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REVIEW

Pollen-mediated intraspecific gene flow from herbicide resistant oilseed rape (*Brassica napus* L.)

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Abstract The cultivation of genetically modified (GM) herbicide resistant oilseed rape (*Brassica napus*) has increased over the past few years. The transfer of herbicide resistance genes via pollen (gene flow) from GM crops to non-GM crops is of relevance for the realisation of co-existence of different agricultural cultivation forms as well as for weed management. Therefore the likelihood of pollen-mediated gene flow has been investigated in numerous studies. Despite the difficulty to compare different experiments with varying levels of out-crossing, we performed a literature search for world-wide studies on cross-fertilisation in fully fertile oilseed rape. The occurrence and frequency of pollen-mediated intraspecific gene flow (outcrossing rate) can vary according to cultivar, experimental design, local topography and environmental conditions. The outcrossing rate from one field to another depends also on the size and arrangement of donor and recipient populations and on the ratio between donor and recipient plot size. The outcrossing levels specified in the presented studies are derived mostly from experiments where the recipient field is either surrounding the donor field (continuous design) or is located as a patch at different distances from the donor field (discontinuous design). Reports of gene flow in *Brassica napus* generally show that the amount of cross-fertilisation decreases as the distance from the pollen source increases. The evidence given in various studies reveals that the bulk of GM cross-fertilisation occurs within the first 10 m of the recipient field. The removal of the first 10 m of a non-transgenic field facing a GM crop might therefore be more efficient for reducing the total level of cross-fertilisation in a recipient sink population than to recommend separation distances. Future experiments should investigate cross-fertilisation with multiple adjacent donor fields at the landscape level under different spatial distributions of rapeseed cultivars and different cropping systems. The level of cross-fertilisation occurring over the whole field is mainly important for co-existence and has not been investigated in agricultural scale experiments until now. Potential problems with herbicide resistant oilseed rape volunteers arising from intraspecific gene flow can be largely solved by the choice of suitable cultivars and herbicides as well as by soil management.

Keywords *Brassica napus* L. · Cross-fertilisation · Pollen flow · Herbicide resistance · Co-existence · Buffer zone

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Introduction

Oilseed rape *(Brassica napus* L.) is the most important oil plant in temperate regions of the world and after soybean it ranks second amongst oilseed crops produced world-wide. Oilseed rape plants produce 9.3 ± 0.5 kg pollen/ha and day over a period of approximately 3–4 weeks. (Becker et al. 2001; Westcott and Nelson 2001). The pollen viability varies with environmental conditions, particularly temperature and humidity. Under natural conditions pollen viability gradually decreases over 4–5 days (Ranito-Lehtimäki 1995). Oilseed rape is largely self-fertilising, but also shows a considerable degree of receptiveness to pollen from other varieties and a tendency to outcross. Under field conditions 12.5–32.8% (Rakow and Woods 1987), 12–47% (Becker et al. 1992) and 41.1% (Lavigne et al. 1998) cross-fertilisation have been reported. The rate differs in relation to the oilseed rape variety and to the environmental conditions and averages around 30%. Oilseed rape pollen grains are large (32–35 μm), heavy and sticky. Under field conditions cross-fertilisation is mediated through physical contact between neighbouring plants as well as by wind and insects. The flowers of oilseed rape produce nectar with relatively high concentrations of sugars and have a colour and structure, which makes them attractive to insects, particularly bees. Insect foraging behaviour is complex, being dependent on a number of factors including spatial arrangement of plants, environmental conditions, plant density and availability of pollen and nectar (Eisikowitch 1981; Rieger et al. 2002). Many studies have shown that a large proportion (up to 80%) of bee flights are less than 1 m in distance, with the majority of pollen being transported by bees less than 5 m (Cresswell 1999; Ramsay et al. 1999; Pierre 2001). Occasionally however, bees may travel much further and studies have measured bee flight distances of 1–2 km (Eckert 1933), up to a maximum distance of 4 km (Ramsay et al. 1999; Thompson et al. 1999). A number of studies have also documented wind dispersal of oilseed rape pollen (Timmons et al. 1995; Simpson et al. 1999; Becker et al. 2001). The vast majority of wind dispersed pollen travels less than 10 m and the amount of pollen decreases as the distance from the pollen source increases (Timmons et al. 1995; Thompson et al. 1999). The dispersal range of rapeseed pollen is variable, from a few meters to several hundred meters (Stringham and Downey 1982). In extreme cases there is evidence of wind transfer of viable pollen up to 1.5 km (Timmons et al. 1995). Air-borne pollen dispersal distances vary with the environmental and topographical conditions. Pollen movement depends on wind direction, wind speed, topography and surrounding vegetation (Gliddon et al. 1999; Thompson et al. 1999). Longer distance pollen transfer (termed ‘regional pollen’) occurs when pollen grains are caught by upward air movements and are transported above the height of vegetation and the local air currents created by surface features. The relative importance of the pollen vectors wind and insects has not been fully resolved and varies regionally and seasonally (Ingram 2000; Treu and Emberlin 2000). Hayter and Cresswell (2003) used an approach, where petals were removed from oilseed rape flowers, to demonstrate insects as primary pollen vectors. Unanimously, Ramsay et al. (2003) showed that covering male sterile plants placed at various distances from a pollen source with a cage of coarse netting largely reduced the number of pollinated flowers. Precise data on the extent of pollination that is mediated through insects or wind, however, are lacking. Generally, it seems to be clear that both factors of pollination are important, and that insects may have a key role for long distance pollination events.

