

A TEST OF THE CROSSPOLL MODEL: SUGGESTIONS FOR FURTHER DEVELOPMENT

Including a first estimation of numbers of expected hybrids for *Beta* and *Brassica* in The Netherlands

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Disclaimer

All suggestions in this report reflect the personal opinions of the authors and may become subject to (scientific) debate. Furthermore, all dispersal values used in this report are valuable only to the obtained data-set and used curve-type. We do not claim to obtain completeness in terms of studies and data cited. The average dispersal values and curve choice do not represent any scientific or political consensus and should therefore not be used as such.

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Aim of this report

AIM OF THIS REPORT

Gene flow from crops to their wild congeners is attracting much attention, due to the increasing commercialization of transgenic (genetically modified) crops worldwide (Gray 2004, Snow *et al.* 2005, Andow and Zwahlen 2006). Evidence for such gene flow has accumulated over the last decades (reviewed in Ellstrand 2003, den Nijs *et al.* 2004), including data on gene flow of concrete transgenes (Mikkelsen *et al.* 1996, Quist and Chapela 2001, Messeguer *et al.* 2001, Massinga *et al.* 2004, Watrud *et al.* 2004, Reichman *et al.* 2006). It has become apparent that if the source crop is transgenic, hybrids containing a copy of the transgene are likely to be formed. As a consequence, predictive frameworks to test case-by-case scenario's prior to possible field testing are increasingly asked for by regulators, for instance in the U.S. (Snow *et al.* 2005), in the UK (Gray 2004) and also by the European Commission (2001).

In 2004, the Netherlands Committee on Genetic Modification (COGEM) of the Dutch Ministry of Spatial Planning, Housing and the Environment (VROM) commissioned a desk top study to analyze the factors influencing crop-wild relatives hybridization rates and to develop a pilot version of a model, which would predict hybridization rates originating from transgenic crop sources. The aimed added value of both report and model was an increased insight in the sensitivity of model predictions for (changes in) model parameters. Such insight would enable improved judgment of modeling results of the pertaining, and other models, and aims at arriving at better scientifically motivated and justified risk assessment procedure rules. We refer to the resulting report of Smith-Kleefsman *et al.* (2005; CGM 2005-03) for further details about this literature research and the rationale of the model development. The obtained model itself is available in pilot version and has been named Crosspoll.

The aim of this report is to evaluate the current pilot version of Crosspoll through intensive use. We aim at providing an estimate of the number of hybrids expected in The Netherlands following from outcrossing of two relevant agricultural species, viz., Oilseed rape (*Brassica rapa*) and Sugarbeet (*Beta vulgaris*) with wild relatives, *Brassica rapa* and *B. napus*, and *Beta vulgaris maritima*, respectively. The original proposal for this project is provided in **Annex 2** (in Dutch). Our goal is to provide suggestions to improve the framework of the model as a whole and, explicitly, not to find small-scale programming errors or perform debugging procedures.

However, we would like to stress that the hybridization rates calculated in this report are preliminary findings only; they result from a model in its testing phase. Also, the variation in parameters and curve-types has especially been chosen to evaluate the basic operation of the model, and does not reflect necessarily real life levels of variation. For these reasons, the obtained model output can not be regarded as accurate and should not be used for policy building.

Aim of this report

CHAPTER 1 AN OVERALL DESCRIPTION OF CROSSPOLL

The Crosspoll model: a general description

The Crosspoll model aims to serve all actors involved in assessing the distance related gene flow between transgenic crops and either their wild relatives or non-GM crops. The latter is often referred to as coexistence. Crosspoll predicts the proportion of hybrid seeds (as a function of distance) produced in a target population after pollination by a GM-source.

Crosspoll has a deterministic mechanistic program design in which two-dimensional dispersal curves of pollen from both source and target population determine the quantities of pollen received by stigmas. Pollen quantities are expressed as fraction of the total pollen amount produced in the pertaining populations. The effect of pollen competition can subsequently be added. For the mathematical features and expressions used, we refer to COGEM-report CGM-005-3 (Smith-Kleefsman et al. 2005).

The program is modeled in C++, but it is provided as executable (.exe) file and can be used without further requirements. Therefore, C++ is not required. Picture files are separate from the main module. We did not check the programming text as such.

The model follows four steps of input of parameters:

1. Calculation of the fraction of pollen as two-dimensional function of distance from the source population. This can be done according to various curve types from which the user has to choose. Included are (i) a negative exponential model, (ii) an inverse power model, (iii) an uniform distribution, and (iv) two custom functions. Please, see Smith-Kleefsman et al. (2005) for mathematical features of these curves. The first two types are parameterized with the average dispersal distance of pollen (\check{D}) of the species to be studied. \check{D} is subsequently transformed into a shape parameter, λ , assuming \check{D} and λ to be inversely proportionate to each other (i.e. $\lambda = 2/\check{D}$). Unless more detailed information is available about the actual dispersal dynamics, equation type based on only a few shape parameters, like (i) and (ii), seem to be the most logical choice of general dispersal curves.

The custom functions have to be fully determined by the user: these are described by maximally two parameters: distance (D) and a shape parameter (λ), similarly to the preset curves, but they are further free in terms of equation. The program does not check the mathematical correctness of the provided equations.

2. Calculation of the fraction of pollen as function of distance from the target population. This procedure is identical to the one applied to the source population. A feature added here is that an “assume same” button is provided, copying all source parameters into the target population calculation.
3. Calculation of the amount of pollen capable of fertilization, recognizing that pollen viability is limited and decreases with time and thus transfer distance. Dispersal takes time, so there is a relation between (decrease of) viability and distance traveled by the pollen. Thus, the dispersal curve outcome must be corrected using a viability curve that reduces proportions of effective pollen according to distance. Two functions are available: (i) a negative cumulative normal distribution function, which is parameterized by the average dispersal

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- distance and a standard deviation from this distance. (ii) A custom function, which is described by one parameter only: distance. This viability correction can be applied to both the source and target independently. Similarly to the pollen dispersal curve no check on mathematical correctness is provided.
4. “Fertilization parameters”. This last step includes three quantitative parameters.
 - a. The population size of both source and target.
 - b. The pollen production per plant or per m² of both source and target. Both parameters can be expressed in actual- and in relative size.
 - c. The “competitive ability”, i.e. the relative probability of source pollen compared to target pollen to fertilize a target ovule. This complex factor can include effects of selfing and partial cross incompatibility but also (partial) male or female sterility, flowering moment asynchronous anthesis, differences in germination time, pollen tube growth rate, the exact place where pollen lands, and further effects of pollen competition.

Subsequently the output of the model is presented in two ways:

1. A graphical depiction of the expected fraction of outcrossed seeds produced in the target population as function of distance, given the parameters entered above.
2. Display of the fraction of hybrid seeds expected at a predetermined distance.

Furthermore, the fraction of hybrid seeds expected and an overview of the added parameters can be saved in MS- Word-format.

What does this model bring additional to existing frameworks?

Crosspoll is certainly not the first modeling study of pollen dispersal resulting in hybridization ratios or quantities. The authors certainly do not claim this. For further modeling studies we like to point among others to the work of Creswell and co-workers (1995, 2002, 2004a, 2004b, 2005), Aylor and coworkers (2003, 2004, 2005a, 2005b), Colbach et al. (2001a, 2001b), Lavigne et al. (1998, 2002), Klein et al. (2003, 2006) and Richter & Seppelt (2004). Therefore, the aim of the program is to provide a practical tool for assessing hybridization based on as few parameters as possible and relatively simple calculations but not to provide a new theoretical framework. Additionally, the program was designed to be species independent and to be specifically adjustable for any species of interest by input of taxon-specific parameter values.

A strong point of the software is that it allows to visually investigate the effect of changing parameter values. This so called “investigate function” provides thus a non-statistical sensitivity analysis of input parameter variation. Furthermore, usage of the software does not require mathematical knowledge as long as no custom functions are used.

The program fulfils the aim it was made for quite well. As far as we are aware of, it is the first species independent ready-made software, which is created for risk assessment of siring effects of a GM source. It might become useful for the industry, regulators, environmental organizations and other third parties. However, Crosspoll is

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simplified in terms of biology and several suggestions of changes and additions are proposed, which we will provide in later chapters.

We would like to add the general remark that users should be aware that the software as presented will not provide or claim to provide objective results. Its input, i.e., the choice of dispersal curves and viability rates with distances, is in principle user dependent and therefore potentially highly subjective. The outcome should be judged accordingly. As our example in **chapter 3** shows, a large variation in results can be obtained by using different pollen dispersal curves. Guidance about the taxon specific choice of (the type of) the dispersal curve, as well as the parameters to be used needs to be given before Crosspoll can be applied to specific situations.

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CHAPTER 2 EMPIRICAL DATA AND TESTING CROSSPOLL

2.1 APPROACH

Evaluation approach

Our evaluation approach can be described as testing by using. By doing so, we aim to reach as output an estimate of the expected number of hybrids expected in The Netherlands between two relevant agricultural species and their wild relatives. The original proposal of this project is provided in **Annex 2** (in Dutch). Explicitly, our goal is not to look for small-scale programming errors or to perform debugging procedures. We also do not provide or claim to provide a full review of data on gene flow between the crops and wild relatives chosen for the testing procedure. Please see for the Dutch situation the recent reports by van Dijk et al. (2004), and Groot et al. (2003: CGM 2003-02). For the well-studied UK situation on (possible) hybridizations, we like to refer to the reports by Norris & Sweet (2000) and Eastham & Sweet (2002). Good general overviews for coexistence data, mainly for *Brassica napus* (Oilseed rape), can be found in Beckie et al. (2003) and Damgaard & Kjellsson (2005). For crop to wild-relatives, good general overviews regarding gene flow are provided in Ellstrand (2003), Stewart et al. (2003), Haygood et al. 2003, Gray (2004), Pilson & Prendeville (2004), Hails & Morley (2005) and Snow et al. (2005).

Wilkinson et al. (2003) and Allainguillaume et al., (2006) presented a UK- wide assessment of hybridization between rapeseed (*Brassica napus*) and *B. rapa* from a combination of sources: population surveys, GIS-information, and pollen dispersal profiles for both crop and wild relative. Using a pollen density dispersal curve and GIS-based distances between crop fields and populations of *B. rapa*, they estimated that 32,000 hybrids form annually in common waterside populations of *B. rapa* across the UK. The less abundant weedy *B. rapa* populations would annually contain 17,000 hybrids. Such findings represent the first step toward quantitative risk assessment on a national scale (Wilkinson et al. 2003), and could help to set targets for strategies to eliminate/reduce hybridization. See **chapter 3** for a mathematical expression of the used model in that study.

Testing Crosspoll, we provide similar, although less elaborate estimates for The Netherlands, asked for by COGEM (subcie Agriculture) d.d. 06 September 2005. This approach includes (1) the gathering of published empirical data in order to parameterize the pertaining dispersal curves, (2) obtaining the current locations of both crops and wild relatives and (3) calculate their intersite distances. By performing this procedure Crosspoll is tested for its user friendliness, the ease of obtaining the necessary parameters and the robustness of its output data.

The testing analysis will be done with two crops relevant for The Netherlands:

- (i) *Brassica napus* (Oilseed Rape/Canola) with its wild relatives *Brassica rapa* and *Sinapis arvensis*.
- (ii) *Beta vulgaris* spp *vulgaris* (Beet) with its wild relative *Beta vulgaris* spp *maritima*.

Additionally, for a third crop, *Zea mays* (Mays/Corn), similar data on crop to crop fertilization between fields will be gathered. This is only relevant for coexistence scenarios. No wild relative is found in The Netherlands of *Z. mays*, therefore no calculations are provided.

The choice of species was determined in consultation with the COGEM staff. Please see **chapter 3.1** for a detailed description of these species.

The results of these calculations are presented in **chapter 3** of this report.

2.2 Literature research

Since empirical data on pollen dispersal distances are rarely found, -many studies provide modeling results only-, we used a more indirect approach to parameterize the dispersal curves of the different species. Similarly to Damgaard & Kjellsson (2005) we calculated the likelihood of “hybrid” pollination based on available published data on among population hybridization rates.

The first step in this process was to perform a meta-analysis of the hybridization for the species combinations:

Brassica napus - *Brassica napus*

Brassica napus - *Brassica rapa*¹

Brassica napus - *Sinapis arvensis*

Beta vulgaris spp *vulgaris* - *Beta vulgaris* spp *maritima*

Zea mays – *Zea mays*

All studies mentioned differ in methodological design with respect to plot size, genotypes, buffer zones and other characteristics, which are known to influence hybridization rates. Please see Van de Wiel & Lotz (2004), Halsey et al. (2005) and DeVos et al. (2005) for discussions about differences in field characteristics.

However, similarly to the meta-analyses of Damgaard & Kjellsson (2005) we assumed that the average of all these studies will give a good indication of the average hybridization rates with distance and the variation around this average.

We included data from following sources:

- Peer reviewed literature with *Web of Science* citation and citations within this literature. As far as feasible, older, literature not available online was collected.
- Existing meta-analyses for outcrossing in *Brassica napus* (Damgaard & Kjellsson, 2005, Beckie et al., 2003).
- Den Nijs et al. (2004): Proceedings of an international symposium on introgression from crops into wild relatives and its consequences.
- Van de Wiel & Lotz (2004): inventory of scientific knowledge about coexistence of Mays, oilseed rape, potato and beet, and references therein.
- Ellstrand (2003): review book on current knowledge on hybridization between crops and wild relatives.

¹ In older literature *B. rapa* may be referred to as *B. campestris*

- Eastham & Sweet (2002): a review and interpretation of literature and ongoing research from the ESF program ‘Assessing the Impact of GM Plants’ (AIGM) program commissioned by the European Science Foundation and the European Environment Agency).
- Norris & Sweet (2000): report of monitoring studies of field scale releases of GM oilseed rape crops in England from 1994 to 2000).

For full citations of literature used, see **Annex 3** and the list of references. The data are described in **chapter 3**. From the survey, a broad range of variation in hybridization rates was obtained from the various sources, likely due to methodological differences (DeVos et al. 2005) and environmental and temporal stochasticity.

