during its transport to and from fields, the redistribution of seed by field equipment (Price et al., 1996; Zwaenepoel et al., 2006; von der Lippe and Kowarik, 2007b; Pivard et al., 2008a,b), or the dispersal of seed, for example by birds and mammals (von der Lippe and Kowarik, 2007a,b; Wichmann et al., 2009). Within agricultural fields, seeds can be lost through the shattering of the seed-bearing pods before and during harvest. At seed maturity the pods become fragile and easily split open, resulting in losses that can reach up to 10% of the seed yield (Thomas et al., 1991; Price et al., 1996; Morgan et al., 1998; Hobson and Bruce, 2002; Gulden et al., 2003a).

### **Occurrence and population characteristics**

Feral oilseed rape has been reported in several regions (see Table 1) and occurs in ruderal habitats such as field margins, road verges, paths, ditches, railway lines, building sites, ports, seed handling, storage and processing facilities, and wastelands. A population can be defined as a single plant or group of plants that is spatially separated from another feral population. The size of such populations ranges from single plants to stands of over 1,000 plants with the majority of populations containing 100 plants or less (Squire et al., 2011). Comparisons of five demographic studies of feral oilseed rape in different EU locations (Denmark, Germany (2), France and the UK), constituting over 1,500 ha and 16 site-years of observations, showed that feral populations generally occur at relatively low densities, with a mean around one population per square kilometre, rising to 15 per square kilometre in areas with a high frequency of oilseed rape cultivation such as the study site at Selommès, Loir-et-Cher, France (Lecomte et al., 2007; Messéan et al., 2009; Squire et al., 2011). The spatial variation in feral populations in part reflects differences in frequency of oilseed rape cultivation and abundance of in-field oilseed rape volunteers in the landscape (Knispel and McLachlan, 2009).

### **Population demography of feral oilseed rape**

Oilseed rape is generally regarded as an opportunistic species, and not as an ecologically hazardous invasive species (Warwick et al., 1999). It can take advantage of disturbed sites due to its early germination potential and capacity to capture resources rapidly. In undisturbed natural habitats, oilseed rape lacks the ability to establish stable populations, possibly due to the absence of competition-free germination sites (Crawley et al., 1993, 2001; Warwick et al., 1999; Hails et al., 2006; Damgaard and Kjaer, 2009). Moreover, in controlled sowings into road verges, field margins and wasteland, very few seedlings survived to maturity due to grazing (e.g., by molluscs) and abiotic stress (Charters et al., 1999).

Once established in competition-free germination sites, feral populations become extinct over a period of years. A 10-year survey (1993-2002), along road verges of a motorway revealed that most quadrats showed transient populations lasting one to four years (Crawley and Brown, 2004). These data and data from other demographic studies indicate a substantial turnover of populations of feral oilseed rape: only a small percentage of populations occurs at the same location over successive years, whereas the majority appears to die out rapidly (Crawley and Brown, 1995, 2004; Charters et al., 1999; Peltzer et al., 2008; Elling et al., 2009; Knispel and McLachlan, 2009; Nishizawa et al., 2009; Squire et al., 2011). However, if habitats are disturbed on a regular basis by anthropogenic activities such as mowing, herbicide applications or soil disturbance, or natural occurrences such as flooding, then feral populations can persist for longer periods (Claessen et al., 2005a; Garnier et al., 2006).

The persistence or recurrence of a population in one location is variously attributed to replenishment with fresh seed spills, to recruitment from seed emerging from the soil seedbank or shed by resident feral adult plants, or to redistribution of feral seed from one location to another. The respective contribution of these input sources is still a matter of discussion.

#### *Replenishment with fresh seed spills*

Because feral oilseed rape is more prevalent in areas with a high frequency of oilseed rape cultivation (Squire et al., 2011), along high-traffic roadsides (Crawley and Brown, 1995, 2004; Knispel and McLachlan, 2009), and in the proximity to seed handling, storage and processing facilities (Yoshimura et al., 2006; Peltzer et al., 2008), repeated seed immigration from both agricultural fields and transport (as fresh seed spills) has been considered the main source contributing to population persistence, countering high extinction rates at a local scale.



Few studies have been able to define the proportion of populations derived from fresh spills, but at the study site of Selommes in France, 15% of feral populations were attributed to immigration through seed transport, potentially including seed imports to the area, as opposed to 35-40% originating from seed from neighbouring fields (Pivard et al., 2008a).

*Recruitment from seed emerging from the soil seedbank or from resident ferals*

The dynamics of feral populations at one location not only depend on seed immigration from fresh seed spills, but also on soil seedbanks and local recruitment from seed produced by resident ferals (Pivard et al., 2008b). Demographic data on feral oilseed rape in different EU locations showed consistently that persistence in the soil seedbank allowed plants to recur after an absence of a year or more, while several populations persisted for two to four years (Squire et al., 2011). For the study site of Selommes, Pivard et al. (2008a) estimated that up to 40% of the observed feral populations persisted mainly through seed emerging from the soil seedbank. There is a large body of evidence from the study of volunteers showing that oilseed rape seed can remain in secondary dormancy for many years in the soil seedbank, and germinate in subsequent years. Under field conditions, the persistence of secondarily dormant seed has been confirmed to be up to five years, but may reach ten years or more (Simard et al., 2002; Gulden et al., 2003b; Lutman et al., 2004, 2005, 2008; Begg et al., 2006; Messéan et al., 2007; Jørgensen et al., 2007; D'Hertefeldt et al., 2008; Gruber et al., 2008; Beckie and Warwick, 2010). Secondary dormancy is complex: it can be induced by a range of factors such as low temperature, soil dryness, and darkness through burial in soil (López-Granados and Lutman, 1998; Squire, 1999; Marshall et al., 2000; Momoh et al., 2002; Gruber et al., 2004, 2010; Gulden et al., 2004a). Recently, dormant oilseed rape seed has been found in the soil seedbank in non-till systems, indicating that seed can fall dormant on the soil surface, and need not to be buried in the dark (Gruber et al., 2010).