As pointed out above, the occurrence and frequency of pollen-mediated intraspecific gene flow (outcrossing rate) can vary according to cultivar, experimental design, local topography and environmental conditions. In addition, the outcrossing rate from one field to another depends also on the size and arrangement of donor and recipient populations and on the ratio between donor and recipient plot size (Eastham and Sweet 2002, Ingram 2000).

Pollen-mediated gene flow and the subsequent fertilisation and establishment of reproductive individuals is an important aspect for setting co-existence measures associated with the cultivation of genetically modified (GM) plants. However, although the focus of co-existence is on ensuring GM free products in conventional agricultural production, co-existence is a two-way process and goes for transgenic crops as well as for conventional and organic crops. This means that while according to European legislation a labelling threshold of 0.9% for the adventitious presence of GM crop products has been set for non-transgenic food and feed, transgenic varieties also have to be protected against foreign gene flow in order to guarantee their specific qualities. *Brassica napus* is one of the most concerning crops in relation to gene flow because it shows considerable outcrossing rates, gives rise to volunteer emergence in the field and can form temporary feral populations. In oilseed rape the dispersal of genes via pollen can cause the adventitious presence of transgenes in neighbouring crops, volunteers and feral populations. Transgenic volunteer and feral plants can serve as a source for further pollen-mediated gene flow (Hall et al. 2000) and may therefore be of relevance for the co-existence of transgenic and conventional crops.

So far, herbicide resistance (HR) is the most common transgenic trait for oilseed rape. It also serves as an excellent marker to follow gene flow. This review focuses on studies on pollen-mediated gene flow from herbicide resistant rapeseed to non HR rapeseed, their impact on containment strategies as well as on the consequences of intraspecific gene flow for agricultural practice.

Studies on the effects of various factors on the level of outcrossing from transgenic to non-transgenic rapeseed

Published world-wide studies on cross-fertilisation in fertile oilseed rape were assigned to one of three specified design classes (Tables 1–3). The tables summarise a number of representative studies which have measured pollen-mediated gene flow from herbicide resistant oilseed rape into a surrounding non HR border area (Table 1), to non HR field patches (Table 2) and to individual fertile plants (Table 3).

Evidence is given in these various studies that there is a considerable potential for oilseed rape pollen to be dispersed by wind and insects and to remain viable over large distances. In general, the frequency of herbicide resistant individuals in the progeny of fertile plants decreases sharply with distance from the source plot, but some resistant progeny are still observed at long distances. All these studies (Tables 1–3) may only provide rough estimates for outcrossing frequencies. They are subject to variation caused by identified factors such as shape, orientation and size of the pollen source and recipient, isolation distance and pollen barriers between the pollen source and recipient, climatic conditions, the number of bees and other insects, local topography, flowering synchrony, genotype and zygosity (Eastham and Sweet 2002; Emberlin et al. 1999; Ingram 2000; Treu and Emberlin 2000).