2.3 Curves used

2.3.1 Meta-analysis curve fit

Pollen dispersal *per se* not necessarily leads to hybridization; many more factors may play a role. However, we assumed an average curve through all data points found in literature would provide us with a reliable estimate for the likelihood of successful outcrossing. On the basis of our meta-analysis, we provide an estimate for the shape parameter, λ , using regression analysis. This curvefit function was developed in cooperation with the authors of Crosspoll and followed a negative exponential curve type:

$$\text{Source: } \frac{\theta * e^{(-\lambda d)}}{\text{Int}}$$

$$\text{Target: } \frac{(1 - \theta) * e^{(-\lambda d)}}{\text{Int}}$$

With θ , the proportion of pollen spreading from a population and therefore $(1 - \theta)$ the proportion pollen staying within a population. Int is the integral calculus of this function.

For this function a non-linear regression analysis was performed with the statistical package SPSS to estimate λ from the meta-analysis data. Such a non-linear regression uses an iteration method for parameter estimation through maximizing the fit of the curve with the data (R^2). Matlab (quadl-function) was used for estimation of the integral calculus, i.e., the total amount/proportion of pollen flowing between 0 and ∞ according to this function. We did this because most functions would not sum up to a cumulative proportion of 1, which is required in Crosspoll. Therefore, a correction with the integral calculus of the function is necessary.

The obtained curve was imported in Crosspoll as custom curve. Although plotting this curve type tends to overestimate the bulk of the curve and to underestimate the tail -i.e. actual average dispersal distances will be larger-, a good fit in the range of 10-500 meters seems to be obtained. Subsequently, for pragmatic reasons, in this testing procedure distances over 500 meters were excluded from the regression analysis.

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The average dispersal distances found and used as input were (data shown in Figures 3.2.A, B and C):

<i>Brassica napus</i> - <i>B. napus</i> :	47 meters
<i>Brassica napus</i> - <i>B. rapa</i> :	40 meters
<i>Beta vulgaris</i> spp <i>vulgaris</i> - <i>B. vulgaris</i> spp <i>maritima</i>	69 meters
<i>Zea mays</i> - <i>Z. mays</i> :	45 meters

Although hybrids between *Brassica napus* and *Sinapis arvensis* can be obtained in low quantities through embryo rescue in laboratory circumstances (Inomata 1994), outcrossing under field conditions is considered to be non-occurring because of genetic incompatibility (Kerlan et al. 1992, Lefol et al. 1996, Chèvre et al. 2003, 2004, Warwick et al. 2000, Moyes et al. 2002).

2.3.2. Other curves used

Apart from the own meta-analysis, we employed three other curves for outcrossing out of *Brassica napus* populations. This was done (a) to test the custom curve fit option and (b) through using three curve types in our example analysis we performed a sensitivity analysis on the outcome (number of hybrids expected). The three curves used were

- Two curves by Damgaard & Kjellsson (2005). Here, the probability distribution that seeds of non-GM-oilseed rape are fathered by foreign pollen grains from a neighboring field of GM-oilseed rape is modeled as a function of the width of the recipient (pollen receiving) field and its distance to the pollen donor fields. Compound exponentially decreasing functions were employed, in which Bayesian statistics were used for fitting parameters. (i) The first curve (“adjacent” fields, Damgaard & Kjellsson (2005), was fitted with data from adjacent fields and therefore contains shorter distances, the curve for bulk and tail was split at 3 meters from the source. (ii) The second curve (“non-adjacent” fields, Damgaard & Kjellsson 2005) contained larger distances and was not split.
- The curve by Wilkinson et al. (2003), which is a pollen density dispersal curve (see above).

Since Crosspoll uses a cumulative fraction of pollen, i.e., summing up to 1, all curves were corrected with the integral calculus of the curve (0 - ∞). Please see **chapter 3** for the actual equations used.

CHAPTER 3
PREDICTED HYBRIDIZATION INTO *BRASSICA RAPA* AND *BETA VULGARIS* SPP *MARITIMA* IN THE NETHERLANDS.

3.1. Goal of this project

The aim of this part of the report is to try out Crosspoll intensively. Having in mind the UK-wide estimate of hybridization between *Brassica rapa* and *Brassica napus* (Wilkinson et al. 2003), we aim to make a similar, although less elaborate estimate for The Netherlands. Our approach was to parameterize the Crosspoll dispersal curves on the basis of published empirical species-specific data. To this end, a meta-analysis of literature was performed (see also **chapter 2** and **Annex 3**). The hybridization assessment will be based on the locations of both crops and wild relatives in 2005. For hybridization of the crops *Brassica napus* and *B. rapa* with the wild relative *B. rapa*, multiple curve-types will be used. Next to our own meta-analysis and the pollen dispersal curve of Wilkinson et al. (2003), two different curves by Damgaard & Kjellsson (2005), describing the probability of foreign pollination by *B. napus*, will be used. Curves were selected on the basis of their applicability to the subject and in order to use the custom functions in Crosspoll throughoutly. Additionally, we will perform a similar analysis using a meta-analysis of published empirical data for hybridization between *Beta vulgaris* spp *vulgaris* (sugar- and fodder beet) and *B. vulgaris* spp *maritima*.

Moreover, an overview of published crop-to-crop outcrossing data for *Zea mays* is provided. However, no predictions are performed for this species since no wild relatives are present in The Netherlands.

3.2. Summary of the results and conclusions for wild *Brassica rapa*

By using our own meta-analysis, an overall hybridization rate of <0.01% was found, representing approximately 360 new hybrids occurring annually. For these calculations, we used an estimated total population size of wild *B. rapa* in The Netherlands of 24 million plants. For reasons explained below, we assume this to be a conservative estimate of the actual abundance of *B. rapa*.

We like to stress the importance of guidance about the dispersal curves and underlying data that will be employed as input in the model. We identified a three orders of magnitude difference between estimates using the different published curves. Consequently, using haphazardly chosen functions, even as they are literature based, could produce widely different rates of hybridization and therefore may rather trouble the debate about risk assessment than facilitate it. In the absence of official guidance of dispersal curve, the numbers of hybrids found in our example are consequently far too preliminary to be considered accurate and should be used for evaluating Crosspoll only.

Small changes in the amount of available pollen, projected to be in the tail of the curve, can have huge impacts on the total amount of hybrids as the amount of potential target populations will strongly increase with distance. Additionally, populations might receive pollen from multiple fields. If summation of all such small fractions would be based on fatter tails this could lead to a substantially higher prediction of hybrid formation.

We think that both the curve by Wilkinson et al. (2003) and Damgaard's "non-adjacent" one (Damgaard & Kjellsson 2005) do overestimate to a large extent the number

of hybrids. The published empirical data hardly show substantial numbers of hybrids at distances of over 500 meters (Damgaard & Kjellsson 2005). Therefore, all data points with separation distances above 500 meters, which are most of the points in our calculation of hybridization between wild populations and crop fields in The Netherlands, could be considered as flawed or at least suspicious. A non-adequate assumption of wind-dispersal instead of insect-dispersal of pollen over larger distances by Wilkinson et al. (2003) may be an additional cause of overestimation.

We conclude several points from this exercise.

Biologically

Guidance for application of the dispersal curves and cross-fertility estimates may be needed for such model to produce sensible and practicable usable predictions. Consequently, much more information regarding the mathematical and biological features of the tail of the dispersal curves to be implemented is needed (Klein et al. 2006).

Practically

Custom functions in Crosspoll are difficult to get accurate without sufficient empirical biological information. It seems more appropriate to use predefined average curves for individual species, and to discourage actors to use ‘own’ custom curve calculations, unless. Prior to using Crosspoll, curves should best be agreed upon by all actors or prescribed by a government body COGEM, or (other) competent body, based on expert consultation. To deal with this, we suggest several options in **chapter 4**. Furthermore, Crosspoll provides a good platform for distance based case by case calculations. However, large numbers of calculations could better be done in (additional) modules, which need to be able to read input files and assign parameters values automatically.

3.3. Methods

3.3.1. Crop species

Preamble:

For privacy reasons we will not provide any information on individual crop fields.

Brassica napus (oilseed rape/canola, koolzaad) is a natural hybrid between *B. rapa* and *Brassica oleracea*. Oilseed rape is a partially self-fertilizing summer or winter annual crop, in which a number of GM varieties have been developed. *B. napus* is grown for its seeds. *B. napus* pollen are wind dispersed on short distances, long-distance dispersal is by bees. The majority of pollen grains is assumed to be dispersed over short distances only (Lavigne et al. 1998, Damgaard & Kjellsson 2005).

Oilseed rape is widely grown in Northern-America, China, India, and parts of Europe, with a total world production area of c. 264,254 km². Main European production areas are in France and Germany, cultivation in The Netherlands is limited. According to the data made available to us by “*Dienst Regelingen*” of the ministry of LNV, about 2100 ha was cultivated in 2005, divided over 653 individual fields. However, it could be expected that cultivation will increase due to foreseen increased demand for biofuel production.

As a crop, *Brassica rapa* (Chinese cabbage/turnip rape, raapzaad) is grown only on a very limited scale in The Netherlands, in total 25 fields (48 ha) are listed in the database by *Dienst Regelingen*. However, the taxon is widely present as wild relative.

Beta vulgaris spp *vulgaris* (sugar & fodder beet). The sugar beet cultivar (grown for its root sugar-content) is a widely cultivated crop in The Netherlands. Sugar beet is only grown to the vegetative stage, since the swollen root is harvested at the end of the first year of this biennial crop. There is usually no flowering in the production period. However, a small proportion of the plants (typically <1%; “bolters”) may flower in the first summer (Eastham & Sweet 2002, Desplanque et al. 2002, Andersen et al. 2005). In Western Europe, seed production takes place in Italy and Southern France, but not in The Netherlands. Sugar beet is self-incompatible and wind-pollinated. Pollen generally stay close to the source, a small proportion is found to be able to pollinate at some distance (Desplanque et al. 1999, 2002, Bartsch et al. 1999, Viard et al. 2002).

In The Netherlands, the *Dienst Regelingen* reports cultivation of sugar beet on over 24,000 fields, with a total surface area of approximately 92,000 ha (920 km²).

The fodder beet cultivar is much less cultivated in The Netherlands, in total 591 fields are reported with a total area of approximately 550 ha.

3.3.2. *Wild relative species*

Brassica rapa (wild Turnip, raapzaad) is a common weed, growing in oilseed rape fields, along road sites and in ruderal habitats. This species is predominantly self-incompatible and pollinated by both insects and wind (Hauser et al. 1998, Wilkinson et al. 2003). From the Floron data-base, we drew a map showing its current distribution in The Netherlands in **Fig 3.1A**. However, there may be a substantial underestimation of its distribution and abundance since: (i) Floron’s rapporteur-volunteers frequently encounter difficulties in distinguishing *Brassica rapa* from *Sinapis arvensis* and *B. nigra* and (ii) it occurs in places (for instance roadsides) which are less often visited by Floron volunteers, and it may therefore be frequently underreported. In case *B. rapa* will be included in any future research of GM effects in The Netherlands, a more reliable survey of its distribution is needed.

Sinapis arvensis (Charlock, Herik) is a common weed in agricultural and ruderal areas. Similarly to *B. rapa*, it is a spring or winter annual. We show its distribution in **Fig 3.1B**. (data by Floron). Similarly to *B. rapa*, its actual occurrence might be uncertain since both species are often confused. Hybridization capability between *B. napus* and *Sinapis arvensis* has been found to be very limited (Kerlan et al. 1992, Lefol et al. 1996, Chèvre et al. 2003, 2004, Warwick et al. 2000, Moyes et al. 2002). Although, under laboratory conditions hybrids can be obtained in low quantities through embryo rescue (Inomata 1994). Therefore, we consider the spontaneous hybridization possibility between *B. napus* and *S. arvensis* to be negligible and will not include this combination in further analyses.

Beta vulgaris ssp. *maritima* (Sea beet, strandbiet) is exclusively found on seaside habitats, although some inland populations are known from southern France. The species is considered the ancestor of the cultivated beet species. It is wind pollinated and considered fully compatible with *Beta vulgaris* ssp. *vulgaris* (Sugar beet and Fodder beet). The wild relative's distribution is shown in **Fig 3.1C**. (data by Floron).

3.3.3. Literature meta-analysis

For parameterizing negative exponential (pollen dispersal) curves based on published data, we performed a meta-analysis of hybridization data, which we list in **Annex 3**. All studies mentioned differ in methodological design with respect to plot size, genotypes, buffer zones, and other characteristics; see Halsey et al. (2005) and DeVos et al. (2005) for discussions of such effects. However, similarly to the meta-analyses of Damgaard & Kjellsson (2005), we assume that the average of all these studies will give a good indication of the mean hybridization rates with distance and of the range of variation around it. The results for *Brassica* and *Mays* are shown in **Fig 3.2A-C**.

3.3.4. Estimates and assumptions used for Crosspoll input parameters

Location of the fields

For the crops, exact locations and size of the individual fields are available from the *Dienst Regelungen*. We transformed individual fields into point-based populations, virtually located in the geometrical center of the original field. The population sizes were subsequently calculated as a relative number of plants compared to the wild relatives.

For the wild relative, occurrences are provided in km²-squares only. For use in Crosspoll, we assumed a single (point-based) population to be present in the geographical center of the km²-square in which presence was recorded. Calculations are done with distances provided in rounded meters.

Further input parameters:

For the crops *Brassica napus* and *B. rapa*, we set flowering plant density at 25 per m² (250,000 ha⁻¹), the initially sown density could be within the range of 100-150 seeds m². For *B. rapa*, being a common weed, we arbitrarily assumed a population size of 10,000 individual flowering plants per km² in which it occurs.

B. napus and *B. rapa* are not fully cross-fertile; rates published range from 88% to 0.3%, depending on cultivar and region of origin (Jørgensen & Andersen 1994). Here, we used a cross-fertility rate of 2.5%, which is admittedly arbitrary, though in line with the 2.9% used by Wilkinson et al. (2003). However, such cross-fertility value is prone to large-scale variation and should be included in any future sensitivity analysis.

We did not include plant-size differences between crop and wild relative species, so for model input, individual plants produce equal amounts of pollen. *B. rapa* plants produce in average about 1,700 ripe seeds per plant (Hauser et al. 1998). For time reasons, we did not perform sensitivity analyses for these parameters; we suggest these to be done after a pertaining module will have been added to Crosspoll facilitating this (see **4.3**).

