

Quantifying the introgressive hybridisation propensity between transgenic oilseed rape and its wild/weedy relatives

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Abstract In order to estimate the introgressive hybridisation propensity (IHP) between genetically modified (GM) oilseed rape (*Brassica napus*) and certain of its cross-compatible wild/weedy relatives at the landscape level, a conceptual approach was developed. A gene flow index was established enclosing the successive steps to successfully achieve introgressive hybridisation: wild/weedy relatives and oilseed rape should co-occur, have overlapping flowering periods, be compatible, produce viable and fertile progeny, and the transgenes should persist in natural/weedy populations. Each step was described and scored, resulting in an IHP value for each cross-compatible oilseed rape wild/weedy relative. The gene flow index revealed that *Brassica rapa* has the highest introgressive hybridisation propensity (IHP value=11.5), followed by *Hirschfeldia incana* and *Raphanus raphanistrum* (IHP=6.7), *Brassica juncea* (IHP=5.1), *Diplotaxis tenuifolia* and *Sinapis arvensis* (IHP=4.5) in Flanders. Based on the IHP values,

monitoring priorities can be defined within the pool of cross-compatible wild/weedy oilseed rape relatives. Moreover, the developed approach enables to select areas where case-specific monitoring of GM oilseed rape could be done in order to detect potential adverse effects on cross-compatible wild/weedy relatives resulting from vertical gene flow. The implementation of the proposed oilseed rape–wild relative gene flow index revealed that the survey design of existing botanical survey networks does not suit general surveillance needs of GM crops in Belgium. The encountered hurdles to implement the gene flow index and proposals to acquire the missing data are discussed.

Keywords Biosafety · Genetic engineering · Geographic information system · Hybridisation · Introgression · Oilseed rape · Post-market environmental monitoring · Risk assessment · Vertical gene flow · Wild/weedy relatives

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Introduction

The deliberate release into the environment of genetically modified (GM) crops raises various concerns about its agricultural and environmental impacts. One of the highly debated and researched topics relates to the escape of transgenes into wild and weedy plant species. Cross-fertilisation between crops and certain of their wild/weedy relatives may lead to the production of viable and fertile interspecific hybrids. When

these hybrids are left uncontrolled in the presence of their parental species over several generations, transgenes may be stabilised into the wild/weedy relatives' genome by introgressive hybridisation (Hansen et al. 2001; Jenczewski et al. 2003; Warwick et al. 2008). Although crops and certain wild/weedy relatives have exchanged genes for centuries, the concern with GM crops is that the acquisition of transgenes may increase the fitness of recipient plants (Ellstrand et al. 1999; Ellstrand 2003). Theoretically, and depending on which transgenes are involved, altered fitness may enable wild/weedy relatives to go extinct, to exacerbate a weed problem, or to expand and invade new habitats, with unwanted effects on other species and agroecosystem integrity. However, there is also the possibility of little or no effect as seems to be the case with the current GM crops being grown commercially since 1995 (Beckie et al. 2006; Sanvido et al. 2007).

To get an indication of the likelihood of escape from crops into wild/weedy relatives and of the agroecological consequences in a given area, gene flow indices were developed as guiding tools. Gene flow indices analyse the potential for spontaneous gene flow between a certain plant species and its wild/weedy relatives through the use of three dispersal variables: dispersal of pollen (Dp), dispersal of diaspores (Dd: fruit and seed) and the distribution frequency of wild relatives (Df; De Vries et al. 1992). To be specifically designed for GM crops, Ammann et al. (2001) extended the existing gene flow indices with a fourth variable (Dg: description of gene) that addresses the potential impact of a transgene on wild/weedy relatives. Using the scores attributed to each variable, transgenic crops were classified into risk categories according to their potential for vertical gene flow. Flannery et al. (2005) further extended the gene flow indices by including seed-mediated gene flow, the role of feral crop populations and pollen-mediated crop-to-crop gene flow.

Here, a conceptual approach is developed to numerically quantify the introgressive hybridisation propensity (IHP) between a GM crop and certain of its cross-compatible wild/weedy relatives at the landscape level. This approach differs from those described above, as the establishment of a ranking of wild/weedy relatives instead of crops is aimed at, based on obtained IHP values. Due to its relatively high propensity for vertical gene flow (Eastham and Sweet 2002; Flannery et al. 2005) and due to the occurrence of various

cross-compatible wild/weedy relatives in areas where it is grown (Chèvre et al. 2004; Devos et al. 2004), oilseed rape (*Brassica napus*) is used as case study. Subsequently, the refined oilseed rape–wild/weedy relative gene flow model is applied to the Belgian situation. Finally, it is discussed how the numerical quantification may serve as a valuable tool in pre-release risk assessments and post-market environmental monitoring of GM oilseed rape deliberately released into the environment.

Description of gene flow index

In order to numerically quantify the propensity for effective gene flow between oilseed rape and certain of its wild/weedy relatives, the introgressive hybridisation process was subdivided in successive steps. All steps have to be completed successfully before transgene introgression and its persistence in the recipient population can be achieved (Table 1). A first prerequisite for hybridisation is that the wild/weedy relative occurs in the viable pollen distribution range of oilseed rape; otherwise, viable pollen grains may not reach the stigma of the wild/weedy relative. Second, there should at least be partial overlap in flowering periods between oilseed rape and wild/weedy relatives, and species should share common pollinators (if insect-pollinated). The success of hybridisation will also be affected by the level of genetic and structural relatedness between the genomes of both species. Embryos need to develop into viable seeds and seeds need to germinate. Third, to continue the introgressive hybridisation process, viable and fertile interspecific (F1) hybrids should be produced. Fourth, the transgene should be transmitted through successive backcross (BC) generations, that is, when F1 hybrids cross with one of the recurrent parent species, albeit selfing can also lead to F2 introgressants (Fig. 1). Fifth, the transgene should be stabilised into the genome of the wild/weedy relative through backcrossing over multiple generations. The final step for effective introgressive hybridisation is that the introgressed transgene persists in natural/weedy populations.

Questions were formulated to capture each sequential step of the introgressive hybridisation process (Table 1). To each question a score was attributed, either a zero in case the answer is 'no' or a number

Table 1 A gene flow index to numerically quantify the propensity for successful (trans)gene flow between oilseed rape and a wild/weedy relative at the landscape level (adapted from Flannery et al. 2005)

| Step | Question | Score |
|------|--|---------|
| S1 | Co-occurrence of oilseed rape and the wild/weedy relative | |
| 1a | Does the interfertile wild/weedy relative of oilseed rape occur in the area of interest? | 0/1 |
| 1b | Is the cross-compatible wild/weedy relative of oilseed rape present within a distance of 200 (1), 400 (2), 1,500 (3) and 4,000 m (4) from a field of oilseed rape? | 1/2/3/4 |
| 1c | Is the abundance of the wild/weedy relative low (1), medium (2) or large (3)? | 1/2/3 |
| S2 | Overlap in flowering period | |
| 2a | Does the flowering period of oilseed rape and its wild/weedy relative overlap? | 0/1 |
| 2b | If flowering occurs, is the wild/weedy relative rated as an obligate inbreeder (0), a partial inbreeder/outbreeder (1) or an obligate outbreeder (2)? | 0/1/2 |
| S3 | Production of viable and fertile F ₁ hybrids | |
| 3a | Are first generation hybrids between oilseed rape and its wild/weedy relative observed under controlled (ideal experimental) conditions (including the use of artificial/hand pollination and embryo rescue techniques)? | 0/1 |
| 3b | Are first generation hybrids between oilseed rape and its wild/weedy relative observed under field (agricultural) conditions? | 0/1 |
| 3c | Are the observed first generation hybrids viable and fertile? | 0/1 |
| 3d | Is the abundance of the first generation hybrids very low (0), low (1) or medium (2)? | 0/1/2 |
| S4 | Production of viable and fertile F ₂ and BC progeny | |
| 4a | Are second generation hybrids and backcross progeny between oilseed rape and its wild/weedy relative observed under controlled (ideal experimental) conditions? | 0/1 |
| 4b | Are second generation hybrids and backcross progeny between oilseed rape and its wild/weedy relative observed under field (agricultural) conditions? | 0/1 |
| 4c | Are the observed second generation hybrids and backcross progeny viable and fertile? | 0/1 |
| 4d | Is the abundance of the second generation hybrids and backcross progeny very low (0), low (1) or medium (2)? | 0/1/2 |
| S5 | Introgression | |
| 5a | Is the introgression of an oilseed rape (trans)gene observed under controlled (experimental) conditions? | 0/1 |
| 5b | Is the introgression of an oilseed rape (trans)gene observed under field (agricultural) conditions? | 0/1 |
| 5c | Is the abundance of introgressants very low (0), low (1) or medium (2)? | 0/1/2 |
| S6 | Persistence of transgenes in natural/weedy communities | |
| 6a | Does the introgressed trait persist in natural/weedy communities? | 0/1 |