Shape, orientation and size of pollen source and recipient field

Two types of experimental designs can be distinguished (Tables 1, 2; visual representation in Table 4): the continuous design, where the recipient plants are spaced around the source plot and the discontinuous design where recipient plants form patches situated adjacent or at various distances from the source. Table 4 shows for the continuous and for the discontinuous design the mean values of cross-fertilisation at several distances, based on all studies where average outcrossing data were available. Using the continuous design, average values of cross-fertilization over different studies (Brown et al. 1996; Dietz-Pfeilstetter and Zwerger 2004; Feldmann et al. 1998, Morris et al. 1994; Scheffler et al. 1993; Staniland et al. 2001) are highest immediately next to the source (1.78%) but are frequently constant around 0.05% over tens of meters. Using discontinuous field trials (Beckie et al. 2003; Champolivier et al. 1999; Dietz-Pfeilstetter and Zwerger 2004; Downey 1999a, b; Scheffler et al. 1995; Simpson, unpublished, cited by Eastham and Sweet 2002) the outcrossing rate declines slowly and steady from a mean value of 0.94% next to the source and is constant around 0.1% over 100 m.

In general the continuous design seems to favour short distance pollen dispersal. This results in high outcrossing frequencies in the first ten meters and in a more rapid decline compared to the discontinuous design. Therefore in the continuous design experiments a clear and high edge effect and a rapid decline of cross-fertilisation over the next 50 m can be observed. In some studies of the continuous design class very high values of cross-fertilisation were detected in 0 m distance from the pollen source (Brown et al. 1996; Scheffler et al. 1993). This might be due to the experimental design or to the kind of sampling (i.e. sampling only plants from the first row

Table 1 Pollen-mediated gene flow from herbicide resistant oilseed rape to non-transgenic border rows (continuous design)

Location, year and experimental design	Isolation distance (m)	Size of pollen donor plot	Width of border area (m)	Outcrossing rates (%) at different distances within the recipient field	Reference
USA, 1993–1995		10 m^2	1993: 8	0 m: 6.3	Brown et al. (1996)
Seasons: 2				7.5 m: 0.5	
Locations: 2					
Data combined over two locations			1994: 30	5 m: 0.1	
Germany, 1999–2001	A: 0.5	0.5 ha each	50	2000/2001:	Dietz-Pfeilstetter and Zwerger (2004)
Seasons: 2				0 m: 0.94/0.67	
Locations: 1				20 m: 0.07/0.11	
				50 m: 0.07/0.05	
2 × 2 transgenic plots (2 different HR types; 2 different isolation distances) data averaged over sample points and wind directions	B: 10	0.5 ha each	50	2000/2001:	
				0 m: 0.24/0.24	
				20 m: 0.08/0.12	
				50 m: 0.04/0.09	
Germany, 1995–1997	6	1360 m^2	8	1996/1997:	Feldmann (2000)
Seasons: 2				0 m: 7.6/9.4	
Locations: 1				8 m: 0.7/1.2	
Data averaged over sample points and wind directions					
USA, 1992		251 m^2	5	Loc. A/B :	Morris et al. (1994)
Seasons: 1				0 m: 2.0/3.5	
Locations: 2				0.3 m: 1.0/1.5	
Data averaged over sample points and wind directions (east and west)				0.6 m: 0.75/1.2	
				3 m: 0.65/0.6	
				4.6 m: 0.5/0.6	
Germany, 1997–2001	15–35	4 × 240 m^2	7.5	0.026–0.13	Hommel and Pallutt (2003)
Seasons: 2					
Locations: 1					
Data averaged over sample points and wind directions					
UK, 1991		64 m^2	47	0.5 m: 4.8	Scheffler et al. (1993)
Seasons: 1				1 m: 1.5	
Locations: 1				3 m: 0.4	
				6 m: 0.11	
				12 m: 0.016	
				24 m: 0.0041	
				36 m: 0.0011	
				47 m: 0.00034	
				70 m: 0	
Canada, 1994–1995	1.5	1800 m^2	15–30	0 m: 0.7	Staniland et al. (2001)
Seasons: 2				2.5 m: 0.3	
Locations: 2				5 m: 0.1	
Data combined over all environments and wind directions				10 m: 0.07	
				15 m: 0.08	
				20 m: 0.07	
				25 m: 0.04	
				30 m: 0.03	

Table 2 Pollen-mediated gene flow from herbicide resistant oilseed rape to non-transgenic fields (discontinuous design)