For *Beta vulgaris* ssp. *vulgaris* (sugar beet & fodder beet), we used a crop density of 90,000 plants ha⁻¹ of which maximally 1% bolts and flowers (Eastham & Sweet 2002,

Andersen et al. 2005). Assumed population size of the pollen source was therefore 900 plants ha⁻¹. This provides a worst case scenario since actual values might be an order of magnitude lower (Desplanque et al. 2002).

For *B. vulgaris* spp *maritima*, we assumed a population size of 100 individuals, since this species occurs generally in small and scattered populations. Wild plants produce approx. 1,000 seeds per plant (Lavigne et al. 2002). Both *Beta* subspecies are assumed to be fully cross-fertile.

3.3.5. Pollen dispersal functions used

For *Brassica*, we used four different functions. The used functions differed in tail “thickness”, i.e., both thin and fat tails were included. Chosen functions are two curves with fat tails (Wilkinson et al. 2003, Damgaard & Kjellsson 2005, “not-adjacent”) and two with shallow tails (Damgaard & Kjellsson 2005 and a simple negative exponential model). For details of the functions, we refer to the original publications. A recent broader discussion of the effect of the tail on predictions of quantity of hybridization can be found in Klein et al. (2006).

All functions were corrected with their integral calculus to assure a cumulative proportion of 1, as required by Crosspoll. Integral calculations were performed in Matlab (quadl-function).

1) The pollen density curve function of Wilkinson et al. (2003), based on empirical values of Timmons et al. (1995). We assigned the parameter values identical as given in Wilkinson et al. (2003). The function was entered as custom function. The pollen dispersal curves of source and target populations were assumed the same. Dissimilarly to the next functions, this function calculates the number of adult F₁-hybrids.

$$\text{Wilkinson: } \frac{e^{(-d/l)}}{\lambda(1+d)^{0.75}} \quad (\text{Eq 3.1})$$

With d = distance from the source population, λ equals 1, l is the exponential loss of pollen with distance with a half-time of 5-hours. Integral calculus for this function is 60.47. l is a parameter describing exponential loss of pollen by death and absorption, and is set on 100,000 m, following Wilkinson et al. (2003).

2) The probability of foreign pollination in adjacent *B. napus* fields as a function of distance from a shared border, following Damgaard & Kjellsson (2005). As a matter of facts, this function is based on coexistence data rather than on outcrossing to wild relatives. A meta-analysis of empirically measured hybridization rates among different *B. napus* fields (n= 286) is at the basis of this function.

This custom function separates the bulk and the tail. Cut-off point between bulk and tail was set at three meters, similarly to Damgaard & Kjellsson (2005). Dissimilarly to the original function, the bulk distribution is set to uniform, since Crosspoll does not provide another option. According to the integral calculus of the sum of the original functions in

Damgaard & Kjellsson (2005), the fraction present in the tail is 0.31. The pollen dispersal curves of source and target populations were assumed the same.

Damgaard adjacent: $\frac{(1-0.84)}{2} * e^{(-0.43\lambda)} * e^{-0.07(d-\lambda)}$ (Eq 3.2)

With d = distance from the source population, λ equals the cut-off point and is set at 3. Integral calculus for the tail function is 0.31; the summed pollen fraction leaving the source (integral $> 0 - \infty$) according to Damgaard & Kjellsson (2005) is 0.45.

3) The average probability of foreign pollination in non-adjacent fields as a function of the distance between fields, following Damgaard & Kjellsson (2005). A meta-analysis of empirically measured hybridization rates among different *B. napus* fields (n= 82) forms the basis here. This function was entered as custom function. The pollen dispersal curves of source and target populations were assumed the same.

Damgaard non-adjacent: $\lambda * (0.00587 * e^{(-0.00601d)})$ (Eq 3.3)

With d = distance from the source population, λ equals 1. Integral calculus for this function is 0.98.

4) The average probability of hybridization among fields of *B. napus*, according to our own meta-analysis (**Fig 3.2A, Annex 3**). A non-linear logistic regression was performed on the meta-analysis data (n = 132), using SPSS non-linear analysis. We estimated λ from these data without inclusion of a constant in the regression. Furthermore, we excluded data beyond 500-m distance, with the assumption that such long-range data were substantially biased towards (few) positive findings.

To estimate the proportion of pollen leaving the source (θ) and the proportion not-leaving the target population ($1 - \theta$), we used the above-mentioned estimates of Damgaard & Kjellsson (2005)-adjacent as estimations of θ . The functions for the source and target population were therefore different.

Source: $\theta * e^{(-\lambda d)}$ (Eq 3.4)

Target: $(1 - \theta) * e^{(-\lambda d)}$ (Eq 3.5)

With d is distance and θ is the proportion pollen leaving the source = 0.45. λ equals 0.043. Integral calculus for this function is 12.96 for the source and 10.41 for the target population.

For outcrossing into *B. vulgaris* spp *maritima*, we used a function based on our own meta-analysis (**Annex 3**). A logistic regression was performed on the meta-analysis data (n = 13) using SPSS non-linear analysis and estimating λ from these data without inclusion of a constant in the regression. Similarly to the above *Brassica*-model, functions depend on the proportion of pollen (θ) leaving the source and the proportion pollen not-leaving the target population. However, we had no data concerning these

values. Consequently, the pollen dispersal curves of source and target populations were assumed the same.

$$\text{Meta-analysis : } \theta * e^{(-\lambda d)} \quad (\text{Eq 3.6})$$

With d is distance and θ is the proportion pollen leaving the source = 0.5. λ equals 0.029. Integral calculus for this function is 11.68.

3.4. Results & Discussion

Contact areas

We listed the number of contact areas (where taxa occur within 3.000 m distance of each other) in **Table 3.1**. In the case of wild *B. rapa*, the number of contact zones is restricted by the limited number of *B. napus* fields. As can be seen in **Table 3.2**, *Brassica* contact zones are found in all provinces.

In the case of *Beta vulgaris* spp *maritima*, only 80 occurrences of the wild relative are listed (**Fig 3.1**). Since this wild relative only occurs on seashores (**Fig 3.1**), a limited amount of contact zones were identified restricted to two provinces (Friesland and Zeeland).

Model output: Brassica napus siring hybrids in B. rapa

Table 3.2 lists the results of the Crosspoll calculation according to the different models. The four different functions tested yielded very different results: between the two extremes more than three orders of magnitude difference was identified in predicted numbers of occurring hybrids.

We split up the results to the province-level. Hybridization with *B. rapa* is expected to occur relatively frequently in Brabant, where wild *B. rapa* is most common (**Fig 3.1**), but see our earlier comments about underestimating of *B. rapa* occurrence in The Netherlands.

We do not present the results in numbers of hybrid seeds. Since every *B. rapa* plant produces around 1,700 seeds (Hauser et al. 1998), those could be easily calculated, though. However, similarly to Wilkinson et al. (2003), we assumed *B. rapa* populations to grow among years at a neutral rate, i.e., showing a population growth rate of 1. Therefore, the hybridization rate can be directly calculated as numbers of hybrid plants present, rather than seeds of which the majority will disappear and not germinate. For instance, with a constant population size of 10,000 and an estimated 1% hybridization rate, the number of hybrid plants in the next year will be predicted at 100. This admittedly implies a neutral selection scenario for the hybrids.

In case Crosspoll will be used for coexistence situations, the amount of hybrid seeds formed needs to be the output, because in that context the assessment of the rate of contamination in the seed crop is the target measure.

Both the Wilkinson et al. (2003) model and the Damgaard's not-adjacent curve (Damgaard & Kjellsson 2005) assume pollen to travel very long distances. However, based on our own meta-analysis, hybridization beyond 500 meters is unlikely. This could be (i) a statistical feature: few data points on large distances bias the tail of the curve, or

(ii) an artifact of estimation of long distance dispersal by wind instead of insects (Creswell 1997), as is the case in Wilkinson et al. (2003). Therefore, we think that these two approaches have a tendency of hybrid formation overestimation. Our own meta-analysis, with an average dispersal distance of 47 meters and the meta-analyses of adjacent fields in Damgaard & Kjellsson (2005), might therefore be a better representation of the actual expected situation than the other two curves.

Klein et al. (2006) found similar estimates of difference in their predictions of pollen flow based on tail properties. In realistic field situations, fatter tails do not only cause seeds and/or pollen to spread further, but as a consequence, also to encounter more target populations. The combined effect of increased distance and more targets leads to an increasing discrepancy between predictions.

Model output: Brassica napus siring hybrids in wild B. rapa

Due to the very limited number of contact areas, hybrids between crop *B. rapa* and wild *B. rapa* are expected to occur only in Zuid-Holland and Flevoland and in low numbers by the models of Wilkinson and Damgaard's not-adjacent, and not at all by the two other models (**Table 3.2**).

Model output: Beta vulgaris vulgaris siring hybrids in B. vulgaris maritima

Although many contact zones could be identified between *B. vulgaris* spp *maritima* and sugar beet, calculations on the basis of the curve with average dispersal distance of 69 meters did not predict many hybrids at long distances. On the basis of the current distribution and abundance patterns and assuming an average wild population size of 100 individuals, a hybridization rate of 1.13% was expected (**Table 3.2**). We used a 1% bolting rate here, which is a worst-case scenario, actual bolting rates might be much lower (Desplanque et al. 2002), potentially lowering this hybridization rate substantially. According to our own model exercises fodder beet would not produce any hybrids.

3.5. Main conclusions

Given the concrete outcome of the model, the following conclusions only refer to hybridization of the type crop *B. napus* x *B. rapa* wild relative.

Biologically

We like to stress the importance of guidance with use of the dispersal curves and cross-fertility estimates, when such model will be used to produce sensible and practical predictions. As seen here, differences of three orders of magnitude between estimates are possible. We think that both the Wilkinson et al. (2003) and Damgaard's non-adjacent curves do overestimate to a large extent the number of hybrids. From published data, hardly substantial numbers of hybrids are found beyond 500 meters (Damgaard & Kjellsson 2005). Therefore, all obtained data points by Crosspoll with separation distances beyond 500 meters, which are most of the contact points (**Table 3.1**), should be considered as flawed or at least suspicious. Since we ran the model with between-site distances of up to 3,000 meters, the numbers of hybrids found in our example are very preliminary and should not be used as such in any debate or included in any official or unofficial statements by all parties involved. In order to reach more robust output, much

more information regarding the mathematical and biological features of the tail of the dispersal curves is needed (Klein et al. 2006). By using our own meta-analysis, an overall hybridization rate of less than 0.01% was found, which represents approximately 360 new hybrids establishing annually (**Table 3.2**).

For these calculations, we assumed a total population size of *B. rapa* in The Netherlands of 24 million plants. However, as shown above, we have to assume this to be an underestimate of the actual population size of *B. rapa*.

Practically

Custom functions in Crosspoll are difficult to get accurate without sufficient empirical biological information. It seems more appropriate to use predefined average curves for individual species, and to discourage actors to use ‘own’ custom curve calculations. Prior to using Crosspoll, curves should best be agreed upon by all actors or prescribed by a governmental body like COGEM, based on expert consultation. To deal with this, we suggest several options in **chapter 4**. Furthermore, Crosspoll provides a good platform for distance based case by case calculations. However, in order to enhance the practical application of Crosspoll, large number of calculations could better be performed in additional modules, which are able to read input files and assign parameter values automatically.

Table 3.1. Estimated number of contact zones between crop fields and wild relative populations, subdivided in distance classes. Please note that the exact values (meters between source and target) are used in Crosspoll calculations. Location of the crop is according to the data of “*Dienst Regelingen*” being located at the geometrical center of the field as a point-based population. Wild relative data are according to the “Floron”-database and are based on occurrences in km²-squares; we assumed a population to be present as a point-based population in the geographical center of the pertaining km²-square.

Distance	<i>Brassica rapa</i>		<i>Beta vulgaris maritima</i>	
	<i>Brassica napus</i> (Oilseed rape)	<i>Brassica rapa</i> (Rapeseed)	<i>Beta vulgaris</i> (Sugar beet)	<i>Beta vulgaris</i> (Fodder beet)
<50	0	0	0	0
50 -100	1	0	0	0
100 – 250	3	0	6	0
250 -500	24	0	6	0
500 – 1000	64	0	61	1
1000 -1500	118	1	104	2
1500- 3000	641	7	691	10
Total	851	8	868	13

Chapter 3 Predicting *Brassica* and *Beta* hybrids

Table 3.2. Estimated number of hybrids, summed up for all contact area distance classes below 3000 meters (**Table 3.1**). Four pollen dispersal functions are used for assessing hybridization of *Brassica napus* with *B. rapa*, including our own meta-analysis. For hybridization between *Beta vulgaris* spp *vulgaris* and ssp. *maritima*, only our own meta-analysis was used.

Hybridization with:		<i>Brassica rapa</i>				<i>Beta vulgaris</i>
Source species province ⁻¹	Function-type:	Wilkinson	Damgaard not-adjacent	Damgaard adjacent	This Meta- analysis	This Meta-analysis
		<u>Source: <i>Brassica napus</i></u>				
Brabant (* x 10 ⁴ applies to all provincial figures)		4.8x10 ^{4*}	5.97	0.005	0.04	c
Drente		0.03	<0.01	<0.01	<0.01	c
Flevoland		<0.01	<0.01	<0.01	<0.01	c
Friesland		0.06	0.01	<0.01	<0.01	0.44
Gelderland		<0.01	0.99	<0.01	<0.01	c
Groningen		0.06	0.04	<0.01	<0.01	c
Limburg		0.06	0.01	<0.01	<0.01	c
Noord-Holland		0.16	0.03	<0.01	<0.01	c
Overijssel		0.04	<0.01	<0.01	<0.01	c
Utrecht		<0.01	<0.01	<0.01	<0.01	c
Zuid-Holland		0.22	0.12	<0.01	<0.01	c
Zeeland		0.03	0.02	<0.01	<0.01	0.69
Predicted # hybrids		56,220 ^b	62,478 ^b	50 ^b	360 ^b	113 ^b
Hybridization % ^a		0.23 %	0.26%	<0.01%	<0.01%	0.14%
<u>Source: <i>Brassica rapa</i></u>						
Predicted # hybrids		10,800	200	<100	<100	
						<u>Source: Fodder beet</u>
Predicted # hybrids						<100

a Based on 2,417 *B. rapa* populations (= km-grid cells occupied, see fig. 31.A), a total 24,170,000 plants was estimated, assumed population size is 10,000. Based on 80 *Beta vulgaris* spp *maritima* populations (= km-grid cells occupied, see fig. 31.C). Consequently a total of 8,000 plants was estimated, assumed population size of 100.

b: Note: adult plants.

c No contact zones.