(≥1) in case the answer is ‘yes.’ Scores higher than one are given when a ranking can be applied to the ‘yes.’ Since introgressive hybridisation can only occur when all steps are realised, further progression in the gene flow index stops when a question is answered with a zero value. Average scores calculated per step (6) were equally weighed and summed, giving IHP values for each oilseed rape wild/weedy relative in the area of interest. As such, a relative ranking of wild relatives could be established based on the geographical proximity between oilseed rape and its wild/weedy related species, the abundance of wild/weedy relatives, and on the ease with which oilseed rape crosses with wild/weedy relatives and forms hybrids and BC progeny. Publicly available data, collected from survey networks and a broad literature base, were used to implement the proposed

oilseed rape–wild/weedy relative gene flow index. More details on the gene flow index and encountered drawbacks are addressed in each successive step. Scores attributed to each question are given in Table 2.

Implementation of gene flow index using oilseed rape in Belgium

Step 1: Co-occurrence of oilseed rape and the wild/weedy relative

Does the interfertile wild/weedy relative of oilseed rape occur in the area of interest?

From the fourteen most closely related and common wild/weedy oilseed rape relatives occurring in the

Fig. 1 Successive steps in the development of F1 and F2 hybrids, and backcross progeny between oilseed rape and wild/weedy related species. Only the following combinations are shown: **a** first generation hybrids formed on the wild/weedy relative after fertilisation by oilseed rape, **b** the second generation hybrids resulting from the selfing of first generation hybrids or from the fertilisation by another first generation hybrid, and **c** backcross progeny ensuing from crosses between first generation hybrids and wild/weedy relative or oilseed rape, which can both act as recurrent parent (thereby, they can either be the pollen donor or receptor)

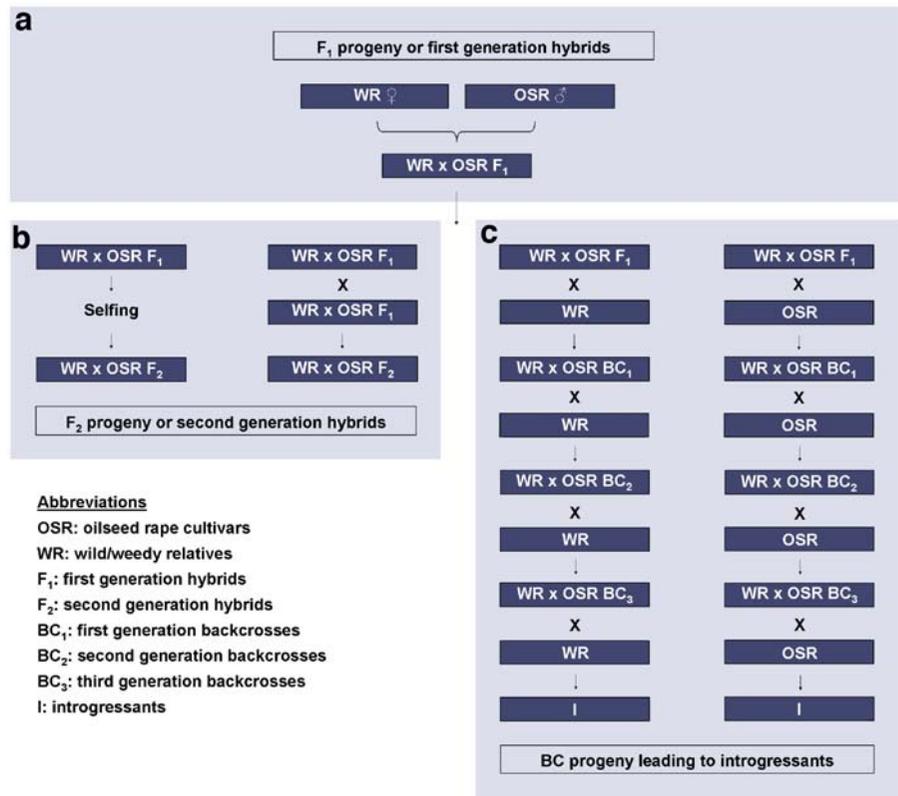


Table 2 Numerical quantification of the introgressive hybridisation propensity between oilseed rape and its wild/weedy relatives in Flanders (Belgium), expressed as introgressive hybridisation propensity (IHP) values

| Wild/weedy relative | Successive steps (S) to achieve introgressive hybridization | | | | | | | | | | | | | | | | IHP value | Rank | |
|--------------------------------|---|----|-----|----|-----|----|-----|----|----|----|-----|----|----|-----|----|------|-----------|------|----|
| | S1 | | S2 | | S3 | | S4 | | | | S5 | | | S6 | | | | | |
| | 1a | 1b | 1c | 2a | 2b | 3a | 3b | 3c | 3d | 4a | 4b | 4c | 4d | 5a | 5b | 5c | | | 6a |
| <i>Brassica juncea</i> | 1 | ? | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | - | - | - | - | - | - | 5.1 | 4 |
| <i>Brassica nigra</i> | 1 | ? | 2 | 1 | 2 | 1 | 0 | - | - | - | - | - | - | - | - | - | - | 4.2 | 7 |
| <i>Brassica oleracea</i> | 1 | ? | 1 | 1 | 1 | 1 | 1 | 0 | - | - | - | - | - | - | - | - | - | 3.2 | 9 |
| <i>Brassica rapa</i> | 1 | ? | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 11.5 | 1 |
| <i>Diplotaxis muralis</i> | 1 | ? | 2 | 1 | 1 | 1 | 0 | - | - | - | - | - | - | - | - | - | - | 2.4 | 10 |
| <i>Diplotaxis tenuifolia</i> | 1 | ? | 3 | 1 | 2 | 1 | 0 | - | - | - | - | - | - | - | - | - | - | 4.5 | 5 |
| <i>Erucastrum gallicum</i> | 1 | ? | 1 | 1 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | 1.6 | 11 |
| <i>Hirschfeldia incana</i> | 1 | ? | 3 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | - | - | - | - | - | - | 6.7 | 2 |
| <i>Raphanus raphanistrum</i> | 1 | ? | 3 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | - | - | - | - | - | - | 6.7 | 2 |
| <i>Sinapis alba</i> | 1 | ? | 1 | 1 | 2 | 1 | 0 | - | - | - | - | - | - | - | - | - | - | 3.9 | 8 |
| <i>Sinapis arvensis</i> | 1 | ? | 3 | 1 | 2 | 1 | 0 | - | - | - | - | - | - | - | - | - | - | 4.5 | 5 |
| Maximum average score per step | 2.7 | | 1.5 | | 1.3 | | 1.3 | | | | 1.3 | | | 1.0 | | - | - | | |
| Weighing factor | 1.0 | | 1.8 | | 2.1 | | 2.1 | | | | 2.0 | | | 2.7 | | - | - | | |
| Maximum IHP value | 2.7 | | 2.7 | | 2.7 | | 2.7 | | | | 2.7 | | | 2.7 | | 16.0 | - | | |

