5.1 Gene flow
Feral nature of alfalfa (*Medicago sativa* L.): Potential barrier for the co-existence of genetically modified and conventional alfalfa in diverse production systems

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**Summary**

Alfalfa (*Medicago sativa* L.), is the major forage crop in North America. Apart from cultivated fields, alfalfa also occurs as feral populations along the road verges and other unmanaged areas. The occurrence of feral alfalfa populations may pose a threat to the co-existence of genetically altered and conventional alfalfa fields. Our experimental results suggest that alfalfa establishes a self-perpetuating population that can persist in the natural environment for several years. The robust nature of feral alfalfa will exacerbate attempts to confine novel traits in this species.

**Introduction**

The Canadian Food Inspection Agency (CFIA) has approved the glyphosate resistant alfalfa events J101 and J163 for unconfined release into the environment and may be available for cultivation any time in Canada (CFIA, 2005). Alfalfa also occurs as feral populations in the road verges and such populations could act as genetic bridges for the movement of novel traits from field to feral alfalfa and vice versa and thereby contaminate conventional and organic alfalfa production fields (Bagavathiannan et al., 2006). Feral alfalfa populations may be a potential hindrance for novel trait confinement and achieving co-existence in alfalfa. Despite the agronomic and environmental significance of feral alfalfa populations, limited information was available on the nature of such populations.

**Methods**

Twelve research sites were identified in three rural municipalities in Southern Manitoba (Western Canada) in 2006. At each site, 30 feral alfalfa plants of different age and size were randomly selected and tagged. Growth and reproductive attributes were measured at monthly intervals and all tagged plants were cut at the end of the season to measure biomass (g) and seed yield (g). Soil samples were taken around the tagged plants and grew out in the greenhouse for seed bank estimation. Number of seedlings recruited around each tagged plant was also recorded. Mowing was done by the rural municipality two times; once at the end of June and again near the end of August.

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Results

In general, non-mowed plants produced higher values for reproductive attributes (Table 1). On average, 93% of non-mowed plants were reproductively successful with about 3.9g of seed plant\textsuperscript{1}. About 29.5% of mowed plants produced seeds (0.8g of seeds plant\textsuperscript{1}). We found a seedbank for alfalfa in our roadside sites (Fig.1). Higher number of seedlings recruited around the non-mowed plants (0.71 seedlings plant\textsuperscript{1}) when compared to the mowed ones (0.26 seedlings plant\textsuperscript{1}) suggesting that the seedbank may be relatively ephemeral.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Mowed</th>
<th>Unmowed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Racemes plant\textsuperscript{1}</td>
<td>35.2 (66.5)</td>
<td>43.8 (73.5)</td>
</tr>
<tr>
<td>Pod clusters plant\textsuperscript{1}</td>
<td>19.7 (47.3)</td>
<td>68.1 (90.3)</td>
</tr>
<tr>
<td>Seed yield (g plant\textsuperscript{1})</td>
<td>0.8 (1.7)</td>
<td>3.9 (4.3)</td>
</tr>
<tr>
<td>Reproductive success (%)</td>
<td>29.5</td>
<td>93</td>
</tr>
</tbody>
</table>

(Values in parenthesis refer to standard deviation)

Table 1. Reproductive parameters of feral alfalfa as affected by mowing

Conclusion

Our study showed that alfalfa populations are reproductively successful in road verges and that they can establish a self-perpetuating population. The existence of an active soil seedbank and the presence of seed dormancy contribute to the survival and persistence of alfalfa in unmanaged environments. Mowing did not prevent many of the feral plants from completing their lifecycle. The robust nature of feral alfalfa will exacerbate attempts to confine novel traits in this species.

References


Short and long distance pollen dispersal in maize under Swiss alpine conditions

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Summary
Data on pollen dispersal under the multiple weather and topographic conditions of the Swiss Alps are scarce. Transgenic cross-pollination was simulated with maize varieties of different grain colours (yellow and white). Short and long distance scenarios were analyzed in 28 on-farm field situations. In general, cross-pollination rates were lower than expected, especially under long distance situations above 50 meters. On short distances, factors such as flowering synchronisation within and between fields and slopes could be proven to have an impact on the cross-pollination rate.

Introduction
The cultivation of transgenic maize varieties has rapidly increased worldwide. Maize is a wind pollinated crop that produces high amounts of pollen and favours cross-pollination by protandry, and has therefore the potential to contaminate neighbouring fields.

Some studies about cross-pollination in maize have been published in the recent years across the world (Ma et al., 2004; Bannert, 2006); experiments are lacking for the Swiss alpine conditions, which are quite diverse with respect to weather conditions and topography.

Methods
Transgenic cross-pollination was simulated with maize varieties of different grain colours. In this visual marker system, yellow grain maize was assumed to be transgenic. Cross-pollination to the white grain receptor hybrid DSP17007 could immediately be detected by the presence of yellow grains on the white-kernelled plant.

In the years 2005 and 2006, 5000 white receptor plants were examined for flower asynchrony at single plant level; especially on small, weak plants with surprisingly high cross-pollination rate.

A model helicopter with special equipment for measuring single plant heights in a 3-D Model was used to examine the effect of slopes on cross-pollination.
Results

Long distance
At long distances the cross-pollination rate of all fields (13 in all) was always below 0.02%. Cross-pollination patterns were in most cases randomly dispersed and happened only on 1.8% of the sampled ears. In four fields (50 m to 370 m in the main wind direction) a low but marked cross-pollination was found at the field border; indicating pollen dispersal by horizontal winds (Figure 1).

Short distance
A strong effect of flower asynchrony on cross-pollination over short distances was observed. The cross-pollination was below 0.9% if the pollen donor field shed pollen five days later than the emergence of silks in the receptor field, even in the neighbouring row at 1 m distance. At single plant level, we found correlations between female flowering and cross-pollination.

Cross-pollination events are enhanced downhill and reduced uphill on the first 5m from the pollen source.

Conclusions
In general, cross-pollination rates over long distances were lower than reported elsewhere, which might be a feature of special Swiss conditions for climate, topography and landscape patterns, or of the special flower biology of the used maize varieties.

On short distance, the correlation of cross-pollination and female flowering could prove a strong impact of the single plant status on cross-pollination. Knowledge about these factors and topography can help predicting cross-pollination events over short distances.

References
Bannert M. (2006) Simulation of transgenic pollen dispersal by use off different grain colour maize. Diss. ETH No. 16508
The Plus-Hybrid system in maize: biocontainment of transgenic pollen and grain yield increase

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Summary

The Plus-Hybrid system, the cultivation of a transgenic cytoplasmic male sterile (CMS) maize hybrid combined with a second unrelated hybrid as pollen donor, has been proposed as a method for biocontainment of transgenic pollen. We aim at defining optimal hybrid combinations leading to higher yields. A European Maize Ring Experiment showed that the average grain yields of specific hybrids were indeed higher, and some very suitable Plus-Hybrids were identified that could enable breeders to offer transgenic maize within this biocontainment system soon.

Introduction

Co-existence of GM and non-GM maize cultivation systems in Europe implies controlling the release and cross-pollination of transgenic pollen. The growth of transgenic CMS maize hybrids in combination with a lower proportion of non-transgenic and unrelated hybrid serving as pollen donor, results in the confinement of the transgene within the CMS-hybrid (Feil et al., 2003). This system presents at least five other advantages: 1- CMS hybrids often yield better than their male-fertile counterparts (Budar et al., 2003; Stamp et al., 2000), 2- the allo-pollination can lead to additional grain yield benefits through the Xenia effect (Bulant and Gallais, 1998; Weingartner et al., 2002), 3- blends of male-sterile Bt-maize and male-fertile non-GM maize may help to delay the development of Bt-toxin-resistant pest populations, 4- several sources of CMS are already available, 5- inexpensive seed of CMS versions of current hybrids can be easily produced in large quantities.

In our field experiments, we aimed at determining the optimal hybrid combinations capable of inducing the Plus-Hybrid effect on yield.

Material and Methods

Small-plot field trials were carried out at 6 locations in Switzerland, Bulgaria, France and Germany. Modern commercial hybrids were tested for their male and female combining ability within the system, taking into account CMS and Xenia effects. Five hybrids, in their CMS- and fertile forms, and three additional pollinators were tested in a factorial design. Yield parameters like total grain yield, thousand kernel weight (TKW) and number of kernels (KN) were investigated.
Results of the European Ring Experiment 2006

The CMS-effect seems to be quite variable and genotype as well as genotype-environment dependent. Four hybrids out of five showed more than 6% yield increase through CMS-effect mainly due to the production of a higher number of grains. Two CMS-hybrids presented an average yield increase through Xenia effect of 9.29% and 4.20% resulting generally from the development of bigger kernels after allo-pollination. The best combinations reached more than 10% gain in yield through Xenia effect over the 6 locations, and up to over 20% in some specific cases. Seven out of eight pollinators acted mostly positively on the grain yield of the mother hybrids; only one hybrid led to a yield decrease. Five pollinators led to an average Plus-Hybrid effect of over 5% and even over 8% for two of them. For 3 combinations, the Plus-Hybrid effect observed over 6 locations was over 10% and up to 15.5%.

Conclusions

Most of the pollinators included in the Ring Experiment showed interesting general pollinating abilities, often inducing a yield increase compared to the isogenic pollination of the CMS-hybrid. It could also be determined that the Plus-Hybrid effect acts on both main yield parameters (i) KN influenced by CMS-effect and (ii) the TKW influenced by Xenia effect. In order to confirm these first results and trends, the European Maize Ring Experiment will be reproduced in 2007 at 6 locations over 4 countries.

The Plus-Hybrid system can reliably be applied as a biocontainment tool to completely avoid the release of transgenic pollen, or at least to reduce the amount of transgenic pollen released into the environment. The right selection of CMS- and fertile counterparts may allow an additional benefit for the farmer by significantly increasing grain yield.

References

Do escaped transgenes persist in nature? Introgression of a transgene in weedy bird rape (Brassica rapa L.), S.I. Warwick, A. Légère M.-J. Simard, and T. James. Agriculture and Agri-Food Canada (AAFC), ECORC, Ottawa, ON, K1A OC6 Canada; (A.L.) AAFC-Saskatoon, 107 Science Place, Saskatoon, SK, S7N 0X2 Canada; (M.J.S.) AAFC, 2560 Hocheleoga, Québec QC, G1V 2J3 Canada; (T.J.) AAFC ECORC, Ottawa, ON, K1A OC6 Canada.

Transgenic bird rape × canola (Brassica napus L.) F1 hybrids were detected under field conditions in 2001 in two Québec bird rape populations (St-Henri and Ste-Agathe). This represented the first documented case of transgene movement into a natural weed population from a commercial transgenic crop. We monitored these two populations in 2002, 2003, and 2005 to determine the fate of the HR transgene under normal agro-environmental conditions. Progeny were grown from three putative hybrid/introgressed plants collected in 2005 from the St-Henri population. All plants were scored for the HR trait (HR+/HR-), presence of species-specific AFLP molecular markers from both parental species, pollen viability, and ploidy level. We observed many hybrid types and identified one plant in 2005 that was diploid, contained the transgenic HR trait, scored negative for canola-specific AFLP markers, and whose progeny had bird rape characteristics and segregated 1:1 for the HR transgene. This confirms that a crop HR transgene, after being introduced into a weedy relative by gene flow, can persist over time in the absence of selection pressure (the herbicide), and in spite of the fitness cost associated with hybridization.
Sympatry and hybridization of canola and bird rape (*Brassica rapa* L.) in Québec. M.-J. Simard, A. Légère, and S.I. Warwick. *Soils and Crops Research and Development Centre, Agriculture and Agri-Food Canada (AAFC), 2560 Hochelaga, Québec, Québec, G1V 2J3, Canada; (A.L.) Saskatoon Research Centre, AAFC, 107 Science Place, Saskatoon, Saskatchewan, S7N 0X2, Canada; (S.I.W.) ECORC, AAFC, Ottawa, Ontario, K1A 0C6, Canada.

Hybridization between herbicide resistant (HR) transgenic canola (*Brassica napus* L.) and weedy bird rape has been documented in Québec. We evaluated the actual hybridization potential based on range overlap and *in situ* rates. We mapped the distribution of canola fields and bird rape herbarium specimens in Québec; collated information on the presence of bird rape in certified canola seed production fields; and surveyed for bird rape in, or close to canola field margins. Progeny from these populations was screened for herbicide resistance (HR) and for the presence of the HR transgene. Significant sympatry was observed in several areas and hybridization occurred in all eight populations (1.1-17.5% hybrid seed) located in field margins and in one (1.1%) out of three populations located less than 10 m from a canola field. Hybridization rates decreased exponentially as bird rape density increased, but inter-plant rates (0 to 68%) were highly variable. At present, there are no compelling data suggesting that the presence of an HR transgene in a wild/weedy relative is inherently risky. However, our current knowledge might not fully describe the risks posed by other transgenes, particularly those that convey fitness-enhancing traits.
Risk assessment of volunteer gm-potatoes in the northernmost European conditions

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Summary

Potatoes (*Solanum tuberosum* L.) have two potential sources of gm-risks in Finnish field ecosystems: volunteers from leftover tubers and those emerging from true potato seed. The first can be controlled by adequate crop rotation and herbicides, while for controlling true potato seed induced risks we suggest that gm-cultivars have intrinsically prevented berry-production.

