



Parameters Affecting Gene Flow in Oilseed Rape

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The transfer of herbicide resistance genes via pollen-mediated gene flow from genetically engineered (GE) crops to non-GE crops is of relevance for ensuring co-existence of different agricultural cultivation forms as well as for weed management. Co-existence in oilseed rape (*Brassica napus*) depends on the development of management strategies to keep the adventitious presence of GE plant material below the EU labeling threshold of 0.9% in non-GE harvest products. Crop-to-crop cross-fertilization is one source of adventitious GE presence. Several field experiments have been conducted to evaluate pollen-mediated intraspecific gene flow from herbicide resistant to nonresistant oilseed rape. We have performed a literature search for worldwide studies on cross-fertilization in oilseed rape¹ to identify the major factors affecting pollen-mediated gene flow.

Pollen-mediated gene flow in oilseed rape

Most of the studies investigated ($n = 16$) could be categorized as having either a continuous design ($n = 7$), in which a donor plot is completely surrounded by receptor plants, or a discontinuous design ($n = 9$), in which the receptor field is on only one side of the donor, either adjacent or at a distance. Studies using individual fertile plants as local pollen traps to measure gene flow were not considered because due to the absence of local pollen competition they do not provide any information on outcrossing rates under the conditions of agricultural production.

Figure 1 shows the mean values of cross-fertilization for continuous and discontinuous design trials at several distances based on all studies in which average outcrossing data were available. Using the continuous design, the average values of cross-fertilization are highest immediately adjacent to the source ($1.78\% \pm 2.48$) but are frequently constant around $0.05\% (\pm 0.05)$ over tens of meters. For discontinuous field trials, the outcrossing rate declines slowly and steadily from a mean value of $0.94\% (\pm 0.51)$ next to the source and is constant around $0.1\% (\pm 0.11)$ over a hundred meters. In general, all studies demonstrate a steep decline in cross-pollination rates with increasing distance and that the bulk of cross-fertilization occurs within the first 10 m of the field. However, various biological and physical parameters, e.g., size, shape, and orientation of the pollen source and the recipient field, isolation distance, wind characteristics, rain, local environment, genotype, and zygosity, influence cross-fertilization in oilseed rape.

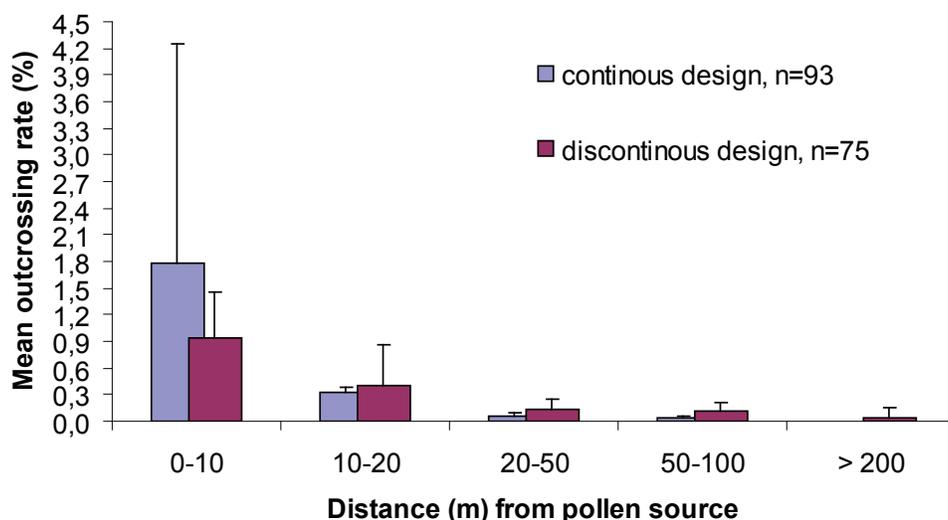


Figure 1. Mean values of cross-fertilization ($\% \pm$ SD) at several distances depending on two specified design classes: continuous design class without/with isolation distance ($n = 93$ data points), discontinuous design class without/with isolation distance ($n = 75$ data points)

• Shape, orientation, and size of pollen source and recipient field

A continuous design seems to favor short distance pollen dispersal; a clear and high edge effect and a rapid decline of cross-fertilization over the next 50 meters is observed. Most field experiments in this design class used small transgenic



plots and relatively wide nontransgenic border areas. It has been observed that large sink populations subsidize the species pool of small source populations via a mass effect. In general, the larger the recipient field compared to the donor field, the lower the probability of cross-fertilization. In the discontinuous design field trials, pollen pressure from both donor and recipient is presumably equal because of similar field sizes. As a consequence, the outcrossing rate in this design class declines slowly and steadily over the first tens of meters and levels off at around 0.1% over a hundred meters. Not only the size but also the alignment of donor and recipient fields is important for levels of outcrossing. With a donor field size of 2 ha, the rate of outcrossing might be much higher than from a 10 ha field, if the long side of a recipient field is facing the source field as compared to the short side. In other words: the deeper the recipient field, the lower the cross-fertilization level of the total harvest product.