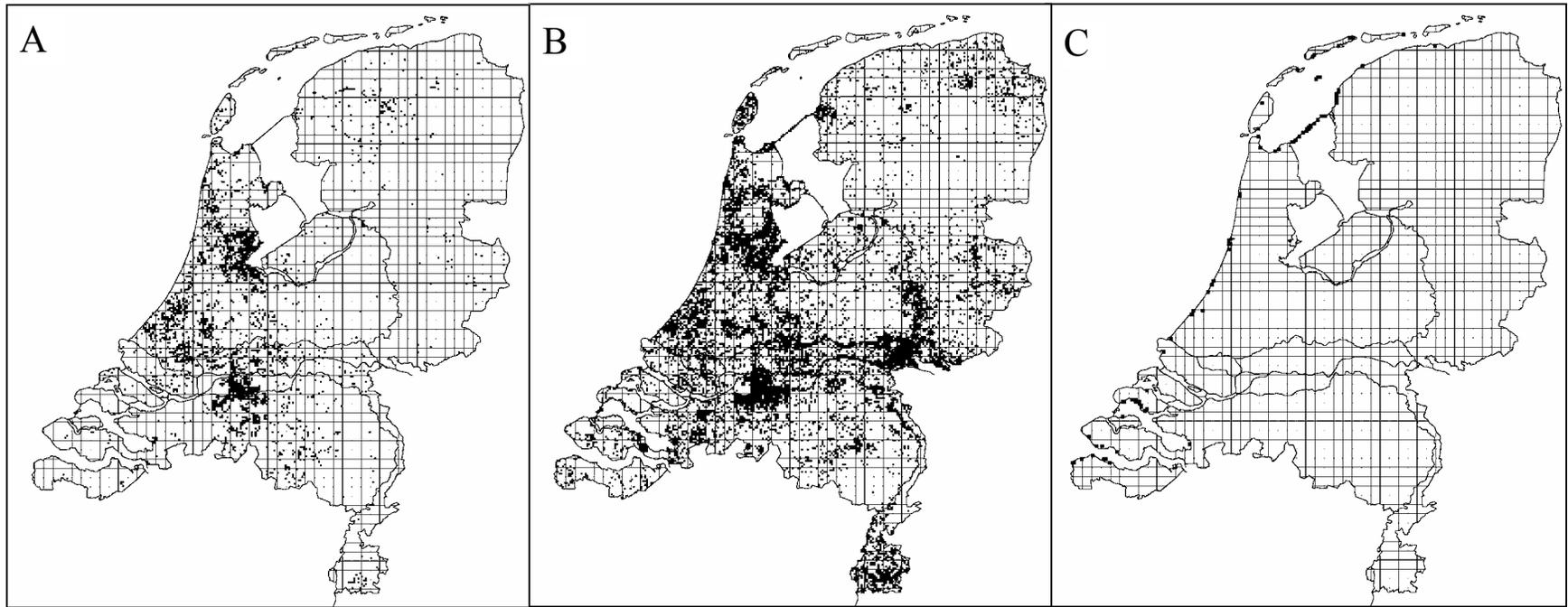


Fig 3.1. Distribution in The Netherlands of: **A)** *Brassica rapa* (2,417 km²-blocks), **B)** *Sinapis arvensis* (8,126 km²-blocks) and **C)** *Beta vulgaris* spp *maritima* (80 km²-blocks). Monitoring grid base: 1 km². Data courtesy by Floron.

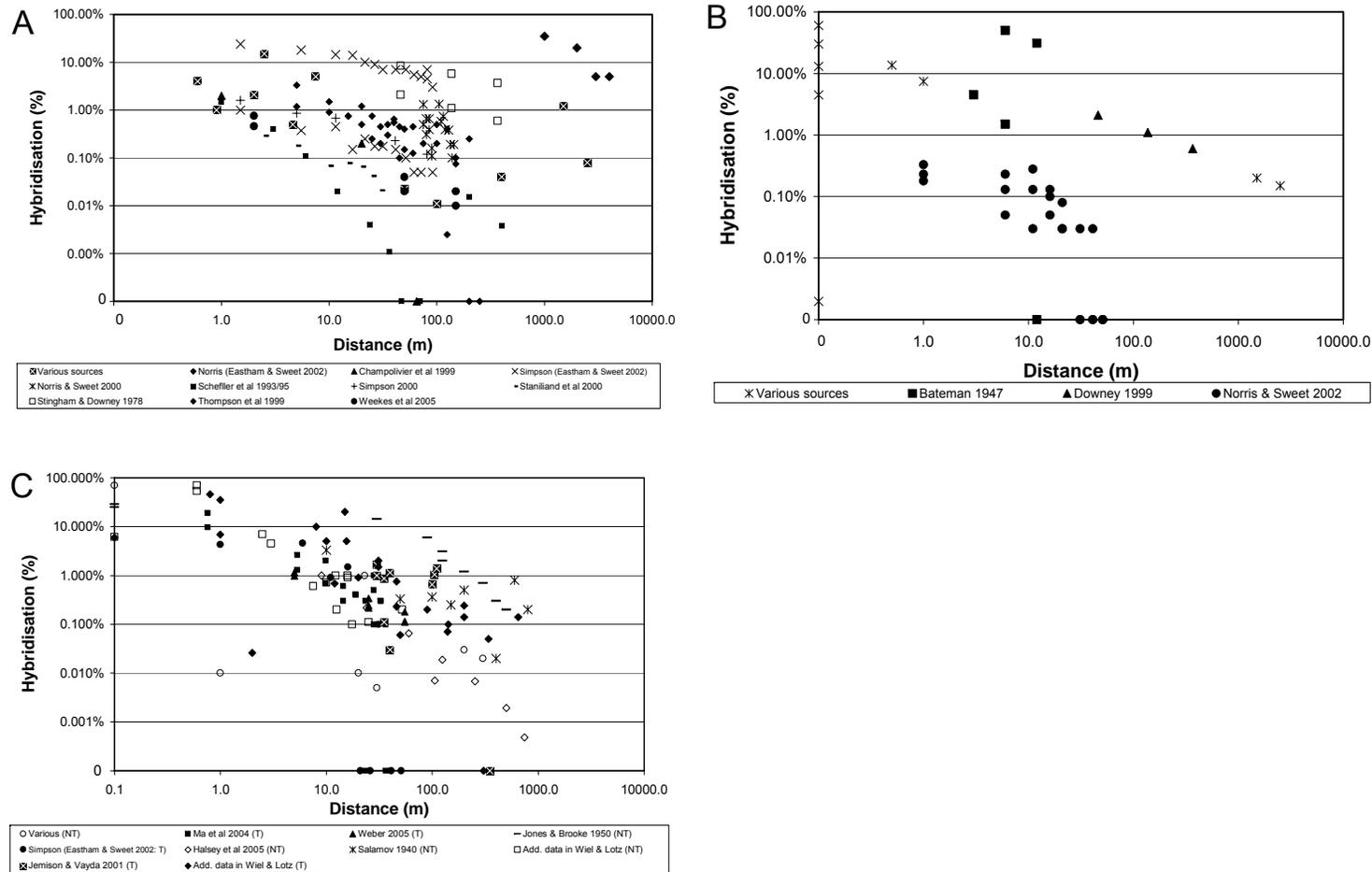


Fig 3.2. Meta-analysis of available literature sources (listed in **Annex 3**), **A**) Crossing among *B. napus* crops (coexistence), **B**) Crossing between *B. napus* and *B. rapa*, **C**) crossing among Maize (coexistence). Main literature sources are given, spurious sources are combined into the category various (sources).

CHAPTER4 SUGGESTIONS TO ADAPT AND FURTHER DEVELOP CROSSPOLL

In this chapter we will present our suggestions for further development of Crosspoll. First we will describe suggestions to develop the current framework (**4.1** & **4.2**). This includes suggestions about:

- improving species specificity of the model and steering an accurate curve-input (**4.1**):
 - Choice of pollen dispersal curves.
 - Pollen viability.
 - Competitive ability (of pollen with respect to fertilization).

- improving the model in general (**4.2**):
 - Translating hybridization data to likelihood of foreign pollination and fertilization.
 - Cumulative proportion in curves.
 - Small scale suggestions to improve the model.

Subsequently, we will give suggestions to extend the current framework (**4.3**). Those suggestions include:

- Spatial scales within populations and among populations.
- Visualization of populations and locations using GIS.
- Changes in the structure of the model.

The last paragraph with suggestions of this chapter will describe future potential directions for development in cooperation with other approaches (**4.4**), those include:

- The size and relative importance of the tail of the distribution curve.
- Competitive ability of pollen.
- Creation of a data-base of available outcrossing and hybridization rates.
- Better monitoring of wild relative species.
- Introgression models.
- Coexistence.

This chapter finishes with a short summary of suggestions (**4.5**).

4.1. Suggestions to strengthen the species specificity of the model

Choice of curves

Choosing among different pollen dispersal curves is a biological problem rather than a mathematical one. Biological knowledge about the shape and type of the dispersal curve, if present at all, will presumably hardly be accessible for users of the model. See also our earlier evaluation for this point (**Annex 1**). Crosspoll or any other model will not solve this problem. Predictions of the model are as good as the **biological quality** of the curves provided. However, instructions could be provided that when no reliable curve estimates are present, the negative exponential curve or inverse power law should be preferred. We have three suggestions:

1. Additional warnings: currently warnings with respect to the consequences of chosen curves are mostly found in the help function. We suggest to add additional

guidance notes to the “preview function” screen, stating that that when no reliable curve estimates are present, the negative exponential curve or inverse power law should be preferred. In case of regulatory use of the software, rationale of curve choice, the used curve and its origin, and accompanying data could be asked for by the judging authority prior to the evaluation of the application.

2. Based on procedures for individual species as above, pre-set curves could be included for important agricultural species or races, although the equations may be subject to political and regulatory debate. Another, less elaborated option would be to provide guidance for choosing the curve type following a species- or crop-specific meta-analysis for the main categories of pollen dispersal, (i) wind dispersal and (ii) animal (insect) dispersal.
3. Changing the features of the preview screen by splitting into multiple menus. One possible idea could be the structure provided in **Fig 4.1** below.

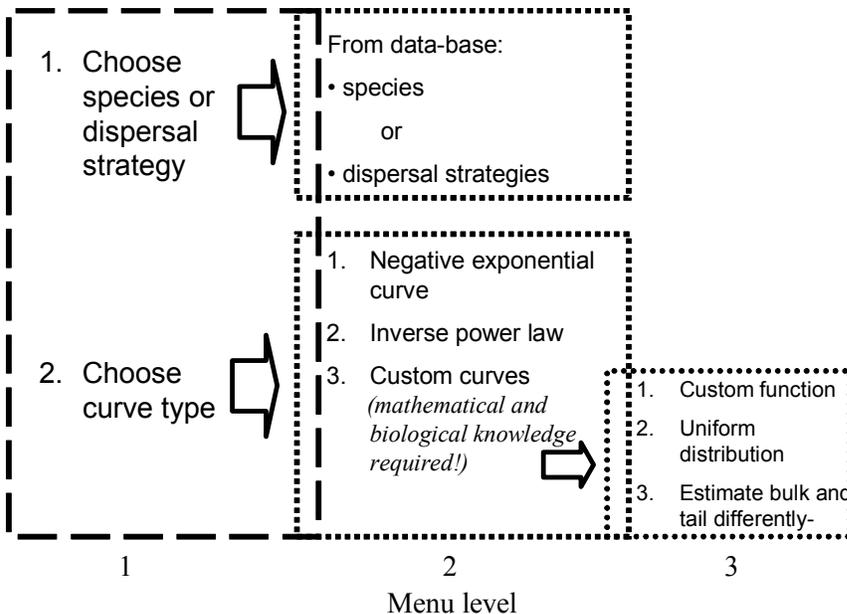


Figure 4.1. Suggested menu structure for choosing the dispersal curve

An important point to realize is that one must be aware that curves are statistical features which mostly tend to overestimate short distance dispersal in the first meters and underestimate long-distance dispersal. Crosspoll rightly warns for this in the help-function. However, as can be seen from the curve provided by Wilkinson et al. (2003), also the opposite can occur. No real solution for this problem currently exists; making the equations more complex will often not derive more significance from meta-analysis data (Damgaard & Kjellsson 2005). In case this needs to be tested, Huisman et al. (1993) provided an useful statistical framework, that tests for added significance with increasing complexity of curves.

Anyhow, prior to using Crosspoll, the choice of curve should be done on biological information and either (i) curve types should be directly admitted by COGEM or another official government body, and be based on expert consultation or (ii) an official agreement

reached by all actors involved.. This could be done in general per species or case-by-case. Additional regulation on this point might be needed.

Pollen viability

In Crosspoll, pollen viability is expressed as a function of distance. Although analogous to dispersal curves, this is incongruent with most literature-based data, since these generally describe time since shedding as viability estimate (Wilkinson 2003, Aylor et al. 2004, 2005a, but see Luna et al. 2001).

For wind-dispersed pollen, we suggest to use time as input parameter and subsequently by using an “average” wind speed or actual windspeed, transforming time to distance. Insect-pollination is more difficult but by using average flight distances of the specific pollinator a similar approach could be used.

Like for dispersal, also for pollen viability pre-assigned species or crop race specific curves are preferred.

Competitive ability

In our previous (Dutch) evaluation (**Annex 1**) we already stated the problem inherent to “competitive ability”. In the model, this single parameter includes the outcome of many processes including (i) outcrossing/selfing rates; (ii) (partial) self-incompatibility; (iii) (partial) sterility; (iv) synchrony in flowering times in source and target; (v) other processes including, e.g., pollen competition on the stigmas.