Introduction

In Finland, potato and oilseed rape (*Brassica napus* L.) are likely to be the first gm-crops adopted by farmers. Regarding coexistence of gm- and conventional production, these two species greatly differ in degree, diversity and management of risks. In Finland, potatoes do not successfully settle into natural habitats. Hence, leftover gm-potatoes are potential risk only in field ecosystem. Due to lack of common, wild *Solanum* species risks for gene flow are negligible. Therefore, our studies focused on evaluating the success of leftover tubers to over-winter in field conditions and to produce tubers during the next season (Lumkes 1981). Also the ability of true potato seed to produce seedlings and mini-tubers was studied. The results will be applied for developing strategies for gm-risk management in northern growing conditions.

Methods

Potato tubers of two cultivars, Saturna and Asterix, both sorted into two size-groups (Ø 30 and 50 mm) originated from same growing conditions and were sown after sorting directly back to the field into two soil depths (10 and 20 cm) in autumn 2004, 2005 and 2006. Soil temperature was measured daily at soil surface and at 10 and 20 cm soil depth till beginning of next growing season. In all studies ‘the worst risk scenario’ was applied. Hence, winter survival and following seedling emergence and tuber production capacity were measured in next season in conditions maximising the risks, i.e. without having any crop sown into the field and by spraying weeds frequently to avoid competition. An additional control condition experiment was arranged to study the effects of critical temperature and its duration combinations on tuber survival rate. Also seedling emergence from true potato seed and their tuber production potential were monitored in the field where berry-producing potato cultivar Saturna was grown in the previous season 2004.
Results

During the three winters, temperature regimes varied greatly. In winter 2004-2005, soil temperatures of -5 °C or occasionally lower occurred for several weeks (February-March), which was lethal for the ‘leftover’ tubers. In 2005-2006, field was covered by up to 40 cm of snow and soil temperatures remained close to zero. It, however, decreased down to -0.9 °C in the late winter, but for so many weeks that it was again lethal for most tubers and only 2% volunteer tubers arose from tubers remaining in the field. This occurred independent on cultivar, tuber size and soil depth. In 2006-2007, fields had no permanent snow cover and despite rather mild winter conditions, soil temperatures decreased close to those in winter 2004-2005. Due to frequent lost of tuber viability in our experiments, and additional experiment was organised to identify the critical and lethal temperature regimes for tubers. When temperatures of -1, -2 and -3 °C were tested for different time periods, low proportion of tubers were always able to stay viable and especially in case -1 °C temperature did not last longer than four days, -2 °C three days and -3 °C two days.

Contrary to cultivar Asterix, Saturna produced true potato seeds, which remained viable through Finnish winter conditions and even may persist in soil seed bank for many years. This proved to be a potential source of gm-risk as emerged seedlings produced mini-tubers. We recorded 3-7 seedlings per 100 square meters, i.e. 300-700 seedlings per hectare. Each seedling produced 5-12 mini-tubers (Ø 5-20 mm) thereby indicating marked reproduction potential. An additional monitoring indicated that seedlings need to emerge till mid-August in order to be able to produce tubers. To avoid such risk in northern growing conditions, breeders need to select for intrinsically prevented berry-production when developing gm-cultivars. According to literature, true potato seed production capacity differs markedly depending on genotype (e.g. Glomsirzaie and Ortiz 2004).

Conclusions

In Finnish conditions, leftover tubers are not a considerable risk for coexistence of gm- and conventional potato production due to the naturally harsh winter conditions and possibilities to control the sporadic volunteers from the next crop. However, true potato seed initiated seedlings were able to produce tubers. This risk can be solved by accepting only non-berry-producing gm-cultivars into cultivation. It is, however, likely that due to climate change risks for winter survival of gm-leftover tubers increase. Future scenarios of gm-risks will be evaluated further as a part of the National Climate Change Adaptation Research Program.

References


EFFECT OF SOWING AND FLOWERING DATES ON MAIZE GENE FLOW

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Introduction. In recent years, several studies have sought to estimate the utility of using separation distances to control or minimize the adventitious presence of GM maize due to cross-pollination. (Bénérix and Bloc, 2003; Henry et al., 2003; Ma et al., 2004; Melé et al., 2004; Messeguer et al., 2006; Ortega Molina 2006; Weber et al., 2007). Applying this strategy may, however, prove difficult in areas where the size of the cultivated fields is relatively small. The aim of this study was to determine to what extent a GM confinement strategy could be successful based on non-synchronous flowering and taking into account the agronomic practices used in temperate areas. A further consideration was whether this strategy could be applied in the regulation of coexistence as has been suggested by some simulation studies (Messean et al., 2006).

Material and methods. The experiment was performed at Pla de Foixà (Girona, Spain). The field (which was 126 m long by 100 m wide) was divided into three equal parts separated by a 9 m border of hybrid white kernel maize (variety PR32Y52), which was homozygous for the white character. In each of those three identical parts, four 3 m bands of GM yellow kernel (variety PR32P76) were sown as pollen donors. The pollen donor bands were separated by three 6 m wide bands (i.e. 8 rows in total) of the white kernel conventional maize PR32Y52 (pollen receptors). The yellow and white kernel varieties were sown on three different dates (31\textsuperscript{st} March 2006, 20\textsuperscript{th} April 2006, and 11\textsuperscript{th} May 2006). The two varieties were sown at each sowing time in order to detect potential differences between plots in which transgenic maize had flowered before, at the same time as, and after, adjacent conventional maize. During flowering, the emergence of silks and their growth until withering was recorded, as was tassel maturation and pollen release. Gene flow was quantified by counting the number of yellow kernels present in 96 white cobs for each sowing date. Real-time PCR analysis was also applied, to confirm the results and also detect any possible gene flow originating outside the field trial.
Results and discussion. As much as a twenty-day delay between the first and second sowing dates resulted in only a three to four day delay in flowering. This was mainly attributable to cool weather during the first three weeks of April. Differences in coincidence of flowering were evident between the second and third sowing dates. A high correlation (R² = 0.99) was found between values obtained with the phenotypic and the molecular method used to quantify the adventitious presence of GM maize in the white plots (also see Pla et al., 2006). Moreover, PCR analyses revealed outcrossing from nearby yellow grain maize fields that were in the direction of the prevailing wind. Gene flow levels were inversely correlated with delays in flowering and a difference of ten days was enough to reduce the gene flow to nearly undetectable levels.

<table>
<thead>
<tr>
<th>Conventional</th>
<th>GM</th>
<th>Flowering Delay</th>
<th>% GM Yellow Kernels</th>
<th>% GM QPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>White 1st sowing,</td>
<td>Yellow</td>
<td>4</td>
<td>5.86±0.51</td>
<td>3.43±0.36</td>
</tr>
<tr>
<td>Flowering 29/06</td>
<td>1st sowing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow 2nd sowing</td>
<td>7</td>
<td></td>
<td>1.48±0.15</td>
<td>0.26±0.06</td>
</tr>
<tr>
<td>Yellow 3rd sowing</td>
<td>20</td>
<td></td>
<td>1.68±0.19</td>
<td>0.05±0.03</td>
</tr>
<tr>
<td>White 2nd sowing,</td>
<td>White</td>
<td>1</td>
<td>16.82±1.11</td>
<td>14.20±0.96</td>
</tr>
<tr>
<td>Flowering 04/07</td>
<td>1st sowing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow 2nd sowing</td>
<td>2</td>
<td></td>
<td>5.05±0.46</td>
<td>4.83±0.57</td>
</tr>
<tr>
<td>Yellow 3rd sowing</td>
<td>15</td>
<td></td>
<td>1.79±0.19</td>
<td>0.34±0.06</td>
</tr>
<tr>
<td>White 3rd sowing,</td>
<td>Yellow</td>
<td>13</td>
<td>0.21±0.05</td>
<td>0.28±0.04</td>
</tr>
<tr>
<td>Flowering 16/07</td>
<td>1st sowing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow 2nd sowing</td>
<td>10</td>
<td></td>
<td>1.87±0.35</td>
<td>0.24±0.04</td>
</tr>
<tr>
<td>Yellow 3rd sowing</td>
<td>3</td>
<td></td>
<td>4.88±0.76</td>
<td>3.76±0.35</td>
</tr>
</tbody>
</table>

In conclusion, in temperate areas where the maize sowing period may extend from early March until early May without any decrease in production yield, a ten-day space between flowering dates seems enough to almost eliminate pollen mediated gene flow.

References


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Effects of field size and physical barriers on GM-pollen mediated gene flow in rice
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Summary. The results of a field trial performed in Spain during the 2005 growing season confirmed that gene flow in rice was an edge phenomenon that decreased with distance and occurred at very low rates. These rates which were almost completely independent of donor field size while the areas of impact were primarily determined by the direction of the prevailing wind during the flowering period. Even though a physical barrier may help to reduce the pollen-mediated gene flow, it would not be necessary to use this strategy to keep transgene levels below the 0.9% EU threshold.

Introduction. Pollen mediated gene flow in rice is generally quite low and decreases with distance (Messeguer et al. 2001, 2004; Rong et al. 2005, 2007). In this study, we used three different transgenic lines to determine the effect of donor field size and differentiate between pollen coming from the inner part of the field and that coming from the edge of the field. We also studied the effect of a physical barrier used as a tool for controlling gene flow from transgenic to non-transgenic rice.

Methods. The field trial was conducted in two 800m² fields, one seeded with conventional rice and the other with transgenic rice. The former (the recipient) was located at a site that was normally down-wind of the latter (donor) (Figure 1). Two rows of maize were sown across half of the field border to establish a physical pollen barrier. To determine upwind cross-pollination, a 2m band of conventional rice was seeded along the upwind border of the donor field. Three transgenic Spanish japonica rice cultivar Senia lines were seeded in the donor field, all of which contained the bar gene conferring tolerance to the herbicide ammonium glufoisinate. The cultivars differed according to an associated marker gene (gusA, encoding B-glucuronidase; gfp, green fluorescent protein; without marker). To evaluate the gene flow from transgenic to non-transgenic rice plants, samples of seeds harvested from each sampling point (taken at distances of 0, 0.25, 0.5, 1, 2, 5, 10 and 20 m from the transgenic plants in question) were sown in a greenhouse in 48x28x7 cm trays containing peat-vermiculite substrate. Seedlings at leaf stage 3-4 were then treated with a commercial herbicide (Finale from AgrEvo Co.) at a dosage equivalent to 800 g of active ingredient/ha. After 3-4 weeks, all surviving plantlets were transferred to individual pots for a Gus-histochemical assay (as described by Jefferson et al. (1987)) and for a GFP evaluation (using a LEICA MZ6 (LEICA) stereomicroscope through a
LEICA GFPplus filter). Plants testing positive were grown in a greenhouse and their progeny were assayed for relevant traits to assure hemizygosity.

Results. A total of about 250,000 seedlings were processed. As expected, the majority of the hemizygous seedlings originated from the closest transgenic (Bar) donors. Outcrossing was greatest at the edge of the receiving field and fell off rapidly with distance (gene flow without a barrier was: 0.22 at 0m; 0.12 at 0.5m; and, 0.04 at 2 m from the pollen source). Gene flow levels were respectively 0.034% and 0.056%, with and without the pollen barrier. We also established the relationship between donor field size and gene flow level (Figure 2). The asymptotic nature of this relationship suggests that gene flow in rice is a localized edge phenomenon. Gene flow between transgenic and non-transgenic rice plants in the opposite direction to the prevailing winds was much smaller than that observed in the downwind direction.

![Figure 1. Field trial design.](image1)

![Figure 2. Relationship between donor field size and gene flow levels.](image2)

Conclusions. The use of three different transgenic lines located downwind from the point of origin allowed us to demonstrate that the gene flow % did not increase in proportion to the increasing of the field size and tended to stabilise at values that were much smaller than the 0.9% threshold established for labelling by the EU. As previously shown (Messeguer et al. 2001, 2004; Rong et al. 2005, 2007) gene flow values are generally very low but could be even lower (reduced by nearly half (0.056% vs. 0.034%)) by using a physical barrier positioned in the path of the prevailing wind.

Acknowledgements
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References
Shed and spread of wheat pollen during the flowering period

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Summary

This study aims to determine the extent to which wheat pollen is shed and spread from a pollen source during the flowering period. The number of pollen grains decreased at increasing distances influenced by the prevailing wind direction and 93% of the pollen remained within 6 m of its source.

1 Introduction

Although wheat (Triticum aestivum L.) is predominantly a self-pollinating crop, outcrossing is possible related to the amount of wind-borne pollen (Bitzer and Patterson 1967). The isolation distance required for preventing outcrossing will depend on pollen quantity and dissemination throughout the flowering period, with a great influence of weather conditions at pollen dehiscence. This study provides data on pollen dispersal during the flowering period in field conditions.

2 Methods

Experiments were carried in Madrid, Spain. The field design consisted of a 3 x 3 m central square plot of a pollen donor, T. aestivum cv Chinese Spring (CS) with receptors at distances of 0, 1, 2, 3, 4, 6, 8, 10, 12 and 14 m for sides A and C. Sides B and D were shorter. The receptors were vaseline-coated slides placed on an incline of 45° at the height of the recipient spikes. Slides were mounted early in the morning and collected daily in the afternoon. The pollen grains were counted using a microscope at low power (10x), in 5 fields across each slide. Data were converted to represent pollen grains in 10 mm², the average of the receptive area of a stigma (Bitzer and Patterson 1967).