Evidence on the contribution of seed from the seedbank is not entirely consistent, however. Biochemical and molecular analyses indicated that feral sites can contain plants with the same varietal profile consistently for at least three years, and can contain varieties last commercially grown three or more years previously (Squire et al., 2011). Since individual varieties of oilseed rape are sown for only a few years before being superseded by new varieties, the existence of markers from previous varieties indicates the possibility they persisted as ferals, provided origins from farm-saved seed or persistent volunteers can be

ruled out (SIGMEA, 2010). Biochemical and genetic analyses, in conjunction with farmer surveys, established the persistence of varieties no longer grown or marketed for at least five years in Austria (Pascher et al., 2006) and eight years in France (Pessel et al., 2001). In a continuation of the study by Charters et al. (1999), it was observed that one population contained, over a period of twelve years, a genetic signature of a variety that had been obsolete for at least ten of those years. In contrast, based on a preliminary analyses of soil samples at feral oilseed rape roadside sites in western Canada in the greenhouse, Knispel et al. (2008) indicated that feral oilseed rape roadside soil seedbanks are small (less than five viable seeds per square metre) and lack substantive dormancy. In total, however, the observations from Europe indicate that feral populations have been sufficiently consistent in their presence and abundance to act as a genetic bridge between past and current oilseed rape varieties.

*Redistribution of feral seed between local populations versus replenishment of the seedbank by resident feral oilseed rape plants*

The feral seedbank could in principle consist of seed brought into the location from outside and seed from plants reproducing on site. Seed brought in from outside could be carried by vehicles, road verge mowers, animals, or by the movement of soil for agricultural and building works (Wilkinson et al., 1995; Garnier et al., 2008; Wichmann et al., 2009). Garnier et al. (2008) showed that wind turbulence behind passing vehicles locally contributed to the secondary dispersal of seed: on average, 20% of the seed was estimated to disperse over a few metres, while 80% of the seed remained at the original place. However, there is little evidence of the contribution of such redistributed seed compared to that of seed deposited by plants reproducing on site. Even though observations from demographic studies across Europe showed that seed yield of feral plants is often much smaller than that of the crop due to the less suitable habitat than agricultural fields, seed from mature plants is still likely to replenish the soil seedbank and contribute to population persistence (Squire et al., 2011). One of the few direct estimates in Europe is by Pivard et al. (2008a) who found that local seed input from resident feral oilseed rape is rare, accounting for less than 10% of subsequent feral populations in the study site of Selommes. Other data, relying on the existence of feral plants bearing seed, are mostly circumstantial and indicate that the proportions of feral plants having pods ranged between 30 to 48% in northwest Germany (Elling et al. 2009). These values are

two to three times higher than those observed in Selommès, while in western Canada, the seed yield from individual feral plants was comparable to that of the crop (Knispel et al., 2008).

### *Conclusion on population demography*

The evidence indicates that oilseed rape is capable of establishing self-perpetuating populations outside agricultural areas. While many feral populations observed over multiple years were transient at a local scale (e.g., Crawley and Brown, 1995, 2004; Knispel et al., 2008), this apparent transience is likely counterbalanced at a landscape scale by repeated seed addition and redistribution from various sources. Local declines or extinctions in feral populations are likely to be temporary and asynchronous at large spatial scales (Charters et al., 1999; Crawley and Brown, 2004; Peltzer et al., 2008; Knispel and McLachlan, 2009; Nishizawa et al., 2009). On a larger scale in the landscape, feral oilseed rape can thus be considered long-lived with a proportion of the populations founded by repeated fresh seed spills from both agricultural fields and transport, and the remainder resulting from the continuous recruitment of seed from local feral soil seedbanks.

## **IS FERAL GMHT OILSEED RAPE A HAZARD OR RISK?**

### **Occurrence of feral GMHT oilseed rape**

Several extensive monitoring surveys, assessing the presence of transgenes in feral populations, have been conducted (see Table 2). In regions where GMHT oilseed rape is widely grown such as western Canada and the USA, monitoring surveys confirmed the widespread occurrence of feral GMHT oilseed rape plants along field margins of agricultural fields, as well as along transportation routes (such as road verges and railway lines). In the study of Yoshimura et al. (2006), approximately 2/3 of the ferals sampled were transgenic, whereas all ferals sampled by Knispel et al. (2008) exhibited the presence of the GLY or GLU tolerance traits (or both). In North Dakota (USA), 347 of the 406 oilseed rape plants collected, tested positive for the GLY or GLU tolerance trait (Schafer et al., 2010). The presence of feral GMHT oilseed rape plants was also detected at the port of Vancouver on the west coast of Canada, where most GMHT oilseed rape seed for export is transported by rail (Yoshimura et al., 2006). These data indicate that feral GMHT oilseed rape will be present along roadsides and other ruderal habitats in areas where GMHT oilseed rape is commercially grown or at points from where it is exported. The frequency of transgenes corresponds

approximately to the proportion of oilseed rape grown or in transit that is transgenic (Yoshimura et al., 2006; Knispel et al., 2008).

In regions where GMHT oilseed rape is currently not grown commercially, surveys, for example performed in and around major ports and along roads leading from these ports to inland processing facilities in Japan, revealed that feral oilseed rape plants can express/contain the GLY or GLU tolerance trait, and to a lesser extent both traits (Saji et al., 2005; Aono et al., 2006; Kawata et al., 2009; Nishizawa et al., 2009). The share of feral plants that is transgenic varied substantially across years and sampling sites, ranging from 0.2% to 100% (Kawata et al., 2009; Nishizawa et al., 2009). Aono et al. (2006) also reported the presence of *barnase* and *barstar* genes in the progeny of some of the sampled oilseed rape plants. Since no GM oilseed rape has been grown for marketing purposes in Japan (Nishizawa et al., 2010), transgene presence could be attributed to the accidental loss and spillage of imported viable GMHT oilseed rape seed.