Our suggestion is to split this single parameter into multiple parameters, which should preferably be preset species-specifically.

However, it should be noted that splitting this parameter should be based on available data. More detailed empirical data might be required to enable this splitting. If such data are not available, this suggestion should be taken with great care. It is apparent that detailed predictions based on high levels of input details could substantially deviate from reality if the parameter values are not correctly chosen. This is largely the result of the stacking of, unintentionally introduced, error margins of these parameters.

4.2. Suggestions to improve the model in general

Translating hybridization data to likelihood of foreign pollination and fertilization

Most pollen dispersal modeling studies contain a wide range of parameters and are often species specific. In contrast, Crosspoll aims at describing pollen flow by two parameters only (λ and D). This approach makes the model in principle species-independent and less likely to suffer from unpredictable noise caused by stacked assumptions of parameter errors. However, since only few empirical data on measured pollen flow in the genera *Beta* and *Brassica* are presented in peer-reviewed literature², we choose a different approach using available hybridization data to calculate the likelihood of foreign fertilization. Although we are aware that parameterizing a model with related output might be prone to circle reasoning, we think this is the best option.

² For *Zea mays* more literature is available describing empirical pollen flow, however hybridization data are still the majority.

Chapter 4 Suggestions for further development of Crosspoll

Users and regulators should realize that no meta-analysis could include all data available, since many are not peer-reviewed and therefore difficult to trace and to evaluate, if accessible at all. On the other hand, one might make an arbitrary choice which data and preferred curve type to use. Different parameter settings will give different results as we clearly showed in **chapter 3**.

However, our approach is not without mathematical problems. Crosspoll dispersal curves are 2-dimensional, and thus pollen disperses equally to all directions according to the shape parameter $\frac{\lambda^2}{2\pi}$. Likelihood of pollination curves do not contain this 2-dimensional shape parameter, causing a potential overestimation of the average dispersal distance, which cannot be easily corrected for. We suggest writing a curve fit protocol as a separate module, which can be put in place as add-in to the Crosspoll main module.

Cumulative proportions in curves

All curves in Crosspoll are constraint by having a cumulative proportion of 1. Most of the published pollen dispersal curve studies present absolute data rather than such proportions. We suggest including an option as well in the model for using such curves or adding a correction function for the integral calculus when entering a curve type.

Practical and small scale suggestions

- We are not sure whether the cumulative fraction as projected in the investigate options for dispersal curves is correct. In cases where we calculated the integral calculus ourselves, cumulative fractions in the graph do not sum up to this value. This needs to be checked.
- When scaling the investigate graphs, the y-axis values are not shown any more.
- Project and species names are not changeable after an initial entry has been entered, could this be changed?
- It would be really nice if the investigate curve of the fertilization function could be printed directly or, even better, integrated in the saved output. This because the hybridization by distance curve is the basis for all output in the review function. See also next point.
- Not only the question “what would be the hybridization rate at distance x?” can be answered with this mathematical framework but also the question “at what distance would the hybridization be y%?” The underlying curve solves both questions. An option box asking this question is suggested.
- There is only an investigate option for the source population dispersal, not for the target population. In case custom functions are used, such investigate functions will be needed, checking the correctness and sensitivity of the target dispersal function as well.
- The help function tends not to start automatically but needs manual search; can this be changed?
- Within the review and output function, much space is given to reviewing the parameter values, whereas the question to be asked to the model (“Distance between source and target”) and the actual output thereof seem a bit underrepresented. It might be clearer to give the latter more emphasis in a

separate window. Such windows could include as well the whole hybridization by distance curve, which is now only given within the investigate option for fertilization (see above).

- The actual introduction of Crosspoll focuses mainly on navigation through the program and not on what will be asked from the user when running Crosspoll. Would it be possible to put in the introduction a more extensive summary of the text such as now available as “Introduction to the program” within the help-function? This way, the user will have a better overview on beforehand, without consulting the help function. This can be done like: “You will be asked to give ..., thereafter you will be asked to choose among etc”.
- Could Crosspoll be made insensitive to Dutch/English setting of computer (“,” or “.”) or could a check be performed at startup with a pop-up like “Error, please change your MS windows language settings to English (UK or US)”.

4.3. Suggestions for extending the model

Spatial scales within and among populations

○ *Multiple populations*

As seen in our meta-analysis (**chapter 3**), and in Wilkinson et al. (2003), target populations can receive pollen from multiple source populations.

Options to include pollen flow from multiple sources/populations could be an important addition to the model.

○ *Wind direction & landscape structure*

Currently, the pollen cloud disperses equally to all sites of the source population. In any landscape and certainly in the Dutch situation this is seldom the case. Wind speed and direction, either increase or decrease the likelihood of foreign pollination and is included in various pollination models (e.g., Klein et al. 2003, Aylor 2004, 2005a).

We suggest including wind as a factor in Crosspoll.

Furthermore, inclusion of wind could be helpful in translating pollen viability time into distance (see **4.2**).

In a further development, multiple sources of effects, which influence turbulence-based dispersal patterns, like hills, forests, etc. might be included. This would make the model more interesting to partners abroad, where landscapes are often less flat and homogeneous in these respects than The Netherlands.

○ *Spatial scale within populations*

The current model is a point-based-population model, i.e., all plants are located on one non-spatial point with unlimited density. This simplification certainly has a strong effect in terms of hybridization by distance. Much pollen produced by plants away from the border of the field will not disperse out of the population, so decreasing the possible amount of hybridization. The meta-analysis of Damgaard & Kjellsson (2005) clearly shows this potential difference between adjacent and not-adjacent receptors. Although many trials have been performed for measuring

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pollen flow among populations, few correction factors for within-population pollen dispersal have been made (Damgaard & Kjellsson 2005).
Therefore we suggest including within-population spatial scales in Crosspoll.

A technical possibility could be to subdivide populations into multiple populations (plots), from which part of the pollen will land in adjacent plots within the source population itself; an approach used in, e.g., the French *Genesys* model (Colbach et al. 2001, 2004). We have to add that *Genesys* is specific to *Brassica napus* and one type of landscape in central France. Crosspoll could provide such opportunity, assuming empirical data are gathered. Likewise, the dimension of the target plays a similar role; plants near the border zone of the target population are more prone to receive pollen than these further away in the population.

Furthermore, DeVos et al. (2005) and Damgaard & Kjellsson (2005) showed that not only distances among sites are important, but also that the shape of the source population determines the actual pollen cloud leaving the source towards the target.

In connection with the previous suggestion, the length of the border of the source population, which faces the target population, could be added to Crosspoll.

- *Border rows and other factors, which could limit gene exchange.*
Borderrows of non-GM catcher-plants and other factors, which influence wind turbulence, like hedge or tree rows, at the edges of fields (DeVos et al. 2005, Damgaard & Kjellsson, 2005) could influence the amount of pollen that is dispersed from the source towards the target. Empirical research on the influence of such mechanism is still mostly absent and much more empirical data are needed.

Crosspoll could steer empirical research, when including such border row options, either along the crop itself or along adjacent target vegetation and populations. Integration of border rows becomes increasingly important when pollen production between source and target is rather similar, such as in coexistence situations. Also in crop-wild relative situations in which severe swamping may occur, border rows might have a strong effect.

Visualization of populations and locations

When including a spatial framework as above, it would be very helpful to have a graphic option, visualizing the (simplified) input landscape.

A second and more elaborate option is to include actual landscapes into Crosspoll. In a very basic fashion we have tried this in **chapter 3**.

Cooperation with GIS based research will be indispensable here.

Structure of the model

- *Make it a model with various add-ins.*

Including the suggested option for spatial aspects, competitive ability, curve choice and curve estimation would increase the model size and therefore limit the usability, since not all modules would be necessary for all users. We suggest building the model in such a way that these modules could flexibly be switched on or off. This will mean that they exist as separate modules next to a main module and could be interchanged with newer versions without changing the main module.

- *Multiple categories reading function*
As we identify in **chapter 3**, using larger data-sets is currently time consuming since all is to be imported manually, like was the case in this test. To conduct sensitivity analysis or for other, larger data-sets, it might be worthwhile to include a C++ module which can read and write data automatically to- and from the model, allowing to run the model on large data-sets.

4.4. Suggestions for possible directions in cooperation with other research

The size and relative importance of the tail of the pollen distribution curve

A more general remark would be, and the authors of Crosspoll do recognize this as well in the Help function, that estimation of the tail of the dispersal curve is problematic, causing either large over or under-estimations. Over the last year, good modeling algorithms for long distance dispersal have been provided, such as the ones mentioned before. However, the strength of all calculations is determined by the data provided. Much more work, based on large samples and solid experimental designs are needed to better underpin our estimate of the tail of the distribution. Only this way we would be able to lower the sensitivity of the results and to provide more robust predictions for distances of more than a few hundreds of meters. Furthermore, as seen in our example in **chapter 3**, apparently inadequate data on mixed insect and wind dispersal could create large discrepancies in the prediction.

Much more robust data are needed to properly separate between wind and insect dispersal on longer distances.

Competitive ability

At present the competitive ability factor in Crosspoll is an oversimplification and includes many factors, that could be split easily, for instance: (i) outcrossing vs. selfing rates; (ii) (partial) self-incompatibility; (iii) (partial) sterility; (iv) synchrony in flowering between source and target; (v) other processes including, e.g. pollen competition on the stigma's. See our remarks in **4.1** providing a suggestion to solve this.

Crosspoll could provide a tool to conduct sensitivity analysis on these separate factors and thus steering empirical research into sensitivity of the mechanisms.

Creation of a data-base of available outcrossing and hybridization rates

For proper functioning of software like Crosspoll, the basic input data are essential; predictions are as good as its underlying empirical data.

Therefore, we suggest that a competent authority (e.g., COGEM) creates and maintains a data-base of all available, validated literature sources (white and gray) of hybridization

between relevant crops and their wild relatives, including details about field and population sizes, distances and border row effects. Only this way Crosspoll can produce accurate estimates of hybridization rates. Moreover, predictions provided by applicants could be compared to such data-base for referencing.

Need for better monitoring of wild relative species.

The Floron database of wild plant species in The Netherlands provides a very good starting point for comparison the distribution and abundance patterns of crops and wild relatives. However, as stated as well by Floron itself, wild relatives of crop species like *Brassica rapa* and *Sinapis arvensis* are mostly non-target species for the volunteers responsible for the field inventories. Consequently, estimates of such species might be severely biased and, additionally, misidentifications among these Brassicaceae are easily possible.

For a proper estimation of risk, more accurate data regarding the distribution and abundance of wild relative species in The Netherlands are needed.

Therefore, we suggest starting a project specifically aiming at censoring wild relatives of crop species. Floron appears the logical partner with respect to personal and database infrastructure.

Introgression models

Formation of hybrid seed is not equal to the occurrence of reproductively viable hybrid plants and certainly not equal to successful introgression of a specific gene into the wild background genome. Many factors might be of importance, which influence the fitness of such hybrid plants and the possibility of introgression of crop specific DNA-elements. For instance Ellstrand (2003), Stewart et al. (2003) and Pilson & Prendeville (2004) gave extensive overviews of this field of research.

In the last years, model-approaches are already under development (e.g., within the European programs “Angel”, QLK3-2001-01657, and “Sigma” FP6-501986) for the prediction of the long-term consequences of hybridization between crops and wild relatives. The two mentioned cases relate to the crops Lettuce and Oilseed rape but the framework is essentially species independent (Hooftman et al. 2007).

We strongly suggest investigating options for integrating a pollen flow model like Crosspoll with introgression modeling. This would provide a good estimate not only of hybridization on the level of seeds formed but also on the long-term perspective of wild populations under an introgression scenario after the original pollen influx.

Coexistence

For coexistence situations, Crosspoll might also become useful, not only in providing current estimates but also by predicting the effects of interventions.

Using a spatial module, predictions can be made about effects of incorporation of catch rows, partial sterility of GM-crops, decreasing/increasing field sizes and altering field dimensions, as well as trying to put GM-fields on safer spots in the landscape.

4.5. Summary

In short, Crosspoll offers an excellent basis to develop further at three different levels

1. The model itself: we suggest to extend Crosspoll with spatial factors, such as field dimensions, environmental parameters such as wind and to link Crosspoll to GIS databases developing the possibility to include actual landscapes. With the aim to create a yet small-sized model, we suggest to build additional modules as separate programs, which can be added to the main framework upon demand.

2. Underlying data. Crosspoll functions as good as the data on which it is based. We suggest several points:

- (a) to generate a data-base including all available data on hybridization by distance from crops to serve as reference for decision making;
- (b) to perform more empirical studies of the tail of distribution curves, acknowledging that mathematical frameworks are available;
- (c) to increase monitoring efforts of the wild relative species to identify areas of risk and provide better estimates of numbers of hybrids that can be expected.

3. Integration with introgression modeling. Models describing this process are under development, we suggest integrating both approaches in order to identify the long-term effects of hybridization and to identify knowledge gaps that need to be filled.

Chapter 4 Suggestions for further development of Crosspoll

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ANNEX 1.

A FIRST EVALUATION OF A PREVIOUS VERSION OF CROSSPOLL (D.D. 25-02-2005. IN DUTCH).

NOTITIE CROSSPOL

Samenvatting: suggesties voor vergroting gebruiksgemak

Als aanvulling op de toelichting tot het agendapunt, naar aanleiding van de discussie tijdens de vergadering van 22 februari en als samenvatting van de hieronder volgende evaluatie volgen hier enkele concrete punten van actie die tot een grotere toegankelijkheid en bruikbaarheid van het model kunnen leiden.

1) Bouw als default-functie in het model in de pollenverspreidingscurves van relevante gewassoorten, zoals Brassica, Beta, etc. Daarover is in de literatuur in ruime mate data voorhanden. De gebruiker van het model zal zo niet hoeven kiezen uit de verschillende wiskundige modellen, hetgeen de discussie en de beoordeling van de uitkomsten veel eenduidiger zal maken.

De betreffende soortspecifieke data zouden automatisch kunnen worden geladen zodra de te onderzoeken soort is ingevoerd.