Location, year and experimental design	Size of pollen donor field	Size of acceptor field	Isolation distance (m)	Outcrossing rates (%) at different distances	Reference
Canada, 1999 Seasons: 1 Locations: 11 Data averaged over locations A: Glyphosate resistant donor B: Glufosinate resistant donor	10–64 ha	10–64 ha		A/B : 0 m: 1.1/1.4 50 m: 0.15/0.22 100 m: 0.13/0.15 200 m: 0.13/0.03 400 m: 0.04 /0.05 600 m: 0/0 800 m: 0/0	Beckie et al. (2003)
France, 1995 Seasons: 1 Locations: 3	1 ha	1 ha		1 m: 2.0 20 m: 0.2 65 m: <0.01	Champolivier et al. (1999)
Germany, 1999–2001 Seasons: 2 Locations: 1 2 different isolation distances; data averaged over transgenic plots with different HR(Glyphosate, Glufosinate)	0.5 ha	0.5 ha	A: 0.5	2000/2001: 0 m: 0.93/1.1 10 m: 0.37/0.56 20 m: 0.19/0.36 40 m: 0.08/0.17 70 m: 0.05 /0.07	Dietz-Pfeilstetter and Zwerger (2004)
	0.5 ha	0.5 ha	B: 10	2000/2001: 0 m: 0.53/0.38 10 m: 0.15/0.29 20 m: 0.07/0.15 40 m: 0.03/0.10 70 m: 0.02/0.11	
Canada, 1998 Seasons: 1 Locations: 2	>16 ha	>16 ha	1	Loc. A/B: 20 m: 1.5/0.01 50 m: 0.4/0 100 m: 0.1/0.4	Downey (1999a, b)
UK Seasons: 1 Locations: 1	10 ha	10 ha		5 m: 1.2–3.3 25 m: 0.25–0.7 40 m: 0.4–0.65 50 m: 0.10–0.25 100 m: 0.11–0.5 200 m: 0.1–0.2	Norris (unpublished, cited by Eastham and Sweet 2002)
Australia, 2000 Seasons: 1 Locations: 63 Samples were pooled per field	25–100 ha	25–100 ha	0–500 500–1000 1000–2000 2000–3000 3000–5000	0–0.16 0–0.11 0–0.2 0–0.15 0	Rieger et al. (2002)

Table 2 Continued

Location, year and experimental design	Size of pollen donor field	Size of acceptor field	Isolation distance (m)	Outcrossing rates (%) at different distances	Reference
UK, 1992 Seasons: 1 Locations: 1 2 different isolation distances; data averaged over two replicates (opposite wind directions)	400 m^2	400 m^2	200 400	0.016 0.004	Scheffler et al. (1995)
UK, 1997–1998 Seasons: 1 Locations: 3 6 m wide non-transgenic pollen barrier between transgenic plot (different HR types) and the adjacent non-transgenic plots (different cultivars)	40 m^2 each	40 m^2		6 m: 0.05 30 m: 0.05 42 m: 0.05–0.33 50 m: 0.05–0.16 10 m: 0.05–0.44 20 m: 0.05 150 m: 0.11–0.22 4 m: 0.16–2 8 m: 0.05–0.33 20 m: 0.16 34 m: 0.05–0.16 50–56 m: 0.05–0.11	Simpson et al. (1999)
UK, 1998 Seasons: 1 Locations: 1	0.8 ha	0.8 ha		1.5 m: 1.0 11.5 m: 0.5 26.5 m: 0.15 51.5 m: 0.1 91.5 m: 0.05	Simpson (unpublished, cited by Eastham and Sweet 2002)

vs. sampling a wider strip), but may also indicate cross-fertilisation levels than can occur when favourable circumstances coincide.

When we have a closer look at the experimental design of the continuous field trials (Table 1), we have to consider that almost all of them used small transgenic plots and relatively wide non-transgenic border areas. Only Feldmann et al. (1998) used an experimental field trial where the ratio between donor and recipient field is close to 1:1; accordingly these authors found high outcrossing rates at the inner edge as well as at the outer edge of the border area. In most cases, however, the ratio between donor and recipient plot size is around 1:4 (Dietz-Pfeilstetter and Zwerger 2004; Hommel and Pallut 2003; Staniland et al. 2001) up to 1:38 (Brown et al. 1996) and 1:129 (Scheffler et al. 1993). Large sink populations are known to subsidise the species pool of small source populations through mass effect. Gliddon et al. (1999) considered most of the outcrossing data reported up to then to be of little use in assessing gene flow because of using small pollen source plots and large recipient populations. Large amounts of non-transgenic pollen over the recipient border area are strong competitors for incoming transgenic pollen. In addition, Damgaard and Kjellsson (2005) show that the probability of cross-fertilisation can be described as a function of distance between fields and field width. In general, the wider the recipient field compared to the donor field, the lower the probability of cross-fertilisation. In the discontinuous design (Table 2) the ratio between donor and recipient is usually close to 1:1 (Champolivier et al. 1999; Dietz-Pfeilstetter and Zwerger 2004; Norris, unpublished, cited by Eastham