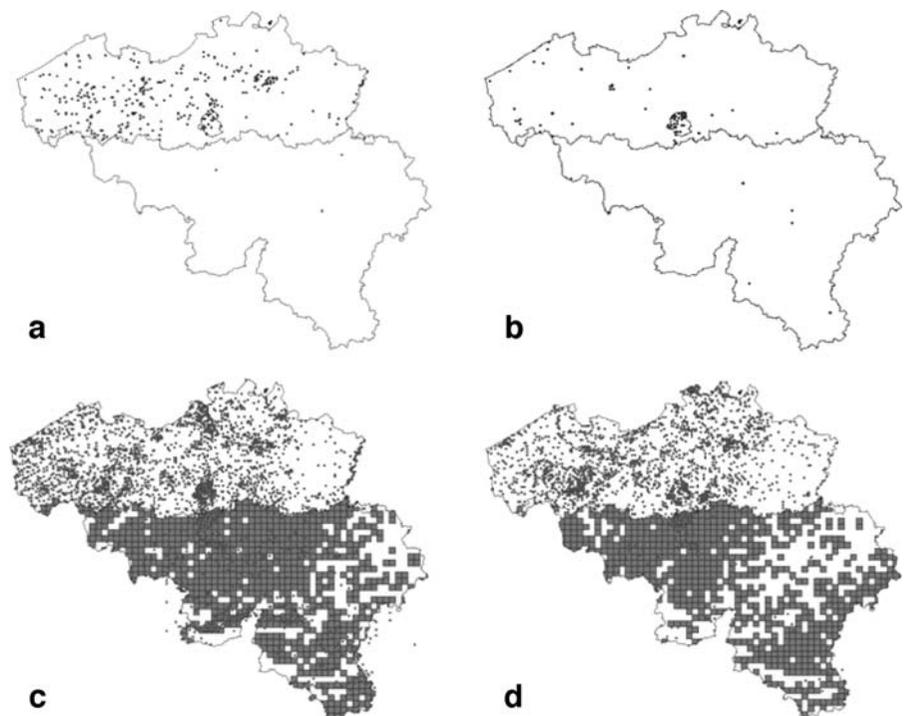
IHP values are the sum of weighed average scores per step. Steps (6) are weighed equally (? : no appropriate experimental data found)

European Union (EU) (Chèvre et al. 2004), the following eleven species are present in Belgium: *Brassica juncea*, *Brassica nigra*, *Brassica oleracea*, *Brassica rapa* (= *Brassica campestris*), *Diplotaxis muralis*, *Diplotaxis tenuifolia*, *Erucastrum gallicum*, *Hirschfeldia incana*, *Raphanus raphanistrum*, *Sinapis alba* and *Sinapis arvensis* (Van Rompaey and Delvosalle 1979; Lambinon et al. 2004). However, the Belgian distribution data of wild/weedy oilseed rape relatives should be looked at soberly. In the absence of national coordination and with variably attributed resources, surveys of the wild flora differ between regions in scale, intensity and wild relatives observed. For instance, in Brussels, survey activities of *B. oleracea* are more intense compared to Flanders and Wallonia (Fig. 2a); the distribution of *B. rapa* is only partially documented in Wallonia (Fig. 2b). Therefore, it is hard to specify whether the absence of a plant species in a sampled area results from a physical absence or from a lower survey intensity. In addition, only the presence or absence of wild/weedy relatives is given per 4-km² before 1980, per 1-km² after 1980 (Fig. 2c,d). Data are not frequently updated and the adding of a wild plant species in existing databases occurs more rapidly than its removal.

Is the cross-compatible wild/weedy relative of oilseed rape present within a distance of 200, 400, 1,500 and 4,000 m from a field of oilseed rape?

Determining the zones of co-occurrence between oilseed rape fields and wild/weedy relatives, requires information on the location of oilseed rape fields and the distribution of wild/weedy relatives in the area of interest (see “Does the interfertile wild/weedy relative of oilseed rape occur in the area of interest?”), as well as on the distribution range of viable oilseed rape pollen. Since fields of oilseed rape provide the pollen that can lead to cross-fertilisation, they were taken as the starting point for the analyses. With crop rotations, oilseed rape fields will inevitably change in location on an annual basis, whereas certain wild/weedy populations may be more stable over a larger number of years. In Belgium, the location and size of oilseed rape fields are generally well known and digitised on an annual basis. Therefore, the gene flow index consists of applying various perimeters concentrically around oilseed rape fields. The perimeters cover the areas in which viable oilseed rape pollen is dispersed and cross-fertilisation observed.

Fig. 2 Geographical distribution maps of **a** *Brassica rapa*, **b** *Brassica oleracea*, **c** *Sinapis arvensis* and **d** *Raphanus raphanistrum* in Belgium (reprinted with permission from Mahy, G., Gembloux Agricultural University). Data sources: Brussels and Flanders= ‘Flora Databank’; Brussels and Wallonia= ‘Amicale pour l’Etude de la Floristique asbl’ (1-km squares after 1980) and ‘Centre de Recherche de la Nature, des Forêts et du Bois’ (4-km squares prior to 1980)



The pollination process in oilseed rape has to be examined prior to the selection of dispersal perimeters of viable pollen. Fertilisation of oilseed rape usually results from self-pollination, although interplant out-crossing rates of 12–47% with an average of 30% have been reported (Beckie et al. 2003; Hüsken and Dietz-Pfeilstetter 2007). These interplant out-crossing rates indicate that external mediators are involved in the transfer of pollen. The transfer of pollen grains, which are large (32–35 μm), heavy and sticky, is not only mediated by mechanical contact between flowers that sway in the wind, but also by wind and insects (e.g. honeybees, bumblebees). When abundant, bees deliver pollen to flowers at least a hundred times more rapidly than any other mode of pollination (Hayter and Cresswell 2006). Other insects, such as the pollen beetle and hover fly might also play a role in mediating the transfer of pollen, but are less efficient pollinators in oilseed rape in comparison to bees. When bees are scarce at the moment of flowering, which generally lasts 3 to 4 weeks for individual oilseed rape plants, the extent of cross-fertilisation with incoming pollen is largely dependent on the rate of self-pollination. Further investigations are still ongoing to better quantify the respective contribution made by wind and insects in the dispersal of oilseed rape pollen (Cresswell et al. 2004; Ramsay 2005; Hoyle et al. 2007).

Various research trials with different designs have been conducted under different geographical and climatic conditions to define out-crossing probabilities between fields, plots or single oilseed rape plants. Most of these studies confirm the rapid exponential or leptokurtic attenuation of airborne pollen concentrations and ultimately cross-fertilisation levels with distance from the source. A large fraction of pollen is dispersed within the 200 m from the field. At distances further than 400 m from the oilseed rape source, dispersion percentages are maintained at very low levels, but do not appear to decrease in any clear manner with distance. In vitro germination experiments indicated that oilseed rape pollen remains viable for 24 h to 1 week, making effective out-crossing over larger distances possible (Mesquida et al. 1987). Hot spots of cross-fertilisation have been measured up to 4,000 m away from the pollen source (Thompson et al. 1999; Rieger et al. 2002; Ramsay et al. 2003), indicating that there is no clear cut-off

distance where levels of out-crossing reach zero (Hüsken and Dietz-Pfeilstetter 2007).

It remains questionable to what extent cross-fertilisation data between oilseed rape fields can predict cross-fertilisation levels between oilseed rape and its wild/weedy relatives. In crop-to-crop studies, experimental conditions are standardised: the spatial distribution of and the distance between pollen donor and recipient plants are exactly known, whilst the spatial arrangement of wild/weedy relatives is stochastic. Wild/weedy relatives are patchily or unequally distributed in the field, and occur in scattered sub-linear populations, on field headlands, along lanes and access routes around fields of oilseed rape.

