Consequences of growing genetically modified (GM) oilseed rape in co-existence with non-GM oilseed rape

Name:	Naja Steen Andersen
University and Department:	University of Copenhagen, Faculty of Life Science, Department of Agricultural Science
Supervisors:	Jesper Rasmussen & Rikke Bagger Jørgensen
Work places:	Biosystems Department, Risø National Laboratory, Technical University of Denmark – DTU, Building 309, DK-4000 Roskilde, Denmark & Department of Agricultural Sciences, Faculty of Life Science, University of Copenhagen, Højbakkegård Allé 30, DK-2630 Taastrup, Denmerk
E-mail/phone:	naja.steen.andersen[a]risoe.dk + 45 46774151(Risø National Laboratory) + 45 35332243 (Falculty of Life Science)
Current scientific degree:	Master in Biology, University of Copenhagen

Sources of funding:	Univerity of Copenhagen/Risø National Laboratory;/SOAR-research scool for organic agriculture and food systems

Background

Varieties of genetically modified oilseed rape will in the nearest future be cultivated in Denmark as well in the rest of the EU countries. Oilseed rape is one of the Danish crops with the greatest possibility of gene flow. Consequently it is important to examine the consequences of coexistence between genetically modified oilseed rape and organic oilseed rape (and non-GM rape). One of the areas that are important to analyse is how much volunteer oilseed rape, wild turnip and impurities in certified seed of winter oilseed rape contribute to the unintended gene flow in different scenarios.

Weedy populations of oilseed rape and wild turnip act like bridges for transgene contamination of organic oilseed rape.

Wild turnip as well as wild radish (*Raphanus raphanistrum*) and charlock (*Sinapis arvensis*) hybridise with oilseed rape (The rate of hybridisation: wild turnip>wild radish>charlock). All three species are common weeds in organic fields. Especially wild turnip and volunteers of oilseed rape are able to act like bridges of transgene flow from GM oilseed rape to conventional or organic oilseed rape. Populations of volunteer oilseed rape are frequent in organic fields with or without oilseed rape production. These volunteer populations of oilseed rape are also frequent in conventionally managed fields with a scantly control of weeds, from where they emerge from the seed bank. Many farmers rent their machines at a machinery station and seeds from all *Brassicáceae* species can be transported over long distance. Even through oilseed rape fields are isolated and the gene flows between them are limited, weedy populations of wild turnip and volunteer oilseed rape can act as an intermediate state in transgene contamination. GM weeds can result from previously cultivations of GM oilseed rape (volunteers/ferals), from crosses between GM weedy populations of oilseed rape and wild turnip/non-GM oilseed rape (F1-hybrids), and from crosses that involves GM F1-hybrids (advanced hybrids).

Part 1: Hybridization between wild turnip and oilseed rape and genetic variation of wild and cultivated *B. rapa* L.

An earlier study showed a high frequency of gene flow between wild turnip and oilseed rape in a natural weedy population of wild turnip and oilseed rape in an organic field (Hansen et al. 2001). The study is based on a single population and it is therefore necessary to study the generality of this scenario. Gene flow between wild turnip and oilseed rape is in this project estimated by the frequencies of hybrids between wild turnip and oilseed rape in selected populations of wild turnip in and outside fields. Wild turnip plants are collected and are analysed by the use of oilseed rape and wild turnip specific DNA-markers (ISSR markers). By comparing the distribution of the specific DNA-markers in the analysed plants to the distribution in varieties of cultivated oilseed rape and known populations of wild turnip, it is possible to identify hybrids between wild turnip and oilseed rape and thereby quantify the gene flow.

In adittion the variation in cultivated and wild material of *B. rapa* L. was analysed with DNA markers (ISSR markers) in order to evaluate if the wild form represents variation not present in cultivars and to develop conservation strategies for future breeding material.

The results from this part of the project contribute to the calculation of the possibility that wild turnip populations in a GM scenario act like intermediate state in transgene contamination of non-GM oilseed rape. An information that, in concert with the information of volunteer oilseed rape, is useful when the adventitious presence of GM oilseed rape in the harvest from fields within part of an agricultural region is estimated. The study will also contribute with knowledge about genetic variation in Danish populations of wild turnip. And if wild turnip seems to be a threatened species, it is possible to use the information for a conservation strategy for wild turnip.

Part 2: Impurities in certified seed

All fields of oilseed rape for seed production are controlled as to variety purity. The control is carried out as a crop inspection *in situ.* In addition 5 % of the seed lots of Danish varieties of oilseed rape are also tested for variety purity after harvest. This is done in control fields by the use of morphological characters of oilseed rape varieties. However, the use of morphological characters is doubtful. In some circumstances the admixture of other varieties must be higher than 10% before the morphological test can recognise them. Impurities in seed can be a consequence of cross-pollination or seed admixture. Risk of cross-pollination is possible when the fields of oilseed rape are flowering. Admixture of other varieties is expected under sowing, harvesting, storing and cleaning. Under such circumstances it is possible that transgenes can spread to the certified seed. In a resent study of variety purity of winter oilseed rape based on DNA-data, four out of fourteen certified winter oilseed rape varieties had more than the allowed 0,3 % admixture of other varieties (Jørgensen, 2005). These certified seed lots were all randomly chosen, and it is not know whether they are among the 5 % of the Danish seed lots of varieties of oilseed rape that are tested for variety purity by the use of morphological markers, or if they are among the 95% that are approved without (in a agreement with the legislation). To analyse if the above frequencies of impurity in Danish certified seed is typical, we analyse several approved seed lots including lots that have undergone morphological analysis in the control fields. In this study, it is also possible to compare the two ways of evaluating impurities in certified seed, DNA-markers and morphological markers respectively.

