Gene Flow from Transgenic Oilseed Rape

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Introduction

Since the introduction of genetically modified (GM) plants for commercial production in 1996, the global area of GM crops has continuously grown to 90 million hectares in 21 countries in 2005.[1] Ninety-six percent of the area grown with GM plants was located in six countries (United States: 55%, Argentina: 19%, Brazil: 10%, Canada: 6%, China: 4%, and Paraguay: 2%). Recently, new EU regulations (Nos. 1829/2003 and 1830/2003) concerning the traceability and labelling of GM food and feed products were passed by the European Parliament and the European Council. Labelling is not obligatory for food and feed products with GM proportions below 0.9% of the ingredients considered individually or products consisting of a single ingredient, provided that this presence is adventitious or technically unavoidable during seed production, cultivation, harvest, transport, or processing. In addition, a recommendation on guidelines for the development of national strategies and best practices was published by the European Commission involving cultivation distances between GM crops and non-GM crops, buffer zones, cropping intervals, the control of volunteer plants, etc., to ensure the co-existence of GM crops with conventional and organic farming. Particularly for oilseed rape, which can be described as a high-risk crop for crop-to-crop gene flow due to cross pollination by the vectors insects and wind, specific rules for cultivation are discussed.

The main objective of this study was the examination of short distance outcrossing of transgenic oilseed rape in the nearest neighborhood. The experimental design allowed the detailed determination of the effects of distance and wind direction on pollination frequencies and distribution. For regulations of co-existence of GM crop cultivation with conventional and organic farming, the relationship between distance and outcrossing is of major interest.

In a three-year field trial, the outcrossing frequencies and distribution from plots with different ratios of transgenic plants (100%, 1.0% and 0.1%) containing the *pat*-gene for resistance towards the broad-range herbicide glufosinate-ammonium were determined in surrounding acceptor plots within a distance of 3–11 m using a biotest.

Materials and methods

Plant material

The oilseed rape (*Brassica napus* L.) varieties used in the field trial were the conventional winter variety 'Falcon' and the isogenic transformation line 'Falcon GS40/90', which is tolerant towards broad-range glufosinate-ammonium herbicides (trade names BASTA® or Liberty®) due to the integration of the synthetic phosphinothricin acetyltransferase (*pat*) gene derived from *Streptomyces viridochromogenes*. The transgenic plants were used in 'contamination plots' as source of transgenic pollen ('donors'). The non-transgenic variety 'Falcon' was used in surrounding plots ('acceptors') to determine the outcrossing frequencies from transgenic oilseed rape caused by transgenic pollen.

Experimental design

The field experiments were carried out on an experimental station near Munich (Germany) in the years 2001 to 2004. Each transgenic donor plot was surrounded by eight acceptor plots with non-transgenic plants. Plot size was 6 m x 6 m with 50 plants/m^2. The distance between donor and acceptor plots was 1.5 m **(Fig. 1)**.

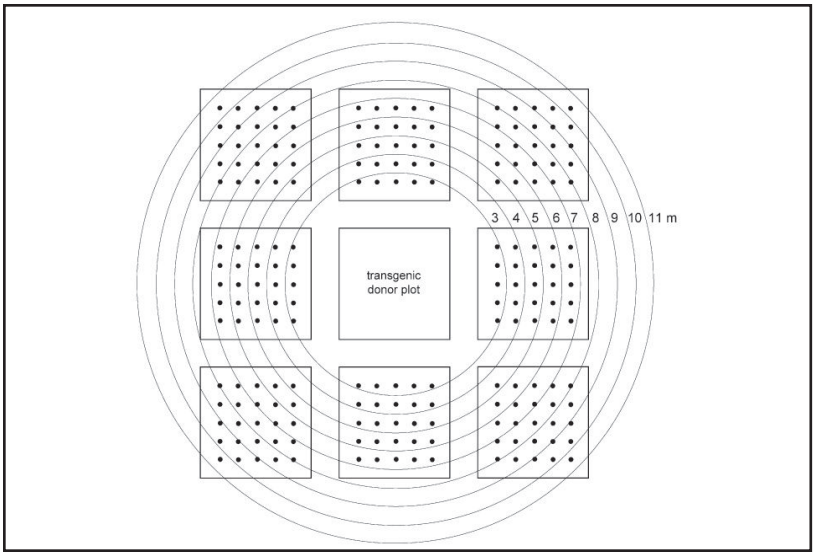

Figure 1: **Schematic representation of the experimental design.** One transgenic donor plot is surrounded by eight non-transgenic acceptor plots. Plot size is 6 m x 6 m, distance between plots is 1.5 m. Dots within the acceptor plots indicate the sampling points. Circles illustrate the distances to the center of the donor plot.

In 2001/2002, the donor plots containing 100% transgenic plants were grown in two replications. In 2002/2003 and 2003/2004, the donor plots contained different ratios of transgenic plants (100%, 1.0% and 0.1%) in order to simulate transgenic seed contaminations. In the vicinity of the field trial, there were several bee hive colonies: about 10 in the north (distance 1 km); 8 in the south (distance 500 m); and 5 in the west (distance 1 km).