### **Imports of (GMHT) oilseed rape commodities to the EU**

To know the actual scale on which losses to the environment of GMHT oilseed rape imports might occur in the EU, the following factors should be considered: the mode of product transfer and transport in the EU countries of destination; the intended uses of oilseed rape imports; the volumes of imported oilseed rape commodities; the share of GMHT oilseed rape in imported commodities; and the country of origin. However, trade statistics do not distinguish between GM and non-GM oilseed rape imports. Since this type of information is considered commercially sensitive and confidential in nature, European operators importing, handling and processing viable oilseed rape commodities are reluctant to provide it (Tamis and de Jong, 2010). Therefore, estimates of potential losses are highly uncertain.

In 2009, approximately 34% of oilseed rape imports to EU countries came from overseas countries, mainly Australia and New Zealand, but also Argentina, Canada and the USA. GMHT oilseed rape is grown commercially in Australia, Canada and the USA (James, 2010; CERA, 2011), and the estimation of the approximate share of GMHT oilseed rape cultivations in these countries gives an indication of the amount of transgenic oilseed rape that could possibly be imported into the EU (see Table 3). The main importers of oilseed rape commodities from outside the Community (including Ukraine) were the Netherlands and France, accounting for 62% of the total volume in 2009. Other significant importers of oilseed

rape commodities in 2009 were Germany, Poland, Belgium and Portugal (Eurostat: <http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home/>). These figures vary annually with the changing domestic production of oilseed rape within EU Member States and market demands.

Import of viable seed for use in the oilseed rape crushing industry is entirely in bulk (i.e., large containers rather than handy-sized bags) and by boat. While most seed is crushed in or near the ports of entry in the EU, a fraction of the imported viable seed can be transported inland to processing (crushing) facilities by boat, truck or rail. Because it is uneconomical to transport imported viable seed inland for processing in landlocked processing facilities, it is mainly transported by boat to river-located ports (EFSA, 2004a), where it is usually unloaded by pneumatic discharge, by crane in sealed crates, or by a screw conveyor in a sealed tube. The unloaded material is deposited on a conveyor belt that takes it to a quayside storage silo from where it is dispatched by truck to a storage site at the processing facility. Evidence indicates that viable oilseed rape is mostly processed on-site and has little travelling distance between the points of entry and processing (Tamis and de Jong, 2010). Smaller independent crushing facilities located inland away from rivers tend to supply themselves from domestic production (EFSA, 2004a), as these facilities market the oil they produce on the basis of locality and provenance. According to Tamis and de Jong (2010), the only route by which small amounts of imported (GMHT) oilseed rape seeds may escape into the wider countryside is during the processing (cleaning) of seed used for the production of pet feed, including seed mixtures for birds. It can be concluded that the use of overseas oilseed rape commodities is minimal in inland processing facilities, and that therefore seed spills of oilseed rape imports possibly containing GM material will be mostly confined to port areas.

Nevertheless, extrapolating the reported instances of feral GMHT oilseed rape in and around major ports and along roads leading from these ports to inland processing facilities in Japan to European environments is problematic. Extensive monitoring surveys, assessing transgene presence in feral populations, as those performed in Japan have not been reported for EU countries (e.g., Mbongolo Mbella et al., 2010). Moreover, the use of inland processing facilities, the origin and volumes of imported (GMHT) oilseed rape commodities, and the habitats into which seed spill may occur (e.g., vegetation density and composition, type and timing of road verge management) may differ in Japan as compared with EU countries. Therefore, as indicated earlier, a worst-case scenario, assessing the potential impact of feral

(GMHT) oilseed rape derived from cultivation (as distinct from import) is used in the following sections to explore the role of feral GMHT oilseed rape potentially originating from seed import spills.

### **Feral oilseed rape as the receptor plant – crop-to-feral gene flow**

#### *Cultivation scenario*

Few direct measurements to quantify crossings between commercially grown oilseed rape and feral plants have been made so far, but the fact that crossing occurs, and hence genomes of old and new varieties combine, was demonstrated at several localities in the EU (Charters et al., 1999; Bond et al., 2004; Dietz-Pfeilstetter et al., 2006; Pascher et al., 2006, 2010; Elling et al., 2009). More generally, the potential for cross-fertilisation of feral plants by the crop plant simultaneously in flower over a range of distances has been demonstrated by the use of small groups of male-sterile recipient plants distributed in the landscape (Ramsay et al., 2003; Devaux et al., 2005, 2007, 2008; Chifflet et al., 2011). Using male-sterile plants (which produce no pollen of their own) as recipients tends to overestimate the actual frequency of cross-fertilisation that would occur between the crop plants and pollen-fertile ferals by more than 10-fold (Ramsay et al., 2003; GR Squire, unpublished data), but demonstrates the potential for its occurrence. This approach combined with modelling work confirmed that cross-fertilisation levels usually decline very steeply with distance from one field to an adjacent or nearby field (Hüsken and Dietz-Pfeilstetter, 2007; Beckie and Hall, 2008), but they occur at low frequency over several kilometres (Rieger et al., 2002; Ramsay et al., 2003; Devaux et al., 2005, 2007, 2008; Chifflet et al., 2011). It is expected that crossing of the order of 1 to 10% will occur to feral plants a few metres from a donor, and of 0.1 to 0.01% to ferals that are 100 m away (SIGMEA, 2010). Since feral plants are widespread in some agricultural regions and occur in close proximity to commercially grown oilseed rape in flower, most feral plants in agricultural landscapes would be exposed to pollen from crops. In the major demographic studies of oilseed rape in Europe, the proximity of feral populations to the nearest flowering field of oilseed rape was measured in four of the study areas: approximately 10% of the ferals were within 10 m; 15% within 100 m (50% at the study side of Selommès) and 80% within 1,000 m (SIGMEA, 2010). This suggests that feral plants, even lasting only one year, can be cross-fertilised by commercially grown oilseed rape and have the potential to accumulate transgenes in areas where GMHT oilseed rape is grown.