The results obtained during this part of the project will generate information about the purity of certified winter oilseed rapeseed today. The information can be used to regulate the guidelines for production and certification of winter oilseed rapeseed in Denmark to ensure GM free certified winter oilseed rapeseed for organic and conventional farming. And thereby ensure farmers and consumers a GM free product.

Part 3: Volunteer oilseed rape in oilseed rape fields

We looked at volunteer populations of oilseed rape in organic fields with different history. It is almost impossible to distinguish between varieties of oilseed rape by the use of morphological characters. Instead we used DNA-markers (ISSR markers), which made it possible to distinguish between the variety presently cultivated and varieties formerly cultivated at the site. We localised potential volunteer oilseed rape in the fields by focusing at the oilseed rape individuals growing between rows in row-sown fields. Oilseed rape between rows is possibly volunteer oilseed rape plant. DNA-marker analysis of plants collected between and in the rows and of the seed presently cultivated makes it possible to identify and estimated the frequency of volunteer oilseed rape in winter oilseed rape fields. Together with the cultivation history of the fields, this will inform about the oilseed rapeseed turnover in the seed bank.

Thus the results obtained during this part of the project will generate knowledge on the extent of volunteer oilseed rape in organic winter oilseed rape fields. This can be used to estimate the possibility of volunteer oilseed rape acting like intermediate states in transgene contamination of non-GM oilseed rape. This is useful when the adventitious presence of GM oilseed rape in the harvest from fields within part of an agricultural region is estimated.

References

Hansen, L.B., Siegismund, H.R. & Jørgensen, R.B. (2001) Introgression between oilseed rape (*Brassica napus* L.) and its weedy relative *B. rapa* L. in a natural population. Genetic resources and Crop Evolution 48: 621-627

Jørgensen T. (2005) Populationsdynamik af spildraps (*Brassica napus* L. ssp. *napus*) og frøbankens betydning for genspredning – Samt sortsrenhedens betydning for

Objective

The project will contribute with knowledge of:

- Population turnover, frequency and distribution of volunteer oilseed rape and wild turnip in organic and conventional fields.
- Frequency of hybrids between wild turnip and oilseed rape in organic and conventional fields.
- Purity of varieties of winter oilseed rape estimated by molecular markers and compared with the purity of varieties found by morphological characters, which is the practice of variety purity testing of today.

The distribution, population turnover and frequency of weedy populations of oilseed rape, wild turnip, hybrids and the detection of variety impurities can be used in the landscape gene flow model GENESYS (model used in the EU-project SIGMEA http://sigmea.dyndns.org/). The model estimates the adventitious presence of GM oilseed rape in the harvest from fields within part of an agricultural region.

The results from the analysis of variety purity can also be used to regulate the guidelines for production and certification of winter oilseed rape in Denmark.

Progress: 01.10.2006-31.09.2007

Part 1: Hybridization between wild turnip and oilseed rape and genetic variation of wild and cultivated *B. rapa* L.
A manuscript with the titel: "Processes affecting genetic structure and conservation - a case study of wild and cultivated *Brassica rapa*" was written and submitted to Molecular Ecology.

Part 2: Impurities in certified seed
The ISSR data were analysed and the writing of a manuscript with the titel: "High levels of impurities in today's certified oilseed rape varieties challenges the Danish seed propagation system" was begun.

Part 3: Volunteer oilseed rape in oilseed rape fields
The ISSR data were analysed.

Stays abroad
I worked 6 months (05.01.2007- 30.06.2007) on a project for Professor Mike Wilkinson at the Institute of Biological Sciences, Edward Llwyd building, The University of Wales, Ceredigion, SY23 3DA, UK. The aim of the project was to develop a method for quantitative detecting of root DNA from *B. rapa* and *B. napus.*

Courses
I participated in the SOAR seminar: "Supervision course for PhD students and supervisors", 2-3 November 2006.

Plans: 01.10.2007-15.07.2008

I am planning to participate in the conference: "Third International Conference on Coexistence between Genetically modified (GM) and non-GM based Agricultural Supply Chains", GMCC Seville (Spain) 29th and 21st November 2007. In addition I am planning to participate in the three PhD courses:

- Management in Science and Innovation 8-11 October 2007: 5 ETCS
- Course on presentation techniques 23-24 October 2007 2 ECTS
- SOAR summer school 2008: 4 ECTS

The paper manuscript about the results from part 2 will be finished and submitted in the end of October 2007.

Data from part 3 will be analysed, whereafter a paper will be written based on the results. Hopefully the manuscript for the paper can be submitted in the end of March 2008.

The PhD-thesis is to be written and is planned to be handed in the 15 of July 2008.

Publications

Andersen, N.S.; Siegismund, H.R.; Meyer, V. and Jørgensen, R.B. (2005) Low level of gene flow from cultivated beets (Beta vulgaris L. ssp. vulgaris) into Danish populations of sea beet (Beta vulgaris L. ssp. maritima (L.) Arcangeli). *Molecular Ecology* 14: pp. 1391-1405

Andersen, Naja Steen (2005) Konsekvenser ved sameksistens mellem økologisk raps og GM raps i Danmark [Consequences of growing genetically modified oilseed rape in coexistence with organic oilseed rape in Denmark]. *Forskningsnytt om Økologisk Jordbrug i Norden* 3: pp. 14-15

Andersen, N.S.(2005) Selvsået raps gav pænt udbytte. *Økologisk jordbrug* 348: p.10

Time schedule details (whole PhD period): 01.01.2005 to 15.08.2008

Part time (fraction): 30/37 hours

(Planned) date of submission of thesis: 15.07.2008

(Planned) date for defence: 15.08.2008