3 Results

The number of wheat pollen grains was monitored to assess the pollen dispersal in the field.

Figure 1. - A - Number of daily collected pollen grains
B - Daily temperatures and rainfall
Pollen shedding lasted about 8 days, from 20th to 28th May. Temperatures were around 25 °C (24.9 ± 2.8 °C), and days were clear during the pollen shedding period (Fig. 1B) which favored anther extrusion. The amount of pollen increased until the 4th day (23 May) when it reached its maximum with a mean of 16.9 grains/10mm² at 0 m (Fig. 1A). The number of pollen grains decreased significantly as distances from the pollen source increased. The amount of pollen collected at the 4 m and 6 m distances was about 10 and 7% of that collected at 0 m, which means that 93% of the pollen remains within 6 m of its source but some pollen can travel greater distances. Wind direction affected pollen dispersal. The effect of primarily NE winds was apparent on the higher average number of pollen grains collected at sides B and C (Fig. 2).

![Graphs showing pollen distribution](image)

**Figure 2.** Number of grains collected in pollen traps placed at four sides of the pollen source.

**References**

Benefit for co-existence of containment strategies through chloroplast transformation

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1. Summary

The work described here originates from the EU STREP project TransContainer (FP6-2006-FQS5; http://www.transcontainer.org/UK/)2. The project aims to develop efficient and stable biological containment systems for genetically modified (GM) plants accompanied by an evaluation of the intrinsic environmental, economic and consumer impact of these new technologies. Assessing the strategy "chloroplast transformation", environmental escape of gm traits via pollen can be minimised, which will benefit co-existence measures.

2. Introduction

Chloroplast transformation is a promising containment system for plant species with strict maternal plastid inheritance by avoiding the out-crossing of foreign genes via the pollen. The technique of has been applied in a lot of model plants like e.g. Arabidopsis thaliana and tobacco (Ruf et al., 2007). The insertion of genes in the chloroplast offers the opportunity for the production of high amounts of new proteins in green plant tissues like leaves e.g. for the production of industrial or pharmaceutical products. On the other hand, these characteristics should be taken into account in the assessment of potential environmental impacts.

3. Methods

The assessment is performed based on the criteria provided by the EFSA Guidance document (2006) for the placing on the market of GM plants knowing that the development of potential crop plants is still in a very early stage. Key issues are (a) a safety assessment focussing on three points: molecular characteristics, ecology of the GM species and consequences of a potential break-down of the containment system. (b) A benefit assessment of the containment system for the co-existence of GM and non-GM plants. The investigation is trait-of-interest independent in this early stage of the project.

4. Results

The targeted insertion of a GM sequence in the chloroplast genome by homologous recombination has three advantages: First, insertional inactivation of unknown functional genes is avoided. Second, endogenic in-situ promoters can be used. Third, even though plastids harbour relatively small replicons, large insertions are tolerated. However, it has to be verified that the transgene is

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2 Acknowledgement: We thank our project partners P. Dix (Maynooth, Ireland) and S. Arcioni (Perugia, Italy) and their colleges for their support. The work is financed by the EU project Transcontainer (FP6-2006-FQS5).
not inserted unintentionally in the nuclear genome since the transformation (e.g. particle bombardment technique) could be unspecific. In any case the impact on the environment will mainly depend on the inserted trait gene and has to be assessed in a case-specific manner. Some general characteristics of insertion of genes in the chloroplast like the possibility of an enhanced amount of GM protein should be taken into account. Our initial assessment should be followed by a detailed analysis for food/feed safety and wider environmental impacts like e.g. effects on (non-) target organisms or biogeochemical processes.

The figure summarises – within a life cycle - the advantages of a chloroplast transformed plant for the coexistence as GM escape through pollen (grey-shaded area) into non-GM plant systems is restricted. However, there is still the opportunity of GM seed escape.

5. Conclusions

GM escape via pollen into adjacent non-GM fields can be substantially limited by chloroplast transformation, but a small likelihood of leakage has to taken into account. The impact of a possible unintended out-crossing via the pollen needs investigation on a case-by-case basis depending on the inserted trait, the plant species and the receiving environment. Regarding the reduction of isolation distances between GM and non-GM fields, the possibility for the loss and spillage of transplastomic seeds during harvest should be considered. GM-free pollen will help to minimise conflicts with bee keepers, either concerning a possible occurrence of gm in the honey as well as with respect to the function of bees as pollinators over long distances.

6. Literature


EFSA Guidance document of the scientific panel of genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed (2006).
Field studies on coexistence in maize: isolation distances, effectiveness of buffer crops, and outcrossing in grain maize in comparison to maize for silage

Maren Langhof, Bernd Hommel, Alexandra Hüsken, Joachim Schiemann, Peter Wehling, Ralf Wilhelm, Gerhard Rühl

Summary

Large-scale field experiments in co-existence of maize in Germany were carried out in 2005 and 2006 to investigate (i) the effectiveness of tall sunflower versus short clover-grass mixture as buffer crop as well as (ii) isolation distances of 24, 51 and 78 m. Two experimental field designs and three different test systems (one GM and two non-GM test systems) were used. The sunflower buffer crop did not effectively reduce outcrossing rates. Climatic conditions and outcrossing rates differed between years. In 2005 data suggest that 78 m might not be sufficient to keep the GM-content reliably below the labelling threshold of 0.9 % if field orientation and size ratio is unfavourable.

1 Introduction

In 2003, the EC proposed a catalogue of measures to facilitate coexistence of GM crops with conventional and organic crops at the farm level. The most discussed ones in view of central European growing conditions are isolation distances and pollen barriers. The current study generally aims at broadening the knowledge on gene flow as well as setting up a reliable data base for the adoption of coexistence measures applicable to maize cultivation in central Europe.

2 Methods

Field trials were carried out in 2005 and 2006 in the northern part of Germany on federal experimental stations at five different sites using approved forage maize varieties. Design 1: The donor maize (pollen source) was planted in the centre, surrounded in all cardinal directions by the recipient maize. Outcrossing was analyzed using three different test systems: a MON810 transgenic event, a non-GM molecular marker polymorphism and a kernel colour polymorphism. In the former two test systems outcrossing rates were assessed quantitatively via Real Time PCR (rtPCR), in the latter visually. Short clover-grass and tall sunflowers were planted as buffer crops alternately in two 12-13 m broad strips between donor and recipient maize, resulting in an inner and an outer recipient maize block. Detailed information on the field designs are given in Langhof et al. (2007). Design 2: The recipient maize was planted downwind from the donor maize plot, separated by either clover-grass or barley stubble as buffer crop with widths of 24, 51 and 78 m. Each isolation distance was
established twice, in a funnel-shaped arrangement with the shortest distance on the left and right side. Total trial size was approx. 8-9 ha. Just the MON810 test system was used; outcrossing was assessed by rtPCR. **Maize flowering:** During the whole flowering period the flowering stages of both pollen donor and pollen recipient maize hybrids were monitored every second day. **Weather recording:** At each site meteorological data were recorded by on-site weather stations during the maize flowering period. **Sampling:** Detailed sampling was carried out to estimate gene flow. Twenty (MON810 and molecular marker test system) and 10 (kernel colour test system) ears each were collected at different distances from the donor within the pollen recipient plots at grain maturity. Number of sample points per site was 92-262. In order to compare GM-contents of whole plant samples versus grain maize samples, whole plants were cut in immediate proximity to cob sample points at two sites at silage maize maturity stage in 2006.

### 3 Results

**Design 1:** Downwind from the donor detectable amounts of outcrossing (> 0.1 %) were noticed at each site up to the farthest distance investigated, i.e. 54 to 66 m. Upwind outcrossing was low (< 0.1 %). Outcrossing rates were not effectively reduced by growing a tall sunflower crop in contrast to a short clover-grass crop between donor and recipient maize. Gene flow into edges of maize recipient fields was generally high (‘edge effect’). **Design 2:** Climatic conditions during maize flowering differed significantly between 2005 (cool/wet) and 2006 (hot/dry). Outcrossing in 2005 was considerably higher than in 2006. Edge effects were very pronounced at all investigated isolation distances. For each isolation distance the GM-content in the harvest of the non-GM field was calculated assuming GM:non-GM field size ratios of 1:0.5, 1:1, and 1:2. Results of 2005 for the unfavourable field size ratios of 1:0.5 suggest that an isolation distance of 78 m might not be sufficient to keep the GM-content reliably always below the labelling threshold of 0.9%. On average, the GM-content of grain maize samples was twice that of whole plant samples.

### 4 Conclusions

No sufficient shielding effect of a tall sunflower crop in comparison to a short clover-grass crop was observed. Climatic conditions and outcrossing rates varied greatly between 2005 and 2006. The ratio of the GM-content of grain samples and whole plant samples was approx. 2:1.

### References

Sixth Framework Programme project TransContainer: Developing efficient and stable biological containment systems for genetically modified plants

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Summary
EU STREP project TransContainer (FP6-2006-FQS5 Contract 023018)\(^1\) aims to develop efficient and stable biological containment systems for genetically modified (GM) plants accompanied by an evaluation of the intrinsic environmental, economic and consumer impact of these new technologies. The project started on May 1\(^{st}\) 2006 and will run until April 30, 2009.

1. Introduction
The spread of transgenes from genetically modified crops to conventional and organic crops or to wild relatives remains a source of public and scientific concern in Europe. Co-existence of GM and non-GM crops can be promoted by the implementation of biological transgene containment strategies. The TransContainer project comprises 13 partners from universities, research and government institutes, SMEs and an industrial partner. The overall goal of the proposal is to lift this major road block to fulfilling the promises of agricultural biotechnology in Europe, and thus increase the competitiveness of that sector.

The Strategic Objectives of TransContainer are:
- Promoting co-existence of GM and non-GM (including organic) agriculture in Europe by using stable, environmentally safe and commercially viable biological containment strategies in crops economically relevant for Europe, and improvement and simplification of rules for co-existence
- Assessing the economic, environment and consumer impact of implementing biological containment strategies in Europe
- Enhancing understanding and acceptance, by stakeholders and the general public, of co-existence through biological containment strategies by invoking dialogue with and between these groups, and by facilitating informed policy and public debates on their consequences for co-existence measures.

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\(^1\) See: [http://www.transcontainer.org/UK/](http://www.transcontainer.org/UK/) For information requests contact Piet.Schenkelaars (pschenkelaars.sbcbiotech@planet.nl)
3. Methods

TransContainer is investigating and developing a number of strategies for biological containment:

- Plastid transformation as a means to prevent transgene spread
- Prevention of flowering as biological containment strategy
- Controlling transgene transmission through pollen and seed

Where necessary, we aim to complement these strategies with tightly controllable switches to restore fertility. The crops used are representative for crops grown for their seeds (oilseed rape), for their fruits (tomato and eggplant), or for their vegetative parts (sugar beet, rye grass, red fescue, poplar and birch). For some of these crops, several strategies are being compared.

Besides developing biological containment strategies, the project also:

- Investigates the impact of the implementation of these strategies on environmental and food safety and possible improvement of co-existence rules
- Assesses the agro-economic effects for European agriculture and compare different scenarios for co-existence
- Invokes stakeholder dialogue on socio-economic and environment issues by holding interviews, and workshops for specific groups (industry, regulators) and one for stakeholders and public
- Communicates co-existence issues and results of the project to stakeholders and general public through workshops, the project’s website, and production of a DVD

3. Results

The project is now running in its second year. In the technical workpackages, the work has concentrated on setting up transformation protocols for some of the plants, selecting varieties for transformation, isolation of genes from the target plants and construction of transformation vectors to be used for implementing the various strategies. Due to the time it takes to transform and regenerate some of the target species, major results are only expected in the last project year. For the environmental impact assessment the individual strategies are being studied. Stakeholder involvement has taken off with a series of interviews, preparation of film portraits of stakeholders for the DVD, and the first of a number of stakeholder workshops, in this case for regulatory officials held in Vienna on April 18 2006. Future workshops specifically for Eastern European countries and the overall final workshop coinciding with presentation of Transcontainer’s results will be held in Bulgaria (May 2008) and in Italy (February 2009), respectively.
Retrotransposon-based markers for detecting gene flow in *Brassica* species

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Summary

In the absence of information on a transgene, gene flow from GMOs may be detected with molecular markers. These marker systems can also then serve as an ideal means of measuring gene flow under local conditions without having to carry out a GMO release. Dominant transposable-element markers specific to the most-planted and most recent varieties of turnip rape (*Brassica rapa*) and oilseed rape (*Brassica napus*) in Finland were developed. These markers are highly sensitive: less than 0.01 % of foreign DNA can be detected on a gel. For quantitative-PCR a retrotransposon-based quantitative-PCR probe was designed. The markers were used to generate data on the potential risks for gene flow under boreal conditions at various distances.

Introduction

Agricultural products marketed in Europe should not be mixed with GMO materials but, in the case that they are, they must be labeled. Although contamination may occur at any stage of the production chain the first step in the chain, the field level, is the most problematic. To be prepared for the potential risk of GMO contamination, methods need to be developed to prevent genetic contamination in the field, as well as to monitor and verify purity and detect the degree of contamination. Gene flow from GMOs can be detected by quantitative PCR targeting the transgene if known, but if not; other molecular markers must be employed. Our key objectives are to develop cost-effective, sensitive, and robust tools for the monitoring of gene flow and contamination in turnip rape (*B. rapa*) and oilseed rape (*B. napus*) and to use these tools to gather data on the parameters for pollen-mediated flow. Our final goal is to prepare recommendation for co-existence under Finnish climatic and agronomic conditions. Here, we present data showing that conserved retrotransposons provide a useful method for detecting gene flow.