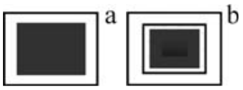
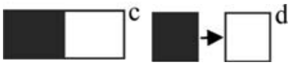
Table 3 Long distance pollen-mediated gene flow from herbicide resistant oilseed rape to individual non-transgenic plants

Location, year and experimental design	Size of pollen donor field	Outcrossing rates (%) at different distances	Reference
Germany, 1995–1997 Recipient: 4–6 fertile plants per sample point Seasons: 2 Locations: 1 Data averaged over sample points and wind directions	1360 m^2 (surrounded by 8 m wide non-GM border)	1996: 200 m: 0.017 1997: 200 m: 0.06	Feldmann (2000)
UK, 1999 Recipient: 10 plants, 4 groups Seasons: 1 Locations: 1 Data averaged over sample points and wind directions	7 ha	0 m : 14.5 10 m : 4.9 50 m : 1.9 225 m: 2.4 550 m: 0.2 800 m: 3.4	Ramsey et al. (2003)
UK Recipient: individual plants Seasons: 2 Locations: 1 Data averaged over sample points and wind directions	9 ha	100 m : 0.03–0.13 200 m : 0.025–0.03 400 m : 0.06	Simpson (unpublished, cited by Eastham and Sweet 2002)

and Sweet 2002; Scheffler et al. 1995; Simpson et al. 1999; Simpson, unpublished, cited by Eastham and Sweet 2002), for which reason the pollen pressure from both sources is presumably equal. As a consequence the outcrossing rate declines in this design class slowly and steady over the first tens of meters and levels off around 0.1% over hundred meters.

In general, the size of the donor and the recipient and the ratio between donor and recipient influence the probability of outcrossing. Irrespective of the

Table 4 Mean values of cross-fertilisation (%) at several distances depending on two specified design classes and the mean values over both designs

Distance from pollen source	Continuous design			Discontinuous design			Mean over both designs		
									
	Mean	Std[e]	*n*[f]	Mean	Std	*n*[e]	Mean	Std[f]	*n*[f]
0–10 m	1.78	2.48	26	0.94	0.51	10	1.54	2.15	36
10–20 m	0.33	0.45	7	0.40	0.47	8	0.37	0.45	15
20–50 m	0.05	0.05	10	0.14	0.11	11	0.10	0.09	21
50–100 m	0.04	0.04	3	0.11	0.11	11	0.09	0.1	14.
>200 m	n.d.[g]	n.d.[g]	n.d.[g]	0.05	0.05	6	n.d.[g]	n.d.[g]	n.d.[g]

[a,b] continuous design class without/with isolation distance

[c,d] discontinuous design class without/with isolation distance (black square: donor, white square: recipient)

[e] standard deviation

[f] number of datapoints

[g] not enough data available

design class a number of experiments used small scale field trials (Brown et al. 1996; Morris et al. 1994; Hommel and Pallutt 2003; Götz and Ammer 2000; Scheffler et al. 1995; Simpson et al. 1999). Small-scale field trials are thought to be inadequate to assess gene flow from GM crops grown on a large agricultural scale (Eastham and Sweet 2002). A theoretical study by Crawford et al. (1999) has examined the effect of increasing donor size on the levels of cross-fertilisation. They concluded that a square plot of 400 m^2 would produce a pollen dispersal characteristic of about ¾ that of a field of 4 ha, but there were indications that the effectiveness of dispersal would decline markedly at plot sizes less than 400 m^2. This means that experiments with small plots may underestimate the level of outcrossing. Also Salisbury (2002) found much smaller values of gene flow from small-scale field trials than from large scale field trials. Only a few studies (Beckie et al. 2003; Downey 1999a, b; Norris unpublished, cited by Eastham and Sweet 2002) carried out large scale experiments. The most realistic one was conducted by Rieger et al. (2002), who are the only ones investigating cross-fertilisation over fragmented landscapes.

But not only the size, also the alignment of donor and recipient field is important for levels of outcrossing. Ingram (2000) emphasized that with a donor field of 2 ha size the rate of outcrossing might be much higher (0.36%) than from a 10 ha field (0.23%), if donor and recipient fields have their long sides abutting.