- **Isolation distance and border crops between pollen source and recipient field**

Isolation distance is one means to ensure seed purity (e.g., 100 m for certified rapeseed). Some outcrossing studies investigated the effectiveness of isolation zones for reducing gene flow compared to the use of nontransgenic buffer areas. 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 pollen due to the low convarietal pollen load of the field margin. When there is no gap between transgenic and nontransgenic fields, the plants located in the contact area act on one side as a pollen trap; on the other side, plants produce additional pollen that dilutes the transgenic airborne pollen, so that at within-field distances comparable to a certain isolation distance lower rates of cross-fertilization are observed.

- **Genotype and zygosity**

Different herbicide resistant plant varieties, in particular with resistance to glufosinate and glyphosate, have been used in outcrossing studies. Each transgenic variety can show different levels of outcrossing under the same experimental and environmental conditions, influenced by differences in flowering time, pollen quantity, and selfing rate. In addition, in the case of homozygous glyphosate and glufosinate resistant plant lines, all pollen carries the herbicide resistance gene. By contrast, in studies of cross-fertilization from glufosinate resistant hybrids, the amount of transgenic pollen was lower, resulting in only about 5/8 of the outcrossing frequencies of homozygous herbicide resistant lines. Moreover when measuring outcrossing via herbicide spray tests, one has to consider that gene dosage effects have been demonstrated in several cases by comparing hemizygous and homozygous transgenic plants with homozygotes usually having higher transgene expression levels. Therefore hemizygous seedlings with low expression levels of the herbicide resistance gene might not always be easily distinguishable from unmodified susceptible plants.

- **Local environment and climatic conditions**

The range of cross-fertilization at a given location is also determined by the narrow range of weather conditions and local topography around the field trial site, and the numbers of bees and other insects that are likely to increase the amount of pollen transfer.

Management strategies to reduce gene flow

Due to additional sources of adventitious GE presence—seed impurity, volunteers, adventitious seed transfer during harvest and transport—a maximal value of 0.5% for crop-to-crop cross-fertilization is relevant within the 0.9% threshold for the adventitious presence of GE crop products in nontransgenic food and feed set by EU labeling legislation. Technical measures for achieving co-existence have to ensure that thresholds will not be exceeded on a long-term basis. The first few oilseed rape rows intercept a high proportion of pollen when open ground or low growing barrier crops separate oilseed rape fields. The removal of the first 10 m of crop along the side of a nontransgenic field facing a GE crop might be more efficient for reducing the total level of cross-fertilization in a recipient sink population than to recommend separation distances. It appears that the use of predominantly self-pollinating, male sterile, or cleistogamous cultivars as a biological containment strategy will also reduce gene flow^{2,3}.

The adventitious presence of GE oilseed rape is not only affected by outcrossing via pollen-mediated gene flow, it is also affected by volunteer populations within fields via seed-mediated gene flow. The dynamics and persistence of volunteer oilseed rape is mainly influenced by field management practices (time of first tillage following oilseed rape, cultivar type, tillage depth, crop rotation)⁴. If volunteers flower, cross-pollination to other oilseed rape plants and fields



can occur. Herbicide resistant volunteers arising from unintended gene flow can be easily managed with herbicides if the subsequent crop is a non-herbicide-resistant cereal⁵ but will require special weed control strategies in the case of crops with resistance to the same herbicide. In order to avoid the formation of multiple herbicide resistant plants, farmers should not grow cultivars with different herbicide resistances in adjacent fields.

Feral populations are widespread at relatively low densities in regions cultivating oilseed rape. Pollen flow from sporadic occurrences of feral oilseed rape to neighboring 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 near an oilseed rape field. Nevertheless, volunteer and feral population dynamics should also be taken into account when assessing sources for adventitious GE presence and feasibility of coexistence.

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