Material and Methods

Gene flow was studied without transgenic lines but instead with conventional cultivars, *Brassica rapa* cvs. Hohto and Kulta, and *B. napus* cv. Wildcat. Field trials were conducted in three adjacent
years, 2004, 2005 and 2006. The field size was 2 ha, typical for Finnish farming. Seed samples were collected from *B. rapa* field at 10 m intervals from area of 100 m² before threshing the field. Furthermore, seed samples were collected from plots at 100 m intervals up to 1000 m from the pollen-source (*B. napus*). DNA was extracted from 3000 seeds per sample site with CTAB and phenol/chloroform extraction. DNA was amplified with conventional PCR using 2 A-genome specific and 4 C-genome specific primer pairs. Quantitative PCR was performed using primers and Taq Man probe specific for C-genome and the ABI Prism 7000 Sequence Detection System.

**Results**

Our marker strategy recognized that the *B. napus* genome, AACC, is comprised of ancestral genomes derived from *B. rapa*, AA, and from *B. oleracea*, CC. Gene flow from a transgenic *B. napus* would be manifested by the presence of genetic material either from the C- or A-genome in the *B. rapa* offspring. We developed in-house procedures for cloning retrotransposons based on universally conserved domains, and used these to amplify sequences derived from elements in *B. oleracea*, the representative of the C-genome, and also to detect the A-genome specific for *B. napus* compared to the A-genome of *B. rapa*. The sequences were used to generate, in turn, new A- or C-genome specific PCR primers. The detection system was simple ethidium bromide staining following agarose gel electrophoresis; for more sensitive results, we used fluorescent labelling for quantitative PCR. Sensitivity is achieved by choosing an element that, while specific either to the A- or C-genome, is present in multiple copies that we expect to be on multiple chromosomes as well. Hence, the retrotransposon marker is considerably more sensitive than a single-locus marker would be. The markers were used to detect gene flow under field conditions in Finland. Contamination was detected all over the 100 m² sampling area. Occasional cross-pollinations were also found at sample plots 1000 m away from the pollen-source field.

**Conclusions**

We have developed cost-effective, robust and sensitive tools for monitoring pollen-flow from *B. napus* to *B. rapa* in field conditions. Results of the contamination depended on the climatic conditions of the growing season. Both humans and pollinators affected the contamination level. Furthermore, marker choice affected the pattern obtained. The data collected from three-year field experiments will be used in modeling. On the basis of the data and models recommendations are prepared for co-existence under Finnish climatic and agronomic conditions.
EFFECT OF VOLUNTEERS ON MAIZE GENE FLOW

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Summary. The presence of GM volunteers in twelve non-GM fields was evaluated and the main factors affecting the rate of cross pollination were determined. Volunteer growth was very poor, scarcely reaching the flowering stage and without cob development, but there was definitely local cross-pollination from volunteers to neighbouring non-GM plants. However, their influence on the final yield must be considered minor, in terms of the regulation of coexistence, because the same farmer subsequently grows GMO and non-GM varieties in a given field.

Introduction. The presence of volunteers could be very usual in maize fields in temperate areas. Crop management and climatic conditions during the post harvest and sowing periods are the main factors that determine their presence. During harvesting, some cobs, cob fragments and/or isolated kernels may remain in the field. If the following autumn is wet, the kernels will germinate and plantlets will die without flowering in winter. In dry conditions, the kernels remain in the field until the next sowing season, when they will germinate and reach the flowering stage. GM volunteers could contribute to the adventitious presence of GMO in the yield through, for example, the production of GM pollen.

Material and methods. The study was performed in the Foixà region of Girona, Spain, where both transgenic Bt and conventional maize varieties are commercially grown. Twelve fields were chosen in which Bt maize had been cultivated in the previous year but conventional maize was sown in the growing season under study. To estimate the number of volunteers, several zones were defined in each maize field (4 to 11 depending on field shape and size). All plants growing outside the seed line were recorded as volunteers and classified according to their distance from the seed line. The cultural practice of making furrows to irrigate the fields removed a large percentage of volunteers and this was also taken into consideration in this study. Volunteers were also classified as isolated plants, grouped plants or clusters, depending on their relative proximity. Four areas of 100 m² were exhaustively monitored to evaluate the proportion of plants belonging to each category that were capable of producing pollen. The Bt nature of the volunteer plants was determined for different leaf samples using a lateral flow strip test (Strategic Diagnostics Inc., Newark, USA). To estimate the potential rate of cross pollination from Bt volunteers, 7 samples were harvested in four different fields (28 samples in total). Each sample consisted of 3 non-GM cobs placed near one Bt
volunteer that had already reached the male flowering stage. These samples were analysed by real-time PCR.

Results and discussion. In the study region, volunteer densities ranged from below 30 to above 7000 plants / Ha (the latter representing almost 10% of the total number of plants in the field) (Table 1). This density varied due to multiple factors, many of which were dependent upon cultural practices. Moreover, as hybrid vigour and uniformity are lost in volunteers, they tend to have different sizes, flowering times, and amounts of fertile pollen, etc. Nevertheless, we determined the capacity of volunteers to cross-pollinate neighbouring non-GM plants and confirmed that the Bt transgene could be transferred from the previous year’s GM crops to the following year’s non-GM plants.

<table>
<thead>
<tr>
<th>FIELD</th>
<th>Irrigation method</th>
<th>Volunteer /Ha after labouring</th>
<th>Flowering rate (%)</th>
<th>Flowered plants/Ha</th>
<th>Cross-pollination (%)</th>
<th>Estimated GMO%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Furrow</td>
<td>97.0 ± 96.9</td>
<td>89.5</td>
<td>86.75</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Furrow</td>
<td>19.2 ± 14.4</td>
<td>89.5</td>
<td>17.18</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Centre-pivot</td>
<td>7111.4 ± 949.0</td>
<td>49.4</td>
<td>3513.47</td>
<td>0.16 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Centre-pivot</td>
<td>5886.3 ± 1349.4</td>
<td>53.5</td>
<td>3147.99</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Furrow</td>
<td>126.6 ± 57.4</td>
<td>74.2</td>
<td>93.95</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Sprinkler</td>
<td>8728.3 ± 1807.4</td>
<td>75.2</td>
<td>6568.01</td>
<td>0.089 ± 1.24</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Sprinkler</td>
<td>1600.0 ± 401.9</td>
<td>82.3</td>
<td>1316.74</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Furrow</td>
<td>154.6 ± 42.2 (1)</td>
<td>66.1</td>
<td>102.20</td>
<td>0.34 ± 0.50</td>
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<tr>
<td>9</td>
<td>Furrow</td>
<td>62.9 ± 59.2 (2)</td>
<td>78.7</td>
<td>49.52</td>
<td>0.001</td>
<td></td>
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<tr>
<td>10</td>
<td>Furrow</td>
<td>1504.7 ± 745.6 (3)</td>
<td>75.1</td>
<td>1129.42</td>
<td>0.30 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Furrow</td>
<td>267.3 ± 267.3 (4)</td>
<td>53.5</td>
<td>142.96</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Furrow</td>
<td>10.4 ± 7.1 (5)</td>
<td>89.5</td>
<td>9.34</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Estimated volunteer development data. Irrigation method and estimated final density of volunteers per Ha in each field. For fields with furrow water, the final density was calculated by visual observation (15) or by considering that 70% of the total field area was dug (16). For fields with centre-pivot or sprinkler irrigation, the number of volunteers initially observed remained unchanged. “Flowering rate” indicates the percentage of plants that developed tassels and was calculated by averaging the % of flowering plants and weighting each type by its frequency in the field. “Cross pollination” indicates the average % of GM obtained from 3 cob samples located close to a GM volunteer. “Estimated GMO%” is an approximate value obtained by multiplying the Field % of cross pollination by the ratio of the plants affected by the transgenic volunteers as a proportion of the total density of maize plants (80,000/Ha).

Under the conditions of this field study, the influence of volunteers on the final GMO yield percentages was moderate. Only when the estimated density of flowering plants was high was the contribution of volunteers to adventitious GMO content potentially relevant: this was the case of field #6. This depended upon the initial density and category of volunteers; and upon the irrigation method applied. We therefore concluded that the mere presence of volunteers in a field is not likely to result in a 0.9 % GMO concentration in the final production (i.e. the threshold established by the EU for labelling), but could contribute to this value, especially at high densities.

This work was supported by the European Union through the SIGMEA (IRTA) and COEXTRA (CSIC) projects.
Coexistence of genetically modified and conventional maize: practical experience on-farm in Slovakia

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Summary

Relevant legislation in Slovakia requires farmers to comply with an isolation distance of 200 m, which can be replaced by border rows of non-GM maize according to the principle that 1 border row replaces 2 m of isolation distance. GM-maize containing the MON 810 event, was drilled at 3 different locations over the Slovakia in the 2006 and during harvest, samples were collected from neighbouring the conventional maize. Real-Time PCR was used to detect potential levels of MON 810 in samples. Data obtained indicate that coexistence of GM and conventional maize planted under defined conditions is possible. An isolation distance of 200 m, respectively 100 border rows of conventional maize represents 3-4 times the effective isolation distance actually needed.

Introduction

The year 2006 was the first year of Bt-maize cultivation in the Slovakia. National coexistence law on growing of GM-plants in agriculture was enforced also in that year. Relevant regulation decree was isuued in the spring 2007. An outercrossing in GM-maize is also considered in this law - isolation distances between conventional and GM-maize were established to 200 m, between organic and GM-maize to 300 m. Farmers can also replace isolation distance by border rows of conventional maize (1 row replaces 2 m of isolation distance). The objective of our study was to study efficiency of isolation distances for GM-maize in relation to requirement of current legislation. The results should provide more rational view on coexistence regulations used in agricultural practice.

Methods

The maize hybrid DKC4442YG containing the event MON 810 (Bt-maize) was drilled in 3 locations – Bajč, Lipové, and Naciná Ves. Bt-maize in Lipové was surrounded by 36 rows of non-GM near-isogenic maize (DK440), in Naciná Ves by 36 rows from 3 sides and by 6 rows from the fourth side, and in Bajč only from the downwind side with 126 rows. Grain samples for DNA analyses were taken from conventional maize in strips during harvest. At Lipové and Naciná Ves samples were sampled from strips of rows: 1-6 (distance 0.7-4.2 m from the GM-plot), 7-12 (4.9-8.4 m), 13-18 (9.1-12.6 m), 19-24 (13.3-16.8 m), 25-30 (17.5-21.0 m) and 31-36 (21.7-25.2 m).
Higher number of downwind samples was taken at Bajč (up to rows 121-126, i.e. 84.7-88.2 m from the GM-field). The influence of wind direction was taken into account by sampling all four sides at two locations (Lipové, Naciná Ves). DNA analyses were performed by Real-Time PCR. Results of adventitious presence were expressed as seed percentage.

Results

Adventitious presence of MON 810 event, irrespective of wind direction, did not exceed 0.25 % of DNA at distance greater than 19-24 rows, i.e. 13.3-16.8 m from the MON 810 plot. Wind direction impact was most pronounced in location Lipové. The calculated and measured results carried out on the bulk grain samples collected from the entire 36 rows (25.2 m) of the conventional field sections neighbouring the central plot of MON 810 fulfilled the legal food and feed labelling threshold of 0.9 % of DNA for grain in all cases. We also observed that required and regulated isolation with 100 border rows of conventional maize (instead of 200 m of isolation distance) exceed the actual need for at least 3-4 times. Moreover adventitious presence of extraneous DNA in harvested conventional grain in practice is further decreased due to simple dilution effects.

Conclusions

Much lower number of conventional maize rows is necessary for effective isolation between GM and other maize fields than is required by current regulation. This confirms results of Gustafson et al. (2006), Devos et al. (2007), and Sandivio et al. (2007) that maize pollen flow and pollen mediated gene flow decline rapidly by distance. Moreover higher isolation distances between GM and conventional maize are not convenient in smaller fields. Reduction of these distances could allow to access benefit of coexistence in agricultural practice for more such farmers in Slovakia.

References


Modeling pollen dispersal between white clover fields

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Summary
For co-existence of some crops like white clover, we need to expand our knowledge about the dispersal routes of transgenes. White clover (Trifolium repens) is dependent on honey bees as pollinators. In the present study a mathematical model was applied to white clover to estimate the amount of gene flow between fields caused by honey bees. The output results of the model will help in quantifying the gene dispersal from transgenic varieties to recipient fields.
This research was partly supported by funding from the Danish Research Centre for Organic Food and Farming (DARCOF III, http://www.darcof.dk).