Isolation distance and border crops between pollen source and recipient field

Isolation zones are used to reduce gene flow between crop populations for wind- and insect-pollinated crops and are common for the production of certified seeds. The required isolation distances to meet certain threshold levels have been reviewed recently (Salisbury 2002). Rapeseed crops were recommended to be separated by at least 100 m for 99.75% certified seed purity and by 200 m for 99.95% basic seed purity. The effectiveness of isolation zones for reducing gene flow from transgenic plants has been investigated in very few studies. Staniland et al. (2001) suggest that outcrossing rates at 30 m into a border crop area were comparable to outcrossing rates for a 200 m isolation distance. Morris et al. (1994) also suggest that the use of a buffer area would be more effective in reducing gene flow than a small isolation distance. Dietz-Pfeilstetter and Zwerger (2004) showed that through a 10 m isolation distance of adjacent plots, gene transfer frequencies were only reduced at the edge facing the transgenic field, but not at sampling points inside the non-transgenic field. When crops are isolated by open ground or low growing crops, it appears that the first rows of the recipient field intercept a high proportion of foreign cross-fertilisation due to the low convarietal pollen load of the field margin (Ingram 2000; Reboud 2003). When there is no gap between transgenic and non-transgenic fields, the plants located in the contact area act on one side as a pollen trap and on the other side they produce additional pollen that dilutes the transgenic airborne pollen, so that at within-field distances comparable to a certain gap width gene flow between fields is decreased. Reboud (2003) suggests that for short distances the strategy of replacing gaps between donor and recipient fields by several rows of non-transgenic oilseed rape plants, which have to be removed after flowering completion would be the most efficient way to limit gene flow. Damgaard and Kjellsson (2005) show that the use of a 5 m discarded buffer zone surrounding the non-transgenic field is expected to reduce cross-fertilisation by one third. In general, the predicted probability of cross-fertilisation between donor and recipient is relatively low if an adequate distance separates them, if the first rows act as a buffer zone and if the recipient field is large.

Genotype and Zygosity

Important factors that can also affect the outcrossing rates are the *genotypes* of the pollen source and sink. The use of different herbicide resistant plant varieties, in particular with glufosinate and with glyphosate resistance as a transgenic marker system, means that different marker systems have different genetic backgrounds (with respect to the cultivar into which the transgene has been incorporated and with respect to the integration site), which can influence the flowering time, the produced pollen quantity and the selfing rate. The differences in reciprocal outcrossing rates using two different transgenic varieties reported by Dietz-Pfeilstetter and Zwerger (2004) indicate that each transgenic variety can show different levels of outcrossing under the same experimental and environmental conditions. A significant donor/recipient effect on the rate of cross-fertilisation between glufosinate resistant and glyphosate resistant oilseed rape varieties was also found by Reboud (2003). In addition, Rieger et al. (2002) and Simpson et al. (1999) emphasized that the variety of the recipient rapeseed has been shown to affect the level of cross-fertilisation. Furthermore, as outlined below, different types of transgenic HR can show different patterns of inheritance. In the case of homozygous glyphosate resistant and glufosinate resistant lines all of the pollen carries the HR gene. In contrast, some studies (e.g. Beckie et al. 2003; Simpson, unpublished, cited by Eastham and Sweet 2002) used the glufosinate resistance hybrid system. The hemizygous female and the homozygous male parent of the glufosinate resistant hybrid oilseed rape system contain the *bar* HR gene at two different loci. In addition, the hemizygous female and the homozygous male parent contain the *barnase* gene for male sterility and the *barstar* restorer gene, respectively. As a result, the hybrid produces transgenic and non-transgenic pollen in a ratio of 5:3, leading to a reduced amount of transgenic pollen as compared to homozygous herbicide resistant lines. Investigation of cross-fertilisation from glufosinate resistant hybrids therefore measures only 5/8 of the actual outcrossing level, resulting in lower outcrossing frequencies compared to homozygous herbicide resistant lines.

Rieger et al. (2002) used a conventional acetolactate synthase (ALS) inhibitor system, the sulfonylurea resistance. Sulfonylurea resistance is conferred by two semi-dominant genes. It might be possible, that the low outcrossing frequencies in this study were due to difficulties in distinguishing between susceptible seedlings and hemizygous seedlings with lower levels of HR, which may not have survived in the field screening test with a lethal discriminating dose of the herbicide chlorsulfuron. Gene dosage effects have been demonstrated in several cases by comparing hemizygous and homozygous transgenic plants, with homozygotes having higher transgene expression levels (Beaujean et al. 1998; James et al. 2002; Richards et al. 2003; Tang et al. 2003).