In western Canada where GMHT oilseed rape is widely grown, pollen-mediated gene flow has resulted in the unintended stacking of HT traits in both volunteer (Hall et al., 2000; Beckie et al., 2003) and feral plants (Knispel et al., 2008). Even though Yoshimura et al. (2006) failed to detect feral plants with both HT traits in western Canada, the authors argued that such plants would likely have been detected with more intensive sampling. Also, in North Dakota (USA), two instances of unintentionally stacked traits have been reported recently (Schafer et al., 2010). It is likely that adjacent plants within feral populations may further contribute to the spread and stacking of HT traits, especially where feral plants with different HT traits occur together (Knispel et al., 2008), as cross-fertilisation rates increase with increasing proximity of oilseed rape plants (Funk et al., 2006).

#### *Import scenario*

Due to the relative scarcity of feral plants, the most plausible source for unintended stacking under an import scenario is through the cross-fertilisation between plants having different HT traits in the country of origin, and the spillage of this unintentionally stacked HT oilseed rape seed subsequently imported in the EU. In Japan, where GMHT oilseed rape is not grown commercially, but viable oilseed rape seed is imported, a portion of the progeny of two feral plants has been shown to contain both the GLY and GLU tolerance traits (Aono et al., 2006). The authors could not conclusively determine whether the double HT progeny resulted from cross-fertilisations between adjacent plants with different HT traits in Japan, or from the import of double HT seed unintentionally stacked in Canada. However, import seems the most reasonable explanation, as the unintended stacking of HT traits in certified seed (Friesen et al., 2003; Demeke et al., 2006) was reported in Canada, whereas other extensive surveys of feral plants conducted in Japan failed to detect feral plants with multiple transgenes (Saji et al., 2005; Kawata et al., 2009; Nishizawa et al., 2009).

### **Feral oilseed rape as the donor plant – feral-to-crop gene flow**

#### *Cultivation scenario*

The contribution of feral plants to pollen flow into agricultural fields has been argued to be extremely small compared to that from the crop plants and volunteers, simply because of the far smaller number of feral plants (Ramsay et al., 2003; Gruber and Claupein, 2007; Messéan et al., 2009; Middelhoff et al., 2011; Squire et al., 2011). The main channel by which HT

traits persist over time in fields would be through volunteers. In the major demographic studies of oilseed rape in Europe, the highest percentage of flowering feral plants was around 0.002% (two flowering feral plants for 100,000 crop plants) and the percentage of seed on feral plants was in all cases estimated to be <0.0001% of the seed produced by the crop, i.e., less than one feral seed for 1,000,000 crop seeds (Messéan et al., 2009; Squire et al., 2011). This estimate for seed can also be taken as an absolute maximum for GM impurity arising through seed in the improbable event that all feral seed was harvested with the crop (Squire et al., 2011). So while several authors have cautioned that feral GMHT oilseed rape might be a significant concern in the management of coexistence of oilseed rape cropping systems (see Introduction), the recent quantitative evidence from demographic studies in Europe shows that its contribution to gene flow should be negligible compared to that from crop plants and volunteers. The only exceptions to this might be where occasionally very large populations of feral plants (e.g., > 10,000 plants) occur in derelict fields or around major construction works, adjacent to very small oilseed rape crop fields or oilseed rape certified seed production fields (SIGMEA, 2010; Squire et al., 2011), or in regions where a ‘zero-tolerance’ policy in terms of GM admixtures is in place (Devos et al., 2008a; Ramessar et al., 2010; Sabalza et al., 2011).

#### *Import scenario*

Since feral plants derived from cultivation (as distinct from import) occur at too low a frequency to affect the tolerance threshold of 0.9% in the EU, even if they were assumed all to be transgenic (Messéan et al., 2009; Squire et al., 2011), it can be concluded that feral GMHT plants resulting from seed import spills will have little relevance as a potential source of pollen or seed for GM admixture. Seed import spills of GMHT oilseed rape will be mostly confined to port areas. In the event that spillage, germination and flowering of a GMHT oilseed rape plant occurred in the ports and associated processing facilities, their location in industrial areas rather than agricultural areas makes it highly unlikely that gene transfer to the oilseed rape crop would occur (EFSA, 2004a). However, in the unlikely event that such gene transfer would occur, the concern may be that HT traits would enter agricultural fields and thus become cultivated unintentionally. Feral plants would in effect become volunteers, subject to the annual cycles of cropping and management. If the herbicides for which tolerance is obtained are applied as the sole agent of weed management in the field, then GMHT plants would not be controlled: HT traits could be amplified, subsequently causing a weed burden, and possibly requiring more stringent weed management. The introduced



GMHT plants may set seed and replenish the soil seedbank. A worst-case scenario would be a persistence of the initial introduced GMHT oilseed rape plants. Therefore, the consequence might be: (1) the unintended cultivation of unapproved GM plants; (2) the subsequent gene flow to crop plants and stacking of HT traits; and (3) harvest admixtures. However, in the unlikely event that spilled seed would enter agricultural fields, the main opportunity of GMHT oilseed rape plants to reach maturity and produce seeds is one in every two to four years of the oilseed rape rotation, because standard herbicides used in oilseed rape do not control volunteer oilseed rape. Moreover, as no GM GLY-tolerant crops are currently approved for cultivation in the EU, the use of GLY is limited to two main timings in arable crops: pre-planting or pre-crop emergence to control a wide range of emerged weed species, and pre-harvest for late weed control or as a harvest desiccant to reduce moisture content (Cook et al., 2010). Therefore, exposure of the hypothesised in-field GMHT oilseed rape plants to GLY is expected to be limited. However, if exposed to GLY, the selective pressure exerted on treated plants will be high.

