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Objektyp: **Article**

Zeitschrift: **Bulletin of the Geobotanical Institute ETH**

Band (Jahr): **67 (2001)**

PDF erstellt am: **20.09.2024**

Persistenter Link: <https://doi.org/10.5169/seals-377841>

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RESEARCH PROJECT

Transgene flow from crops to wild plants, consequences for associated insects and implications for hybrid fitness

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Summary

1 There is consensus that hybridisation between transgenic *Brassica napus* and related wild species (family Brassicaceae) is likely to occur eventually. Resulting transgene flow from cultivated to wild populations might have detrimental consequences for associated organisms and ecosystem functioning. However, the hybridization rate likely to be found in natural and agro-ecosystems is controversially debated. Knowledge on potential rates of hybridisation between transgenic *B. napus* and wild relatives, on the fitness of hybrids, and on their effects on associated organisms is needed for the ecological risk assessment of transgenic *B. napus*.

2 In this project we will investigate the spatio-temporal patterns of co-occurrence of *B. napus* and its wild relatives in two oilseed producing areas in Switzerland to assess the potential for hybridisation. In addition, seeds of wild relatives will be collected, and their progeny will be screened with molecular methods to see whether hybridisation with *B. napus* has already occurred.

3 The fitness of hybrids between transgenic *Bacillus thuringiensis* (Bt) *B. napus* and wild relatives will be assessed in laboratory studies comparing the seed production, seed germination and seedling development of hybrids between either transgenic or non-transgenic *B. napus* and wild relatives.

4 To evaluate potential effects of transgene flow on insect species diversity, the insect fauna will be surveyed in *B. napus* fields and on wild Brassicaceae in surrounding areas. Moreover, in the laboratory, several herbivorous insect species will be placed on transgenic or non-transgenic hybrids between *B. napus* and wild Brassicaceae in single- as well as multiple-species experiments. Developmental time and survival of the insects will be recorded.

5 The results of these experiments will be useful to develop a testing procedure for the assessment of consequences of gene flow from transgenic crops to wild relatives.

Keywords: *Brassica napus*, gene flow, herbivory, hybridisation, introgression, risk assessment, transgenic plants

Bulletin of the Geobotanical Institute ETH (2001), 67, 89–95

Introduction

Hybridisation between cultivated *Brassica napus* and its wild relatives has been identified as a major risk factor associated with the large scale commercial release of transgenic crops (Crawley *et al.* 1993). Hybridisation is a possible escape route for a transgene into natural populations (Ellstrand & Hoffman 1990; Hancock *et al.* 1996; Rissler & Mellon 1996). The introgression of foreign genes constitutes an economic as well as an environmental threat. Introgression might lead to plants with enhanced weediness (Ellstrand & Hoffman 1990) requiring higher expenses to control. Introgression might also disrupt natural ecosystem functioning with possible repercussions on associated organisms. This could possibly lead to species extinction and, therefore, to loss of biodiversity (Daniels & Sheail 1999).

Theory indicates that introgression of a foreign gene depends on gene flow and on the relative selective advantage of the foreign gene (Manasse 1992). In case of a transgene the selective advantage depends on the selective value of the transgenic trait (Pohl-Orf *et al.* 1998). For the risk assessment of transgenic plants, knowledge on both gene flow and the relative selective advantage of the transgene is required (Manasse 1992).

Considering the high proportion of hybrids present in floras of various regions, gene flow between different plant species or even genera seems to be a common phenomenon (Ellstrand *et al.* 1996; Stace 1975). Experience with conventionally bred crop indicates that hybridisation between crop plants and wild relatives has occurred spontaneously (Ellstrand *et al.* 1999; Raybould & Gray 1993). However, the frequency of hybridisation is not evenly distributed within the plant kingdom. Hybridisation is common within some groups of plants and rare in others (Daniels & Sheail 1999).

B. napus (n=19) is an amphidiploid hybrid between *B. oleracea* (n=9) and *B. rapa* (n=10) (Song *et al.* 1988). Many studies have reported spontaneous hybridisation between *B. napus* and closely related Brassicaceae species (Table 1). Hybrids with other Brassicaceae species, such as *B. nigra*, *Diplotaxis erucoides*, *D. muralis*, *D. tenuifolia*, and *Sinapis alba* have been achieved by manual pollination and/or by techniques such as ovule or embryo culture (Scheffler & Dale 1994).

There is consensus that gene flow from transgenic *B. napus* to wild relatives will occur (Lefol *et al.* 1996; Davenport *et al.* 2000). However, it is unknown how fast the process will be since observed rates of hybridisation between *B. napus* and wild relatives vary greatly among studies (Table 2). Besides the amount of overlap in flowering time these differences may be due to differences in spatial structure (Landbo *et al.* 1996). Ellstrand *et al.* (1989) showed that the size of the pollen source relative to the pollen sink plays an important role for the gene flow rate. Therefore, gene flow may be greater in isolated populations consisting of only few plants that receive large relative amounts of pollen from *B. napus*.