Introduction
Genetically modified crops are a reality. For some crops like clover our knowledge on gene flow is scanty, and we need to expand our knowledge about the dispersal routes of transgenes, so control measures for co-existence can be set.
White clover (Trifolium repens) is important to the forage production in clover grass mixtures; it is also used by organic farmers as green manure and cover crop. Denmark has a considerable seed production of white clover, up till 80 % of the total European production. Approximately 8 % of the total Danish agricultural area is covered with clover grass mixtures.
The self-incompatible white clover is dependent on its pollinators, which predominantly are honey bees (Apis mellifera). In the present study a mathematical model, the “Portion-dilution model” (PDM) (developed by Cresswell et. al. 2002), was applied to white clover (Trifolium repens) to model the amount of gene flow between fields caused by pollinators.
Material and methods

The Portion-dilution-model (PDM)

The model is based on three parameters, which can relatively easy be estimated in the field.

\[
\sum_{v=1}^{m} f(v)
\]

\( E \) = the fraction of pollinators that arrive in a recipient field from an extrinsic pollen donor source. It is not possible to observe the fraction of pollen which arrives from an extrinsic source; instead \( E \) is estimated from general flight patterns of honeybees, simple scenarios of the spatial distribution of white clover fields, and some simplifying assumptions on the admixture of pollen in a bee colony, this done to include secondary pollen transfer, which is not included in the original model of Cresswell et. al. 2002, but are include in the model of Damgaard et al. (in prep).

\( \psi \) = the paternity shadow, which is the cumulative function of offspring produced by donor pollen in a recipient flower, as the number of visits in the recipient population increases. This can be calculated by 
\( \psi = \sum_{v} f(v) \) where \( v \) is the sequence of flowers, and \( f(v) \) is the proportion of ovules fertilized by donor pollen, and \( m \) is the maximum number of visited flower heads.

\( b \) = mean number of flower heads a pollinator visits in one foraging bout. Because it is the first time the model is applied to white clover and honeybees, the foraging behavior of honeybees has necessitated some changes of the model.

Model modification and results

In original the model by Cresswell et. al. 2002, a bee was followed in the field and the visitation bout was acknowledged as finished, when the bumblebee flew high up and left the field. Honeybees do not have such a “finishing behavior”, and therefore it was not possible to determine, when the visitation bout was over. An alternative estimation method of this parameter was developed and will be presented. This estimated value of \( b \) is minimum 10 times higher then the \( b \) value based upon observations in the field. The consequences of the new estimation method to the model output will be tested against the results of the traditional method.

When defining mitigating measures for co-existence of white clover, part of the base line information on pollen dispersal will be generated by the output from the PDM.

References

Maize pollen viability: an important factor to consider in coexistence studies.

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Abstract

The evaluation of the risk of cross pollination between fields, in particular between GM and conventional maize, must take account of the viability of pollen. All the maize coexistence studies show that the rate of outcrossing fecundation decreases drastically as the distance from the GMO source increases. These results are due to the very low quantities of pollen in competition with the pollen of the conventional field and its low viability. This study confirms that the pollen is selected at its emission, as a result of the non viable pollen falling more slowly and flying furthest. The viable pollen, being very wet, is heavier and falls relatively quickly. Therefore, to consider both pollen quantity and quality, a dispersal model must take account of the viability.

Introduction

Maize pollen quantities decrease, and viability drops drastically with distance: from 70% in the source field to 17% at 250 m (Audran 2003; Foueillassar GMCC05). The objective of this experiment was to show that maize pollen transported by the wind away from the source is least viable.

Materials and methods

Two varieties of maize (15 plants) were used for this study as sources of pollen. For 2 hours (8 U.H. - 10 U.H.), the pollen was trapped on germination media (50 mm diameter) placed at different distances (0.5 m, 2 m, 4 m) and heights (0.10 m; 0.90 m - ear level - and 2.10 m - panicle level -). The trapped pollen grains were analysed using an optical microscope to evaluate the percentage of viable pollen for each distance x height combination. Viability is measured by the percentage of pollen grains which germinate on the medium.

Results

Out of the eleven trials carried out, only five trials were retained, the amount of pollen captured in two hours having been considered sufficient for each distance x height combination (30 grains minimum). These 5 validated experiments took place under a minimum wind speed of 2m/s. Most of the pollen fell between 0.5 and 2m from the source. For these distances, maximum quantities of pollen are found at heights of 0.1 and 0.9 m, i.e. near the ground and at ear level.
Maximum pollen viability in the field is never 100% at its emission. In these experiments it was on average 60% viable. This viability decreases during the day depending on climatic conditions.

Pollen viability decreases when the distance from the source increases. Moreover, for each distance, pollen viability decreases as the height from the ground increases. Viable and not-viable pollen is sorted according to the distance from the source. We also note that viable pollen falls to the ground closer to the pollen source, regardless of the test carried out and of climatic conditions. The proportion of viable pollen decreases not only when the distance from the source increases, but also when the height from the ground increases. Viable pollen falls down faster than not viable pollen.

Jarosz (2003) showed that dry pollen is lighter and falls more slowly than wet pollen, which is more likely to be viable.

A variance analysis shows the significant effect of the distance and height factors, with a probability of 5%. Conversely, there would be no significant effect with a probability of 5% with variety.

Viable pollen is found in greater quantity near the source whereas non-viable pollen is found further from the source (92% at 0.5 m and 49% at 4 m at ground level). Moreover, it is the non-viable pollen which is more likely found at a height of 2.1 m. (59% at 0.5 m and 28% at 4 m)

Conclusion

The pollen is selected immediately after release. A minimum wind speed of 2 m/s is necessary to transfer the pollen outside the field. The non-viable pollen is often the lightest and that is why it flies further. The risk of significant outcrossing fecundation between GMO fields must be considered as potentially limited because it is expected that non-viable pollen will travel further than more viable pollen. If these preliminary results are confirmed in other experiments, it will make sense to incorporate the proportions of viable and non viable pollen in pollen dispersion models.

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Specific coexistence measures for sweet corn

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Abstract
The purity of sweet corn becomes a crucial objective in GMO fields. Cross fertilisation rate between commodity maize and sweet corn pollen can be easily determined by the different aspect of the kernel after drying.
The trials carried out during two consecutive years (16 locations in 2001 - 15 locations in 2002) on the rates of cross fertilisation between sweet corn fields and commodity corn crops were designed to give some answers on the possibilities of coexistence between GM maize and sweet corn (intended for human consumption). The rates of cross fertilisation in 2002 were lower than in 2001 (0.014 %–0.084 %) depending on wind speed (2 m/s in 2002 and 5 m/s in 2001 on average). But harvest conditions do not lend themselves to mixing the kernels and some ears from the outside of the field (first 6 rows) can have high outercross fertilisation levels.

Introduction
Sweet corn has been produced for many years in the South of France (25 000 ha). With the development of GM crops, the sweet corn industry is expecting coexistence rules to ensure maximum purity. The objective of this study is to evaluate the potential rate of cross fertilisation between commodity maize and sweet corn located in the vicinity.

Materials and methods
25 days after flowering, 60 ears (cobs) were sampled from 12 defined points distributed in the six outer rows of the sweet corn crops (2 x 5 ears/row). The same number of ears was collected from each row in order to measure cross pollination for each of the outer rows of the field. Beyond the first 24 outer rows, the remainder of the field was sampled in order to obtain an average level of cross pollination in this part of the field, by sampling 5 consecutive ears from 24 defined points across the field, giving a total of 120 ears. After collecting and drying the ears, sweet corn kernels are recognizable due to their wrinkled appearance. Kernels produced from outcrossing with commodity maize are firm and smooth and can be easily counted to measure
the level of outcrossing. Sweet corn fields are not isolated from neighbouring grain maize fields and the criteria used in choosing the study fields were flowering stage synchronicity and the presence of a single conventional grain maize source. The selected source and recipient crops had flowering dates from - 6 days to + 5 days and a range of different separation distances. The recorded parameters included: crop areas, front length, separation distance, wind direction and wind speed.

Results

In the majority of cases, pollen production is higher in sweet corn than in commodity maize due to the structure of sweet corn which has multiple tillers and flowers.

In 2002, the rates of cross pollination were lower than in 2001 (0.014 % - 0.084 % resp.) probably due to the lower wind speed (2 m/s in 2002 and 5 m/s in 2001 on average).

A sharp decrease in the level of cross pollination between the first outer six rows and the next six rows, showed the barrier function of the outer maize rows. Cross pollination subsequently decreased exponentially away from the source and occurred randomly at low levels for distances from 200 to 300 meters.

Conclusion

Sweet corn has low levels of outcrossing due to high levels of pollen production. For sweet corn production, outer rows are not taken into account because they are generally discarded at harvest. For coexistence, it will be important to measure the level of cross pollination in the outer rows before harvesting, when GM maize is produced in the vicinity.

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Low-dormancy oilseed rape genotypes – what can they contribute to co-existence?

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Summary

Seed persistence in the soil of a high-dormancy oilseed rape genotype was 50% over 30 months. This number was only 9% for seeds of a low-dormancy genotype. There was no difference for seed persistence between the soil depths of 10, 20 and 30cm. Since there currently exist several varieties with low dormancy, growing these genotypes can be an essential element in co-existence practices.

Introduction

Oilseed rape seeds are able to persist in the soil for many growing seasons (Lutman et al. 2003). Post-harvest tillage is a relevant factor for induction of seed persistence and vertical distribution of the soil seed bank (Gruber et al. 2005). There are additionally clear indications that the genotype has an important effect on dormancy induction and on GM and non-GM seed persistence (Pekrun et al. 1997, Gulden et al. 2004, Gruber et al. 2004). Vertical distribution of seeds and genotypic seed persistence should be combined in one experimental approach in this study. The results will contribute to the quantification of GM seed persistence and seed mortality in the soil.

Materials and Methods

Seeds of the winter oilseed rape varieties Express (low dormancy, 9% dormant seeds, 96% viability) and Smart (high dormancy, 91% dormant seeds, 99% viability) were enclosed in fabric bags, buried in the field in a depth of 10, 20 and 30cm in August 2004, and dug up periodically. The seed lot was 500 seeds per bag. All seeds were tested for germination after removal from the soil. The statistical analysis was performed by the GLM procedure in the SAS programme.

Results

While the number of persistent seeds declined by 91 % for the low-dormancy variety Express over 30 months, this decline was only about 50% for the high-dormancy variety Smart (Fig. 1). The viability of the persisting seeds was 99%. There were nearly no significant differences and no genotype–depth interactions in seed persistence between the soil depths.
Fig. 1. Number of viable seeds of the oilseed rape varieties Express (A) and Smart (B) persisting over a period of 30 months after artificial burial in the soil, significance by Fisher’s LSD (p=0.05)

Conclusions

Genotypic variation of seed persistence offers the option for farmers to select suitable varieties for growing oilseed rape. Moreover, this variability can be used for breeding new varieties with low seed persistence. Seed loss by fatal germination or other mortality factors in soil horizons equal to or below 10cm are obviously low once the seed bank has been established. The more important is the effect of the genotype of oilseed rape for the long-term existence of a seed bank. If seed persistence of varieties significantly varies under same soil and tillage conditions, this potential should be used for co-existence purposes.

References


Using the SIGMEA data base to provide an overview of the persistence of seeds of oilseed rape in the context of the coexistence of GM and conventional crops

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Summary
Comparison of 84 data sets provided by partners in the SIGMEA project from France, Germany and the UK, that recorded seed rain at harvest of winter rape crops, concluded that mean seed loss from non-transgenic crops was 4102 seeds m$^{-2}$ and from GM crops 6039 seeds m$^{-2}$. Evaluation of 63 data sets on seed persistence indicated a decline rate of 80% in the first 12 months, but a slower decline thereafter. Mean decline rates were similar for GM and non-transgenic rape. After five years it was estimated that 135-224 seeds m$^{-2}$ were still present. Tillage immediately after harvest resulted in greater seed persistence than delayed tillage, and ploughing caused more persistence than tine cultivation. A further important effect was the influence of genotype on seed persistence.

Introduction
Seed persistence, volunteers and intermixture of seeds are issues of concern in the debate about coexistence of genetically modified (GM) and conventional (non-GM) oilseed rape (Brassica napus). Oilseed rape seeds can persist in the soil for more than 10 years (Lutman et al. 2003), and GM volunteers can occur within subsequent non-GM oilseed rape crops posing coexistence problems. The EU funded SIGMEA project (http://sigmea.dnyds.org) enhanced information on seed rain and seed persistence from a number of research groups in the EU, by generating new data, collating existing results and producing overall conclusions, that can inform decisions on coexistence. The focus of this paper is the overall analysis of the data base on: a) the losses of rape seeds at harvest, b) the decline rates of GM and non-GM rape seeds in the soil, c) the effects of post-harvest cultivations on seed persistence, and d) the effects of genotype on seed persistence.

Materials and Methods
Data sets have been provided by partners in France, Germany and UK. Some were from randomised block experiments, some from half field comparisons and some from multiple plot samples. The
data provided by the partners have been verified and appropriate statistical analyses have been used to combine the data sets (GENSTAT; seed rain: linear mixed model in the REML procedure; seed persistence: spline model in REML; tillage practices: analysis of variance).

Results

a) Seed rain (84 data sets): Mean seed rain at harvest from non-transgenic crops was $\log_{10} 3.61$ seeds m$^{-2}$ (ESE 0.078) (detrans 4102 seeds m$^{-2}$) and $\log_{10} 3.78$ (ESE 0.088) (detrans 6039) for GM crops. Thus seed rain appears greater for GM than for non-transgenic crops.

b) Seed persistence (63 data sets): Approximately 80% of the shed seeds disappeared within 12 months after the harvest of a rape crop. Subsequent decline was slower with 5% remaining after 5 years. This equated to 135 seeds m$^{-2}$ for the non-transgenic and 224 seeds m$^{-2}$ for the GM rape ($\log_{10} 2.133 & 2.155$ resp. (ESE 0.106)). Although the initial seed rain was higher for the GM rape, decline rates for GM and conventional rape crops were not statistically different.

c) Post harvest soil tillage (12 data sets): The result was that tillage immediately after the harvest of rape crops resulted in 7% (ESE 2.02) - 14% (ESE 3.43) seed survival in the soil in the following spring with ploughing and tine cultivation, respectively, as the main tillage operation. When tillage was delayed for 4 weeks survival was only 0.5 (ESE 0.18) - 2% (ESE 0.80), using the same two cultivation practices. Any management advice aimed at minimising seed persistence must stipulate no cultivation immediately after harvest, with fields preferably left for at least 20 days, to encourage maximum seed germination and to avoid dormancy induction.

d) Genotype: A further result from the research has been the variability in persistence between cultivars. Seeds of some cultivars persisted in the soil much longer than others, although there was little evidence that GM ones were more persistent than comparable non-GM ones. A long-term strategy for resolution of problems from volunteer rape could be breeding non-dormant cultivars.