The extrapolation of cross-fertilisation levels ensuing from crop-to-crop configurations to crop-to-wild/weedy relative ones may also be hampered by different degrees in attractiveness between oilseed rape and its wild/weedy relatives for insects, which may serve as pollinators. For example, *R. raphanistrum* is preferentially visited by small insects such as solitary bees and flies, and by certain bumblebee species such as *Bombus lapidaries*. Honeybees and *Bombus terrestris* show high flower constancy to oilseed rape and avoid *R. raphanistrum* when it occurs in a patch surrounded by oilseed rape, as it provides lesser quantities of food (Pierre 2001). Pollinators' behaviour is also affected by field sizes, field distribution and amount of rewards (Cresswell and Osborne 2004).

Given the lack of alternative data on wild/weedy relatives, recent empirical and predictive crop-to-crop gene flow studies were used to select the perimeters to be implemented in the present study. Data obtained in small-scale experiments (Scheffler et al. 1993, 1995; Paul et al. 1995; Timmons et al. 1995, 1996; Lavigne et al. 1998; Bilsborrow et al. 1998; Simpson et al. 1999, 2006; Walklate et al. 2004; Funk et al. 2006) were combined with those collected at the landscape level (Thompson et al. 1999; Rieger et al. 2002; Beckie et al. 2003; Ramsay et al. 2003; Damgaard and Kjellsson 2005; Devaux et al. 2005, 2007, 2008; Weekes et al. 2005; Klein et al. 2006; Shaw et al. 2006). The former provide valuable information on the decay of cross-fertilisation levels close to the source, whilst the latter are more suitable to observe long-distance dispersal events. On the basis of these data, the following perimeters were selected, reflecting the decline in cross-fertilisation levels with increasing

distance from the source: 200, 400, 1,500 and 4,000 m. The presence of a wild/weedy relative within a set perimeter adds a one to the score of question 1b (Table 1), with four being the highest score when the wild/weedy relatives are present in all four perimeters.

Using geographic information system (GIS) software, the digital distribution maps of wild/weedy relatives can be overlaid with those of oilseed rape fields taking into account the perimeters in which viable oilseed rape pollen leads to cross-fertilisation. As such, the zones of co-occurrence and their frequency of occurrence can be calculated at the landscape level (Graef et al. 2005). However, due to the low resolution of distribution maps of wild/weedy relatives in Belgium, it was not possible to reliably quantify zones of sympatry. In the UK, remote-sensing technology (satellite imagery) was used in combination with digital river systems information to identify oilseed rape fields that occurred in the vicinity of wild *B. rapa* populations along riverbanks and canals (Davenport et al. 2000; Wilkinson et al. 2000; Elliott et al. 2004).

Is the abundance of the wild/weedy relative low, medium or large?

Once the zones of co-occurrence between oilseed rape and its wild/weedy relatives are identified, the density of wild/weedy relatives is to be quantified in these zones. In theory, more wild/weedy relatives will be exposed to oilseed rape pollen when they are more abundant in a certain area. The question of abundance of wild/weedy relatives in Belgium could not be answered due to the inappropriate distribution maps of wild/weedy relatives. To have an indication about the density of oilseed rape wild/weedy relatives in the zones of co-occurrence, relative abundances of wild/weedy relatives estimated in Flanders were used (Table 3).

Ground surveys are the sole means to properly define the density of wild/weedy relatives in the zones of co-occurrence with oilseed rape fields. Within this frame, valuable work has been done in the UK, which is currently pursued (Allainguillaume et al. 2006). Wilkinson et al. (2003a) surveyed some waterways containing wild *B. rapa* populations and found that *B. rapa* was more common on riverbanks (0.755 plants/m²) than on canals (0.004 plants/m²). Using these data, they predicted the distribution of *B. rapa* water-

Table 3 Relative abundances of wild/weedy relatives of oilseed rape in Belgium

| Plant species | Region | |
|------------------------------|----------|----------|
| | Flanders | Wallonia |
| <i>Brassica juncea</i> | + | – |
| <i>Brassica nigra</i> | ++ | +++ |
| <i>Brassica oleracea</i> | + | + |
| <i>Brassica rapa</i> | ++ | + |
| <i>Diplotaxis muralis</i> | ++ | + |
| <i>Diplotaxis tenuifolia</i> | +++ | + |
| <i>Erucastrum gallicum</i> | + | + |
| <i>Hirschfeldia incana</i> | +++ | +++ |
| <i>Raphanus raphanistrum</i> | +++ | +++ |
| <i>Sinapis alba</i> | + | ++ |
| <i>Sinapis arvensis</i> | +++ | +++ |

+: rare; ++: abundant; +++: very abundant; –: not present or no appropriate experimental data found

Data sources: Flanders=‘Flora Databank’; Wallonia=‘Amicale pour l’Etude de la Floristique asbl’ and ‘Centre de Recherche de la Nature, des Forêts et du Bois’

side populations, including in the zones of sympatry with oilseed rape fields. If zones of co-occurrence can easily be identified, it may be worthwhile to simulate various densities of wild/weedy relatives in these areas, based on surveyed ones. For example, Monte Carlo simulations could be performed to randomly allocate transgenic oilseed rape to agricultural fields falling within the zones of co-occurrence (Devos et al. 2007, 2008).

Step 2: Overlap in flowering period

Does the flowering period of oilseed rape and its wild/weedy relative overlap?

To ensure spontaneous hybridisation between oilseed rape and its wild/weedy relatives, there has to be some overlap in flowering periods. The pollination potential increases with the time during which the flowering periods of species overlap (more pollen is available for the fertilisation of wild/weedy relatives).

Oilseed rape is grown as either a winter-sown or a spring-sown crop, which leads to two flowering periods in the EU: winter oilseed rape flowers from late March to early May, whilst spring oilseed rape blooms in June/July in Belgium. The range of flowering dates of wild/weedy relatives is generally less

well quantified, since it is largely affected by the habitat and genetic variability (Simard and Légère 2004). Lefol et al. (1996a) observed that wild/weedy *S. arvensis* plants occurring within or adjacent to oilseed rape flowered earlier than in distant plots. Usually, wild/weedy relatives display a wider range of flowering dates than oilseed rape. In general, most of the wild/weedy relatives show at least partial overlap in flowering periods of winter and spring oilseed rape (Table 4).

If flowering occurs, is the wild/weedy relative rated as an obligate inbreeder, a partial inbreeder/ outbreeder or an obligate outbreeder?

B. nigra, *B. rapa*, *D. tenuifolia*, *H. incana*, *R. raphanistrum*, *S. alba* and *S. arvensis* are obligate out-crossers, because these species are self-incompatible. Therefore, a score of two is attributed to question 2b (Table 1). *B. juncea* is self-compatible, but in the field up to 30% of the seeds can be derived from out-crossing (Frello et al. 1995). *B. oleracea*, *D. muralis* and *E. gallicum* are predominantly selfing species, albeit low levels of out-crossing cannot always be excluded. Because successful hybridisation with oilseed rape has been reported for *B. oleracea* and *D. muralis* under controlled (ideal experimental) conditions, a score of one is given.