Even if gene flow from transgenic *B. napus* to wild relatives under crop growing conditions is a rare event, ecological consequences will primarily depend on the fitness of hybrids, which may be modified by the transgene. Hardly anything is known on the fitness of hybrids between transgenic *B. napus* and wild relatives. For transgenic *Bacillus thuringiensis* (Bt) *B. napus*, Stewart *et al.* (1997) found increased fitness under insect selection pressure in the field. It is very likely that hybrids between Bt *B. napus* and wild relatives will under certain circumstances also have an increased fitness due to the Bt transgene.

Table 1. Brassicaceae species occurring in Switzerland for which spontaneous hybridisation with *Brassica napus* has been detected (offspring of seeds from these species being hybrids)

Species	Bloom in Switzerland	Reference
<i>Brassica juncea</i>	June to September	Scheffler & Dale (1994)
<i>Brassica oleracea</i>	April to May	Chèvre <i>et al.</i> (1998)
<i>Brassica rapa</i>	April to May	Jørgensen & Anderson (1994) Scheffler & Dale (1994) Mikkelsen <i>et al.</i> (1996) Hauser <i>et al.</i> (1998)
<i>Eruca sativa</i>	May to August	Schulte & Jacot (1994)
<i>Hirschfeldia incana</i>	June to September	Lefol <i>et al.</i> (1996)
<i>Raphanus sativus</i>	May to August	Schulte & Jacot (1994)
<i>Raphanus raphanistrum</i>	May to October	Darmency <i>et al.</i> (1998)
<i>Sinapis arvensis</i>	April to June	Schulte & Jacot (1994)

Table 2. Frequencies of hybridisation between *B. napus* and *B. rapa* (hybrid percentage of the offspring germinating from *B. rapa* seeds) found in studies in different European countries

Sampled population	% hybrid offspring	Reference
<i>B. napus</i> and <i>B. rapa</i> sown as a 1:1 seed mixture in 8-m ² plots	13	Jørgensen & Andersen (1994)
Individuals of <i>B. rapa</i> planted at intervals of 25 m in a <i>B. napus</i> field	93	Jørgensen & Andersen (1994)
Wild <i>B. rapa</i> in a <i>B. napus</i> field	60	Jørgensen & Andersen (1994)
Wild <i>B. rapa</i> in <i>B. napus</i> fields or at ruderal sites	0 to 3	Landbo <i>et al.</i> (1996)
Wild <i>B. rapa</i> next to two large <i>B. napus</i> fields	0.4 and 1.5	Scott & Wilkinson (1998)
Wild <i>B. rapa</i> (89 populations)	~0	Wilkinson <i>et al.</i> (2000)

Research plan: questions and methods

In our project, we will investigate the spatio-temporal patterns of co-occurrence of cultivated *B. napus* and wild relatives in Switzerland and determine the frequency of hybrids. We will also survey the insect biodiversity in commercial *B. napus* fields and on wild relatives. The fitness of hybrids between transgenic *B. napus* and wild relatives as well as their impact on associated insect species will be determined in climate chamber trials.

PART A. FIELD STUDIES

Which wild relatives co-occur spatially and temporally with cultivated B. napus in two B. napus production areas of Switzerland?

Spatial and temporal co-occurrence of related species is a crucial prerequisite for hybridisation. The family of Brassicaceae includes 167 wild or adventive species in Switzerland, of which around 65% occur in the "Mittelland" region, where *B. napus* is cultivated (Lauber & Wagner 1996). With the exception of *B. rapa*,



Fig. 1. *Raphanus raphanistrum* in a *B. napus* field in the "Rafzerfeld" (photograph: M. Meier)

all Brassicaceae species known to hybridise spontaneously with *B. napus* (Table 1) are found in the cultivation area of *B. napus* (Lauber & Wagner 1996), although *H. incana* and *E. sativa* are unlikely to be found in the study areas (see below) since both species prefer particularly warm habitats.

The purpose of this part of the field study is to determine which of the potentially co-occurring Brassicaceae species are indeed frequently found in close proximity to *B. napus* fields and flower at the same time, so that they are potential outcrossing partners for *B. napus* in Switzerland (Fig. 1).

How big is gene flow from commercially grown B. napus to wild Brassicaceae species in two B. napus production regions in Switzerland?

Assuming that the spread of transgenic crops and their hybrids with wild relatives can be

modelled in the same manner as for genetically unmodified crops (Raybould & Gray 1993) gene flow rates between conventionally grown *B. napus* and wild relatives can provide an estimate of transgene flow as needed for the risk assessment of transgenic *B. napus* (see Bergelson *et al.* 1998 for restrictions). Gene flow rates will be inferred from the natural genetic structure (Ellstrand 1992).