### **Feral oilseed rape as the donor plant – feral-to-wild relative gene flow**

#### *Cultivation scenario*

Oilseed rape is known to spontaneously hybridise with certain of its sexually compatible wild relatives (Scheffler and Dale, 1994). Several oilseed rape x wild relative hybrids have been reported in the scientific literature, but under field conditions transgene introgression has only been confirmed for progeny of oilseed rape x *B. rapa* hybrids (Hansen et al., 2001, 2003; Warwick et al., 2003, 2008; Norris et al., 2004; Jørgensen, 2007). Due to ecological and genetic barriers, not all relatives of oilseed rape share the same potential for hybridisation and transgene introgression (Jenczewski et al., 2003; Chèvre et al., 2004; FitzJohn et al., 2007; Wilkinson and Ford, 2007; Devos et al., 2009a; Jørgensen et al., 2009). For transgene introgression to occur, both species must occur in their respective distribution range of viable pollen. This requires at least partial overlap in flowering in time and space, and sharing of common pollinators (if insect-pollinated). Sufficient level of genetic and structural relatedness between the genomes of both species also is needed to produce viable and fertile oilseed rape x wild relative hybrids that stably express the transgene (e.g., Heyn, 1977; Kerlan et al., 1993). Genes, subsequently, must be transmitted through successive backcross generations or selfing, so that the transgene becomes stabilised into the genome of the

recipient. As no or only very low numbers of viable and fertile hybrids are obtained between oilseed rape and most of its wild relatives under ideal experimental conditions (e.g., through the use of artificial pollination and embryo rescue techniques in laboratory conditions (see FitzJohn et al., 2007)), Wilkinson et al. (2003) concluded that exposure under real conditions is likely to be negligible, and the probability of transgene introgression is extremely small in most instances, with the exception of *B. rapa* in areas where it occurs close to (feral) oilseed rape. Transgene introgression is likely to take place when oilseed rape and *B. rapa* grow in close proximity over successive growing seasons, especially if no significant fitness costs are imposed to backcross plants by transgene acquisition (Snow et al., 1999). Recent observations in Canada confirmed the persistence of a GLY tolerance trait over a period of six years in a population of *B. rapa* in the absence of herbicide pressure (with the exception of possible exposure to GLY in one year) and in spite of fitness costs associated with hybridisation (Warwick et al., 2008). A single GM *B. rapa* x *B. napus* hybrid was also reported along a road in Vancouver (Yoshimura et al., 2006), confirming the hybridisation possibility between these two *Brassica* species, albeit at very low frequencies (see also Elling et al., 2009 for the detection of triploid hybrid offspring of a single *B. rapa* mother plant with intermediate morphology and oilseed rape microsatellite alleles).

#### *Import scenario*

Surveys and analyses conducted in Japan did not detect transgenes in seed collected from wild relatives (*B. rapa* and *Brassica juncea*) sampled at several ports and along roadsides and riverbanks (Saji et al., 2005; Aono et al., 2006). There have been very few others attempts to measure the transfer of genetic material from ferals to wild relatives. Thus while theoretically possible, the combined probabilities of spilled feral GMHT oilseed rape germinating, surviving, hybridising with its wild relatives, hybrids surviving and containing the transgene were below the levels of detection in these two studies.

#### **Impact – would HT traits alter fitness, persistence and invasiveness?**

##### *GMHT oilseed rape*

The evidence on fitness, persistence and invasiveness of feral GMHT oilseed rape is derived from the following sources: (1) transplant or seed sowing experiments; (2) ecophysiological experiments and models on comparative fitness; and (3) observations or monitoring to see

whether feral oilseed rape invades semi-natural habitats. Field studies in the first category have confirmed that HT traits in oilseed rape do not confer a fitness advantage, unless the herbicides for which tolerance is obtained are applied. In these studies, the invasive potential of GM plants was assessed directly by releasing them into natural habitats and by monitoring their fitness in subsequent generation(s). GMHT oilseed rape introduced into twelve different habitats at three sites across the UK failed to persist in established vegetation: in none of the natural plant communities considered was oilseed rape found after three years even when vegetation had been removed in the first year of sowing (Crawley et al., 1993, 2001). These experiments demonstrated that genetic engineering *per se* does not enhance ecological fitness (although seed survival was reduced for these particular transgenic lines (Hails et al., 1997)). While studies of the first category provide good indicators of invasiveness potential, they have some disadvantages. They are labour intensive, would need to be conducted for each crop x transgene combination, are inevitably restricted to a few environments, and do not provide insight into the mechanisms behind any changes in fitness (Hails and Morley, 2005).

Experiments and models on fitness differences between the GM plant and its non-GM counterpart (category 2 above) are usually inferred from a composite measure of relative plant germination, emergence, growth, survivorship, biomass and fecundity (Fredshavn et al., 1995; Warwick et al., 1999, 2004, 2009; Norris and Sweet, 2002; Claessen et al., 2005a,b; Garnier and Lecomte, 2006; Garnier et al., 2006; Simard et al., 2005; Londo et al., 2010). Beckie et al. (2004) showed that GMHT oilseed rape with single or multiple HT traits is not more persistent (weedier) than non-GMHT plants. Also greenhouse studies, in which the fitness of oilseed rape volunteers with no, single, or multiple HT was assessed, have shown no or little difference in fitness among oilseed rape plants in the absence of herbicide pressure (Simard et al., 2005). However, the danger of the latter approach is that fitness differences, which are restricted to the conditions in which the test were done, may only become apparent in the field, where trade-offs between growth and reproduction may be more acute (Hails and Morley, 2005). There is also no evidence that tolerance to GLY or GLU enhances seed dormancy, and thus the persistence of GMHT oilseed rape plants, compared to its conventional counterpart (Hails et al., 1997; Sweet et al., 2004; Lutman et al., 2005, 2008; Messéan et al., 2007). Seed dormancy (secondary dormancy, since there is little primary dormancy at seed shed), is more likely to be affected by the genetic background of parental genotypes than the acquisition of HT traits (López-Granados and Lutman, 1998; Lutman et

al., 2003; Gulden et al., 2004a,b; Gruber et al., 2004; Messéan et al., 2007; Baker and Preston, 2008).