Seed sampling and testing

A grid with 25 sampling points was laid over each of the surrounding non-transgenic acceptor plots **(Fig.1)**. Thirty mature pods were collected randomly at each sampling point, resulting in a total of 750 pods per plot. For analyzing the seed samples by means of a biotest, 320 seeds from each sampling point were sown in plastic trays in the greenhouse. Considering a germination capacity of approximately 95%, the technically detectable outcrossing frequency at each sampling point was 0.33% (1 transgenic out of 300 seedlings). After one week, the seedlings were sprayed with a 1% BASTA® solution (200 g glufosinate-ammonium/l). Resistant plants could be distinguished after two weeks from non-transgenic plants. In order to exclude false-positives (i.e., non-transgenic survivors), all putative transgenic seedling were retested by qualitative PCR.

For the construct-specific detection of the *pat*-gene, the primer pair pat_f 5'-CAC AAT CCC ACT ATC CTT CGC-3' and pat_r 5'-TGC TGT AGC TGG CCT AAT CTC A-3' were used to target the 35S-*pat* junction region. PCR control reactions were performed with the primer pair s_gt-f 5'-CAA AGA CGA TAA AGG CTA CGG C-3' and s_gt-r 5'-TAA TGC TCC GAT CAG AGC TTC C-3' for the *Brassica* specific nucleotide sequence of the S-glucosyltransferase gene.[2]

Results and Discussion

In 2001/2002, outcrossing frequencies and distribution from 100% transgenic plots were investigated, whereas in 2002/2003 and 2003/2004, outcrossing was additionally determined from the 1% and 0.1% transgenic plots in order to simulate transgenic seed contamination.

In total, 630,000 seedlings from surrounding acceptor plots were tested in the greenhouse for glufosinate-ammonium resistance. PCR reactions for the exclusion of false-positives were carried out with DNA from all surviving seedlings and resulted in the amplification of a *pat*-specific DNA fragment with the expected size of 141 bp in 87.2% of the seedlings, while an average of 12.8% of the seedlings showed no signals and were therefore classified as false-positives. The combination of biotest and subsequent PCR analysis was very suitable for the detection of outcrossing events because large numbers of seeds could be tested quickly and cost effectively, and therefore a high resolution of the distribution of single outcrossing events could be obtained. The average gene flow within a distance of 3–11 m from the 100% transgenic plots ranged from 0.25% to 0.31% **(Table 1)**. Regarding the 1.0% and 0.1% transgenic plots, a marginal and randomly distributed average gene flow amounting to 0.01% and 0.00083% to 0.0065%, respectively, was determined (**Table 1**).

Figure 2 A–C exemplify the spatial distribution of the outrcossing events around 100%, 1%, and 0.1% transgenic plots. The peaks represent the extent of outcrossing in the surrounding acceptor plots. For all transgenic plots a random distribution with isolated pollination events became apparent.

Transgenic donor plots	2001/2002		2002/2003		2003/2004	
	Outcrossing rates from single plots [%]	Average outcrossing rates [%]	Outcrossing rates from single plots [%]	Average outcrossing rates [%]	Outcrossing rates from single plots [%]	Average outcrossing rates [%]
0,1 %	—	—	0,005	0,0065	0,0017	0,00083
	—		0,008		0,0000	
1 %	—	—	0,002	0,01	—	—
	—		0,018		—	
100 %	0,29	0,29	0,25	0,25	0,31	0,31
	0,29		—		—	

Table 1: Outcrossing rates from transgenic donor plots with different ratios of transgenic plants in 2001/2002, 2002/2003, and 2003/2004.

The transgenic contamination in neighboring oilseed rape crops therefore was clearly below the EU labelling threshold of 0.9%. The experimental design and the extensive sampling in the acceptor plots allowed a detailed analysis of the outcrossing pattern with respect to the wind and distance parameters.

According to a χ^2-test, no signifcant influence of wind on the distribution of transgenic outcrossing was found. Random and undirected gene flow can be explained by insect activity, since honey-bees (*Apis mellifera* L.) and bumble-bees (*Bombus terrestris* L.) play an important role for cross-pollination. Our observations are supported by results of other outcrossing studies with oilseed rape[3,4] which observed no directional effects that could be ascribed to wind activity. Several studies showed that pollen will predominantly be deposited by bees on plants close to the pollen source.[5,6] The overall means of outcrossing events from the 100% transgenic donor plots were plotted against the distance. The pollination declined exponentially with increasing distance **(Fig. 3)**. Applying the fitted curve, outcrossing remains even in the nearest vicinity below the actual threshold of 0.9 % for GMO contaminations of food and feed, thus making recommendations postulating cultivation distances of 200 - 500 m questionable. The effect of the distance on the number of outcrossing events was found to be highly significant ($P < 0.0001$) by correlation analysis. Nevertheless, as shown in this study the effects of the pollination vectors, wind and insects, are often interacting, especially at short distances, and cannot be predicted completely.