Individual pollen acceptor plants

Some studies have used individual fertile plants (Table 3) as local pollen traps to measure gene flow. Using this method, outcrossing rates up to 0.83% were detected at a distance of 200 m from the pollen source (Feldmann et al. 1998); in another study 3.4% outcrossing was found at 800 m (Ramsey et al. 2003). However, it is important to realise that the use of individual plants as local pollen traps tends to overestimate the gene flow due to the absence of extensive pollen competitions. Beside this a number of studies have reported maximum distances up to 1.5 km for wind mediated pollen dispersal by using mechanical pollen traps (Timmons et al. 1995) and between 2.5 km and 4 km for pollination by using male sterile bait plants (Timmons et al. 1995; Thompson et al. 1999). These studies are not reviewed here because they only represent the potential for gene flow, i.e. pollen travelling a certain distance, but due to the absence of pollen competition do not provide any information on actual outcrossing.

Local environment and climatic conditions

Experiments were often made in one season at one location. It is likely that some of the results overestimate or underestimate gene flow levels because of the absence of replications. Over years and locations different climatic conditions can occur in different local topographies. Factors such as frictional turbulence and thermal convection can transport large numbers of pollen to higher altitudes where they can be dispersed over long distances. Long-range transport occurs most efficiently in dry conditions with limited mixing of moderate to high wind speeds. Apart from climatic conditions pollen flow is also directed or filtered by topography and structures as vegetation and buildings (Treu and Emberlin 2000). In general, the range of cross-fertilisation at a given site is coupled with the narrow range of weather conditions and the local topography around the field trial and the numbers of bees and other insects, which are likely to increase the amount of pollen transfer.

Recommended measures to reduce GM presence in a non-GM crop caused by cross-fertilisation

Besides the so far described factors affecting crop to crop cross-fertilisation additional sources of adventitious GM presence in a non-GM crop are seed impurities, GM volunteer plants, and GM seed transfer via machinery. Estimated average potential rates of adventitious presence occurring at various stages during farm production are 0.3% for seed impurity and 0.1% for all other sources (volunteers, machinery etc.). Therefore a maximal value of 0.5% for crop-to-crop cross-fertilisation is relevant within the 0.9% threshold set by the EU labelling legislation (Scientific Committee on Plants 2001). Furthermore, it is important to realise, that the overall level of outcrossing from transgenic plots occurring over the whole recipient field will generally be much lower than the average value obtained from different distances as described in various studies. Due to the high interception of foreign cross-fertilisation of the field margin (edge effect) and the negative exponential outcrossing function, the mean level of GM cross-fertilisation occurring over the whole field is mainly determined by outcrossing beyond a certain distance in the recipient field. Although the presented studies have limitations and should not be extrapolated outside the observed range, evidence is given that the bulk of outcrossing occurs within the first 10 m of the recipient field. This means in general, that the predicted probability of cross-fertilisation over the whole recipient field is relatively low if the first rows act as a buffer zone. The removal of the first rows of a non-transgenic field facing a GM crop might be more worthwhile for reducing the total level of cross-fertilisation in the recipient sink population than to recommend separation distances. The buffer zone of the non-transgenic field could be discarded prior to the harvest serving for e.g. bioethanol production or it could be sold as a GM crop in cooperation with GM growers. Alternatively, to ensure co-existence with organic rapeseed producers, large isolation distances have to kept.

Based on the average levels of cross-fertilisation across the studies (Table 4), a 10 m buffer zone at the edge of a non-transgenic field facing a GM field is expected to reduce cross-fertilisation over the whole field as required to reach the maximum cross-fertilisation value of 0.5%. For organic rapeseed production the recommended plant breeder isolation distances of 200 m (ensuring maximal outcrossing of 0.1%) should be sufficient. So far, no clear cut-off distance where levels of outcrossing reach zero, could be determined. To be sure, organic producers could also discared or sell the first rows of their recipient fields.

Possible consequences of intraspecific gene flow for agricultural cultivation

Another concern over agricultural settings may be the occurrence of multiple herbicide resistant oilseed rape volunteers as a consequence of cross-fertilization between different herbicide resistant varieties. Volunteer oilseed rape in subsequent crops is a general problem in crop rotations, which usually can be met by the choice of suitable selective herbicides. Moreover, the emergence of volunteer oilseed rape in subsequent crops can be reduced by delayed and shallow soil cultivation (Gruber et al. 2004). The cultivation of herbicide resistant crops is a special case with respect to volunteer management, in that herbicide resistant volunteer rape cannot be combated in subsequent crops with the same resistance, when using the complementary herbicide. However, control can be gained of herbicide resistant oilseed rape volunteers by avoiding crops with resistance to the same herbicide in the rotation.