Conclusions

The re-analyses of multiple data sets that has been possible in SIGMEA, has enabled us to reach sounder conclusions as to the behaviour of rape seeds shed at harvest and thus to contribute positively to the debate on the temporal coexistence of GM and conventional rape.

REFERENCES

High levels of adventitious presence in certified oilseed rape varieties and organic oilseed rape fields

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Summary. DNA fingerprinting of oilseed rape revealed high frequencies of impurities in certified seed lots and a high level of volunteers in organic fields. These findings support a recent study by Jørgensen et al (2007) and suggest that in a GM scenario there could be a high frequency of GM in both organic and conventional oilseed rape harvests. It might therefore be necessary to change the procedures, control and management currently applied to certified oilseed rape to ensure farmers and consumers a low content of GM in non-GM oilseed rape in the future.

Introduction. Coexistence regulations are to some extent based on the knowledge obtained from propagation of conventional varieties, because it is believed that the present control measures for seed propagation will ensure a high level of purity in the harvest. All Danish fields of oilseed rape for seed production are monitored for variety purity by the Danish Plant Directorate. This control is a crop inspection in situ and, furthermore, 5% of these seed lots are also tested for variety purity post harvest in control fields by the use of morphological characters. A study of certified oilseed rape lots in Denmark showed that 3 out of 14 seed lots had adventitious presences above the quality requirement threshold of 0.3% (Jørgensen et al, 2007). Therefore, in order to evaluate the effectiveness of the morphological test and to find the purity of today's certified seeds, 8 seed lots representing 5 varieties of winter oilseed rape and their standards (the basis for the acceptance on the list of varieties) were analyzed by 169 ISSR markers.

Volunteers derived from the seed bank are predicted to contribute the most to adventitious presence of GM in a non-GM scenario (Damgaard et al., 2006). About 2000 - 10,000 seeds m⁻² are lost to the ground before or during oilseed rape harvest (Gulden et al., 2003) and if incorporated in the soil seed bank, the seeds can survive up to 17 years (Jørgensen et al., 2007). Populations of volunteer oilseed rape can be frequent in conventional oilseed rape fields and fields with other crops. Using ISSR markers we analyzed the genetic composition of volunteer populations in 11 organic oilseed rape fields each having different history of cultivation.

Materials and methods. For the purity test, 8 seed lots and their standards were analyzed. 3 of them were collected from control fields of the Danish Plant Directorate and had therefore undergone a morphological analysis as well. Potential volunteer oilseed rape was localized in 11 organic fields by focusing on oilseed rape individuals between rows. ISSR-marker analysis of plants collected from the fields - both from within and between rows - and from the seed lots made it possible to identify and
estimate the frequency of volunteers and in some instances even the variety of the volunteer. DNA extraction and ISSR analysis were performed according to the procedure by Doyle & Doyle (1990) and Charters et al. (1996), respectively. The primers 834 ([AG]n-GYT) and 890 (VHV-[GT]n) (Chao-zhi et al. 2003) were used. AFLPop version 1.1 (Duchesne & Bernatchez, 2002) was used to perform the assignment of plants.

**Results and discussion.** The results show that all the certified seed lots had a few plants that did not allocate significantly to any of the varieties presented and as expected they showed a different ISSR fingerprint when compared to other analyzed individuals and standards. Results from the morphological analysis showed that only 1 of the tested seed lots contained individuals with a different morphology (tall and late flowering), but at an acceptable contamination level (3 out of 2000). The remaining seed lots were found to be pure based on their morphology. There was no agreement between the molecular and morphological test results. In conclusion, all the certified seed lots had a higher level of adventitious present than the quality requirement threshold of 0.3% for food. Most of the organic oilseed rape fields had high frequencies of oilseed rape volunteers, which had emerged from the seed bank. Hoeing between rows is often performed when the distance between rows is sufficiently large. Thus, most volunteers outside the rows are controlled. However, results indicate that many fields also have volunteers within the rows.

The incidence of high frequencies of adventitious presence in certified seed and high levels of volunteers in organic oilseed rape fields, coupled with the longevity of seeds in the soil seed bank, suggest that the co-existence of future cultivated GM oilseed rape varieties might be problematic. It may therefore be difficult to guarantee a GM content below the threshold value of 0.9% in food and feed from oilseed rape without changing the procedures used today.

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Influence of flowering heterogeneity on cross-pollination rates in maize: experiments and modeling

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Summary

Male and female flowering of maize has been measured in adjacent fields of Bt-maize (Donor) and isogenic varieties of conventional maize (Recipient). Cross-pollination rates in the recipient field were simulated from pollen competition considering the in-field heterogeneities of flowering over time. It could be shown that flowering synchronicity plays an important role in determining cross-pollination rates. The variation in cross-pollination rates caused by heterogeneity in flowering is quantified with Monte-Carlo techniques.

1 Introduction

In order to manage adventitious presence in non-GM harvest cross-pollination needs to be quantified. Limitations in scenario variation of field studies can be overcome by using simulation models. Flowering synchronicity has been identified as one of the major factors determining the frequency of cross-pollination (Messeguer et al., 2006). Models predicting cross-pollination in maize, usually consider flowering phase to be homogeneous throughout the whole field (Angevin et al., 2001; Lipsius et al., 2007). This simplification neglects the fact that in-field heterogeneity of flowering can be considerable (Basetti et al., 1994). We investigated the effect of heterogeneous flowering on cross-pollination rates.

2 Methods

In 2006 male flowering was determined in two 2.4 ha Bt-maize (donor) and in adjacent fields with conventional maize (recipient), 5.3 ha each. Female flowering dynamic was determined only in the recipient. At 293 sampling points 20 plants were analyzed every day during the flowering period.

The model to predict cross-pollination rates was based on the transfer function model introduced by Lipsius et al. (2007). The probability of cross-pollination was calculated by weighting the hourly cross-pollination predictions with the number of female flowers susceptible at each time step. To quantify the uncertainties in cross-pollination rates due to in-field heterogeneity of flowering we
used Monte-Carlo simulations. For each run the flowering distribution was determined using random distribution for the temporal synchronicity and the variograms identified in the field trials for geostatistical simulation.

3 Results

Male and female flowering showed significant heterogeneity within the fields. On average male flowering of the recipient all but ended on July 31st. In contrast female flowering lasted until August 8th. The male flowering in the GM donor exhibited long tailing matching the delayed female recipient flowering. Therefore the model predicts high probabilities for cross-pollination during the later stages of the flowering period. The Monte-Carlo simulations for a wide range of possible flowering situations show that in-field heterogeneity in flowering has a strong impact on expected cross-pollination rates. However, independent of the flowering patterns the cross-pollination rates are highest at the field edges facing the donor plots.

4 Conclusions

In contrast to the assumptions of most simulation models (Angevin et al., 2001; Lipsius et al., 2007) maize plants within a field do not flower synchronously. The in-field heterogeneity in flowering affects the probability cross-pollinated kernels. If during female flowering the pollen shed from the same field is low, the chances for cross-pollination increase. Field experiments and application of Monte-Carlo simulations to a cross-pollination model help to quantify uncertainties due to heterogeneity in flowering.

References


“Erprobungsanbau 2005”: Different crops as spacers to minimize cross fertilization between GM and non-GM maize on field scale level
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Key words: Zea mays– MON810 – GM DNA content – coexistence – quantitative real-time-PCR

Summary
The impact of different crops used as spacers between GM and non-GM maize fields on the out crossing rate was measured at 11 sites in Germany situated in the North (Mecklenburg-Vorpommern), the Centre (Saxony-Anhalt) and the South (Bavaria) regions that differ in climate and wind intensity. All three regions also took part in the “Erprobungsanbau 2004”. No specific influence of the different spacer crops was detectable. Nevertheless, due to extreme wind conditions, most of the pollen was transferred to the eastern maize field in all three regions. After a 20-50 m belt of variable crops and 25 m maize the GM content was below the threshold of 0.9%. Hence, the proposed distance of 20 m to maintain the out crossing below the threshold level, might not be sufficient in extreme situations.

Introduction
Several field trials to analyze the out crossing of transgenic maize pollen into conventional lines have been carried out during the last years in Europe (e.g. Brookes et al., 2004; Melé et al., 2004). One example is the “Erprobungsanbau” that took place in Germany 2004 at 30 different sites (Weber et al., 2006). As expected from earlier studies a 20 m belt of non GM maize was sufficient to reduce the out crossing rate below the threshold of 0.9%. In contrast to 2004, where the GM fields were placed directly adjacent to non GM maize, different buffer crops were used to separate the GM and non-GM fields in 2005.

Methods
The study was performed at 11 sites in Germany in the federal states Mecklenburg-Vorpommern, Saxony-Anhalt and Bavaria. Field sizes of Bt maize varied between 1 and 5 ha. GM hybrids based on the event MON810 (Bt maize) were drilled in fields surrounded by a 20 or 50 m belt of different crops like potatoes, grass, summer barley, peas, or even a street. These plots were surrounded by near isogenic maize fields of at least 60m width. Single cobs were
taken from the conventional maize by hand at distances of 50 m, 55 m and 75 m from the Bt
fields in all directions, dried and ground to a particle size of 0.5 mm. To compare the influence
of sample collection at 3 sites whole maize plants were harvested. Genomic DNA of samples
and Certified Reference Material (CRM) MON810 maize (IRMM 413d and 413f, Fluka) were
isolated using the High Pure GMO Sample Preparation Kit (Roche). Quantitative PCR was used
to detect the event specific MON810 DNA sequence and the maize endogenous control high
mobility group protein gene (hmg) in sampled materials (DIN EN ISO 21570). The analysis
was conducted by a certified independent diagnostic testing company and the University of Rostock.

Results and Discussion

The flowering period of Bt and conventional maize was synchronous. In contrast to the
"Erprobungsanbau 2004" (Weber et al., 2006), where due to mixed wind conditions similar out
crossing levels were observed over all directions, in 2005 there was a pronounced preference for
west wind, hence the probes collected in the east contained considerably more GMO DNA than
probes from all other directions. Nevertheless, after the 20 or 50 m spacer and 25 m
conventional maize GM levels were always below 0.9 %. The choice of buffer plants did not
influence out crossing rates.

Conclusions

In conclusion, the data indicate that even under extreme wind conditions like 2005 coexistence
of GM and conventional maize is possible. A distance of 50m and a maize belt of 25 m either
next to the GMO plot or labeling the harvest of the first 25m strip of the adjacent field is
sufficient to keep out crossing below the threshold of 0.9%.

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Pollen-mediated gene flow in maize under agronomical conditions representative for The Netherlands


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Summary

In 2006, field trials were performed to address the effects of two isolation distances indicated by the Dutch Coexistence Committee, i.e. 25 m between GM and conventional maize cultivation, and 250 m between GM and deliberately non-GM (e.g. organic) maize cultivation, on pollen-mediated gene flow (PMGF) under agronomical conditions representative for The Netherlands in 2006. Levels of the transgene in the grains were measured at the DNA level using a validated real-time PCR quantification method for the MON810 event. Transgene levels ranged from 0.01-0.3% in the 25 m receptor fields and 0-0.04% in the 250 m receptor fields. The trials will be repeated in 2007.

1. Introduction

In 2004, the Dutch Coexistence Committee produced a report on measures allowing coexistence between GM and non-GM cultivation in The Netherlands. For maize, separate isolation distances were proposed for conventional and deliberately non-GM (e.g. organic) cultivations, i.e. 25 m and 250 m, respectively. Additional research on the effectiveness of these distances was deemed necessary. Thus, in 2006, field trials were performed addressing the effects of these two isolation distances of 25 and 250 m on pollen-mediated gene flow (PMGF) under agronomical conditions representative for The Netherlands in 2006. Each isolation distance was implemented as an “empty” distance, i.e. without maize cultivation, since at the time of the Coexistence Committee’s report only few such large-scale trials had been published (Van de Wiel & Lotz 2006).

2. Methods

For each isolation distance, trials were performed at three different locations across The Netherlands. For the 25 m isolation distance, a MON810 variety (DKC3421YG) source field of 100x100 m (1 ha) was surrounded at all four sides by equally sized non-GM receptor fields at a
distance of 25 m. For the 250 m isolation distance, a 1 ha source field of the same MON810 variety was surrounded by 50x50 m (0.25) ha receptor fields in four different directions at 250 m. A maize cultivar near-isogenic to the GM cultivar (DKc3-420) was grown on the receptor fields to obtain optimal synchronicity of flowering between GM and non-GM maize. For sampling, the 250 m receptor fields were divided into 16 quadrants and samples were taken from each quadrant. With the 25 m receptor fields, samples were taken along three transects starting at the border row closest to the GM source field and moving into the field perpendicularly to that row. Each sample consisted of five ears. Levels of the transgene in the grains were measured using a validated real-time PCR quantification method for the MON810 event. For quantification of field levels of GM presence in the 25 m receptor fields, an inverse power regression (Generalized Linear Model with Reciprocal Link and Gamma distribution) was applied to the GM levels versus distance from the field border (cf. Henry et al. 2003).