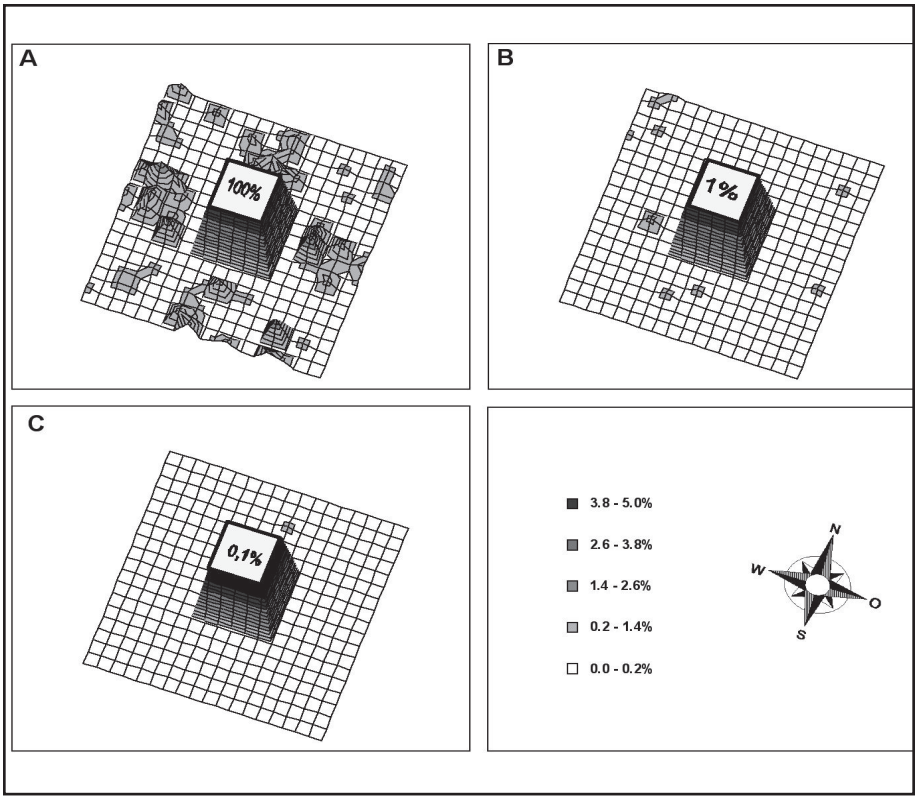

Figure 2: Distribution of the outcrossing events in non transgenic acceptor plots. (A) 100% transgenic donor plot; (B) 1.0% transgenic donor plot; (C) 0.1% transgenic donor plot

Another objective of this field trial was to measure the persistance of

transgenic seeds in the soil. By measurements of 'good agricultural practice' (reduced soil cultivation, application of common herbicides, crop rotation), the number of germinable seeds in the soil seedbank could be reduced by 99.7% to 100% in comparison to the input during harvest within a period of two years.

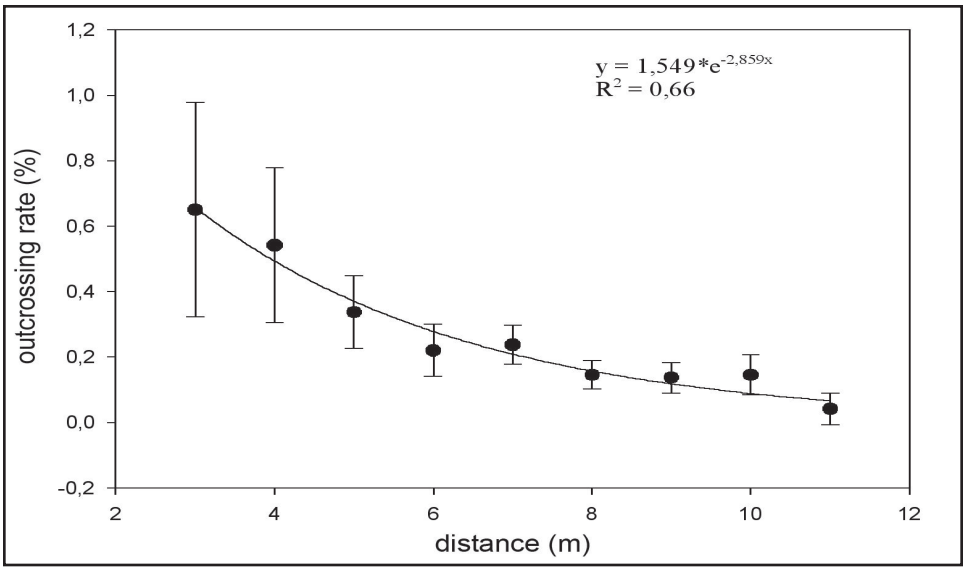

Figure 3: Average outcrossing events as a function of distance from the 100% transgenic plots. The best linear least-squares regression curve fit for the relationship is shown. Standard deviations are represented with error bars.

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