Step 3: Production of viable and fertile F1 hybrids

Although interspecific hybridisation is usually more frequent and successful when oilseed rape serves as the maternal parent (Scheffler and Dale 1994; Rieger et al. 1999; Chèvre et al. 2004; Jørgensen et al. 2007), only the development of hybrids on wild/weedy relatives as the maternal parent is considered in the present paper for a number of reasons (Fig. 1). First, GM oilseed rape already contains the transgene. Secondly, the more abundant oilseed rape plants in fields will produce overall many more flowers and pollen than the wild/weedy relative. Third, in some situations, oilseed rape shows a strong selection against foreign pollen. When oilseed rape stigmas are pollinated by pollen mixtures of oilseed rape and *B. rapa* pollen, *B. rapa* pollen has a lower fitness, compared to oilseed rape pollen (Hauser et al. 1997). Fourth, wild/weedy relatives occurring outside agricultural fields may be pollinated by oilseed rape pollen, which leads to the formation of hybrids that may escape control. These uncontrolled wild/weedy relatives are potentially prone to interspecific cross-fertilisation (Simard and Légère 2004). Fifth, an important fraction of hybrid seeds will be harvested and thus be removed from the field with oilseed rape. Finally, hybrid seeds that fell to the ground prior and during harvest will undergo the same agricultural

Table 4 Generic flowering periods for some wild/weedy related species of oilseed rape in Belgium according to Lambinon et al. (2004)

| Plant species | Month | | | | | | | | | | | |
|------------------------------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
| Winter oilseed rape | | | + | + | + | | | | | | | |
| Spring oilseed rape | | | | | | + | + | | | | | |
| <i>Brassica juncea</i> | | | | | | + | + | + | + | + | | |
| <i>Brassica nigra</i> | | | | | | + | + | + | | | | |
| <i>Brassica oleracea</i> | | | | | + | + | + | + | + | | | |
| <i>Brassica rapa</i> | | | + | + | + | + | + | + | | | | |
| <i>Diplotaxis muralis</i> | | | | | | + | + | + | + | | | |
| <i>Diplotaxis tenuifolia</i> | | | | | | + | + | + | + | + | | |
| <i>Erucastrum gallicum</i> | | | | | | + | + | + | | | | |
| <i>Hirschfeldia incana</i> | | | | | | + | + | + | + | | | |
| <i>Raphanus raphanistrum</i> | | | | | + | + | + | + | + | | | |
| <i>Sinapis alba</i> | | | | | + | + | + | + | + | + | | |
| <i>Sinapis arvensis</i> | | | | | + | + | + | + | + | + | + | |

management practices as oilseed rape, aiming at controlling oilseed rape volunteers.

In case of transplastomic oilseed rape, it will be important to focus on wild/weedy relatives as the pollen donor. As plastid inheritance is strictly maternal in oilseed rape, the wild/weedy relative may give rise to transplastomic first generation hybrids (Scott and Wilkinson 1999; Johannessen et al. 2005).

In the following section, it is assessed whether interspecific hybrids were observed under controlled (ideal experimental) conditions (including the use of artificial/hand pollination and embryo rescue techniques) and/or field (agricultural) conditions, and whether they are viable, fertile and abundant (cf. steps 3a, 3b, 3c and 3d of Table 1). Attributed scores are presented in Table 2.

Brassica juncea Spontaneous hybridisation between *B. juncea* being interplanted in oilseed rape stands was reported at low frequencies under field conditions. Up to 3% of *B. juncea* offspring were hybrids, depending on the interplant ratio between oilseed rape and *B. juncea* (Frello et al. 1995; Bing et al. 1996; Jørgensen et al. 1998). When the ratio between oilseed rape and *B. juncea* was increased from 1:3 to 1:15, hybridisation frequencies decreased from 2.3 to 0.3% (Jørgensen et al. 1998). Hybrids had low pollen fertility (0–28%) and low seed set (Frello et al. 1995; Bing et al. 1996).

Brassica nigra Bing et al. (1996) failed to detect any spontaneous hybridisation between oilseed rape and *B. nigra* when grown together in the field. Under controlled conditions, detected hybridisation levels were extremely low (Raybould and Gray 1993; Scheffler and Dale 1994).

Brassica oleracea Although hybrids between oilseed rape and *B. oleracea* can be obtained after hand pollination of *B. oleracea* with oilseed rape pollen, these crosses generate very few hybrids (Scheffler and Dale 1994; Eastham and Sweet 2002). Moreover, hybridisation is unlikely under field conditions, because wild populations of *B. oleracea* naturally grow on coastal cliffs such as in the UK. Therefore, *B. oleracea* is rarely sympatric with oilseed rape (Chèvre et al. 1997, 2004; Wilkinson et al. 2000). However, in sympatric sites, hybridisation has been observed, albeit at very low frequencies: from the 842 sampled seeds,

Ford et al. (2006) detected one hybrid in one of two sympatric coastal sites in the UK.

B. rapa occurs as a weedy, wild and ruderal type. The weedy form occurs in agricultural fields and is also present along roadsides, in fallow and in other disturbed areas. Weedy *B. rapa* does not generally adopt the biennial habit, which is predominant among riverside populations. The wild type forms stable populations and grows exclusively on riverbanks where it rarely spreads beyond the limits of flooding. The ruderal type very occasionally occurs on disturbed land (Jørgensen et al. 1998, 2007; Wilkinson et al. 2003a; Chèvre et al. 2004; Daniels et al. 2005; Simard et al. 2006).

Hybridisation frequencies between oilseed rape and weedy *B. rapa* vary between studies. Within *B. napus* fields, hybridisation frequencies range from 0 to 69% of the seeds harvested on *B. rapa* plants depending on the ratio of parental species, parental genotypes, synchrony in flowering times, chromosome location of the (trans)gene, spatial distribution of species, environment and agricultural practices (Jørgensen and Andersen 1994; Jørgensen et al. 1996; Landbo et al. 1996; Mikkelsen et al. 1996b; Warwick et al. 2003; Halfhill et al. 2004; Norris et al. 2004; Leflon et al. 2006; Simard et al. 2006). Although *B. rapa* is suggested to be widespread at low levels of infestation in oilseed rape fields in the UK, Wilkinson et al. (2003a) estimated that – for a mean of 876,000 weedy *B. rapa* plants – 17,000 hybrids would be formed annually in agricultural habitats. Hybridisation frequencies of *B. rapa* plants when grown in field margins are low (Warwick et al. 2003; Halfhill et al. 2004; Simard et al. 2006).

Since pollen of *B. rapa* and *B. napus* have a similar fitness when applied in a mixture on *B. rapa* stigma, they are equally likely to fertilise *B. rapa* (Hauser et al. 1997). Hence, *B. rapa* × oilseed rape hybrids occur frequently when isolated *B. rapa* plants are surrounded by oilseed rape: a large amount of oilseed rape pollen is produced for the self-incompatible *B. rapa* plants. Where *B. rapa* occurs in dense and large patches, hybrids are less frequent due to lower pollen competition from oilseed rape (Jørgensen and Andersen 1994; Jørgensen et al. 1996; Landbo et al. 1996; Simard et al. 2006).

Fitness parameters, including seed dormancy, germinability and male/female fertility, of *B. rapa* ×

oilseed rape hybrids have been studied. Because fitness parameters are affected both by the genotype of hybrids (Mikkelsen et al. 1996a) and by growth conditions such as plant densities (Linder and Schmidt 1995; Pertl et al. 2002; Hauser et al. 2003), divergent, sometimes contradicting, observations have been made. Some data indicate that the dormancy parameters of weedy *B. rapa* × oilseed rape seeds resemble more those of the maternal parent (Adler et al. 1993); other emphasize that F1 seeds resemble their crop parent with respect to dormancy (Landbo and Jørgensen 1997; Linder 1998).

As weedy *B. rapa* × oilseed rape hybrids produced many more flowers than *B. rapa* in Danish field experiments, especially when grown at low densities, the total number of pollen produced was higher than that of *B. rapa* (Hauser et al. 1998b; Pertl et al. 2002). However, pollen had a depressed viability, leading to an overall lower male fitness of F1 hybrids relative to its parents (Jørgensen and Andersen 1994; Jørgensen et al. 1996). Reduced pollen viability (on average ranging between 32% and 55%) was also found for interspecific hybrids collected from oilseed rape plots and fields in Canada (Warwick et al. 2003). In paternity studies, Pertl et al. (2002) observed, on average, that only few *B. rapa* seeds – 1.5% (14/957) and 2.5% (22/888) at low and high density plantings, respectively – were sired by pollen of F1 hybrids, indicating a low paternity potential.

Female fitness parameters of weedy *B. rapa* × oilseed rape hybrids grown in mixed plots tend to be as high or even higher than those of the Danish weedy parent: on average, F1 hybrids produced fewer fully developed seeds per pod, but many more pods per plant (Hauser et al. 1998b, 2003; Pertl et al. 2002). However, a lower seed set per plant compared to *B. rapa* was observed for F1 hybrids found in a commercial oilseed rape field in the UK (Norris et al. 2004). Reduced reproductive fitness was observed for *B. rapa* × oilseed rape hybrids, but these hybrids were at times as fit or nearly as fit as their parents (Hauser et al. 1998a, b, 2003; Norris et al. 2004).