What is the pollinator and herbivore biodiversity on the wild relatives of B. napus and in the conventional cultivated B. napus fields?

Besides wind, pollinating insects play an important role for the dispersal of pollen and may therefore facilitate hybridisation. In addition, pollinators might be affected as non-target insects by transgenic Bt *B. napus* and transgenic hybrids. To date little is known about pollinators occurring in *B. napus* and wild relative species.

Herbivores feeding on *B. napus* are well known (König & Heitefuss 2000), whereas this is much less the case for herbivores found on wild relatives. The herbivores associated with wild relatives will be of importance for the invasive potential of transgenic insecticidal hybrids.

Methods

The occurrence of wild Brassicaceae species will be determined in the surroundings of conventional *B. napus* fields in two oilseed production areas in Switzerland, "Rafzerfeld" (Canton of Zurich) and "Siggenthal" (Canton of Aargau). The identified plants will be marked using differential GPS. Phenological development and health status of the marked plants will be monitored until seed harvest. The seeds will be collected for molecular analysis of the progeny and for the climate chamber experiments.

During monitoring of the wild Brassicaceae species, the plants will also be checked for presence and abundance of herbivores and pollinators. Herbivore and pollinator biodiversity in the cultivated *B. napus* fields will be surveyed with sweep net sampling and yellow pan sampling techniques. Four fields (1–2 ha) will be surveyed in the production area “Rafzerfeld”, and two fields (0.75 and 1.2 ha) in the production area “Siggenthal”. Sampling will be carried out for at least two years in the same production areas. Laboratory colonies of at least one lepidopteran, one coleopteran, and one homopteran species collected during the field ecological studies will be established for the climate chamber trials.

PART B. CLIMATE CHAMBER TRIALS

Does the introduction of an insecticidal transgene like Bt improve hybrid fitness?

Gene flow from crops to wild relatives has a dual effect on biodiversity, affecting the plant species itself and the organisms associated with these plants and their hybrids. In the case of an insecticidal transgenic trait the interactions between transgenic hybrids and associated insects have important implications for the invasive potential of a plant.

Often the F1 generation of hybrids shows low survival. The introduction of an insecticidal transgenic trait like Bt might improve hybrid fitness substantially. However, the effect of Bt on plant fitness will likely differ between various wild species because wild relatives differ strongly in their content of secondary compounds such as glucosinolates and erucic acid, which play a role in defence against herbivory and the attraction of pollinators. In order to obtain information on the invasive capacity, we will carry out insect feeding and competition trials with different herbivores and transgenic hybrids between *B.*

napus and wild relatives. Performance of insects and plants will be monitored.

Methods

Seeds of the wild relative species harvested during the field studies will be germinated, and seedlings will be grown in the laboratory until bloom. The flowers of these plants will be cross-pollinated by hand with pollen from transgenic Bt *B. napus* plants or isogenic Bt-free *B. napus*. Seeds from the resulting F1 plants will be cultivated, and plant fitness parameters such as seed production, seed germination rates, and plant development time will be recorded. Successful hybridisation will be verified by screening the F1 progeny with different molecular markers (AFLP markers from the A- and C-genome of *B. napus*, inter-microsatellite markers from the C-genome of *B. napus*, and cDNA markers specific to the plastids of *B. napus*). This part of the project will be accomplished in collaboration with Dr. Rikke Bagger-Jørgensen in her laboratory at Risø National Laboratory, Roskilde, Denmark.

For the insect trials, at least one coleopteran, one lepidopteran and one homopteran species will be tested. In the single species experiments, neonates of one of the three herbivore species will be placed on transgenic and non-transgenic hybrids. In the multiple species experiments neonates of two or three species will be placed simultaneously on transgenic and non-transgenic hybrids. In both experiments developmental times and survival will be recorded.

Relevance of the project

This project is concerned with the ecological consequences of gene flow from transgenic *B. napus* to wild relatives and thereby addresses

a problem of national and international importance. With the prospect of the commercial release of transgenic *B. napus* in Europe, concern about transgene flow from crops to wild relatives has intensified and interspecific gene flow from *B. napus* to weedy species is currently discussed within the ESF Scientific Programme "Assessing of the Impacts of Genetically Modified Plants (AIGM)".

Even though effects of transgenic crops that hybridise with wild relatives have to be assessed on a crop-by-crop and construct-by-construct basis (Raybould & Gray 1993), the project will help to establish a model system for the pre-release safety testing of transgenic plants. Further, results and experiences gained from the field ecological studies may be relevant for the monitoring of transgenic *B. napus* after commercial release.

Funding

This project is funded by a grant from the Swiss Federal Institute of Technology (ETH Zürich).

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Received 23 March 2001

Revised version accepted 24 May 2001