Observations in semi-natural habitats (category 3) have concluded that feral oilseed rape is confined to ruderal habitats (see Table 1 and references therein).

In conclusion, therefore, GMHT oilseed rape is neither more likely to survive, nor be more persistent or invasive than its conventional counterpart in the absence of GLY or GLU. The ability of oilseed rape to successfully invade ruderal habitats appears to be limited principally by the availability of seed germination sites and interspecific plant competition, and there is no evidence that genes conferring HT significantly alter its competitive ability. Since GMHT oilseed rape has no altered survival, multiplication or dissemination characteristics, it is concluded that the likelihood of unintended environmental effects due to the establishment and spread of GMHT oilseed rape will be no different from that of conventional oilseed rape.

#### *Wild relatives*

There is no evidence to suggest that HT traits in a wild relative changes its behaviour (Scheffler and Dale, 1994; Eastham and Sweet, 2002; Chèvre et al., 2004; Warwick et al., 2003, 2004, 2008; Jørgensen, 2007; Jørgensen et al., 2009), or the scale and nature of its interactions with associated flora and fauna (Wilkinson et al., 2003; Wilkinson and Ford, 2007). Progeny from hybrids of oilseed rape and wild relatives that bear the HT trait do not show any enhanced fitness, persistence and invasiveness, and behave as conventional counterparts, unless the herbicides for which tolerance is obtained are applied (Londo et al., 2010).

#### *Other traits than HT*

A trait that is expected to exert a negative effect on the fitness of feral GM oilseed rape is male sterility (i.e., the absence of pollen-producing anthers) which occurs in a proportion of seed produced by MS8 x RF3. Progeny may be male fertile or male sterile and have a variable number of copies of the *bar* gene, while a small proportion will have no *bar*, *barstar* or *barnase* genes. Male-sterile plants still produce stigmas and will set seed by pollen from another plant. They can therefore receive genes, but not transmit them. However, the effect of such male sterility on the fitness of feral individuals and populations has not been investigated in the field.

Other traits, designed to reduce susceptibility to herbivores or pathogens (Hails and Morley, 2005; Raybould and Cooper, 2005; Wilkinson and Tepfer, 2009), or confer drought or salt tolerance, may theoretically enable a GM plant to grow or spread beyond the geographical range of its conventional counterpart, and to occur in new areas close to wild relatives from which it was previously isolated (Warwick et al., 2009). Fitness advantages due to reduced plant damage resulting from insect pest attack (herbivory) cannot be seen independently from other ecological factors that limit plant fitness, and may be offset by deleterious effects of hybridisation or expression of resistance, and will only be of ecological significance if suitable competition-free germination sites are available for recruitment from seed (Crawley and Brown, 1995; Hails et al., 2006; Sutherland et al., 2006). Theoretical studies predict that insect resistant plants might only be able to invade semi-natural habitats in situations where infestation by target insect pests is sufficiently high and the habitat for plant establishment suitable (Damgaard and Kjaer, 2009). Since these ecological factors rarely influence plant fitness independently and vary in frequency and intensity over space and time (Kareiva et al., 1996), it remains difficult to predict over longer timeframes whether fitness-conferring transgenes will alter the invasive potential of feral plants (Hails and Morley, 2005). Semi-natural habitats under the temperate climatic conditions, which are widespread in the EU, mainly consist of perennial and competitive species. Consequently, to behave as a successful invading species, any annual, partially domesticated and poorly competitive plant has to change its behaviour fundamentally; otherwise, it will be restricted to frequently disturbed ruderal habitats.

## **MANAGEMENT**

### **Management recommendations and feasibility**

There is a large body of opinion that feral oilseed rape arising from GM sources should be managed at the points of entry and processing, and subsequently if feral populations become established at and in between those points in the EU. For example, EFSA advised the implementation of appropriate management systems to minimise seed spillage and accidental loss of imported GMHT oilseed rape. Especially in EU oilseed rape cultivation areas, management systems have been recommended to be put in place to restrict seed of GM oilseed rape to enter cultivation (EFSA, 2004a,b, 2005, 2006, 2008, 2009a,b). In the annex of its approval decision for the marketing of GT73, the European Commission requested the

implementation of appropriate management measures to prevent any damage to human and animal health and the environment in case of accidental spillage of GM oilseed rape (EC, 2005). EU Member States that consider seed import spills of GMHT oilseed rape as a major concern also required monitoring to confirm that populations of feral GMHT oilseed rape do not emerge, and to identify areas where feral populations become established to undertake remedial measures (EC, 2004).

European operators importing, handling and processing viable oilseed rape commodities have joined with the European Association of Bioindustries (EuropaBio) in developing monitoring systems for imported GM oilseed rape at the main points of entry and along distribution and processing networks (Lecoq et al., 2007; Windels et al., 2008). These monitoring systems are HACCP (Hazard Analysis and Critical Control Points) compliant, and aim to ensure that appropriate procedures are in place to avoid, report and to clean up potential seed spills, as spills can result in fines and the revocation of operating licenses.

At present, however, feral oilseed rape is not usually the specific target of road verge management in Europe, but in some areas most roadside verges are likely to be sprayed with herbicides or mown as part of general control of vegetation by municipal or highway authorities (Charters et al., 1999; Knispel and McLachlan, 2009). A range of studies concluded that targeted control of roadside feral plants can be achieved chemically or mechanically (e.g., mowing) at a local scale (Beckie et al., 2004; Warwick et al., 2004; Simard et al., 2005; Gruber et al., 2008; Lutman et al., 2008), provided that monitoring systems are in place to detect where significant populations of feral oilseed rape exist (Beckie et al., 2010) and that any control measures taken are timely (Yoshimura et al., 2006). GMHT oilseed rape with single or multiple transgenes can be controlled by the application of currently used herbicides with alternative modes of action (Beckie et al., 2004; Dietz-Pfeilstetter and Zwerger, 2009), or by mowing or cutting.