Compared to weedy *B. rapa*, observed hybridisation frequencies are much lower in wild *B. rapa* occurring beside commercial oilseed rape fields. Because wild *B. rapa* receives more pollen from conspecifics and is generally more separated from oilseed rape, hybridisation frequencies range from 0% to 1.6% (Scott and Wilkinson 1998; Wilkinson et al.

2000; Elliott et al. 2004; Allainguillaume et al. 2006). Based on survey data in the UK, it was calculated that 26,000 hybrids would be formed annually within wild *B. rapa* populations of approximately 1.8 million plants co-existing with oilseed rape fields. The length of riverbanks occurring close to oilseed rape fields was multiplied with the mean density of wild *B. rapa* and hybridisation frequency. An additional 5,600 wild *B. rapa* × oilseed rape hybrids resulting from long-range pollen dispersal from oilseed rape fields was estimated, giving a total of 31,500 hybrids annually formed in the UK (Wilkinson et al. 2003a).

The fecundity of wild *B. rapa* × oilseed rape hybrids is severely reduced (Pallett et al. 2006). Despite similar amounts of viable pollen produced per hybrid plant compared to wild *B. rapa*, only 2.8% of the offspring on *B. rapa* was sired by hybrids. On average, hybrids showed a selfing rate of 6.5% resulting in 9.8–39.2% self-pollinated F2 hybrid seeds. Also female fitness parameters were diminished: *B. rapa* × oilseed rape hybrids overall yielded approximately half as many germinable seeds per plant (Allainguillaume et al. 2006).

Diplotaxis muralis and *Diplotaxis tenuifolia* Studies suggest that only very low numbers of viable F1 hybrids are produced under controlled conditions, and that these plants are male-sterile (Scheffler and Dale 1994).

Erucastrum gallicum No hybrids were detected during a 2-year field study in Canada during which approximately 22,000 *E. gallicum* seedlings were tested (Warwick et al. 2003). Lefol et al. (1997) failed to obtain *E. gallicum* × oilseed rape hybrids under controlled conditions by hand pollination.

Hirschfeldia incana After hand pollination and/or embryo rescue, 1.3 *H. incana* × oilseed rape hybrids were observed per 100 flowers (Kerlan et al. 1992; Chèvre et al. 1996). Low numbers of hybrids were also obtained under field conditions where herbicide resistant (HR) oilseed rape was interplanted with low frequencies of *H. incana*: hybrids between oilseed rape and *H. incana* spontaneously occur at a frequency of 2×10^{-5} . On average over 3 years, 0.6 hybrids were formed per plant, representing 0.4% of the seeds (Lefol et al. 1995, 1996b; Darmency and Fleury 2000). *H. incana* × *B. napus* hybrids had a low

fertility: most pollen grains were not viable and less than one seed per plant was produced under greenhouse conditions (Lefol et al. 1996b).

Raphanus raphanistrum In field plots in France where *R. raphanistrum* was present at low frequencies within and around transgenic oilseed rape fields, only a few hybrids were detected on *R. raphanistrum* at frequencies of 3×10^{-5} to 10^{-7} (Darmency et al. 1998; Chèvre et al. 2000; Thalmann et al. 2001). In Australia, Rieger et al. (2001) found no hybrids among 25,000 seedlings grown from seeds collected from *R. raphanistrum* occurring in fields of imidazolinone resistant oilseed rape. A survey done in the UK also failed to detect any transgene in *R. raphanistrum* growing nearby HR oilseed rape fields (Eastham and Sweet 2002; Norris and Sweet 2002). Similarly, no interspecific hybrids were found among the seedlings of 4,059 *R. raphanistrum* plants collected next to commercial HR oilseed rape fields in Canada (Warwick et al. 2003). The low hybridisation frequencies observed under controlled conditions can be attributed to the wide variability in the ability of oilseed rape pollen to germinate on the *R. raphanistrum* stigma (Guéritaine and Darmency 2001).

The seed dormancy of hybrid seeds fell within the range of their parents (Chadoeuf et al. 1998). Under field conditions, *R. raphanistrum* × oilseed rape hybrids showed a reduced seedling emergence, a significant delay in emergence and a lower survival rate, compared to both parents (Guéritaine et al. 2003). Hybrids have a very low fertility: pollen viability is largely depressed and, on average, they produce less than two seeds per plant (Chèvre et al. 1998; Darmency et al. 1998).

Sinapis alba Successful hybridisation with *S. alba* under controlled conditions is only reported in older studies (Raybould and Gray 1993; Scheffler and Dale 1994). Recent monitoring studies carried out in the UK failed to detect any interspecific *S. alba* × oilseed rape hybrids in sites of co-occurrence (Norris and Sweet 2002; Daniels et al. 2005).

Sinapis arvensis In spite of a single transgenic HR hybrid found in the year after a field trial in the UK (Daniels et al. 2005), no *S. arvensis* × oilseed rape hybrids have been observed under field conditions (Bing et al. 1996; Chèvre et al. 1996; Lefol et al.

1996a; Moyes et al. 2002; Norris and Sweet 2002; Warwick et al. 2003). Lefol et al. (1996a) failed to detect a hybrid among the approximately 2.9 million analysed seeds produced on *S. arvensis* grown in the presence of transgenic HR oilseed rape in France. In Canada, Warwick et al. (2003) did not detect hybrids among the nearly 43,000 tested seedlings under experimental and commercial field settings. Among the *S. arvensis* seeds collected from 102 populations across the UK – within and outside oilseed rape growing areas – no hybrids were found (Moyes et al. 2002). Even under controlled conditions, there is a limited potential to produce hybrids: Bing et al. (1995) did not obtain hybrids through hand pollination, whilst Moyes et al. (2002) found *S. arvensis* × oilseed rape hybrids at a frequency of $\leq 0.0015\%$ of the potential seed output. Pollen fertility of these hybrids was always low or zero (Moyes et al. 2002).

Step 4: Production of viable and fertile F2 and BC progeny

Here, it is discussed whether viable and fertile F2 hybrids and BC progeny were observed under controlled (ideal experimental) conditions and/or field (agricultural) conditions, and whether they are abundant (see steps 4a, 4b, 4c and 4d of Table 1).

Brassica juncea Although hybrids had low pollen fertility (0–28%) and seed set, transfer of oilseed rape markers to the first backcross generation with *B. juncea* was observed under controlled conditions. Pollen fertility of BC1 plants improved (24–90%), compared to that of hybrids (Frello et al. 1995). These results suggest that introgression is possible, but until now no information is available to confirm if introgression will occur under agricultural settings and how persistent it will be.

Brassica rapa Introgressive hybridisation beyond the F1 generation has been observed both under controlled (Halfhill et al. 2004) and field conditions (Mikkelsen et al. 1996a; Hansen et al. 2001; Warwick et al. 2008). When transgenic *B. rapa* × oilseed rape hybrids were grown in plots with weedy *B. rapa*, backcross progeny – that carried the transgene – with a morphology and chromosome number corresponding to the weedy species was obtained within two plant

generations (Mikkelsen et al. 1996a; Halfhill et al. 2002). Average backcrossing frequencies of 0.074% were observed when maternal *B. rapa* plants were pollinated by transgenic hybrids, and of 47.6% when F1/BC1 served as maternal plants and *B. rapa* as pollinator (Halfhill et al. 2004).

Fitness parameters of F2 and backcross progeny vary highly with local plant densities and genotype, which complicates reviewing and summarising fitness data. Focus is put on the main observations, although without neglecting the different observations made. Seed dormancy that was largely absent for hybrid seeds was restored over backcross generations. First and second backcross generations to *B. rapa* produced seeds with a dormancy pattern similar to the weedy parent (Mikkelsen et al. 1996a; Landbo and Jørgensen 1997; Norris et al. 2004).