De wiskundige curves kunnen in het model blijven zitten, vanzelfsprekend, en zullen de mogelijkheid blijven aanbieden voor onderzoeks- en gevoeligheidsanalyses.

2) Bouw als default-functie in het model de gegevens in over pollenlevensvatbaarheid per soort (eventueel op de basis van geconstateerde bevruchtingsafstanden uit de literatuur).

De betreffende soortspecifieke data zouden automatisch kunnen worden geladen zodra de te onderzoeken soort is ingevoerd.

3) In module 2, fertilisation: splits de functie van de "competetive pollen ability".

De parameter η is een samengestelde eenheid, die beter in een aantal

afzonderlijke kan worden uiteengehaald. Per soort zijn tenminste een aantal van de samenstellende factoren bekend, dan wel uit de literatuur te halen, voorbeelden: outcrossing rate, self-incompatibility, manlijke steriliteit, fenologische synchroniciteit.

Voorzover beschikbaar de betreffende soortspecifieke data automatisch kunnen worden geladen zodra de te onderzoeken soort is ingevoerd.

Na het toekennen van de per soort/gewas en omstandigheid relevante waarde blijft een restfactor over, waarin dan bijvoorbeeld de de echte pollencompetitie op de stempel zal zitten. Voor deze restcomponent zou dan een "worst case" scenario als default kunnen worden ingevuld, met als onderzoeksmogelijkheid een gevoeligheidsanalyse.

Deze verandering zal de aanvechtbaarheid van de uitkomsten sterk verminderen.

4) Voordat een opschaling van het model naar expliciete ruimtelijkheid plaatsvindt, zou een parameter moeten worden toegevoegd die rekening houdt met de grootte van de verschillende populaties. De (grote) donorpopulatie zal in de regel zelf ook een zodanige dimensie hebben dat aannahme van een puntbron de schatting van de in de target aankomende hoeveelheid pollen niet correct zal schatten. Hier dient op een of andere wijze een aanpassing voor te komen.

5) Enkele verdere suggesties staan in de hieronder volgende evaluatiebevindingen als mogelijke extra verfijningen en experimenteermogelijkheden.

Evaluatie van het CROSSPOLL MODEL V 1.0.0

Dit is een goed uitziend model, het werkt goed.

De geschreven tekst is soms wat "wordy", een native check-up zou kunnen helpen, maar dan wel van iemand met kennis van modellen.

Het functioneren lijkt stabiel, het heeft niet de neiging tot vastlopen.

Het model is deterministisch, d.w.z. het houdt geen rekening met variatie, bij dezelfde input parameters zal er altijd dezelfde output uit komen.

Het model lijkt verder te doen wat het belooft, namelijk een simpele interpreteerbare uitkomst te geven ten aanzien van het percentage (GM) "outcrossed" zaad dat via pollen flow in een naburige populatie zal ontstaan.

Het model is in voorname mate gebouwd op de basis van mathematische verspreidingscurves. Dit is enerzijds een goed gegeven, zonder duidelijke wiskunde kunnen er nooit eenduidige voorspellingen worden gegeven, dit wordt vaak onderschat. Maar dit laatste is ook het belangrijkste nadeel, er wordt niet vanuit de ecologie van soorten geredeneerd en de link tussen de mathematische curves en soorten of soortsgroepen –dus de vraag: welke curve?– wordt geheel aan de gebruiker overgelaten. Wij kunnen ons erg goed voorstellen dat de doelgroep (beleidsmakers, ecologen, boeren zelf, NGO's) in mindere mate deze wiskundige kennis bezit. Hieronder zijn enkele suggesties gedaan om aan dit probleem tegemoet te komen.

Wij hebben in het bijzonder veel waardering voor de "investigate" opties, die in feite een vorm van (handmatige) sensitiviteits analyse mogelijk maken.

Suggesties:

Pollen dispersal curves:

De bottleneck is dat de gebruiker uit wiskundige modellen moet beslissen, iets wat erg lastig is omdat het een zeer grote en specialistische voorkennis vereist. Meer leidraad zou hier op zijn plaats zijn, gebaseerd op soortspecifieke informatie.

Hier zijn enkele suggesties, sommige kunnen inhouden dat er modules moet worden bijgebouwd:

- 1) Een aantal algemene (default) dispersal curves aanbrenen, materiaal hiervoor zal uit de literatuur gehaald kunnen worden, er zou gekeken moeten worden of per gewas een eigen curve zou kunnen worden opgebouwd en in het model opgenomen. Alternatief valt te denken aan een organisatie naar bestuivingsmechanisme (wind-, insectenbestuiving, een combinatie van beide en eventueel minder waarschijnlijke zoals niet-intentionele bestuiving door andere dieren). Bij windbestuiving zou een subverdeling mogelijk zijn tussen hoge en lage gewassen.
- 2) Een advanced of custom functie waarin een eigen model kan worden ingevoerd; overwogen kan worden om hier in de toekomst nog meer parameters aan te bieden. De gescheiden invoer van de "bulk" en "tail" is ook een zeer goede optie onder deze advanced setting, die voor specialisten erg bruikbaar kan zijn .
- 3) Een plotfunctie waarin een beperkt aantal bekende waarnemingen kunnen worden ingevoerd door de gebruiker, waarna er via een zo simpel mogelijke functie (één van de nu genoemde) een meest waarschijnlijke dispersal curve wordt gegeven. Deze nieuwe module toe te voegen aan het model zou tegemoet komen aan het feit dat er weinig empirische gegevens zijn. Eventueel kan dit als losse module (een soort "add-in" functie) worden aangeboden.
- 4) Verder is het gebruik van lambda verwarrend voor populatiebiologen, bovendien maakt het het gebruik van het model nodeloos ingewikkeld, want lambda moet eerst worden uitgerekend. Vervang Lambda door de "average dispersal distance", laat in feite de huidige keuzemogelijkheid er maar uit. Bij invoeren van een soortspecifieke curve vervalt voor gemiddeld gebruik dit punt sowieso al.
[in de investigate optie lijken Lambda en average distance ontkoppeld, ze kunnen onafhankelijk van elkaar worden veranderd; dat is lijkt ons niet de bedoeling]

Pollen viability

Het probleem hier is het gebrek aan empirische data in de literatuur voor veel soorten en dus onmogelijkheid om schattingen van halfwaardetijd te doen. Ook hier is het dus belangrijk om een aantal defaultfuncties aan te bieden, onder te verdelen naar bestuivingsmechanisme. Hiernaast natuurlijk de meer advanced opties zoals hierboven gesuggereerd.

Uit de grote invloed die de in te stellen StandaardDeviatie heeft op het verloop van de curve (zie de investigate optie) blijkt wel dat informatie over de pollenvitaliteit van relatief groot belang is. Daar zullen dus ook de problemen bij het gebruik van het model voor een deel mee te maken gaan hebben. Ook hier moeten we iets aan (laten) doen. Het “Nijmegen-rapport” zal hier geen oplossing brengen; wel is duidelijk dat (ook hier) soortspecifieke data moeten beschikbaar komen.

Vooralsnog zou een oplossing kunnen zijn het voorschrijven van een grote SD, of aan te nemen dat het pollen gedurende de hele dispersal-periode/afstand (of een groot deel daarvan) levensvatbaar blijft.

Pollen cloud distributie (Fertilization).

Population size and pollen production per populatie zijn identieke parameters. De parameter, die beter in plaats van de tweede kan worden gebruikt, is pollenproductie per plant. Dan is pollen productie per plant* population size = production per population. [wellicht zit het al zo in het model, maar de tekst zou daar dan aan moeten worden aangepast omdat dat nu niet duidelijk is en de gebruiker eigenlijk niet goed ziet wat hij/zij moet invullen].

Omdat de (relatieve) pollenproductie van zo'n groot belang is, zou er wat meer data van de gebruiker kunnen worden gevraagd. Individuele relatieve verschillen in pollenproductie tussen source en target planten op basis van basale gegevens zoals het aantal bloemen per plant, maar ook (partiële) mannelijke sterilitet in

een van de populaties zou zeker een rol kunnen spelen. Deze gegevens zouden dan in een wat meer uitgewerkte “submodule” moeten worden ingevoerd.

Bij competitive pollen ability zien we (verwachtbaar) problemen. Uit het rapport bleek al dat deze factor een gecompliceerde is, daar over de afzonderlijke factoren vaak moeilijk een kwantitatieve uitspraak zal zijn te doen. Het lijkt me verstandig om alsnog te proberen dit aspect wat inzichtelijker te maken door bijvoorbeeld enkele van de factoren afzonderlijk op te vragen. Bijvoorbeeld: de selfing rate van de soorten in kwestie beïnvloedt het resultaat aanzienlijk. Deze is wellicht redelijk bekend, dus kan apart gevraagd en ingevoerd worden; voor self-incompatabiliteit geldt hetzelfde.

Zaken als verschillen in pollenbuisgroei, die natuurlijk in feite wel degelijk van belang zijn, kunnen niet/zeer moeilijk geschat worden. Dat soort factoren kan wellicht beter expliciet op een “worst case” default worden gesteld, zijnde .

De optie om de afstand te bepalen tussen beide populaties, nu aanwezig op de resultaatpagina, lijkt ons op deze pagina thuis te horen.

Verder zou op deze pagina een aparte module kunnen worden overwogen die rekening houdt met de heersende windrichting en snelheid in betrekking tot de ligging van de velden t.o.v elkaar. Hierdoor wordt de verspreidingscurve ingedrukt danwel uitgerek.

Investigate functions

Het is een heel goed idee om deze analyse mogelijk te maken, het is een soort sensitiviteitsanalyse. Probleem is wel ook hier dat het een bepaalde wiskundige kennis vereist om in te schatten welke schattingen van Lambda danwel halfwaardetijd en afstand normaal gezien als reëel kunnen worden beschouwd. Juist in deze functies moet een leidraad vermijden dat foute input een "eigen leven" kan gaan leiden.

Eindresultaat, de output:

De review tekst is zeer dominant aanwezig, maar de feitelijke berekende uitkomst (in %) is relatief klein onderin weergegeven.

Suggestie: geef de berekende uitkomst samen met de betreffende curve van afstand tegen % zaad als apart window weer; breng een link aan naar de parameterreview tekst (met printfunctie), voor het geval de gebruiker deze zou willen bekijken.

[Kleinigheid: het percentage hybride zaad wordt nu uitgedrukt in 5 decimalen, dat geeft een verkeerde indruk van de nauwkeurigheid, twee is ruim genoeg]

Overall

Het model functioneert uitstekend. Het lijkt naast stand-alone functies ook zeker mogelijkheden te hebben om gekoppeld te worden aan reeds elders in ontwikkeling zijnde expliciet-spatieële modellen aan de ene kant en populatiemodellen (met de introgressie-effecten daarin) aan de andere kant. De brug naar deze beide contexten zou wel uitvoerig getest moeten worden.

Een nadeel van het huidige model is dat van de gebruikers inzicht in wiskundige formules in relatie tot de biologie van de plantensoorten wordt verwacht bij hun keuzes van de parameters/functies.

Veel gebruikers zullen waarschijnlijk niet over genoeg empirische gegevens beschikken om uit de aangeboden functies een reële keuze te maken, waardoor invoer van irreële (foutieve) waarden tot de mogelijkheid behoort. Het aanbieden van meer default-functies op basis soortspecifieke informatie, b.v. naar aanleiding van het type bestuiving, outcrossing rates, SI-systemen, al of niet uit de literatuur, zou daar uitkomst kunnen bieden.,

Daarnaast zijn de advanced functies (“investigate”) zoals nu aanwezig natuurlijk van groot nut voor meer wetenschappelijke en experimentele benadering.

Deterministische modellen hebben als groot voordeel dat hun uitkomst éénduidig is, dit zou op vele terreinen genoeg zijn. Maar voor een wetenschappelijke

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benadering zou een mogelijkheid tot het verkrijgen van confidence intervallen eventueel door bootstrapanalyse met behulp van (geschatte) standaarddeviaties erg nuttig bij. Dit zou ook als een additionele losse module (een soort "add-in" functie) kunnen worden aangeboden.

Hans den Nijs
Danny Hooftman

Amsterdam, 25 februari 2005

ANNEX 2.

PROPOSAL FOR EVALUATION OF CROSSPOLL (D.D. 03-11-2005. IN DUTCH).

.....

EEN TEST VAN HET CROSSPOLL-MODEL

Cogem heeft kortgeleden als produkt van een desk study een eerste versie van het model CrossPoll ontvangen. Dit programma is bedoeld om aan de hand van een aantal (soort- en site-gebonden) parameters de (relatieve) hoeveelheid kruisbevruchtingen te schatten die het gevolg zijn van pollenverplaatsing van een (GM-) gewas naar een populatie van wilde verwanten. De eerste exercities met het model hebben veelbelovende resultaten laten zien, maar maakten ook duidelijk dat een nadere analyse van het CrossPoll-model moet leiden tot suggesties ter vergroting van de helderheid van in- en output en van de gebruikersvriendelijkheid. Ook is het nodig om aan de hand van empirische data de output van het model te valideren.

Kortgeleden is in Groot Britannië een grootschalige schatting gemaakt van de hoeveelheid uitgekruist zaad dat jaarlijks door *Sinapis arvensis* wordt gevormd na bestuiving met pollen van Koolzaad (o.a. Wilkinson *et al.* 2003: *Science* 302: 457-459). In de vergadering van de subcie Landbouw d.d. 6 september 2005 is gesuggereerd met CrossPoll een soortgelijke schatting voor de Nederlandse situatie te maken.

Dit voorstel beschrijft de werkzaamheden die nodig worden geacht om deze beide doelen te bereiken: enerzijds een gedetailleerde validatie en kritische beschouwing van de huidige versie van het model, en anderzijds een schatting van de *Sinapis arvensis* hybriden die op grond van de huidige teelt van Koolzaad in ons land zouden kunnen ontstaan.

De data die nodig zijn zullen worden verkregen uit de literatuur, uit relevante internetbronnen en van terzake deskundigen. De test zal worden gedaan voor drie voor Nederland relevante landbouwgewassen:

- (i) Koolzaad (*Brassica napus*) met als wilde verwant *Sinapis arvensis*; insectenbestuiver;
- (ii) Biet (*Beta vulgaris* subsp *vulgaris*) met als wilde verwant *B. vulgaris* subsp *maritima*; windbestuiver;
- (iii) Maïs (*Zea mays*) zonder in Nederland voorkomende wilde verwant; windbestuiver.