GLY is frequently used for the control of vegetation along railway tracks and in arable land, open spaces, pavements or in industrial sites (Monsanto, 2010). In these areas, the GLY-HT trait is likely to increase the fitness of GMHT plants (be it feral plants or progeny from hybrids of oilseed rape and wild relatives) relative to non-GLY-HT plants when exposed to GLY (Londo et al., 2010). To avoid that GLY functions as a selective agent that will contribute to an increased persistence of GLY-HT plants, mowing may be the primary option.

Repeated mowing during the season may be necessary to limit flowering and seed set by asynchronously developing populations (Garnier et al., 2006), but will similarly affect a broader range of non-target wild plant species. Since feral populations generally consist of a mixture of different (including spring and winter-sown) varieties (Pascher et al., 2010), varying in morphology and phenology, with seedlings emerging and flowering at various rates and times in the season, management would need to be in tune with the feral life cycle (Crawley et al., 1993; Claessen et al., 2005a,b; Knipsel and McLachlan, 2009).

However, such control measures are not likely to be sufficient to drive feral oilseed rape populations to extinction in the short-term, and may even be counterproductive. The pattern and timing of mowing may vary, as a result of which the potential effects on the reproductive success of feral plants will vary considerably. Moreover, Wilkinson et al. (1995) reported that none of the 15 feral populations that were either mown (5) or sprayed (1), or that underwent both control treatments (4) in their study were entirely eliminated by the implemented control measures. Ecological models predicted that the regular mowing of vegetation encourages the establishment of annual weed species including oilseed rape due to the creation of competition-free germination sites where new seed can establish and contribute to new feral plants (Claessen et al., 2005a,b; Garnier et al., 2006).

### **Necessity or desirability of management?**

The possible reasons for managing feral GMHT oilseed rape, as put forward in the Introduction, can now each be considered. The first reason is consequent on there being a change in the fitness of GMHT oilseed rape compared to its conventional counterpart. A change in fitness might allow ferals to invade semi-natural vegetation, but evidence described above points to this being a negligible risk for GMHT oilseed rape.

The second reason for managing feral GMHT oilseed rape is the only one that appears to have justification. The reason would be to prevent HT traits from entering agricultural fields following movement of seed or pollen and thus the cultivation of unapproved GM plants, as this requires specific market approval. GM material transmitted by feral GMHT oilseed rape plants to commercially grown oilseed rape may challenge low-level tolerance thresholds for unapproved GMOs. Moreover, a change in fitness might allow feral GMHT oilseed rape to cause a greater weed problem, for example through stacking of HT traits. However, since seed import spills will be limited mainly to port areas, this scenario is considered unlikely;

and even if it occurred, a range of options for managing HT plants in fields are available in European agriculture. Unapproved GM oilseed rape events are far more likely to enter cultivation through impurities in certified seed (Friesen et al., 2003; Demeke et al., 2006; Damgaard et al., 2007; Jørgensen et al., 2007).

The third reason would be to prevent transmission of GM material by feral GMHT oilseed rape plants to commercially grown oilseed rape that would challenge coexistence thresholds. This scenario may occur only if the GM oilseed rape events imported for food and feed uses and processing are also approved for cultivation, as the EU operates a coexistence policy towards GM plants that are approved for cultivation (EC, 2003a, 2010; Devos et al., 2009b). As indicated above, long-term studies in the EU have shown that feral plants derived from cultivation (as distinct from import) occur at too low a frequency to affect the tolerance threshold of 0.9% (Messéan et al., 2009; Squire et al., 2011), so routine control of feral GMHT oilseed rape derived from seed import spills would not be relevant in ensuring coexistence between oilseed rape cropping systems. The only circumstances in which special attention may be required for the purpose of managing coexistence would be where occasionally very large populations of feral GMHT oilseed rape plants occur, but such populations would be visible and therefore an obvious target for local control. Since GMHT oilseed rape is not grown commercially in the EU and because of the mode of product transfer and transport of oilseed rape commodities between points of entry and processing in EU countries, seed import spills will be mostly confined to port areas. Therefore, in principle, the management of feral GMHT oilseed rape resulting from seed import spills is neither necessary, nor desirable in order to achieve coexistence. Admixture between oilseed rape cropping systems is far more likely to arise from other sources such as the sharing of farm machinery and the occurrence of volunteers (see General characteristics of feral oilseed rape).

It can therefore be concluded that where *routine* management measures for feral oilseed rape are recommended or put in place for any of these reasons, they have a precautionary basis, rather than because there is strong scientific evidence they are necessary. Moreover, the act of managing feral populations could itself have adverse consequences. Management could be counterproductive for four main reasons. First, it could promote the establishment of annual ruderal species, including new GM oilseed rape, by creating germination sites and removing competitive perennials. Second, it could destroy ruderal communities that contain uncommon plants or useful plant functional types such as those supporting pollinators. Third, it consumes



human effort and fossil fuel resources. And fourth, the use of chemicals such as herbicides could lead to persistence in soil and surface and ground water pollution. These potential adverse effects of routinely managing feral oilseed rape have not been quantified, and accordingly the environmental costs of management in relation to any environmental benefit are uncertain. In the absence of such information, this paper concludes that there is good scientific justification for revising decisions to routinely manage feral oilseed rape.

## **DISCLAIMER**

Opinions and views expressed in this paper are strictly those of the authors, and do not necessarily represent those of the organisations where the authors are currently employed.