Although pollen viability has been shown to be highly variable for hybrid progeny derived from Danish weedy *B. rapa* populations, on average, pollen viability of F2 (48%) and BC1 (43%) plants is severely depressed compared to *B. napus* and *B. rapa* (Hauser et al. 1998a). In contrast to F1 hybrids, female fitness parameters of F2 and BC generations grown in mixtures in the field were lower than those of *B. rapa*: on average, they produced fewer pods and viable seeds (Hauser et al. 1998a, 2003). Due to an inferior vegetative vigour and photosynthetic capability, backcross plants strongly reduced their seed production (Hauser et al. 2003; Ammitzbøll et al. 2005). In general, one can say that F2 and BC progeny have an overall lower fitness relative to their parental species and F1 hybrids.

According to Hauser et al. (2003) first generation hybrids are most likely to transfer genes to the next generation via seeds if grown at low frequencies among *B. rapa* plants, which is a common situation in agricultural fields. In the rather uncommon situation where hybrids occur in much higher frequencies than *B. rapa*, hybrids may pollinate *B. rapa*.

Hirschfeldia incana Due to the low fertility of hybrids and genome incompatibility, backcross plants were rarely observed under controlled conditions. Moreover, the likelihood of transgene loss in subsequent generations was shown to be high (Darmency and Fleury 2000).

Raphanus raphanistrum Due to the reduced seedling emergence of *R. raphanistrum* × oilseed rape hybrids,

a significant delay in emergence, a lower survival rate, and very low fertility, gene introgression from oilseed rape to *R. raphanistrum* is unlikely. The decreasing percentage of HR plants obtained during successive backcrossing under controlled conditions confirmed that the transgene used as marker did not recombine into the *R. raphanistrum* genome (Chèvre et al. 1997, 1998; Al Mouemar and Darmency 2004). Moreover, cytoplasmic incompatibility markedly reduced the vigour and viability of BC5 and BC6 plants (Guéritaine et al. 2002; Benabdelmouna et al. 2003).

Step 5: Introgression

Several interspecific hybrids between oilseed rape and certain of its wild/weedy relatives have been described, but under field conditions, transgene introgression is only confirmed for *B. rapa* × oilseed rape hybrids (Hansen et al. 2001, 2003; Warwick et al. 2003, 2008; Norris et al. 2004; Jørgensen 2007). The low average fitness of BC progeny probably slows down introgression, but does not hinder it: many of these plants do survive and reproduce, and some were as fit as their parents (Hauser et al. 1998a, b, 2003). Moreover, transgene introgression is likely to take place when both species are present in the same area over several growing seasons, since the acquisition of certain types of transgenes does not necessarily seem to impose fitness costs in backcrossed plants (e.g. Snow et al. 1999).

Step 6: Persistence of transgenes in natural/weedy communities

In agricultural fields, management practices will largely affect the extent of the introgressive hybridisation process and subsequently the persistence of transgenes. When weed management is effective, there may be a higher potential for hybridisation between oilseed rape and *B. rapa*, because individual *B. rapa* plants that escaped herbicide treatment are very prone to oilseed rape pollen. Due to their reduced dormancy, most F1 hybrid seeds germinate after harvest. F1 hybrids re-appearing in cereal crops – usually grown for 2 years after oilseed rape – will be killed by broadleaf herbicides before reaching the adult stage. In contrast to F1 hybrid seeds, BC seeds have a restored dormancy, allowing them to persist success-

fully in the soil, and have a more *B. rapa*-like scattered germination pattern. These plants may not only re-appear in cereals, but also in oilseed rape regrown as a break crop in year four of the rotation. Because standard herbicides used in oilseed rape control hybrid progeny poorly, their only opportunity to reach maturity and produce seeds is one in every 4 years. Accordingly, the development of transgenic hybrid progeny is expected to be slow in conventionally managed fields. Only two introgressed plants beyond the F1 stage were found in 2,450 plants from eight populations of weedy *B. rapa* in conventionally managed oilseed rape fields in Denmark (Jørgensen et al. 2004). Weedy relatives growing in less intensively managed fields or in fields that are left fallow in the years following the growing of oilseed rape, may, however, escape weed control. In a large population of volunteer oilseed rape and weedy *B. rapa* that occurred in a Danish field (4 ha) under organic cultivation for 11 years, Hansen et al. (2001, 2003) observed recombined oilseed rape DNA into *B. rapa* genome. From 102 plants screened, 44 appeared to be introgressed beyond the F1 generation. Outside agricultural fields (e.g. field margins, road verges) wild/weedy relatives may be more or less successfully controlled depending on the type of management used and its timing of application (Garnier et al. 2006).

In addition to management practices, the introgressive hybridisation process and persistence of transgenes in natural/weedy communities will be influenced by pest pressure and plant competition (Hails et al. 2006). Empirical studies – which investigated the fitness impact of transgenes through the manipulation of the selection pressure of targeted pests – have demonstrated enhanced fitness for certain transgenic plants under high selection pressure (Stewart et al. 1997; Halfhill et al. 2002, 2005; Mason et al. 2003). *B. rapa* × oilseed rape hybrids containing *Bacillus thuringiensis* (Bt) transgenes had a fitness (e.g. fecundity) advantage under high insect herbivore pressure: hybrids produced 1.4 times more seeds than *B. rapa* plants, and their seeds had similar germination rates (Vacher et al. 2004). However, in the absence of pest pressure, it has been suggested that hybrids might have a lower fitness than the wild/weedy relative, due to potential deleterious effects of hybridisation and resistance expression. If the transgene acting as a dominant allele – and genes linked to it – reduce

fitness, then transgenes are expected to decline in frequency, or even to be lost in the natural/weedy community. In contrast, if these genes increase fitness of recipient plants, then the frequency of transgenes will increase over time, ultimately leading to adaptive/persistent introgression. Neutral alleles or alleles that have a weak effect (positive or negative) on fitness are expected to introgress into natural communities and to persist as rare alleles at low levels (Mercer and Wainwright 2008). Recent observations confirmed the persistence of a transgene from a GM HR oilseed rape over a period of 6 years into a population of *B. rapa* in the absence of herbicide selection pressure (with the exception of possible exposure to the active substance glyphosate in 1 year) and in spite of fitness costs associated with hybridisation (Warwick et al. 2008). Whether ecological fitness traits will alter specific life-history parameters and the invasive potential of wild/weedy relatives and their hybrid progeny in natural communities remains unclear (Hails and Morley 2005). Under natural conditions, pest pressure and plant competition will be variable in space and time. Fitness advantages enjoyed by plants that escaped pest attack in one generation may be reduced in the following one, due to the absence or low number of available competition-free germination sites (Crawley and Brown 1995; Hails et al. 2006).

Discussion of gene flow index

By attributing a score to each successive step in the introgressive hybridisation process, a conceptual approach was developed that allowed estimating the introgressive hybridisation propensity (IHP) of each cross-compatible oilseed rape wild/weedy relative. Information on the presence and abundance of wild/weedy relatives growing sympatrically with oilseed rape should ideally be obtained at the landscape level. Surveys in Belgium have shown that eleven cross-compatible species were found in areas where oilseed rape is grown, although their absolute abundances are unknown. Information on step 2 to 6 can be retrieved from literature and has been reviewed in the present paper for oilseed rape relatives found in Belgium. Next to flowering characteristics of oilseed rape and wild/weedy relatives, focus was put on hybridisation frequencies, fitness parameters – such as dormancy, germination, survival and fecundity – of interspecific

hybrids and their progeny, the transgene and management practices, as these all play a crucial role in achieving introgression. From the eleven studied wild/weedy oilseed rape relatives, *B. rapa* had the highest introgressive hybridisation propensity (IHP value=11.5), followed by *H. incana* and *R. raphanistrum* (IHP=6.7) in Flanders. Due to its high potential to produce some interspecific hybrids under field conditions, *B. juncea* was ranked in fourth position, although it is rare in Flanders and is a partial inbreeder. Rank five was taken by *D. tenuifolia* and *S. arvensis*. That *D. tenuifolia* and *S. arvensis* are obligate outbreeders and show a relative high abundance in Flanders was counterbalanced by their very low potential to produce interspecific hybrids under controlled conditions. Being less abundantly distributed *B. nigra* was ranked as seventh. Because *S. alba* is quite rare in Flanders and because successful hybridisation between oilseed rape and *S. alba* has not been detected under field conditions yet, it was ranked as eighth. IHP values of the obligate inbreeders *B. oleracea*, *D. muralis* and *E. gallicum* were the lowest, compared to those of *B. rapa*.