Deze soortkeuze is tot stand gekomen in overleg met het COGEM-secretariaat.

Hierbij zal voor de combinatie *Sinapis*/Koolzaad een test gedaan worden ter voorspelling van het mogelijk te verwachten aantal hybride zaden per jaar in Nederland. Voor *Beta*-soorten zullen literatuurdata ten aanzien van crop-wild relative uitkruising worden gebruikt als model-input. Voor maïs zal alleen met crop-to-crop uitkruisingsdata worden gewerkt.

In detail zal het project de volgende fases bevatten (voor de geschatte tijdsinvestering zie de begroting):

- 1) Bijeenbrengen van verspreidingsgegevens van zowel de verwante wilde soort als de teeltgebieden van gewassen uit relevante bronnen. Op de basis van deze gegevens zal een schatting worden gemaakt van de betreffende aantallen planten.
- 2) Bijeenbrengen van empirische data over de soorten uit literatuur en relevante databases betreffende de uitkruisingsratio's binnen en tussen populaties.
- 3) Het vaststellen van de ruimtelijke match van teelt en wilde voorkomens van Koolzaad en *Sinapis* (indien mogelijk GIS-gebaseerd). Deze analyse volgt in principe de methodiek van Wilkinson *et al.* 2003).
- 4) Het runnen van CrossPoll op de gegevens van de drie soorten en het beoordelen van de uitkomsten m.b.v. literatuurgegevens. Vervolgens zal er een sensitiviteitsanalyse gemaakt worden van de in het model gebruikte parameters.
- 5) Een schatting van de te verwachten hoeveelheid hybride zaad dat tussen de soorten *Sinapis arvensis* en Koolzaad in de huidige situatie in Nederland zou kunnen worden gevormd.
- 6) Een uitvoerige analyse van het model en zijn uitkomsten, leidend tot concrete suggesties voor verbetering.

De resultaten zullen schriftelijk gerapporteerd worden aan de COGEM.

Het project zal uitgevoerd worden door Dr. Danny Hooftman, tot aan 01-11-2005 Postdoc aan de Universiteit van Amsterdam, samenwerkende samen met Dr. Hans den Nijs aan de effecten en het modelleren van de consequenties van hybridisatie tussen landbouwgewassen en wilde verwante planten.

ANNEX 3.
USED LITERATURE SOURCES FOR META-ANALYSIS PER SPECIES

Table A3.1. Sources used for assessment of hybridization between *B. napus* and *B. napus*, including distances and hybridization rates. In case of indirect citations, this is indicated. We added the experimental method used: ‘receptor plants’ in generally point to planted rows or single plants. The difference between ‘inter-plot’ and ‘inter-field’ is the size of the experimental set-up, e.g., experimental or crop field scale. Some scarcer methods are: natural hybridization in volunteer populations and spraying seedlings in the field after cropping. Main categories of detection method for hybrids used are spraying plants to detect (GM) herbicide resistance hybrids, employing a variety of molecular markers and the detection of seed-set on emasculated plants.

<i>Brassica napus</i> to <i>B. napus</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenes used (y/n)	Detection method
Becker et al. (1992)	0	12-47	Receptor plants	No	Molecular markers
Cresswell (2005)	2	2.1	Receptor plants	No	Molecular markers
Cuthberth & McVetty (2001) ^a	0.6	4.0	Inter-plot	Yes	Herbicide resistance
Huehn & Rakow (1979) ^a	2.5	15.0	Inter-plot	Yes	Herbicide resistance
Huehn & Rakow (1979) ^a	7.5	5.0	Inter-plot	Yes	Herbicide resistance
Manasse & Kareiva (1991) ^a	50	0.022	Volunteer populations	No	Herbicide resistance
Manasse & Kareiva (1991) ^a	100	0.011	Volunteer populations	No	Herbicide resistance
Morris et al. (1994) ^a	0.9	1.0	Inter-plot	Yes	Herbicide resistance
Morris et al. (1994) ^a	4.6	0.5	Inter-plot	Yes	Herbicide resistance
Timmons et al. (1995)	1500	1.2	Inter-plot	No	Seed set on emasculated plants
Timmons et al. (1995)	2500	0.08	Inter-plot	No	Seed set on emasculated plants
Beckie et al. (2003)	0	1.4	Seedlings found in fields	Yes	Herbicide resistance
Beckie et al. (2003)	400	0.04	Seedlings found in fields	Yes	Herbicide resistance
Champolivier et al. (1999) ^a	0	1.6	Inter-plot	Yes	Herbicide resistance
Champolivier et al. (1999) ^a	0	7.0	Inter-plot	Yes	Herbicide resistance
Champolivier et al. (1999) ^a	1	2.0	Inter-plot	Yes	Herbicide resistance
Champolivier et al. (1999) ^a	20	0.2	Inter-plot	Yes	Herbicide resistance
Champolivier et al. (1999) ^a	65	0	Inter-plot	Yes	Herbicide resistance

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<i>Brassica napus</i> to <i>B. napus</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenes used (y/n)	Detection method
Eastham & Sweet (2002)	5	1.175	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	5	3.3	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	10	0.9	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	10	1.5	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	15	0.75	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	15	0.75	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	20	0.5	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	20	1.2	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	25	0.25	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	25	0.75	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	30	0.2	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	30	0.45	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	35	0.3	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	35	0.5	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	40	0.55	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	40	0.65	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	45	0.1	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	45	0.45	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	50	0.15	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	50	0.4	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	60	0.125	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	60	0.45	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	75	0.2	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	100	0.2	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	100	0.5	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	125	0.0025	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	125	0.4	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	150	0.075	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	150	0.1	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	200	0	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	200	0.25	Inter-field	Yes	Herbicide resistance
Eastham & Sweet (2002)	250	0	Inter-field	Yes	Herbicide resistance

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<i>Brassica napus</i> to <i>B. napus</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenes used (y/n)	Detection method
Eastham & Sweet (2002)	1.5	1.0	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	1.5	24.0	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	5.5	0.375	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	5.5	18.0	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	11.5	0.45	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	11.5	14.5	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	16.5	0.15	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	16.5	14.0	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	21.5	0.25	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	21.5	10.0	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	26.5	0.175	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	26.5	9.0	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	31.5	0.175	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	31.5	7.0	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	41.5	0.15	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	41.5	7.0	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	51.5	0.10	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	51.5	7.0	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	61.5	0.05	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	61.5	5.5	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	71.5	0.05	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	71.5	5.0	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	81.5	4.5	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	81.5	7.0	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	91.5	0.05	Receptor plants	Yes	Herbicide resistance
Eastham & Sweet (2002)	91.5	3.0	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	75	0.5	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	75	1.31	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	80	0.31	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	80	0.66	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	85	0.39	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	85	0.66	Inter-field	Yes	Herbicide resistance

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<i>Brassica napus</i> to <i>B. napus</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenes used (y/n)	Detection method
Norris & Sweet (2002)	90	0.11	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	90	0.16	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	105	1.33	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	110	0.58	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	115	0.74	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	120	0.39	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	129	0.38	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	134	0.19	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	139	0.10	Inter-field	Yes	Herbicide resistance
Norris & Sweet (2002)	144	0.19	Inter-field	Yes	Herbicide resistance
Scheffler et al. (1993)	0	4.8	Receptor plants	Yes	Herbicide resistance
Scheffler et al. (1993)	1	1.5	Receptor plants	Yes	Herbicide resistance
Scheffler et al. (1993)	3	0.4	Receptor plants	Yes	Herbicide resistance
Scheffler et al. (1993)	6	0.11	Receptor plants	Yes	Herbicide resistance
Scheffler et al. (1993)	12	0.02	Receptor plants	Yes	Herbicide resistance
Scheffler et al. (1993)	24	0.0041	Receptor plants	Yes	Herbicide resistance
Scheffler et al. (1993)	36	0.0011	Receptor plants	Yes	Herbicide resistance
Scheffler et al. (1993)	47	0	Receptor plants	Yes	Herbicide resistance
Scheffler et al. (1993)	70	0	Receptor plants	Yes	Herbicide resistance
Scheffler et al. (1995)	200	0.0156	Seedlings found in fields	Yes	Herbicide resistance
Scheffler et al. (1995)	400	0.0038	Seedlings found in fields	Yes	Herbicide resistance
Simpson (2000) ^a	1.5	1.6	Inter-plot	Yes	Herbicide resistance
Simpson (2000) ^a	5	0.86	Inter-plot	Yes	Herbicide resistance
Simpson (2000) ^a	11.5	0.68	Inter-plot	Yes	Herbicide resistance
Simpson (2000) ^a	41	0.23	Inter-plot	Yes	Herbicide resistance
Simpson (2000) ^a	81	0.12	Inter-plot	Yes	Herbicide resistance
Staniland et al. (2000)	0	0.69	Receptor plants	Yes	Herbicide resistance
Staniland et al. (2000)	2.5	0.29	Receptor plants	Yes	Herbicide resistance
Staniland et al. (2000)	5	0.18	Receptor plants	Yes	Herbicide resistance
Staniland et al. (2000)	10	0.068	Receptor plants	Yes	Herbicide resistance
Staniland et al. (2000)	15	0.078	Receptor plants	Yes	Herbicide resistance
Staniland et al. (2000)	20	0.0657	Receptor plants	Yes	Herbicide resistance

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<i>Brassica napus</i> to <i>B. napus</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenes used (y/n)	Detection method
Staniland et al. (2000)	25	0.042	Receptor plants	Yes	Herbicide resistance
Staniland et al. (2000)	30	0.021	Receptor plants	Yes	Herbicide resistance
Stringham & Downey (1978)	46	8.5	Receptor plants	No	Mutant strains
Stringham & Downey (1978)	137	5.8	Receptor plants	No	Mutant strains
Stringham & Downey (1978)	366	3.7	Receptor plants	No	Mutant strains
Stringham & Downey (1982) ^a	46	2.1	Inter-plot	No	Mutant strains
Stringham & Downey (1982) ^a	137	1.1	Inter-plot	No	Mutant strains
Stringham & Downey (1982) ^a	366	0.6	Inter-plot	No	Mutant strains
Thompson et al. (1999) ^a	0	88.0	Inter-field	Yes	Seed set on emasculated plants
Thompson et al. (1999) ^a	1000	35.0	Inter-field	Yes	Seed set on emasculated plants
Thompson et al. (1999) ^a	2000	20.0	Inter-field	Yes	Seed set on emasculated plants
Thompson et al. (1999) ^a	3000	5.0	Inter-field	Yes	Seed set on emasculated plants
Thompson et al. (1999) ^a	4000	5.0	Inter-field	Yes	Seed set on emasculated plants
Weekes et al. (2005)	2	0.46	Receptor plants	No	Molecular markers
Weekes et al. (2005)	2	0.76	Receptor plants	No	Molecular markers
Weekes et al. (2005)	50	0.02	Receptor plants	No	Molecular markers
Weekes et al. (2005)	50	0.04	Receptor plants	No	Molecular markers
Weekes et al. (2005)	150	0.01	Receptor plants	No	Molecular markers
Weekes et al. (2005)	150	0.02	Receptor plants	No	Molecular markers

^a: indirect citation via Beckie et al. (2003), indirect citations are not mentioned in the literature cited.

Table A3.2. Sources used for assessment of hybridization between *B. napus* (source) and *B. rapa* (target), including distances and hybridization rates. In case of indirect citations, this is indicated. We added the experimental method used: ‘receptor plants’ in generally point to planted rows or single plants. Greenhouse pollinations and interplanting are used to get low-distance hybridization rates. Main categories of detection method for hybrids used are spraying plants to detect (GM) herbicide resistance hybrids, employing a variety of molecular markers and the detection of seed-set on emasculated plants.

<i>Brassica napus</i> to <i>B. rapa</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenics used (y/n)	Markers type
Bateman (1947) ^b	3	4.5	Receptor plants	No	Seed set on emasculated plants
Bateman (1947) ^b	6	1.5	Receptor plants	No	Seed set on emasculated plants
Bateman (1947) ^b	6	50.0	Receptor plants	No	Seed set on emasculated plants
Bateman (1947) ^b	12	0	Receptor plants	No	Seed set on emasculated plants
Bateman (1947) ^b	12	31.0	Receptor plants	No	Seed set on emasculated plants
Downey (1999) ^b	46	2.1	Receptor plants	No	Seed set on emasculated plants
Downey (1999) ^b	137	1.1	Receptor plants	No	Seed set on emasculated plants
Downey (1999) ^b	366	0.6	Receptor plants	No	Seed set on emasculated plants
Norris & Sweet (2002)	1	0.18	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	1	0.23	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	1	0.33	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	6	0.05	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	6	0.13	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	6	0.23	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	11	0.03	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	11	0.13	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	11	0.28	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	16	0.05	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	16	0.1	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	16	0.13	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	21	0.03	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	21	0.03	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	21	0.08	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	31	0	Receptor plants	Yes	Herbicide resistance

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<i>Brassica napus</i> to <i>B. rapa</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenics used (y/n)	Markers type
Norris & Sweet (2002)	31	0	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	31	0.03	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	41	0	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	41	0	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	41	0.03	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	51	0	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	51	0	Receptor plants	Yes	Herbicide resistance
Norris & Sweet (2002)	51	0	Receptor plants	Yes	Herbicide resistance
Hauser et al. (1997)	0	30.0	Greenhouse pollinations	No	Seed set on emasculated plants
Jorgensen & Andersen (1994)	0	13.0	Interplanted	No	Molecular markers
Jorgensen & Andersen (1994)	0	60.0	Interplanted	No	Molecular markers
Brown & Brown (1996)	0	4.47	Greenhouse pollinations	No	Herbicide resistance
Rieger et al. (2002)	1500	0.2	Receptor plants	Yes	Herbicide resistance
Rieger et al. (2002)	2500	0.15	Receptor plants	Yes	Herbicide resistance
Warwick et al. (2003)	0	13.6	Interplanted rows	Yes	Herbicide resistance
Warwick et al. (2003)	1	7.4	Interplanted rows	Yes	Herbicide resistance
Wilkinson et al. (2000)	0	0.002	Receptor plants	No	Molecular markers

^b: indirect citation via Ingram (2000), indirect citations are not mentioned in the literature cited.