3. Results

The pattern of GM presence in the receptor fields at 25 m distance from the GM source field was according to the usual pattern of PMGF into agricultural fields: the highest levels were found at the side of the field closest to the source field, and levels dropped quickly moving further into the field. GM levels found per field ranged from 0.01-0.3%. In the 250 m fields, these values ranged form nil to 0.04%.

4. Conclusions

GM levels in the receptor fields as a result from PMGF were not different from results published previously (cf. reviews by Van de Wiel & Lotz 2006 and Gustafson et al. 2006). As results are highly dependent on meteorological conditions, trials will be repeated in 2007.

References


A simple biophysical model to simulate variations in pollen release from a maize crop during the day.

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Summary

Based on various experiments between 2000 and 2006, we developed a simple model of daily variations in maize pollen release. The purpose of the model is to provide a source term for physically-based 2-D or 3-D pollen dispersal model.

Introduction

To quantify cross-pollination in maize physically-based models have been developed (Jarosz et al., 2005), but actually are limited by the lack of knowledge on the source term modeling. Our study consists in modelling daily pattern of pollen release and daily amount of pollen during the flowering period in relation to meteorological conditions.

Methods

Field experiments were done between 2000 and 2006 on four sites in various parts of France (west and south of Paris area, south-west of France) during July and early August (Huber et al., 2005). For each experiment three variables linked to pollen release were measured during the flowering period: flowering percentage, pollen concentration in the air (Burkard trap, reading every 15 mn) and pollen deposition on the ground (hourly). In addition, weather variables such as relative humidity, rainfall, wind speed, temperature and global radiation were measured every 15 mn above the canopy.

Quantities of pollen caught continuously near the top of the canopy by the Burkard trap allowed to simulate a nondimensional index based on the time variation of the mean number ($Q_d(t)$) of pollen grains trapped per minute. The index equation is a combination of two Gauss curves, the first one occurring during mid-day and the second in the late afternoon, using the following equation:

$$I_d(t) = \frac{Q_d(t)}{Q_d} = \frac{F_d}{P1_d \times \sqrt{\pi/2}} \times \exp \left( -2 \left( \frac{t - t_{\text{max}1_d}}{P1_d} \right)^2 \right) + \frac{(1 - F_d)}{P2_d \times \sqrt{\pi/2}} \times \exp \left( -2 \left( \frac{t - t_{\text{max}2_d}}{P2_d} \right)^2 \right)$$
Where $Q_d$ is the daily amount of pollen caught by the Burkard trap (number of grains trapped per day), $F_d$ is the fraction of $Q_d$ released during the first Gauss curve, $P1$ and $P2$ are the periods (in hours) between both occurrences of pollen release being equal to half the maximum for each Gaussian curve, $t_{max1}$ and $t_{max2}$ are the peak times. The five parameters are assumed to be independent and obtained by either regression trees or regression analysis by least-square method using microclimate as predictor variables. Assuming that deposition can be used as first-order estimate of pollen release at field scale, pollen release can be estimated as the product of the index $I_d(t)$ and the total daily amount of pollen deposited $D_d$ (grains.cm$^{-2}$.d$^{-1}$) whose value is directly linked to the flowering percentage. Based on plant density and flowering percentage, pollen release by an average plant during the day can be inferred.

Results

Depending on parameter $t_{max1}$, 3 situations were identified. Strong wind speed ($>3.3$ m.s$^{-1}$) during the beginning of emission accelerates the emission process ($t_{max1} \sim 8h15$) whereas lower speeds determine late emission ($t_{max1} \sim 10h25$ for morning temperature $< 14.4^\circ C$, $t_{max1} \sim 9h55$ otherwise). The duration parameter $P1$ is comprised between 2 and 7h and is modelled by regression using a sigmoid function decreasing with daily average vapour pressure deficit (VPD). An exponential regression model is used for $F1$ which varies opposite to average global radiation. The values of parameters $t_{max2}$ and $P2$ are assumed to be constant and estimated as 16h15 and 4h05, respectively. The cumulated deposit $D_d$ is obtained by an exponential regression against flowering percentage.

Conclusions

The coupling of emission and dispersal models is expected to lead to a methodology for quantifying pollen transfer between neighboring maize crops aiming at the assessment of source-sink relationships at landscape scale. It is intended to incorporate rainfall occurrence in the modeling approach and more importantly the effect of genetic variability on daily pollen release.

References


Monitoring of feral canola (*Brassica napus*) populations: distribution of GM and non-GM canola originating from spilled seeds around Kashima Port, Japan

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**Summary**: We researched the distribution of feral canola populations along highway and main cross-points in the port of unloading canola and its vicinity. The results showed the feral canola populations are restricted to the along routes. And no differences in habitat preference were detected between GM and non-GM canola.

1 **Introduction**

Japan is one of the largest importers of agricultural products in the world. Most of the imported canola (*Brassica napus*) is from Canada, where more than 80% of the canola cultivated is thought to be genetically modified (GM) herbicide-tolerant varieties (Yoshimura *et al.*, 2006). In 2004, feral GM canola specimens were discovered in Kashima Port, a major receiving port in Japan. These were identified as imported canola based on seed constituent analysis and the detection of the transferred gene by PCR. Given that no canola is cultivated in the vicinity of Kashima Port, and since the distribution of canola is primarily restricted to the main traffic routes, these specimens of canola are not escaped from fields but originated from seeds spilled during transportation. Although the Ministry of Agriculture, Forestry and Fisheries have certified GM canola as being suitable for cultivation in Japan, consumers are concerned about whether GM canola will become widely dispersed and have adverse effects on the ecosystem and the cultivation of related rapeseed varieties (*B. rapa* and *B. juncea*). This study assessed the ecological effect posed by feral canola including herbicide tolerant GM plants (both glyphosate- and glufosinate-tolerance) by focusing on their ability to become established in various habitats.

2 **Methods**

We researched the distribution of feral *Brassica napus* populations along highway and main cross-points in Kashima Port and its vicinity within a 5-kilometer radius of the Minato park. At
19 sites selected, we investigated ecological conditions of habitats, fluctuations of number of individuals and phenology of marking individuals. And glyphosate tolerance (RR) protein (CP4 EPSPS protein) and glufosinate tolerance (LL) protein (PAT protein) induced by transgenes were detected by the test strips (Strategic diagnostics Inc.) in the laboratory.

3 Results
Feral canola populations have been found in three types of habitat, each of which varied with respect to the soil depth, spreading of population (linear, planar or isolated) and vegetation cover. These habitats were disturbed land and the ridge of paddy fields (Type 1), road islands and the areas beneath trees on sidewalks (Type 2), and the spaces on curbs and sidewalks (Type 3). Various sizes of canola plants were found throughout the year in Type 1 habitat while few canola plants were found throughout the year in Type 2 habitat. Fluctuations in the distributions of canola populations were very high in Type 3 habitat because of frequent disturbance due to weed and mud removal. No differences in habitat preference were detected between GM and non-GM canola because the total number of plants and the proportion of GM to non-GM canola in mixed populations exhibited differences in each of the habitats. It is suggested that the population dynamics of both GM and non-GM canola were more highly dependent upon habitat and ecological conditions than differences in herbicide tolerance. Furthermore, some stacking plants were detected by PCR analysis, however their origin were not identified in this study.

4 Conclusions
Feral canola populations originated from spilled seeds are restricted to the along traffic routes, where wasteland, road island, spaces on curts and sidewalk are main habitats. It is postulated that the population dynamics of both GM and non-GM canola were more highly dependent upon habitat and ecological conditions than differences in herbicide tolerance.

References
Evaluation of the GENESYS-RAPE gene flow model: Case of reconversion from a genetically-modified to a conventional oilseed rape

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1. Summary

Gene flow between oilseed rape (OSR) varieties cannot be solely studied on field experiments. A model, GENESYS-RAPE, was therefore developed to predict the effects of cropping systems on gene flow in farming regions. In the present work, the model was confronted to experimental data from situations of return from genetically modified (GM) to conventional OSR varieties. The model was shown to produce satisfactory predictions except that some varietal effects probably related to seed dormancy and survival are not yet sufficiently taken into account by GENESYS.

2. Introduction

Gene flow in rape is due to pollen and seed dispersal between fields and over the years, and greatly interacts with the cropping systems. Gene flow models like GENESYS-RAPE (Colbach et al., 2001) have been developed for some years to integrate all these phenomena at a large spatio-temporal scale. GENESYS has already been evaluated for predicting gene flow in the case of co-existing GM and non-GM varieties (Colbach et al., 2005). In the present paper, we focused on gene flow in time, from GM varieties to later non-GM crops grown in the same fields. For this purpose, we used experimental data obtained in cases of return from GM OSR to non-GM OSR.

3. Methods

The aim of GENESYS-RAPE, is to rank cropping systems according to their risk of gene flow between OSR cultivars. The model integrates the effects of crop succession and crop management at the level of a region as well as the management of uncultivated areas such as road verges. The main output variable which was compared to experimental observations was the harvest impurity of non-GM OSR (i.e. the percentage of GM seeds in the harvest of the non-GM OSR).

Experimental data were collected on two GMO platforms where three GM herbicide-tolerant OSR varieties were cultivated from 1996 to 2004 (Messéan et al., 2007). From 2002 onwards, non-GM varieties were also used and each year, 35 samples were collected in their harvests. Harvest impurity was measured in greenhouse by sowing sub-samples and spraying groups of at least 1500 resulting seedlings with the three herbicides corresponding to the GM varieties.
4. Results

On the GMO platforms, harvest impurity in non-GM harvests ranged from 0 to 25.8% (105 observations). The evaluation of GENE SYS-RAPE was made comparing the predicted percentage of GM seeds in the harvest of non-GM OSR to the percentage observed on the GMO platforms. Modelling efficiency ($r^2$) was 0.24 and RMSEP 3.29%. But 94% of the error was due to only 12 observations coming from samples clustered in one field located at the edge of the Toulouse platform in 2002. Without these points, modelling efficiency and RMSEP were respectively 0.36 and 0.80%. The prediction bias (i.e. the means of residuals) was -0.12% and not significantly different from 0. But GENE SYS-RAPE did not take correctly into account the complete range of observed variability as observed variance significantly exceeded the simulated variance. The ranking capacity was bad (Kendall correlation $K=0.37$) The analysis of residual values showed that the region, the GM variety and the harvested non-GM variety (i.e. semi-dwarf or tall) had significant effects. These factors are not yet sufficiently integrated into GENE SYS-RAPE and are probably related to varietal differences in seed dormancy and survival.

5. Conclusion

The confrontation of GENE SYS-RAPE to independent field data showed that the model is adequate to predict gene flow in time, to crops grown after GM varieties, as a function of cropping systems. However, the differences between OSR varieties are not yet sufficiently taken into account in the model.

6. References


Seed loss at sowing and harvesting time as a risk for voluntary plants and gene flow for oilseed crops in the Northern conditions

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Summary

In this study, four experiments for spring sown oilseed crops were generated in Jokioinen for specifying the viability of potential seed loss at sowing time, seed shattering during harvesting, and effect of crop management on the exist of voluntary plants. Seed shattering during harvesting was 4.8% of harvested yield for rape, and 4.5% for turnip rape. Thus it is the most prominent source of voluntary plants for spring oilseed crops. Immediately after harvesting, 14 – 18% of seed spill germinated on soil surface. The number of seedlings found in the following years decreased rapidly, and after two years only 1-2 seedlings per square meter were found, and after three years none. On the soil surface, seeds lost their viability more quickly than in the deeper layers of soil.

1 Introduction

In Finland, spring oilseed rape (Brassica napus var. oleifera L.) and potato are likely to be the first GM-crops adopted by farmers. Both spring rape and spring turnip rape (B. rapa ssp. oleifera L.) are grown in south and western Finland. They exist as weeds commonly in spring cereals as well as several wild species belonging to Brassicaceae family (Salonen et al. 2001). The occurrence of rape outside the field ecosystem is exceptional, but turnip rape can grow occasionally on roadside or loading places in Finland (Hämälä-Ahti et al. 1998). In the field conditions, the source of volunteers can be seed spill at sowing or seed shattering before or at harvesting. The seed loss occurred before harvesting is dependent on species (0.1-0.6% of harvested yield for turnip rape and 0.1-1% for rape), the growing year and weather conditions (Pahkala and Sankari 2001). At harvesting, seed loss for oilseed rape reported by Lutman (2003) can be as high as 5000 seeds per square meter.

Our key objective in this study was to gather data on the potential sources for gene flow from volunteer plants to specify it separately at sowing and harvesting time, and finally, on the basis of the data to prepare recommendation for co-existence under Finnish conditions.