A particularly unique situation with respect to volunteer management is the outcrossing of a HR gene to adjacent fields with non-transgenic oilseed rape or with oilseed rape being resistant to a different herbicide. Multiple herbicide resistant plants as a result of pollen flow have already been reported (Downey 1999a, b; Dietz-Pfeilstetter and Zwerger 2004), and in some cases were also found to serve as a source for further outcrossing to adjacent fields (Beckie et al. 2003; Hall et al. 2000). Herbicide resistant volunteers arising from unintended gene flow can be easily managed with herbicides if the following crop is a non herbicide resistant cereal (Downey 1999a), but will require special weed control strategies for instance in transgenic herbicide resistant sugar beet (Dietz-Pfeilstetter et al. 1999). Therefore particular care has to be taken with regard to crop rotation and herbicide management. In order to avoid the formation of multiple herbicide resistant plants, farmers should not grow cultivars with different HRs in adjacent fields. However, as mentioned above, even multiple resistant oilseed rape volunteers are no ‘super weeds’ and can be controlled by alternative herbicides and by appropriate crop rotation.

Transgenes can also be spread through pollen-mediated gene flow to feral oilseed rape growing in semi-natural habitats. Whether GM feral oilseed rape can be expected to have a fitness advantage depends on the transgenic trait. While herbicide resistant feral rape is not more invasive than conventional oilseed rape (Crawley et al. 1993), the situation may be different for oilseed rape with other traits like for instance pest resistance (Stewart et al. 1997). Pollen flow from sporadic occurrences of feral oilseed rape to neighbouring rapeseed fields can be considered a rare event due to the high amount of competing field pollen. Therefore feral plants as a source for further transgene flow may only be a realistic scenario if large feral populations are present close to an oilseed rape field.

Conclusions

The main result from the various studies (Tables 1, 2), independent from all parameters affecting cross-pollination, is that the *bulk of outcrossing* occurs within very short distances, less than 10 m from the source. Lavigne et al. (1998) suggested that approximately 50% of the pollen produced by an individual plant falls down within 3 m and that the probability of cross-fertilisation beyond this distance decreases gradually along a negative exponential curve. Hot spots of cross-fertilisation have been measured up to 3 km away from the donor field (Rieger et al. 2002) in fields with fertile rapeseed plants. So far, no clear cut-off distance where levels of outcrossing reach zero, could be determined.

Due to the existence of other sources for adventitious GM presence a maximal value of 0.5% for crop to crop cross-fertilisation is relevant within the 0.9% threshold for the adventitious presence of GM crop products in non-transgenic food and feed set by the EU labelling legislation. It is important to realise, that the overall level of outcrossing from transgenic plots occurring over the whole recipient field will be much lower than the average value obtained from different distances. The bulk of GM cross-fertilisation occurs within the first 10 m of the field. The removal of a 10 m wide strip at the field side facing a GM crop might be more worthwhile for reducing the total level of cross-fertilisation in a recipient sink population below 0.5% than to recommend separation distances.

Currently there are some knowledge gaps in relation to cross-fertilisation from field to field, which should be filled. The outcrossing levels specified above are derived mostly from experiments where the recipient field is around or adjacent to a single donor field. Future experiments should investigate cross-fertilisation with multiple adjacent fields at the landscape level under different spatial distribution of rapeseed cultivars and different cropping systems. The level of cross-fertilisation occurring over the whole field is most relevant for co-existence and until now has not been predicted in agricultural scale experiments. In addition, in agricultural practice adventitious seed mixing may occur within a field during sowing and harvest. This is also an important factor for co-existence.

A concern over agricultural settings may be the occurrence of multiple herbicide resistant oilseed rape volunteers. In order to avoid the formation of multiple herbicide resistant plants, farmers should not grow cultivars with different HRs in adjacent fields. But even multiple resistant oilseed rape volunteers can be controlled by alternative herbicides and by appropriate crop rotation.

It has to be kept in mind, that management strategies to prevent gene flow from GM oilseed rape are physical approaches. They are useful in order not to exceed specific thresholds, e.g. for seed production, but they are probably an ineffective way to prevent gene flow under commercial agricultural conditions. Molecular strategies, like gene containment, could provide an additional solution, if gene flow needs to be prevented completely. Molecular gene containment strategies have been reviewed recently (Daniell 2002; Devos et al. 2004). Most of the methods for gene containment will not be feasible for rapeseed, but some might offer a solution to prevent pollen-mediated gene flow, like cleistogamic or male sterile rapeseed.

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