Table A3.3. Sources used for assessment of hybridization between *Beta vulgaris (vulgaris)* and *B. vulgaris (maritima)*, not distinguishing among crop and wild type. We included the distances and hybridization rates. In case of indirect citations, this is indicated. We added the experimental method used: ‘receptor plants’ in generally point to planted rows or single plants. Inter field points to hybridization among fields of crops. Main categories of detection method for hybrids used are spraying plants to detect (GM) herbicide resistance hybrids, employing a variety of molecular markers and using colour varieties of the crop.

<i>Beta vulgaris</i> to <i>B. vulgaris</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenics used (y/n)	Markers type
Viard et al. (2004)	1000 - 10,000	3.0	Inter-population	No	Molecular markers
Dark 1971 ^c	0	5.0	Inter-field	No	Beet colour
Dark 1971 ^c	90	0.3	Inter-field	No	Beet colour
Dark 1971 ^c	180	0.1	Inter-field	No	Beet colour
Jensen & Borgh (1941) ^c	400	0.42	Inter-field	No	Fodder vs. sugar beet
Jensen & Borgh (1941) ^c	600	0.11	Inter-field	No	Fodder vs. sugar beet
Jensen & Borgh (1941) ^c	600	0.12	Inter-field	No	Fodder vs. sugar beet
Alibert et al. (2005)	10	0.0491	Receptor plants	Yes	Herbicide resistance
Alibert et al. (2005)	50	0.0296	Receptor plants	Yes	Herbicide resistance
Alibert et al. (2005)	100	0.0102	Receptor plants	Yes	Herbicide resistance
Alibert et al. (2005)	200	0.0051	Receptor plants	Yes	Herbicide resistance
Arnaud et al. (2003)	1500	0.01290	Inter-population	No	Molecular markers
Champolivier et al. (1999) ^d	3	10.0	Receptor plants	No	Beet colour
Champolivier et al. (1999) ^d	15	1.0	Receptor plants	No	Beet colour

^c: indirect citation via Eastham & Sweet (2002), indirect citations are not mentioned in the literature cited.

^d: indirect citation via van de Wiel & Lotz (2004), indirect citations are not mentioned in the literature cited.

Table A3.4. Sources used for assessment of crop to crop hybridization between *Z. mays* and *Z. mays*, we included distances and hybridization rates. In case of indirect citations, this is indicated. We added the experimental method used: ‘receptor plants’ in generally point to planted rows or single plants. The difference between ‘inter-plot’ and ‘inter-field’ is the size of the experimental set-up, e.g. experimental or crop field scale. Some scarcer methods are: natural hybridization in volunteer populations, spraying seedlings in the field after cropping. Main categories of detection method for hybrids used are spraying plants to detect (GM) herbicide resistance hybrids, employing a variety of molecular markers, and using different grain properties.

<i>Zea mays</i> to <i>Zea mays</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenics used (y/n)	Markers type
Airy (1950) ^d	52	0.2	Inter-plot	No	Grain color
Airy (1950) ^d	16	0.9	Inter-plot	No	Grain color
Airy (1950) ^d	3	4.5	Inter-plot	No	Grain color
Bateman (1947b) ^d	12.2	1.0	Receptor plants	No	Grain color
Bateman (1947b) ^d	15.8	1.0	Receptor plants	No	Grain color
Bateman (1947b) ^d	0.6	54.0	Receptor plants	No	Grain color
Bateman (1947b) ^d	0.6	70.0	Receptor plants	No	Grain color
Foueillassar & Fabie (2003) ^d	25	0.11	Receptor plants	No	Grain color
Foueillassar & Fabie (2003) ^d	10	0.72	Receptor plants	No	Grain color
Foueillassar & Fabie (2003) ^d	0.1	6.2	Receptor plants	No	Grain color
Meijers (1937) ^d	17.5	0.1	Inter-plot	No	Grain color
Meijers (1937) ^d	12.5	0.2	Inter-plot	No	Grain color
Meijers (1937) ^d	7.5	0.6	Inter-plot	No	Grain color
Meijers (1937) ^d	2.5	7.0	Inter-plot	No	Grain color
Benetrix (2004) ^d	10	5.0	Inter-field	Yes	Grain color
Byrne et al. (2003) ^d	305	0	Inter-plot	Yes	Grain color
Byrne et al. (2003) ^d	46	0.23	Inter-plot	Yes	Grain color
Byrne et al. (2003) ^d	46	0.75	Inter-plot	Yes	Grain color
Byrne et al. (2003) ^d	0.8	46.0	Inter-plot	Yes	Grain color
Chilburt & Tabshnik (2004) ^d	31	1.5	Receptor plants	Yes	Bt concentration
Chilburt & Tabshnik (2004) ^d	31	2.0	Receptor plants	Yes	Bt concentration
Chilburt & Tabshnik (2004) ^d	15.5	5.0	Receptor plants	Yes	Bt concentration

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<i>Zea mays to Zea mays</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenics used (y/n)	Markers type
Chilburt & Tabshnik (2004) ^d	8	10.0	Receptor plants	Yes	Bt concentration
Chilburt & Tabshnik (2004) ^d	15	20.0	Receptor plants	Yes	Bt concentration
Chilburt & Tabshnik (2004) ^d	1	35.0	Receptor plants	Yes	Bt concentration
Henry et al. (2003) ^d	2	0.026	Inter-field	Yes	Molecular markers
Henry et al. (2003) ^d	50	0.06	Inter-field	Yes	Molecular markers
Henry et al. (2003) ^d	142	0.1	Inter-field	Yes	Molecular markers
Henry et al. (2003) ^d	200	0.14	Inter-field	Yes	Molecular markers
Henry et al. (2003) ^d	650	0.14	Inter-field	Yes	Molecular markers
Henry et al. (2003) ^d	200	0.24	Inter-field	Yes	Molecular markers
Henry et al. (2003) ^d	20	0.9	Inter-field	Yes	Molecular markers
Henry et al. (2003) ^d	0.1	6.0	Inter-field	Yes	Molecular markers
IRTA et al. (2004) ^d	340	0.05	Receptor plants	Yes	Molecular markers
IRTA et al. (2004) ^d	140	0.07	Receptor plants	Yes	Molecular markers
IRTA et al. (2004) ^d	90	0.2	Receptor plants	Yes	Molecular markers
IRTA et al. (2004) ^d	12	0.68	Receptor plants	Yes	Molecular markers
IRTA et al. (2004) ^d	1	6.86	Receptor plants	Yes	Molecular markers
Paterniani & Short (1974) ^e	30	0.005	Receptor plants	No	Grain color
Paterniani & Short (1974) ^e	1	0.01	Receptor plants	No	Grain color
Paterniani & Short (1974) ^e	20	0.01	Receptor plants	No	Grain color
Bateman (1947) ^f	23	1.0	Inter_fields	No	Empty kernels
Bateman (1947) ^f	0.1	70.0	Inter_fields	No	Empty kernels
Stevens et al. (2004)	300	0.02	Inter-plot	No	Grain color
Stevens et al. (2004)	200	0.03	Inter-plot	No	Grain color
Eastham & Sweet (2002)	21	0	Inter_fields	Yes	Herbicide resistance
Eastham & Sweet (2002)	26	0	Inter_fields	Yes	Herbicide resistance
Eastham & Sweet (2002)	41	0	Inter_fields	Yes	Herbicide resistance
Eastham & Sweet (2002)	51	0	Inter_fields	Yes	Herbicide resistance
Eastham & Sweet (2002)	31	0.1	Inter_fields	Yes	Herbicide resistance
Eastham & Sweet (2002)	11	0.9	Inter_fields	Yes	Herbicide resistance
Eastham & Sweet (2002)	16	1.5	Inter_fields	Yes	Herbicide resistance
Eastham & Sweet (2002)	1	4.3	Inter_fields	Yes	Herbicide resistance
Eastham & Sweet (2002)	6	4.6	Inter_fields	Yes	Herbicide resistance

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<i>Zea mays to Zea mays</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenics used (y/n)	Markers type
Halsey et al. (2005)	743	0.0005	Receptor plants	No	Grain color
Halsey et al. (2005)	500	0.0019	Receptor plants	No	Grain color
Halsey et al. (2005)	254	0.0068	Receptor plants	No	Grain color
Halsey et al. (2005)	106	0.007	Receptor plants	No	Grain color
Halsey et al. (2005)	125	0.0188	Receptor plants	No	Grain color
Halsey et al. (2005)	60	0.065	Receptor plants	No	Grain color
Halsey et al. (2005)	24	0.217	Receptor plants	No	Grain color
Halsey et al. (2005)	9	1.0	Receptor plants	No	Grain color
Jemison & Vayda (2001)	350	0.0001	Inter-plot	Yes	Molecular markers
Jemison & Vayda (2001)	40	0.03	Inter-plot	Yes	Molecular markers
Jemison & Vayda (2001)	35	0.11	Inter-plot	Yes	Molecular markers
Jemison & Vayda (2001)	100	0.65	Inter-plot	Yes	Molecular markers
Jemison & Vayda (2001)	35	0.86	Inter-plot	Yes	Molecular markers
Jemison & Vayda (2001)	30	1.0	Inter-plot	Yes	Molecular markers
Jemison & Vayda (2001)	105	1.04	Inter-plot	Yes	Molecular markers
Jemison & Vayda (2001)	40	1.14	Inter-plot	Yes	Molecular markers
Jemison & Vayda (2001)	110	1.38	Inter-plot	Yes	Molecular markers
Jemison & Vayda (2001)	30	1.65	Inter-plot	Yes	Molecular markers
Jones & Brooke (1950)	500	0.2	Inter-plot	No	Grain color
Jones & Brooke (1950)	400	0.3	Inter-plot	No	Grain color
Jones & Brooke (1950)	300	0.7	Inter-plot	No	Grain color
Jones & Brooke (1950)	200	1.19	Inter-plot	No	Grain color
Jones & Brooke (1950)	125	2.0	Inter-plot	No	Grain color
Jones & Brooke (1950)	90	6.0	Inter-plot	No	Grain color
Jones & Brooke (1950)	30	14.3	Inter-plot	No	Grain color
Jones & Brooke (1950)	0.1	28.62	Inter-plot	No	Grain color
Jones & Brooke (1950)	125	3.1	Inter-plot	No	Grain color
Jones & Brooke (1950)	0.1	25.0	Inter-plot	No	Grain color
Ma et al. (2004)	23.56	0	Receptor plants	Yes	Grain color
Ma et al. (2004)	36.48	0	Receptor plants	Yes	Grain color
Ma et al. (2004)	40.04	0	Receptor plants	Yes	Grain color
Ma et al. (2004)	28.12	0.1	Receptor plants	Yes	Grain color

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<i>Zea mays to Zea mays</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenics used (y/n)	Markers type
Ma et al. (2004)	36.48	0.1	Receptor plants	Yes	Grain color
Ma et al. (2004)	14.44	0.3	Receptor plants	Yes	Grain color
Ma et al. (2004)	23.56	0.3	Receptor plants	Yes	Grain color
Ma et al. (2004)	32.76	0.3	Receptor plants	Yes	Grain color
Ma et al. (2004)	32.76	0.3	Receptor plants	Yes	Grain color
Ma et al. (2004)	19	0.4	Receptor plants	Yes	Grain color
Ma et al. (2004)	19	0.4	Receptor plants	Yes	Grain color
Ma et al. (2004)	28.12	0.5	Receptor plants	Yes	Grain color
Ma et al. (2004)	14.44	0.6	Receptor plants	Yes	Grain color
Ma et al. (2004)	9.88	0.7	Receptor plants	Yes	Grain color
Ma et al. (2004)	28	1.0	Receptor plants	Yes	Grain color
Ma et al. (2004)	5.32	1.3	Receptor plants	Yes	Grain color
Ma et al. (2004)	9.88	2.0	Receptor plants	Yes	Grain color
Ma et al. (2004)	5.32	2.6	Receptor plants	Yes	Grain color
Ma et al. (2004)	0.76	9.7	Receptor plants	Yes	Grain color
Ma et al. (2004)	0.76	19.0	Receptor plants	Yes	Grain color
Salamov (1940) ^c	800	0.2	Inter-plot	No	Grain color
Salamov (1940) ^c	200	0.5	Inter-plot	No	Grain color
Salamov (1940) ^c	600	0.8	Inter-plot	No	Grain color
Salamov (1940) ^c	10	3.3	Inter-plot	No	Grain color
Salamov (1940) ^d	400	0.02	Inter-plot	No	Grain color
Salamov (1940) ^d	150	0.25	Inter-plot	No	Grain color
Salamov (1940) ^d	50	0.33	Inter-plot	No	Grain color
Salamov (1940) ^d	100	0.36	Inter-plot	No	Grain color
Weber et al. (2005)	55	0.11	Inter-field	Yes	Molecular markers
Weber et al. (2005)	55	0.115	Inter-field	Yes	Molecular markers
Weber et al. (2005)	55	0.18	Inter-field	Yes	Molecular markers
Weber et al. (2005)	25	0.217	Inter-field	Yes	Molecular markers
Weber et al. (2005)	25	0.24	Inter-field	Yes	Molecular markers
Weber et al. (2005)	25	0.34	Inter-field	Yes	Molecular markers
Weber et al. (2005)	5	0.98	Inter-field	Yes	Molecular markers
Weber et al. (2005)	5	1.12	Inter-field	Yes	Molecular markers

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<i>Zea mays to Zea mays</i> Source	Distance (meters)	Hybridization rate (%)	Method	Transgenics used (y/n)	Markers type
Weber et al. (2005)	5	1.146	Inter-field	Yes	Molecular markers

^d: indirect citation via van de Wiel & Lotz (2004), indirect citations are not mentioned in the literature cited.

^e: indirect citation via Emberlin (1999), indirect citations are not mentioned in the literature cited.

^f: indirect citation via van de Halsey et al. (2005), indirect citations are not mentioned in the literature cited.