2 Methods

Four field experiments were generated in Jokioinen (60°49'N, 23°28'E); 1) For specifying the viability of potential seed loss at sowing time, 100 rape seeds were sown at 0, 2.5, 5.0, 10.0 and 20.0 cm depth of soil in spring 2005. The viability of seeds was detected by counting established seedlings weekly
during the summer and autumn. 2) Effect of crop management on the viability of rape seed was evaluated between 2004 and 2007. Seed rate of 63 kg ha\(^{-1}\) was sown in autumn 2004 on untilled field using four replicates. One third of the replicate was ploughed and sown with barley in the following spring, one third was direct sown, and one third was left untreated. The crop management was repeated in 2005, 2006 and 2007. Seedlings were counted on two circles of 0.25 m\(^2\) in autumn 2004, and thereafter weekly during the growing period of 2005, 2006 and 2007. 3) Seed loss during threshing was measured for rape and turnip rape from straw walkers, sieves and drum of the harvester according to ISO 8210 standard, and from the table using metal collectors between the wheels. 4) Viability of rape seeds was studied at three soil depths (0, 10 and 20 cm) in 2004-2007 using polyethylene bags with 100 seeds per each, and by germinating the seeds two times per year in Jacobsen pool. Conventional varieties, Wildcat for rape and Kulta for turnip rape, were used in this study, because the invasiveness or persistence of transgenic rape plants has not been more than that of the conventional varieties (Crawley et al. 1993).

3 Results

After sowing in spring, less than 40% of seeds germinated, if they were left on the soil surface, whereas 96% of seeds germinated at the depth of 2.5 cm. At the depths of 5 cm, 10 cm and 20 cm germination was reduced to 70%, to 2%, and to 0%, respectively. In the trial of crop management, the number of rape seedlings was lower in the direct sown treatment than in the ploughing treatment. In autumn 2004, 14 – 18% of simulated seed spill germinated immediately on soil surface. The number of viable seedlings decreased rapidly in the following years. In 2006, only 1-2 seedlings were found per square meter, and in 2007, none. From the seeds placed at different depths in autumn 2004, on soil surface less than 5% germinated in 2006, at depth of 10 cm 10%, and less than 10% at the depth of 20 cm. Harvesting loss was 4.8% of the harvested yield for rape, and 4.5% for turnip rape.

References


Effect of Seeding Date and Density on Flowering Synchrony of Volunteer and Crop Canola (*Brassica napus*) in Western Canada and Impact on Novel Trait Movement

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**Summary:** The effect of seeding dates and densities on flowering synchrony, and the impact of flowering synchrony on the relative amount of trait movement from volunteer to crop canola (*B. napus*) were investigated. Data from the first year of field experiments showed that flowering synchrony and relative trait movement varied with seeding dates depending on the densities of volunteers.

**Introduction:** Novel trait movement within a species will occur at higher rates and can potentially be more detrimental than interspecific movement because of the extensive production of novel crops, and minimal barriers to prevent genetic exchange (Ellstrand 2003). Flowering synchrony between source and sink populations is key to intraspecific trait movement (Augspurger 1983). While the individual effects of seeding dates and densities on flowering periods for winter canola have been studied (Kimber and McGregor 1988), there is a data gap on the interaction of these two factors on spring canola and volunteers in western Canada. This is relevant given the range of densities and emergence of volunteers in commercial fields, and their role in trait movement (Reagon and Snow 2006). This study aims to determine the effect of seeding dates and densities on flowering synchrony of volunteer and crop spring canola, and the impact of flowering synchrony on the relative amount of trait movement using herbicide resistance as a marker. A volunteer-crop gene flow model will also be developed.

**Methods:** Field experiments followed a RCBD factorial of treatments consisting of three volunteer seeding dates, four volunteer seeding densities and two crop seeding dates. Each plot had six alternate rows of volunteer (F2 seed of glufosinate-tolerant commercial variety collected from farmers’ bins) and crop (hybrid sulfonyl-urea herbicide tolerant canola), spaced 6 inches apart. Flowering counts were collected every second day on three randomly tagged volunteer and crop plants from each treatment to determine timing of first flowering and duration of flowering. Flowering synchrony indices (FSI) were calculated using the equation of Augspurger (1983), where complete overlap corresponds to a FSI = 1 and no overlap corresponds to a FSI = 0. Harvested crop seed from the field experiments were screened for the glufosinate-tolerant trait to assess relative amount of trait movement (%TM).

**Results:** Table 1 shows: (a) there was less flowering synchrony and trait movement with a later crop seeding date and when volunteers were seeded simultaneously; (b) in treatments where volunteers were seeded 7 days before the crop, flowering synchrony decreased with early crop seeding dates, and increased with late crop seeding dates, but did not decrease trait movement in treatments.
with higher volunteer densities for both crop seeding dates; and, (c) staggered seeding dates as far as 14 days apart produced flowering synchrony and trait movement.

<table>
<thead>
<tr>
<th>Early CSDate, Simultaneous VSDate</th>
<th>Late CSDate, Simultaneous VSDate</th>
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<tbody>
<tr>
<td>VDe 1 FSI = 0.38 %TM = 1.1</td>
<td>VDe 1 FSI = 0.28 %TM = 0.9</td>
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<tr>
<td>VDe 2 FSI = 0.65 %TM = 4.0</td>
<td>VDe 2 FSI = 0.33 %TM = 1.8</td>
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<tr>
<td>VDe 3 FSI = 0.63 %TM = 5.1</td>
<td>VDe 3 FSI = 0.34 %TM = 2.3</td>
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<td>VDe 4 FSI = 0.62 %TM = 6.4</td>
<td>VDe 4 FSI = 0.44 %TM = 3.1</td>
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<tr>
<th>Early CSDate, VSDate 7 days before crop</th>
<th>Late CSDate, VSDate 7 days before crop</th>
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<tbody>
<tr>
<td>VDe 1 FSI = 0.38 %TM = 1.1</td>
<td>VDe 1 FSI = 0.28 %TM = 1.9</td>
</tr>
<tr>
<td>VDe 2 FSI = 0.46 %TM = 3.3</td>
<td>VDe 2 FSI = 0.39 %TM = 2.4</td>
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<tr>
<td>VDe 3 FSI = 0.24 %TM = 3.4</td>
<td>VDe 3 FSI = 0.44 %TM = 2.6</td>
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<tr>
<td>VDe 4 FSI = 0.15 %TM = 3.4</td>
<td>VDe 4 FSI = 0.58 %TM = 2.7</td>
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<tr>
<th>Early CSDate, VSDate 7 days after crop</th>
<th>Late CSDate, VSDate 14 days before crop</th>
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<tbody>
<tr>
<td>VDe 1 FSI = 0.58 %TM = 5.2</td>
<td>VDe 1 FSI = 0.36 %TM = 3.3</td>
</tr>
<tr>
<td>VDe 2 FSI = 0.36 %TM = 3.7</td>
<td>VDe 2 FSI = 0.29 %TM = 2.0</td>
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<tr>
<td>VDe 3 FSI = 0.88 %TM = 2.0</td>
<td>VDe 3 FSI = 0.19 %TM = 0.7</td>
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<tr>
<td>VDe 4 FSI = 0.84 %TM = 4.4</td>
<td>VDe 4 FSI = 0.00 %TM = 0.0</td>
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**Conclusions:** Seeding dates, densities and their interaction affect flowering synchrony of volunteer and crop canola, and impacts trait movement because of competition produced, and amount of pollen available versus number of pollen recipients. Further statistical analysis is being done to relate other biology and phenological factors with these results. These relations will be used to develop a simple deterministic model to facilitate identifying the potential for and probability of novel trait movement and provide guidance regarding the need for volunteer management for trait confinement.

**Acknowledgements**
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**References**
Minimum isolation distance for GM sugar beet in its production areas

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Summary

Quantitative estimates of the gene flow from fields with GM-crops to fields with non-Gm crops are needed in order to suggest isolation distance to keep GM-pollination below the acceptable threshold. One possible approach is to use some mathematical model describing the transport and dispersion of particulate matters of the same qualitatively and quantitatively parameters as pollen grains of genetically modified plants.

Introduction

In Europe concerns have led the European Union to promote the concept of coexistence to allow the freedom to plant conventional and genetically modified varieties and minimise the presence of transgenes within conventional crops. The main strategy adopted in order to protect varietal purity in crops has been isolation by distance. Other important factors, that affect the level of cross-pollination at the landscape level are the spatial arrangement of plants and the size of the source and sink populations (Ceddia et al., 2007). Available models in beets for gene flow mainly focus on the small-scale field level (e.g. Alibert et al., 2005, Saeglitz et al., 2000) and provide a very wide range which is due to the great variety of the affecting factors.

Depending on the permitted maximum threshold of GMOs in the harvest, researchers have come up with different recommendations, reaching from 25 to 250 metres for maize (Devos et al., 2005). For other crop species, little data is available at the moment. In the European Community in seed beet production, the recommended minimum distance from neighbouring pollen sources of the genus Beta is at least 1000 m (Council Directive 2002/54/EC). The question is, whether this isolation distance is sufficient to prevent the transgene dispersal in sugar beet production areas, as well.

Material and Methods

The frequency of pollinated plants (weed beets carrying no anthocyanic colour) placed at different distances (1.5, 3, 6, 12, 25, 50, 75 and 100 m) and directions (E, W, S, N) from a pollen donor source (red beets) was tested to investigate the pollen movement under realistic conditions. After the harvest at the end of the vegetation period in September, seeds were dried and later let to germinate. The frequency of gene flow from the red beets was calculated as the percentage of individuals having the red colour of its root, hypocotyl or even stem. The data from this experiment were compared with approach based on standard Gaussian dispersion model.
Results and Conclusions

The influence of insects as pollinators, intensity and dynamics of pollination process during the flowering period, the impact of moisture of the pollen grains and their weight, although it can play an important role in pollination process, were from the model excluded due to insufficient information.

![Graph showing the probability of pollination at distances up to 300 m as modelled under three wind speeds in prevailing west-wind flow.](image)

**Figure 1.** The probability of pollination at the distances up to 300 m as modelled under three wind speed in prevailing west-wind flow.

The distance 800 m from pollen source (data not shown) can be considered as safety, even if the least favourable conditions were taken into account. As shown on Fig. 1, higher wind speed suppressed vertical elevation, however the pollen could be than dispersed on a longer distance.

Based on our studies, suggestions have been submitted to the Czech national GMO regulatory authorities (Ministry of Agriculture) to broaden the science-based platform for the preparation of co-existence rules.

References


Acknowledgement

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Persistence of feral oilseed rape populations

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Summary

Feral populations of oilseed rape (OSR) are widespread outside fields on field margins, roadside verges or waste ground. In the case of introduction of GM crops, their presence could increase the risks of unintentional spread of genes from crop to crop, making the coexistence of GM and non-GM crops difficult. Investigations on the persistence abilities of feral OSR populations have been mainly conducted to-date through correlation analyses of spatial data. Here we present the first results of a study based on multilocus genotypes considering the evolution of cultivars across years. These results confirmed the key ability of feral populations to persist and thus strengthened their potential behaviour as reservoirs of (trans)genes.

Introduction

Many cultivated species can escape from fields and colonize semi-natural habitats as “feral populations”\textsuperscript{1}. Of these, feral OSR is a widespread feature of field margins and roadsides. The processes leading to its presence outside fields are increasingly better understood. First suspected to rely only on seed immigration from neighbouring crops and seed transport, they were shown to result also from processes of persistence, through the constitution of seed bank or local recruitment (Charters, Robertson & Squire, 1999; Pivard et al., 2007).

Most of results on feral OSR to-date were obtained by analysing their co-occurrence in a local surrounding of the feral populations to explain the possible local seed sources. In this study we scrutinised the possible origin of feral populations, using microsatellite markers. Because most of these populations result directly (crops), or more and less indirectly (ferals) from the sowing of certified seeds, we studied them in terms of cultivars. This approach provided useful information on the origin and the persistence ability of feral populations. For instance, while the presence of recently introduced cultivars in feral populations suggests recent seed immigration, the presence of old cultivars in feral populations suggests persistence and an effective seed bank.

\textsuperscript{1} By opposition to the populations escaped within fields, called “volunteers”
Material and methods

The study area was a 42 km² production area of winter OSR centred in the village of Selommes (central France), where we conducted observational studies every year since 2000 including a collection of the GPS coordinates of all cultivated OSR fields, volunteers and feral plants present along the road and track network (Pivard et al., 2007).

Seven microsatellite markers revealing eight independent polymorphic loci were selected to allow a good discrimination among 50 cultivars likely to have been cultivated in the area between 1991 and 2005: We genotyped an average of 24 certified seeds per cultivar to establish a reference list of cultivar genotypes. In July 2004 and 2005, leaves were collected on max. 10 plants in each crop and feral population to be genotyped. Their genotypes were then assigned to the reference list of cultivar genotypes with several goals: (1) comparing the relative proportions of cultivars in crops and feral populations across years; (2) considering these cultivar compositions in relation to the last known year of sowing for each cultivar, being careful to the presence and the proportion of old cultivars.

Results

The cultivars found in crops varied greatly in terms of presence and relative proportion from one year to the next. Thanks to this fast turn-over, cultivars proved to be good informers about the likely origin of plants to which they were assigned. For instance, in addition to cultivars recently sown, a large proportion of cultivars sown before 2002 were also found in the feral populations sampled in 2005, confirming that these populations rely on both seed immigration and seed persistence. The finding of a substantial number of very old cultivars suggested more than seven years of persistence outside fields.

Conclusion

These first results provide valuable information proving the role of seed immigration and persistence processes in the presence of feral populations outside fields. Seed bank was confirmed to be crucial for these populations considering that old and very old cultivars were identified in these populations at high proportions.

Reference