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**Table 1.** Demographic studies of feral oilseed rape

Country	Type of study	Surveyed/sampled area	Period	Aim	Proportion of oilseed rape in agricultural area	Reference
Austria	Genetic variation analysis	Roadsides, railway lines, fallow land, excavated soil and ruderal sites in Burgenland, Waldviertel and Innviertel	1998-1999	Study of genetic relationship between oilseed rape varieties and ferals through molecular markers	Moderate	Pascher et al. (2006, 2010)
Canada*	Field survey	Roadsides nearby and field margins of cropped fields in southern Manitoba (central Canada)	2004-2006	Study of population parameters, long-term dynamics and factors affecting persistence and spread of feral oilseed rape	High	Knispel and McLachlan (2009)
Denmark	Field survey	Roadsides nearby and field margins of cropped fields in Mid-Jutland/Bjerringbro	2005-2006	Study of population parameters and dynamics of feral oilseed rape	Moderate	SIGMEA (2010); Squire et al. (2011)
France	Field survey and biochemical variation analysis	Roadsides in Selommes (Loir-et-Cher)	1996-1997	Study of population parameters and dynamics of feral oilseed rape, and its genetic relationship with oilseed rape varieties through biochemical profiles (origin and persistence analysis)	High	Pessel et al. (2001)

	Field survey	Roadsides nearby and field margins of cropped fields in Selommès (Loir-et-Cher)	2000-2005	Study of population parameters and dynamics of feral oilseed rape	High	Deville (2004); Pivard et al. (2008a,b); SIGMEA (2010); Squire et al. (2011)
Germany	Field survey	Roadsides and field margins of cropped fields in northern Germany (Bremen)	2001-2003, 2005	Study of population parameters and dynamics of feral oilseed rape	Moderate	Menzel (2006); Reuter et al. (2008); SIGMEA (2010); Squire et al. (2011)
	Field survey and biochemical and genetic variation analysis	Roadsides and field margins of cropped fields in northern Germany (Braunschweig)	2001-2004	Study of population parameters and dynamics of feral oilseed rape, and its genetic relationship with oilseed rape varieties through molecular markers (origin and persistence analysis)	Moderate	Dietz-Pfeilstetter et al. (2006); SIGMEA (2010); Squire et al. (2011)
	Field survey and genetic variation analysis	Roadsides and semi-natural habitats in northwest Germany (Lower Saxony)	2004-2007	Study of population parameters and dynamics of feral oilseed rape, and its genetic relationship with oilseed rape varieties through molecular markers (origin and persistence analysis)	Moderate	Elling et al. (2009)
	Field survey	Roadsides, railway lines and semi-natural habitats in oilseed rape cultivation areas, and the ports of Rotterdam and Amsterdam	2008-2009	Study of the distribution of feral and naturalised <i>Brassica</i> populations at a landscape level	Low	Luijten and de Jong (2010)
New Zealand	Field survey	Road verges, drainage ditches, channels,	2003, 2005	Study of the distribution of feral and naturalised <i>Brassica</i>	High	Heenan et al. (2004); Peltzer et al. (2008)

		natural watercourses, shelterbelts and wasteland in several plots in the region of Canterbury (South Island)		populations at a landscape level		
United Kingdom	Field survey	Roadside (M25) in southern England	1993-2002	Study of population parameters and long-term population dynamics of feral oilseed rape	Low	Crawley and Brown (1995, 2004)
	Field survey and genetic variation analysis	Roadsides nearby and field margins of cropped fields in the Tayside region (Scotland)	1993-1995, 2004	Study of population parameters and dynamics of feral oilseed rape, and its genetic relationship with oilseed rape varieties through molecular markers (origin and persistence analysis)	Moderate	Wilkinson et al. (1995); Charters et al. (1999); SIGMEA (2010); Squire et al. (2011)
	Genetic variation analysis	Field margins of a cropped field in the Tayside region (Scotland)	Not specified	Study of genetic relationship between oilseed rape varieties and ferals through molecular markers (origin and persistence analysis)	Moderate	Bond et al. (2004)
	Field survey	Field margins, hedges, roadsides and watercourses nearby cropped fields across the UK	1994-2000	Study of the distribution of feral oilseed rape plants	Moderate	Norris and Sweet (2002)

\* Country where GMHT oilseed rape is grown commercially

**Table 2.** Surveys to monitor transgene presence in feral oilseed rape populations

Country	Surveyed area	Period	Transgene detection	Sampled material	Reference
Belgium	Roadsides nearby and field margins of cropped fields in Wallonia	2007-2008	DNA analysis	Leaf	Berben (2008, 2009)
	Port areas (Antwerp, Gent, Izegem and Kluisbergen)	Not specified	DNA analysis	Leaf	Mbongolo Mbella et al. (2010)
Canada*	Roadsides nearby and field margins of cropped fields in southern Manitoba (central Canada)	2004-2006	Herbicide screening, biochemical (protein) analysis	Seed, leaf	Knispel et al. (2008)
	Roadsides and railway lines in Saskatchewan and at the port of Vancouver	2005	Biochemical (protein) analysis	Leaf	Yoshimura et al. (2006)
Japan	Port areas (Kashima, Chiba and Yokohama), roadsides and riverbanks in the Kanto district	2004	Herbicide screening, biochemical (protein) analysis, DNA analysis	Seed	Saji et al. (2005)
	Port areas, roadsides and riverbanks in western Japan (Shimizu, Yokkaichi, Sakai-Senboku, Uno, Mizushima, Kita-Kyusyu and Hakata)	2005	Herbicide screening, biochemical (protein) analysis, DNA analysis	Seed	Aono et al. (2006)
	Port areas and roadsides in the area of Yokkaichi	2004-2007	Biochemical (protein) analysis	Leaf	Kawata et al. (2009)
	Roadside (Route 51) in eastern Japan	2005-2007	Biochemical (protein) analysis, DNA analysis	Leaf	Nishizawa et al. (2009)
USA*	Roadsides (interstate, state and country roads) in North Dakota	2010	Biochemical (protein) analysis	Leaf	Schafer et al. (2010)

\* Country where GMHT oilseed rape is grown commercially

Table 3. Area cropped to and adoption rate of GM oilseed rape in main GM oilseed rape growing and exporting countries in 2009 and 2010  
(based on James, 2010)

<b>Country</b>	<b>Year</b>	<b>Area cropped to GM oilseed rape (ha)</b>	<b>Adoption rate of GM oilseed rape (%)</b>
Australia	2009	41,000	3
	2010	133,000	8
Canada	2009	6,400,000	93
	2010	6,700,000	94
USA	2009	335,000	85
	2010	616,000	88