IHP values will vary according to differences in distribution and abundance of wild/weedy relatives in the zones of co-occurrence. This confirms the importance of collecting information at the landscape level in order to cover regional variability. The proposed ranking might also change in the light of new empirical data collected under agriculturally and/or ecologically more realistic conditions over several years (see Warwick et al. 2008). Certain oilseed rape wild/weedy relatives (such as *B. rapa*, *H. incana*, *R. raphanistrum*, *S. arvensis*) and transgenic traits (such as herbicide resistance) have attracted much more research efforts on introgressive hybridisation than others. More data on the latter are needed to refine the IHP values.

The developed gene flow index is a semi-qualitative one: scores were either a zero in case the answer to a question is 'no' or a number (≥ 1) in case the answer is 'yes.' Consequently, each of the six successive steps was considered of equal importance in the introgressive hybridisation process. A quantitative index in which scores are based on probabilities (provided that these become available via experimental research or modelling work) might enhance the discriminative power of the gene flow index. Probabilities would automatically weigh each step in the introgressive

hybridisation process differently, emphasising their absolute or relative importance.

Implications for pre-release risk assessment and post-market environmental monitoring of GM oilseed rape

Ranking cross-compatible wild/weedy relatives of oilseed rape according to their introgressive hybridisation propensity is valuable in the frame of pre-release risk assessments (Wilkinson et al. 2003b; FitzJohn et al. 2007). Potentially adverse effects on the environment, resulting from the vertical gene flow between GM oilseed rape and certain of its cross-compatible wild/weedy relatives, are generally subjected to evaluation before release. The gene flow index may complement the exposure term of the risk equation [$\text{risk} = f(\text{hazard}, \text{exposure})$]. On the one hand, it considers how far pollen can be dispersed, if hybridisation occurs, how abundant the wild/weedy relative is, whether the resulting hybrids and backcross progeny are viable, fertile and abundant, and whether introgressants do appear and persist. On the other hand, it takes into account that the frequency with which interspecific hybrids and backcross progeny would be formed largely varies among the wild/weedy relatives of oilseed rape. However, since the estimation of vertical gene flow per se is not considered to be a complete risk assessment, exposure data need to be combined with those on the potential adverse consequence (=hazard). In this respect, the potential establishment and spread of hybrids and backcross progeny should be considered, since their fitness and invasive potential may be enhanced by transgene uptake. Due to the low IHP values of *D. tenuifolia*, *S. arvensis*, *B. nigra*, *S. alba*, *B. oleracea*, *D. muralis* and *E. gallicum* in Flanders, compared to *B. rapa*, the exposure term of the risk equation is expected to be very low. As no or only very low numbers of hybrids were obtained between oilseed rape and some of these wild/weedy relatives under worst-case conditions (e.g. through the use of artificial pollination and embryo rescue techniques under ideal experimental conditions), it is highly likely that exposure under real conditions will be negligible. The probability that the successive steps in the introgressive hybridisation process will be completed to realise the hazard will be extremely small. As such, the gene

flow index offers the possibility to systematically eliminate improbable hazards (and subsequently risks), and thus may serve as a predictive tool in risk assessment.

Besides risk assessment, the gene flow index provides valuable information for post-market environmental monitoring of GM oilseed rape. In the EU, monitoring is composed of case-specific monitoring and general surveillance. In the case-specific monitoring, anticipated potentially adverse changes are related to the cultivation of specific GM crops, whereas in the general surveillance the detection of unforeseen changes without known specific cause is aimed at. Case-specific monitoring is mainly triggered by scientific uncertainties that were identified in risk assessment. A hypothesis is established that can be confirmed or rejected on the basis of newly collected data. In contrast, the general status of the environment being associated to the uses of GM crops is monitored without any preconception in general surveillance (Sanvido et al. 2005; European Food Safety Authority 2006).

Since cross-compatible wild/weedy relatives that acquired a transgene may have unwanted effects on other species and ecosystem integrity, they deserve special monitoring attention. This may be even more relevant for wild relatives that occur in natural communities with conservational significance or that possess a greater scope to harm species of conservational importance. To select representative areas where potential adverse effects are most likely to be detected and thus where case-specific monitoring should be done, one may rely on the geographical distribution maps of agricultural fields and wild plant species. By overlaying these maps with geographic information system (GIS) software – taking into account the perimeters in which viable oilseed rape pollen is distributed in significant amounts – areas of co-occurrence between oilseed rape fields and its wild/weedy relatives can be identified and their frequency of occurrence be defined at the landscape level for successive years. As such, the location and number of areas to be followed up, as well as their distribution can be set. In other words, our approach not only allows defining the monitoring intensity in and over different geographical areas, reflecting varying levels of conservational importance, but also ensures adequate spatial and temporal replications. Moreover, ranking wild/weedy relatives according to their IHPs determines the monitoring priorities within the pool of

cross-compatible wild/weedy relatives. Focussing on the scientific uncertainties and knowledge gaps identified in each successive step of the introgressive hybridisation process for each wild/weedy relative also allows prioritising future monitoring and research efforts case-by-case in a holistic manner.

Since general surveillance aims at tracing unforeseen and cumulative changes related to the large-scale growing of transgenic crops, any unexpected shift in the abundance of wild/weedy relative plant populations in and outside agricultural habitats should be surveyed. Once an unexpected change in plant abundances or population structure has been detected, it has to be determined whether this change is adverse and whether it is associated to the use of transgenic crops. At the European level, the collaboration with existing surveillance networks is currently suggested for general surveillance of GM crops. The reason is that these networks were designed to obtain information on the state of the receiving environment as a result of what they might provide good baseline data for comparison purposes. Farm-based surveys using farmers' questionnaires may deliver first hand data on the occurrence and relative abundance of wild/weedy relatives within agricultural fields: farmers have experience in estimating their crops and surroundings for variance and deviation in performance (Schiemann et al. 2006). Networks that survey wild plant species may supply distribution and abundance data in non-agricultural habitats. However, due to the poor survey quality and unsuited survey design of wild/weedy oilseed rape relatives (e.g. recording of only presence/absence, non-systematic scale and time of data collection, random selection of survey sites, update of the information, use of voluntary workers), botanical survey networks seem to be of limited help for general surveillance in Belgium (Legg and Nagy 2006). Most programmes are not designed to characterise variation in populations or their sources or to distinguish 'unusual' from 'usual' variation at the landscape level. To suit the collected data to general surveillance needs, the abundance of wild oilseed rape relatives should be surveyed with a higher spatial accuracy and on a more frequent and systematic basis. In practice, however, such intensive surveys may be unfeasible at the landscape level. Considering that monitoring should be cost-effective and proportionate to the extent of market introduction of GM crops (Neemann et al. 2006), survey activities should con-

centrate on wild/weedy relatives that can form hybrids under field (agricultural) conditions, and in areas where potential sympatry is the highest and conservational significance the highest. The selection of such areas can be achieved by overlaying the digital distribution maps of areas where oilseed rape is commonly grown over multiple years with those of areas with conservational importance. Within this context, botanical surveys can serve to define areas where monitoring and/or surveillance for hybrids should be done in priority (Simard et al. 2006). This could be one step forward in ensuring long-term coherent and reliable data collection that could meet general surveillance needs of GM crops in Belgium.

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